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Abstracts

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EUROPEAN SOCIETY OF HUMAN GENETICS

European Human Genetics Conference

June 8 - 11, 2013
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Abstracts

European Society of Human Genetics

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Future European Human Genetics Conferences

European Human Genetics Conference 2014, joint with EMPAG 2014
Milano, Italy
May 31 – June 3, 2014

European Human Genetics Conference 2015
Glasgow, United Kingdom
June 6 – 9, 2015

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ESHG Plenary Lectures

PL1.1
Integrating chromosome structure and function during X-chromosome inactivation

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Using the mammalian process of X-chromosome inactivation as a model, we are interested in understanding how the differential treatment of identical DNA sequences in the same nucleus can be achieved during early mammalian development. The establishment of X inactivation involves a complex locus, the Xic, which produces the non-coding Xist RNA that is the trigger for chromosome-wide silencing. X inactivation is also accompanied by numerous epigenetic modifications that ensure stability and heritability of the inactive state. By investigating the regulatory landscape of the Xic locus using chromosome-conformation capture technologies and super-resolution microscopy, we recently uncovered a new level of chromosome folding into topologically associating chromosome domains (TADs), each spanning hundreds of kilobases (Nora et al, 2012). TAD organization was shown to be highly conserved, genome-wide phenomenon in mammals (Dixon et al, 2012). Sequences within TADs tend to interact more frequently and may thus provide a scaffold for privileged interactions between genes and their regulatory sequences. We demonstrated that within the Xic, TADs enable the precise coordination of gene expression dynamics during early differentiation and also underlie the partitioning of epigenomic landscapes, such as histone modifications. In our recent work we have further explored the functional relevance of TADs using physical modeling, as well as genetic engineering approaches at the *Xic* locus. We have also investigated the extent to which TAD organization is predictive of coordinated gene expression and epigenomic dynamics at the genome-wide scale.

Dixon, J.R., Selvaraj, S., Yue, F., Kim, A., Li, Y., Shen, Y., Hu, M., Liu, J.S., and Ren, B. (2012). Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature 485, 376-380

Nora, E.P., Lajoie, B.R., Schulz, E.G., Giorgetti, L., Okamoto, I., Servant, N., Piolot, T., van Berkum, N.L., Meisig, J., Sedat, J., Barillot E., Blüthgen N., Dekker J.* and Heard E*. (2012). Spatial partitioning of the regulatory landscape of the X-inactivation centre. Nature 485, 381-385.

E. Heard: None.

PL1.2
Signaling transcription factors explode dogmas in brain development and disease

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The concept of homeoprotein transduction, first proposed in 1991, is now well established in several biological systems. Because homeoprotein signaling is active in plants and animals it is proposed that it has predated the separation between animals and plants and is thus very ancient. This may explain why the basic phenomenon of homeoprotein transduction is so minimalist, requiring no specific receptors or specialized transduction pathways. Indeed complexity has been added in the course of evolution and the conservation of homeoprotein transduction can be discussed in the context of its synergy with bona fide signaling mechanism that may have added robustness to this primitive cell communication device. The same synergy possibly explains why homeoprotein signaling is important both in embryonic development and in adult functions fulfilled by signaling entities (e.g. growth factors) themselves active throughout development and in the adult. Several functions have been identified, including morphogenesis, axon and cell guidance and plasticity of the cerebral cortex. In addition some homeoproteins have been used as therapeutic proteins in animal models of human diseases including glaucoma and Parkison disease. Although it is clear that many questions are still in want of answers, it appears that the sequences responsible both for secretion and internalization are in the DNA-binding domain and very highly conserved among most homeoproteins. On this basis, it can be proposed that this signaling pathway is likely to imply as many as 200 proteins that participate in a myriad of developmental and physiological pathways.

PL1.3
Life-threatening infectious diseases of childhood: single-gene inborn errors of immunity?

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"The hypothesis that inborn errors of immunity underlie infectious diseases is gaining experimental support. However, the apparent modes of inheritance of predisposition or resistance differ considerably between diseases and between studies. A coherent genetic architecture of infectious diseases is lacking. We suggest here that life-threatening infectious diseases in childhood, occurring in the course of primary infection, result mostly from individually rare but collectively diverse single-gene variations of variable clinical penetrance, whereas the genetic component of predisposition to secondary or reactivation infections in adults is more complex. This model is consistent with (i) the high incidence of most infectious diseases in early childhood, followed by a steady decline, (ii) theoretical modeling of the impact of monogenic or polygenic predisposition on the incidence distribution of infectious diseases before reproductive age, (iii) available molecular evidence from both monogenic and complex genetics of infectious diseases in children and adults, (iv) current knowledge of immunity to primary and secondary or latent infections, (v) the state of the art in the clinical genetics of non-infectious pediatric and adult diseases, and (vi) evolutionary data for the genes underlying single-gene and complex disease risk. With the recent advent of new-generation deep resequencing, this model of single-gene variations underlying severe pediatric infectious diseases is experimentally testable."

PL2.1
Mutations of TCF12, encoding a basic-helix-loop-helix partner of TWIST1, are a frequent cause of coronal craniosynostosis

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Craniosynostosis is the premature fusion of cranial sutures. It is a heterogeneous disorder with a prevalence of approximately 1 in 2,200 and in ~22% of cases, a specific genetic cause can be identified. This includes mutations of TWIST1, which encodes a class II basic helix-loop-helix (bHLH) transcription factor, causing Saethre-Chotzen syndrome, typically associated with coronal synostosis. We exome sequenced 7 patients with bilateral coronal synostosis, identifying loss-of-function mutations in TCF12 in 3 cases. TCF12 encodes a class I E-protein that heterodimerizes with class II bHLH proteins such as TWIST1. Further sequencing of individuals with craniosynostosis identified a total of 38 heterozygous TCF12 mutations in 347 unrelated samples. Furthermore, 14 cases arose de novo. The mutations predominantly occurred in patients with coronal synostosis, accounting for 32% and 10% of subjects with bilateral and unilateral pathology, respectively. Comparing surgical trajectory of TCF12-mutation positive patients with other genotypes reveals a favourable post-operative course. 14% had associated learning disability. A significant level of non-penetrance of craniofacial features (53%) was found in 34 mutation-positive individuals from 23 families also tested. Relevant cephalometric measurements were normal and there was no evidence of somatic mosaicism. TCF12 lies in a region of strong linkage disequilibrium and initial haplotype investigation demonstrates phe-

notypic variability may be due to genotypes that protect from or predispose to craniosynostosis. TCF12 and TWIST1 act synergistically in a transactivation assay, and mice doubly heterozygous for loss-of-function mutations have severe coronal synostosis, demonstrating that normal coronal suture development critically depends on heterodimer dosage.

V.P. Sharma: None. **A.L. Fenwick:** None. **M.S. Brockop:** None. **S.J. McGowan:** None. **J.A.C. Goos:** None. **A.J.M. Hoogeboom:** None. **A.F. Brady:** None. **O. Jeelani:** None. **S. Lynch:** None. **J.B. Mulliken:** None. **D.J. Murray:** None. **J.M. Phipps:** None. **E. Sweeney:** None. **S.E. Tomkins:** None. **L.C. Wilson:** None. **S. Bennett:** None. **R.J. Cornall:** None. **J. Broxholme:** None. **A. Kanapin:** None. **D. Johnson:** None. **S.A. Wall:** None. **P.J. van der Spek:** None. **I.M.J. Mathijssen:** None. **R.E. Maxson:** None. **S.R.F. Twigg:** None. **A.O.M. Wilkie:** None.

PL2.2

C-terminal deletions of the *AUTS2* locus cause distinct syndromic features and cognitive impairment

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Translocations involving 7q21.22 have been associated with autism and intellectual disability (ID). Discordant evidence has suggested that these clinical phenotypes might be driven by a number of genes (*AUTS2, WBSCR17, CALN1*). Here, we examined whether *AUTS2* disruptions are causal for neurocognitive defects.

An international cohort of ~50,000 patients and 16,000 controls was examined for CNVs in *AUTS2* by array CGH and we performed detailed phenotypic analyses. 5'RACE experiments were performed to test for alternative transcripts in human brain. A zebrafish knock down model was generated to test the potential of different *AUTS2* splice isoforms to induce some of the anatomical phenotypes seen in patients.

We found 44 *AUTS2* deletions. Microdeletions disrupting the coding sequence were causal to a complex syndromic ID/autism phenotype including short stature, microcephaly, cerebral palsy and distinct facial dysmorphisms. The exonic *AUTS2* deletions occur with a frequency comparable to *NSD1* deletions causing Sotos syndrome. We also discovered a novel, highly conserved, C-terminal *AUTS2* isoform in human brain. Clinical and functional studies showed this isoform to be a key contributor to the human phenotype because (a) patients with C-terminal deletions exhibited significantly more severe and pleiotropic aspects of *AUTS2* syndrome, and (b) the significant microcephaly and craniofacial defects seen in *auts2* knockdown zebrafish embryos were rescued by this C-terminal isoform.

We identified a hitherto unappreciated syndromic phenotype caused by deletions in *AUTS2,* required for both brain and craniofacial development. The C-terminal portion of the gene contributes significantly to the phenotype, demonstrating how transcriptional complexity can underpin human pathology.

E. Voorhoeve: None. **G. Beunders:** None. **C. Golzio:** None. **L. Pardo:** None. **J. Rosenfeld:** A. Employment (full or part-time); Significant; Perkin Elmer inc.. **M. Talkowski:** None. **I. Simonic:** None. **A. Lionel:** None. **S. Vergult:** None. **R. Pyatt:** None. **J. van de Kamp:** None. **A. Nieuwint:** None. **M. Weiss:** None. **P. Rizzu:** None. **D. Posthuma:** None. **L. Verwer:** None. **H. Meijers-Heijboer:** None. **B. Menten:** None. **G. Mortier:** None. **S. Scherer:** None. **E. Eichler:** F. Consultant/ Advisory Board; Modest; Pacific Biosciences, SynapDx, and DNAnexus. **S. Girirajan:** None. **N. Katsanis:** None. **A. Groffen:** None. **E. Sistermans:** None.

PL2.3

MED4: a suicide gene to explain low penetrance in retinoblastoma patients

C. Dehainault[1], *A. Garancher*[2], *L. Castéra*[1], *I. Aerts*[3], *F. Doz*[3], *L. Lumbroso*[4], *R. Montes-de-Oca*[5], *G. Almouzni*[5], *D. Stoppa-Lyonnet*[1,6], *C. Pouponnot*[2], *M. Gauthier-Villars*[1], ***C. Houdayer***[1,6];
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Monoallelic germline loss of function mutations in the *RB1* gene predispose to a high risk of bilateral retinoblastoma, following loss of heterozygosity of the second *RB1* allele in the tumor. Surprisingly enough, complete germline *RB1* deletions are associated with low or no tumoral risk . This low penetrance might be explained by the homozygous contiguous deletion of an unknown gene adjacent to *RB1*, essential for survival when LOH occurs and without which the *RB1* -/- cell cannot survive.

To test this hypothesis, we mapped an entire germline *RB1* deletion interval in an unaffected carrier and extended this analysis to a series of tumors carrying two full *RB1* deletions. By comparing the mapped germline and somatic intervals that included or excluded the gene, respectively, and by eliminating genes with poor expression in retinoblastoma cells, we selected two gene candidates : *NUDT15* and *MED4*. We tested our 'suicide gene' hypothesis by downregulating *MED4* and *NUDT15* in two retinoblastoma cell lines. Briefly, we ruled out *NUDT15* as a candidate gene. In contrast, reduced *MED4* expression decreased cell proliferation and anchorage independent growth and induced apoptosis as measured by FACS analysis using caspase 3 as a marker. Together our results suggest that retinoblastoma *RB1* -/- cells cannot bear the loss of *MED4* expression, thereby explain the low penetrance in patients with deleted *RB1*. These new results may offer novel perspectives for therapeutic intervention adapted to patients with cancers where *RB1* is inactivated.

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PL2.4

Van Maldergem syndrome is caused by defective cadherin receptor-ligand interactions leading to dysregulation of neuroprogenitor cell proliferation and differentiation

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The orchestration of proliferation and differentiation of neuronal stem cells prior to their radial migration to the cerebral cortex is central to brain development in humans. Periventricular neuronal heterotopia (PH), the mislocalisation of grey matter centrally within the brain, can indicate a failure of neuronal progenitors to negotiate some aspect of this developmental process. Using combinations of autozygosity mapping and exome sequencing, bi-allelic mutations in genes encoding a pair of giant cadherin proteins that operate as a receptor-ligand pair are shown to lead to van Maldergem syndrome (VMS; MIM 601390) a recessive multiple malformation syndrome in humans characterized by intellectual disability, craniofacial, auditory, renal, skeletal and limb malformations in addition to a partially penetrant PH phenotype. In 6 unrelated families with the disorder both truncating and missense mutations were characterized, indicating a loss of function mechanism in the genesis of VMS. To understand the mechanism behind compromised cadherin receptor-ligand engagement in the genesis of impaired neuronal migration in the mammalian brain, we employed in utero shRNA directed knockdown of both genes in mouse embryos. A reduction in expression of both genes resulted in an increase in neuroprogenitor cell number combined with a block in differentiation of neuronal precursors, resulting in the heterotopic accumulation of cells in the subcortex. These findings, which are reminiscent of the human phenotype, underscore the existence of links between neuroepithelial layer integrity and the control of proliferation and differentiation of neuronal precursors during human cortical development.

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PL2.5
BMN111, a CNP analogue, potential novel investigational therapy for achondroplasia

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Achondroplasia (ACH), the most common form of dwarfism, is an inherited autosomal dominant chondrodysplasia caused by a gain of function mutation in the fibroblast growth factor receptor 3 (FGFR3). Among the possible targets to antagonize FGFR3 signaling, we chose the C-type natriuretic peptide (CNP) strategy. CNP acts as a key regulator of longitudinal bone growth by down-regulating the MAPK pathway. The constitutive activation of this same pathway, due to a FGFR3 gain-of-function mutation, is the cause for impaired bone growth in ACH patients.
We characterized the pharmacological activity of BMN 111, a CNP analogue peptide that has an extended plasma half-life compared to that of the native CNP due to its resistance to neutral endopeptidase degradation. In ACH growth plate chondrocytes, we demonstrated that BMN 111 decreased MAPK pathway activation. In Fgfr3Y367C/+ mice mimicking ACH, daily subcutaneous administration of BMN 111 led to the attenuation of the dwarfism phenotype. We observed a significant increase in the axial and appendicular skeleton length with improvements in ACH related clinical features including flattening of the skull, increase in the size of the paws and digits, straightening of the tibias and femurs. A rescue of the height and architecture of the different zones of the growth plate was also observed. BMN 111 treatment led to the largest improvement in skeletal parameters observed to date in an Fgfr3 mouse model.
Our results support further development of BMN 111 for the treatment for ACH.

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PL2.6
Sequencing-based GWAS on peripheral blood monocyte counts in the SardiNIA cohort

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Monocytes are a subset of white blood cells belonging to the innate immune system. They can differentiate into macrophages and dendritic cells to elicit key functions (phagocytosis and antigen presentation, respectively) at sites of inflammation. Genome-wide association studies (GWAS) have identified several loci but, in most cases, the specific causal variants and their relative impact on the immune system remain unknown.
Here, we measured circulating monocyte counts in 5,894 individuals enrolled in the SardiNIA project. Samples were genotyped using four different Illumina Beadchip arrays: OmniExpress, Cardio-MetaboChip, ImmunoChip and ExomeChip. To increase the genomic resolution, we imputed ~17 Million variants from a reference panel of 1,488 unrelated Sardinian samples sequenced at an average of 4x coverage. We searched for additive effects using a mixed association model accounting for population structure and samples' relatedness, and we observed 5 significantly associated loci (pvalue<5x10-8), of which 3 are novel. Among the two loci known to influence monocyte counts, we replicated the signal at the ITGA4 gene (r2=0.98 with previously reported SNP); moreover, fine mapping suggests a novel variant near LPAR1, which likely regulates the expression levels of the gene (r2=0.53 with a reported eQTL). Notably, the novel loci include strong biological candidates, such as a variant highly correlated (r2=0.97) with a missense mutation located in the gene coding for suppressor of activated monocytes with critical role in inflammation and tumorigenesis.
Our results suggest that sequencing-based GWAS contribute to a better understanding of the genetic basis of monocyte counts regulation and function.

M. Steri: None. **A. Mulas:** None. **M. Zoledziewska:** None. **C. Sidore:** None. **G. Pistis:** None. **F. Danjou:** None. **E. Porcu:** None. **M. Marongiu:** None. **F. Busonero:** None. **M.G. Piras:** None. **M. Lobina:** None. **F. Reinier:** None. **R. Berutti:** None. **M.F. Urru:** None. **A. Angius:** None. **C.M. Jones:** None. **D. Schlessinger:** None. **G.R. Abecasis:** None. **S. Sanna:** None. **F. Cucca:** None.

PL3.1
Deciphering Developmental Disorders Project

M. Hurles;
Cambridge, United Kingdom.

No abstract received as per date of production. Please check http://www.eshg.org/abstracts2013.0.html for possible updates.

PL3.2
Duke Fetal and Neonatal Cohort Study

N. Katsanis;
Durham, NC, United States.

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PL3.3
Ethical implications of Whole Genome Sequencing in medicine

J. Kaye;
Oxford, United Kingdom.

No abstract received as per date of production. Please check http://www.eshg.org/abstracts2013.0.html for possible updates.

PL4.1
Mendel Lecture: Rett syndrome and *MECP2* Disorders: From the Clinic to Genes and Neurobiology

H. Zoghbi;
Investigator Howard Hughes Medical Institute Professor, Baylor College of Medicine and Director, Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital, Houston, TX, United States.

Rett Syndrome, a postnatal neurological disorder that causes a broad range of severe neurological and behavioral disabilities, is fascinating in that its symptoms appear after a period of normal development and point to disturbances in most brain cells and regions. The quest for the gene revealed that the disease is caused by mutations in MECP2. The path from gene discovery to therapy, however, is not a straightforward one and requires deep understanding of pathogenic mechanisms and key molecular and anatomical determinants of various symptoms and pathologies. We have used genetic, behavioral, physiological and molecular approaches to interrogate the pathogenesis of Rett and MECP2 disorders.Recent discoveries suggest that MeCP2 is critical for many neuronal functions, especially for the ability of neurons to respond to change. Moreover, the findings reveal functions of the protein that were not suspected previously.

H. Zoghbi: None.

PL5.1
ESHG Award Lecture

F. Mitelman;
Lund, Sweden.

Immense amounts of data on neoplasia-associated chromosomal aberrations have been collected during the last three decades. Chromosome abnormalities have been described in more than 60 000 human neoplasms, and a substantial number of the cytogenetic changes have now also been characterized at the molecular genetic level. The most important fact that has emerged from the cytogenetic studies is the realization that every tumor type that has been studied in a sufficient number to permit conclusions may be subdivided on the basis of characteristic, often specific, and sometimes even pathognomonic, rearrangements. An increasing number of the recurrent aberrations, in particular balanced changes, are with remarkable specificity associated with distinctive morphological and/or clinical disease characteristics. The identification of these recurrent changes has several important implications. First, cytogenetics has become an increasingly important tool in the clinical management of cancer patients to help establish a correct diagnosis, to predict prognosis, and to select the most appropriate treatment. Second, the cytogenetic information has provided invaluable help to identify genes of importance in the carcinogenic process by focusing the attention to chromosomal sites that may harbor genes which when rearranged lead to neoplasia. So far, all balanced structural rearrangements

that have been characterized at the molecular level have been found to exert their action through one of two alternative mechanisms: deregulation of a seemingly normal gene in one of the breakpoints, or the creation of a hybrid gene through fusion of parts of two genes, one in each breakpoint. Presently, almost 3,000 recurrent balanced chromosomal rearrangements and more than 1,500 gene fusions have been reported, each of potential pathogenetic and/or clinical significance. It is reasonable to assume that the achievements obtained to date, albeit impressive, probably only represent the tip of the iceberg.

Concurrent Symposia

S01.1
Evolutionary pressures and gene expression regulation

Y. Gilad;
The University of Chicago, Department of Human Genetics, Chicago, IL, United States.

Changes in gene regulation are thought to play an important role in adaptation and speciation, notably in primates. However, the extent to which changes in different regulatory mechanisms underlie gene expression evolution is not yet known. To address this gap, we comparatively characterized gene expression (using RNA sequencing) and genetic and epigenetic regulatory mechanisms in humans, chimpanzees, and rhesus macaques, using LCLs from 8 individuals from each species. Specifically, we used ChIP-seq to obtain genome-wide profiles of H3K4me3, H3K4me1, H3K27me3 and H3K27ac histone modifications, as well as binding of RNA polymerase II. We also collected DNaseI-sequencing from the same LCLs, and by using the CENTIPEDE algorithm we measured the strength of transcription factor binding for over 200 transcription factors in all three species. These data allowed us to identify both conserved and species-specific enhancer and repressor regulatory elements, as well as characterize similarities and differences across species in transcription factor binding to these regulatory elements. We found that that transcription factor binding and histone modifications in more than 67% of regulatory elements in putative promoter regions is conserved across the three species. In turn, by considering sequence conservation at genomic locations that showed differences in regulatory mechanisms across species we were able to better understand the extent to which changes in transcription factor binding are due to either cis- or trans- differences across species. Finally, we analyzed correlations between inter-species differences in the genetic and epigenetic regulatory mechanisms and variation in gene expression levels across species using a system of logistic regression models. Assuming that these correlations do imply a causal regulatory relationship, we estimate that up to 50% of inter-species gene expression differences can be accounted for by corresponding changes in transcription factor binding and/or the presence of histone modification marks.

Y. Gilad: None.

S01.2
Chromosomal rearrangements and gene expression

A. Reymond;
Lausanne, Switzerland.

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S01.3
Variation in Gene Regulation, Chromatin States and Protein Levels across Human Individuals and Populations

M. Kasowski[*1], *S. Kyriazopoulou-Panagiotopoulou*[*2], *F. Grubert*[*1], *J. B. Zaugg*[*1], *A. Kundaje*[*2,3], *L. Wu*[*1], *S. Candille*[*1], *Y. Liu*[4], *L. Jiang*[*1], *D. Xie*[*1], *A. Boyle*[1], *Q. Zhang*[1], *F. Zakharia*[1], *D. V. Spacek*[1], *J. Li*[1], *L. M. Steinmetz*[1,5], *J. B. Hogenesch*[6], *M. Kellis*[3], *S. Batzoglou*[2], *H. Tang*[1], ***M. Snyder****[1], *contributed equally;
[1]*Department of Genetics, Stanford University School of Medicine, Stanford, CA, United States,* [2]*Department of Computer Science, Stanford University, Stanford, CA, United States,* [3]*Department of Computer Science, Massachusetts Institute of Technology, Cambridge, MA, United States,* [4]*Department Of Chemistry, Stanford University, Stanford, CA, United States,* [5]*Genome Biology EMBL Heidelberg, Heidelberg, Germany,* [6]*Department of Pharmacology, University of Pennsylvania, Philadelphia, PA, United States.*

The vast majority of disease-associated variants lie outside protein-coding regions, suggesting that variation in regulatory regions may play a major role in disease predisposition. We mapped differences in transcripton factor binding and chromatin states using six histone modifications, cohesin, Pol2 and CTCF in lymphoblastoid lines from 10-19 individuals of diverse ancestry. We find extensive signal variation in regulatory regions as well as switches of chromatin state across individuals, most frequently between active and repressed states. Enhancer activity is particularly diverse among individuals, and is strikingly divergent across populations despite a lack of similar structure in gene expression. Population specific enhancer activity often correlates with genetic divergence and is associated with signals of positive selection. Consistently, transcription factor binding and chromatin marks show strong inheritance in trios. To understand how gene regulation corrlates with protein levels, we also measured protein levels in LCL lines of 90 individual, We found that protein levels are also heritable and the approximately one half of the loci that control protein levels are independent of those that affect gene

expression Overall, our results provide fundamental insights into molecular differences in humans and how they are genetics controlled.

M. Kasowski*: None. **S. Kyriazopoulou-Panagiotopoulou*:** None. **F. Grubert*:** None. **J.B. Zaugg*:** None. **A. Kundaje*:** None. **L. Wu*:** None. **S. Candille*:** None. **Y. Liu:** None. **L. Jiang*:** None. **D. Xie*:** None. **A. Boyle:** None. **Q. Zhang:** None. **F. Zakharia:** None. **D.V. Spacek:** None. **J. Li:** None. **L.M. Steinmetz:** None. **J.B. Hogenesch:** None. **M. Kellis:** None. **S. Batzoglou:** None. **H. Tang:** None. **M. Snyder*:** Other; Modest; founder and member of the SAB for Personalis.

S02.1

The search for bone and joint genes, and what to do with them?

***A. G. Uitterlinden**[1,2,3];*

[1]Department of Internal Medicine, Erasmus MC, Rotterdam, Netherlands, [2]Department of Clinical Chemistry , Erasmus MC, Rotterdam, Netherlands, [3]Departments of Epidemiology, Erasmus MC, Rotterdam, Netherlands.

Height, osteoporosis (OP) and osteoarthritis (OA) have strong genetic influences and efforts try to identify the causal genes. Knowledge of these gene variants can help in understanding biology of the disease process and benefit development of interventions, but also have potential for diagnostics. Genome-Wide Association studies (GWAS) builds upon (1) human genetic variation, (2) genotyping technology, (3) bio-banks, and (4) collaboration among researchers in consortia. For height this is the GIANT consortium, for osteoporosis GEFOS/GENOMOS, and for osteoarthritis TREAT-OA.
GWAS has proven to be widely successful and for height, OP, OA, and Paget's Disease several GWAS have been published. For example, 80 loci have been identified for bone mineral density (BMD), ~600 loci for height, and several loci for Paget's and OA. Please note that a) GWAS identifies DNA variants rather then genes, and b) the effects per variant are generally modest, e.g., with Odds Ratios ranging from 1.1 -1.7, and explained variance of combined common variants, e.g., for height being 25% and for BMD being 3-7 %. While dozens of new loci have been discovered in GWAS, translation takes more time because a) discoveries are recent, b) there are many to choose from, and c) the conservative nature of "non-hypothesis-free" scientists.Together with studies on rare genetic syndromes, the GWAS approach clarifies the genetic architecture of the skeleton and bone and joint disease. GWAS based on SNP arrays are assessing only a small part, i.e., 0.2%, of the base pairs constituting the human genome. Next Generation Sequencing technology and exome arrys now allows to asses all coding parts of the genome (~5% of bp) and even the majority of base pairs by full-genome sequencing (~95% of bp). These approaches are now underway in Mendelian and complex disease, and for the latter again involve association approaches in large consortia.

A.G. Uitterlinden: None.

S02.2

Inflammatory bowel disease: From genes to clinical impact?

***C. A. Anderson**;*

The Wellcome Trust Sanger Institute, Hinxton, United Kingdom.

163 independent genomic regions have now been associated with inflammatory bowel disease (IBD), an immune-mediated disease that affects the gastrointestinal tract. I will highlight a series of analyses that, when applied across a large number of disease associated loci for a given trait, allow additional insights to be drawn regarding disease relevant cell types, pathways and evolutionary selective pressures. I will also discuss on-going projects within the International IBD Genetics Consortium to identify genetic loci associated with clinically relevant disease subphenotypes such as disease location and severity. I will finish by describing the identification of a non-coding polymorphism in FOXO3 that is significantly correlated with disease severity in IBD and other TNF-alpha driven diseases such as rheumatoid arthritis and malaria infection. I will show that this locus regulates monocyte production of TNFα and IL-10 through control of TGFβ1 production. This work highlights a pathway that could, through improved patient stratification or targeting with novel agents, contribute to the development of personalised medicine in TNFα-driven diseases.

C. Anderson: None.

S02.3

From genetics to translation in SCD

***R. Graham**;*

South San Francisco, CA, United States.

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S03.1

Lynch syndrome as a model of mutations and epimutations in cancer

***P. Peltomäki**;*

University of Helsinki, Helsinki, Finland.

The DNA mismatch repair (MMR) system is crucial for cancer avoidance through the correction of replication errors, DNA damage surveillance, and other functions. Failure to properly accomplish these functions may promote cancer development. It is therefore not surprising that inherited defects in the MMR system, MLH1, MSH2, MSH6, and PMS2 genes, underlie one of the most prevalent cancer syndromes in man, Lynch syndrome (LS), previously known as hereditary nonpolyposis colorectal cancer (HNPCC). LS is estimated to account for 5% of all colorectal cancers in the population and possibly a similar share of endometrial, ovarian, and other cancers. LS not only serves as a model for cancers arising through MMR defects and microsatellite instability, which concerns 10 - 25% of all colorectal, endometrial, and other cancers, but also highlights the significance of the epigenetic component in cancer development.
Knudson's hypothesis postulates that tumor suppressor gene inactivation requires two hits, the first of which may occur either in somatic cells, giving rise to sporadic cancer, or in the germline, giving rise to hereditary cancer. The second hit is always somatic. Cumulative evidence suggests that, besides genetic mutations, either hit may be epigenetic. Increased methylation of selected tumor suppressor gene promoters is an acquired property of many tumors developing in LS, and the patterns in part mirror changes seen in the corresponding sporadic cancers[1]. Hypomethylation of LINE-1 is often less prominent in LS tumors, in accordance with their near-diploid chromosome content[2]. Like genetic instability phenotypes, epigenetic phenotypes may be associated with a preferential inactivation or activation of certain growth-regulatory genes and pathways and thereby influence prognosis and response to therapy. Importantly, inactivation of the susceptibility genes MLH1 and MSH2 by epigenetic mechanisms may occur already in the germline. Constitutional epimutations may explain around 10% of LS-suspected families which lack MLH1 or MSH2 protein expression in tumor tissue and show no genetic mutations in MMR genes in the germline[3].
Knowledge of genetic and epigenetic alterations associated with cancer susceptibility and tumor development provides the basis for improved diagnostics, prevention and therapy in the respective cancers.
1. Lotsari JE et al., Breast carcinoma and Lynch syndrome - Molecular analysis of tumors arising in mutation carriers, non-carriers, and sporadic cases. Breast Cancer Res 14: R90 (2012).
2. Niskakoski et al., unpublished data.
3. Gylling A et al., Large genomic rearrangements and germline epimutations in Lynch syndrome. Int J Cancer 124: 2333 - 2340 (2009).

P. Peltomäki: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; ERC-FP7-232635.

S03.2

Epigenetic Programming of the Cancer Phenotype

***J. Issa**;*

Fels Institute, Temple University, Philadelphia, PA, United States.

The epigenome is reset during embryogenesis and matures around the end of development. Large scale genomic studies have now shown considerable proliferation dependent epigenome changes in aging cells (DNA methylation instability, chromatin instability). Comparison of rodent, primate and human aging shows that DNA methylation instability is conserved, depends primarily on chronologic age, and can be predicted to a certain degree by local genomic features (e.g. retrotransposons). It can therefore be argued that this epigenomic instability is a necessary result of the evolution of complex genomes that lack reprogramming capabilities in adult cells. Epigenetic instability creates gene expression variation in aging tissues that serve as an enabler of Darwinian evolution at the tissue level. Selective pressures result in cells with unique epigenetic programs that lead to diseases such as cancer or atherosclerosis. Importantly, epigenetic variation can be modulated by exposures (inflammation and perhaps diet), providing a mechanistic link between lifestyle and disease. In turn, epigenetic reprogramming could be useful for prevention and treatment of age-related pathology. In leukemias, reprogramming by DNA methylation inhibitors has gained acceptance as effective therapy for myeloid leukemias, and drugs for other epigenetic targets are rapidly proceeding towards clinical trials.

J. Issa: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Astex. F. Consultant/ Advisory Board; Modest; Astex, Janssen.

S03.3
Insights into oncogenesis from cancer predisposition genes
N. Rahman;
Institute of Cancer Research, London, United Kingdom.

Over the last 30 years nearly 100 predisposition genes associated with increased risks of cancer have been identified. These discoveries have resulted in considerable insights into the mechanisms underlying cancer causation and the many, diverse biological pathways that can be involved in oncogenesis. In this talk I will present an overview of known cancer predisposition genes and will consider the current and future clinical and scientific potential of their discovery.

N. Rahman: None.

S04.1
The role of microglia in synaptogenesis
C. Gross;
Monterotondo, Italy.

Microglia are phagocytic cells that infiltrate the brain during development and play a role in the elimination of synapses during brain maturation. Changes in microglia morphology and gene expression have been associated with autism. However, it remains unknown whether these changes are a primary cause or a secondary consequence of neuronal deficits associated with the disorder. Here we tested whether a primary deficit in microglia was sufficient to induce autism-related behavioral and functional connectivity deficits. Mice lacking the microglia chemokine receptor Cx3cr1 showed a transient reduction of microglia in the brain during the early postnatal period and a consequent deficit in synaptic pruning. Deficient synaptic pruning was associated with a failure to strengthen excitatory synaptic transmission, decreased functional connectivity across brain regions, deficits in social interaction, and increased repetitive behavior, all hallmarks of autism. These findings open the possibility that deficits in microglia function could contribute to key features of autism.

S04.2
The role of glia in neurodegenerative diseases
D. W. Cleveland;
Ludwig Institute, Univ. of California at San Diego, La Jolla, CA, United States.

The great biologists of the 19th century, especially Virchow and Bernard, established the pivotal idea that individual cells function autonomously, while being part of the whole organism. Since then many the major neurodegenerative diseases have traditionally been considered mechanistically cell autonomous, meaning that damage within a selective population of affected neurons alone suffices to produce disease. Most of the genes whose mutation is now known to cause the major neurodegenerative diseases are widely or ubiquitously expressed, however, including superoxide dismutase (SOD1) whose mutation causes an inherited form of the fatal, adult motor neuron disease ALS. Modeling in mice has demonstrated that disease mechanism is through an acquired toxicity unrelated to dismutase activity. Selective gene excision has demonstrated that toxicity is non-cell autonomous, with mutant SOD1 within motor neurons and oligodendrocytes driving disease onset, while damage within neighboring astrocytes and microglia accelerates disease progression. Non-cell autonomy is a general feature of human neurodegenerative disease, including Parkinson's and frontal temporal dementia, where disease has been demonstrated to spread from cell to cell in a prion-like manner, and in Huntington's disease (HD), an inherited disease caused by tri-nucleotide expansion.
An approach to therapy is suppression of disease causing genes following infusion of DNA antisense oligonucleotides (ASOs) to direct RNase H-dependent destruction of the target mRNA widely within the nervous system. Such an approach slows disease progression in inherited examples of ALS-like disease in rodents. Moreover, infusion of ASOs targeting huntingtin mRNA effectively lowers huntingtin levels in the striatum and cortex, the primary brain targets of HD pathology. Transient infusion into already symptomatic HD mouse models mediates a sustained reversal of disease phenotype that persists for much longer than the huntingtin knockdown. These findings establish a therapeutic strategy for sustained HD disease reversal from a "Huntingtin holiday" produced by transient ASO therapy.

D.W. Cleveland: F. Consultant/Advisory Board; Modest; Isis Pharmaceuticals.

S04.3
Molecular genetics of axon degeneration
*J. Gilley[1], L. Conforti[2], R. Adalbert[1], S. Milde[1], **M. Coleman**[1];*
[1]The Babraham Institute, Cambridge, United Kingdom, [2]University of Nottingham, Nottingham, United Kingdom.

The Slow Wallerian degeneration protein (WldS) is an aberrant protein that arose in mice and protects distal axons after injury. Ectopic expression of WldS exerts the same protective effect in transgenic rats, flies and zebrafish, and in transfected human neurons. It also preserves axons in some neurodegenerative disease models, including inherited models of peripheral neuropathy and motor neuron disease.
Structure-function studies of WldS show that its intrinsic NAD synthesizing (Nmnat) activity is critical for axon survival. Of the three normal mammalian Nmnat isoforms, only Nmnat2 has been confirmed to be in axons. Nmnat2 is essential for axon survival and has a short half-life, so axons require its constant replenishment by axonal transport. When delivery of Nmnat2 is prevented by nerve injury, or by knockdown or knockout of Nmnat2, axons degenerate or fail to grow. WldS can rescue them by replacing the Nmnat enzyme activity and does so for a prolonged period as it has a much longer half-life.
Nmnat2 lies at the head of a signaling pathway regulating axon degeneration, and this pathway implicates several genes in axon survival. Nmnat2 degradation is at least partially controlled by ubiquitination, and the Drosophila ubiquitin ligase Highwire and its mammalian orthologue Phr1 are candidates for this. Downstream of Nmnat2, we identify a rise in the metabolic intermediate NMN as a key event, and inhibition of Nampt, the enzyme catalyzing NMN synthesis, preserves injured axons. Further downstream Sarm1, a Toll-like receptor adapter, is necessary for axons to degenerate in both Drosophila and mice. Evidence of further genes regulating axon survival is emerging from screening in Drosophila and there is a strong likelihood that these functions will be conserved in mammals.
Identification of further axon survival proteins, and their organization into a pathway, should facilitate rational decisions as to the most promising targets for axon degeneration disorders.

J. Gilley: None. **L. Conforti:** None. **R. Adalbert:** None. **S. Milde:** None. **M. Coleman:** None.

S05.1
Analytical challenges of using next-generation sequencing to unlock complex disease
M. Daly;
Boston, MA, United States.

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S05.2
Diagnostic exome sequencing in genetically heterogeneous disease
L. E. L. M. Vissers;
Dept of Human Genetics, Radboud University Medical Centre, Nijmegen, Netherlands.

Exome sequencing is of great use in a diagnostic setting to establish a genetic diagnosis, especially for those diseases where the current diagnostic yield is low due to the genetic heterogeneity of the disease. The analysis and interpretation of exome data in a diagnostic setting is however challenging, and requires methods for quality control, minimizing the possibility of incidental findings but simultaneously allowing for easy and flexible interpretation of exome sequencing data for diagnosing patients.
During this presentation I will discuss results that were obtained in our diagnostic lab by analyzing patients with intellectual disability using family-based sequencing. Additionally, I will show data obtained in patients with *e.g.* blindness, deafness, ataxias and metabolic disorders that were analyzed using "gene packages", an approach in which exome sequencing is followed by a targeted analysis of known disease genes. To further expand the possibilities of diagnostic exome sequencing for mutation detection we have recently validated automatic CNV detection on exome data and compared its performance to that of high resolution genomic microarrays. This analysis shows that exome sequencing can reliably detect the large majority of pathogenic *de novo* CNVs. Together, these data provide further arguments to use exome sequencing as a first tier test for genetically heterogeneous diseases.

L.E.L.M. Vissers: None.

S05.3

Prioritizing disease-causing variation by genomic data fusion

Y. Moreau;
University of Leuven, Leuven, Belgium.

NGS has rapidly increased our ability to discover the cause of many previously unresolved rare monogenic disorders by sequencing rare exomic variation. However, after standard filtering against nonsynonymous single nucleotide variants (nSNVs) and loss-of-function mutations that are not present in healthy populations or unaffected samples, many potential candidate mutations are often retained and we need predictive methods to prioritize variants for further validation. Several computational methods have been proposed that take into account biochemical, evolutionary and structural properties of mutations to assess their potential deleteriousness. However, most of these methods suffer from high false positive rates when predicting the impact of rare nSNVs. A plausible explanation for this poor performance is that many of these predicted variants are mildly deleterious, but in no way specific to the disease of interest. We therefore propose a genomic data fusion methodology that integrates multiple strategies to detect deleteriousness of mutations and prioritizes them in a phenotype-specific manner. A key innovation is that we incorporate into our strategy a computational method for gene prioritization, which scores mutated genes based on their similarity to known disease genes by fusing heterogeneous genomic information. We also integrate haploinsufficiency prediction scores that predict the probability that the function of a gene is affected if present in a functionally haploid state. To integrate or fuse these data sources, we develop a machine-learning model using the Human Genome Mutation Database (HGMD) of human disease-causing mutations compared to three control sets: common polymorphisms and two independent sets of rare variation. Benchmarking on HGMD demonstrates that this integrative phenotype-specific variant prioritization significantly outperforms state-of-the-art predictors, such as SIFT or PolyPhen-2.

Y. Moreau: E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Cartagenia n.v..

S06.1

Identification of the gene for mixed polyposis syndrome: the end of a 50-year journey

I. Tomlinson;
Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom.

Hereditary mixed polyposis syndrome (HMPS) was first described in the early 1960s in a Jewish family from St Mark's Hospital, London. Affected individuals develop unusual, large-bowel polyps of several different types and individual polyps can have more than one morphology. Many individuals have colorectal cancer and disease dominantly inherited. After identifying several more HMPS families. all of Ashkenazi origin, we mapped the HMPS gene to a shared haplotype of ~2Mb on chr15q13.3. Sequencing found no protein-coding mutations, but we eventually found a constitutional 40kb copy number gain upstream of Gremlin 1 that is unique to HMPS patients. The HMPS duplication affects a genomic control region, leading to massive over-expression of Gremlin and relocation of its expression from the colorectal mesenchyme to the epithelium. The HMPS mutation provides a very rare example of a Mendelian condition caused by a non-coding duplication that has profound effects on gene expression. We have recently shown that HMPS tumours probably initiate with *KRAS* or *BRAF* mutation that does not cause senescence. *APC* mutations then follow, leading to dysplasia and cancer. A *Vill-Grem1* mouse recapitulates the principal aspects of the human disease.

I. Tomlinson: None.

S06.2

The role of IL7R in childhood T-cell acute lymphoblastic leukemia

J. T. Barata;
Instituto de Medicina Molecular, Lisboa, Portugal.

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive subtype of the most frequent childhood cancer. Interleukin 7 (IL-7), a cytokine produced in the bone marrow, thymus and other organs, is absolutely essential for T-cell development. However, there is evidence that IL-7 may also partake in leukemia development. We showed that IL-7 activates PI3K/Akt/mTOR signaling pathway, thereby mediating viability, cell cycle progression and growth of human T-ALL cells *in vitro* and that IL-7 can accelerate human T-ALL *in vivo*. The evidence that IL-7 can impact on leukemia maintenance drove us to evaluate whether genetic alterations involving the IL-7 receptor (*IL7R*) could exist in malignant T-cells. We eventually found that around 9% of T-ALL patients display somatic gain-of-function mutations in exon 6 of *IL7R*. The mutations create, in most cases, a *de novo* unpaired cysteine in the transmembrane to extracellular juxtamembrane region, resulting in disulfide bond-dependent homodimerization of two mutant receptors and consequent constitutive activation of downstream signaling with ensuing cell transformation *in vitro* and tumorigenic ability *in vivo*. Is the oncogenic potential of deregulated IL7R-mediated signaling restricted to mutated receptor? Our unpublished studies showing that conditional tetracycline-inducible IL7R transgenic mice eventually develop leukemia upon treatment with doxycline, suggest otherwise and indicate that continued expression of the wild type receptor can also display oncogenic potential. Overall, our results reveal that IL-7/IL-7R-mediated signaling is an important oncogenic axis in T-cell leukemia.

J.T. Barata: None.

S06.3

The role of exosomes in cancer-cell communication, dissemination, and therapy-resistence

L. O'Driscoll;
Trinity College Dublin, Dublin, Ireland.

No abstract received as per date of production. Please check http://www.eshg.org/abstracts2013.0.html for possible updates.

S07.1

iPS and their therapeutic potentiel for keratinizing disorders

D. Roop;
Aurora, CO, United States.

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S07.2

Netherton syndrome and links to eczema

A. Hovnanian[1,2,3];
[1]INSERM U781, Paris, France, [2]Department of Genetics, Necker hospital, Paris, France, [3]University Paris Descartes - Sorbonne Cite, Imagine Institute, Paris, France.

Netherton syndrome (NS) is a rare autosomal recessive orphan disease with severe skin inflammation and scaling, a specific hair shaft abnormality and constant allergic manifestations with high serum IgE levels. NS results from loss-of-function mutations in *SPINK5* (serine protease inhibitor of Kazal type 5) encoding LEKTI-1 (lympho-epithelial Kazal type related inhibitor type 5), a 15-domain protease inhibitor expressed in stratified epithelia and in Hassall's corpuscles in the thymus. *In vitro* and *in vivo* studies in murine models and in NS patients have implicated unopposed activity of kallikrein (KLK) 5, KLK7 and elastase 2 with dual consequences on skin homeostasis. The skin barrier is severely disrupted secondary to stratum corneum detachment, increased profilaggrin processing and abnormal lipid lamellae. This leads to increased skin permeability, enhanced allergen and microbe penetration which favor skin allergy and inflammation. Danger signals are also generated, resulting in the production of IL-1 beta which contributes to skin inflammation. In parallel, KLK5 activates PAR2 which leads to the activation of the NF-kappa B pathway and the production of pro-allergic (Thymic Stromal Lymphopoietin) and pro-inflammatory (TNF-alpha) cytokines. These molecules, in concert with TARC and MDC, induce skin allergy and inflammation with severe itching and systemic involvement.
These results have identified major pathways and therapeutic targets for NS. Importantly, they have also established a link with the frequent and more complex disease atopic dermatitis, which shares several common features with NS. These include enhanced proteolytic activies, skin barrier defect through filaggrin mutations, abnormal lipid composition, PAR-2 mediated allergy and inflammation. Recent functional studies reporting defective protease inhibition caused by the frequent E420K LEKTI variant, further support the notion that *SPINK5* plays a role in eczema through a complex network involving multiple genes and/or interactions with the environment, with potential therapeutic implications.

A. Hovnanian: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; GSK.

S07.3
Artificial Skin
M. del Rio[1,2,3];
[1]*Department of Bioengineering. Universidad Carlos III de Madrid, Madrid, Spain,* [2]*CIEMAT, Madrid, Spain,* [3]*Centre for Biomedical Research on Rare Diseases (CIBERER), Madrid, Spain.*

The skin is the subject of more than 400 rare inherited diseases or genodermatoses which together represent a significant part of the dermatological clinical practice. Our group is devoted to diagnostic, modeling and development of advanced therapies for some of the most relevant genodermatoses. Among that group, Epidermolysis Bullosa (EB) comprises a set of skin fragility disorders characterized by defective adherence of the epidermis to the underlying connective tissue. We have mainly focused in the recessive dystrophyc form of EB (RDEB) due to mutations in *COL7A1* coding for type VII collagen. Our studies led us to the disclosure of one of the world's most recurrent mutation, c.6257insC, affecting nearly half of the Spanish RDEB population and due to a founder effect. Recent work led us also to demonstrate revertant mosaicism within the RDEB patient population which tends to be more common than previously expected. Using optimized skin bioengineering approaches we have been able to establish skin humanized mouse models of EB suitable for cell and gene therapy testing. In addition, we have shown the feasibility of using naturally gene revertant EB cells as a source for autologous skin transplantation. My talk will also cover the use of analogous strategies, as those described for EB, to the modeling and treatment of other genodermatoses including Lamellar Ichtyosis and Xeroderma Pigmentosus as paradigmatic examples of epidermal differentiation and DNA repair defects, respectively. We hope we can contribute to the goal of having 200 new treatments for rare genetic diseases by the year 2020.

M. del Rio: None.

S08.1
Dissecting the effects of selection in the human genome: the case of immunity to infection
L. Quintana-Murci;
Institut Pasteur-CNRS, Paris, France.

Because infectious diseases have been paramount among the threats to health and survival throughout human history, natural selection is expected to act strongly on host defence genes, particularly on innate immunity genes. Searching for evidence of selection targeting innate immunity represents therefore an indispensable complement to clinical and epidemiological approaches for identifying functionally important genes involved in host immunity to infection and disease outcome. In the past years, we have initiated an evolutionary dissection of genes and pathways involved in innate immunity in humans. We focus on major families of innate immunity receptors, such as TLRs, RLRs and NLRs, and other molecules involved in the signalling pathways triggered by them, such as adaptors and effectors molecules, such as TIR-containing adaptors and IFNs. Although the biological role in host defence for some members of these families has been well characterised, the biological function of many others (e.g. most NALP members of the NLR family) remain poorly understood, with little knowledge about their immunological relevance. I will present different cases of how some of these genes have been targeted by strong purifying selection, attesting to their essential and nonredundant functions, while others evolve under much relaxed selective constraints, suggesting higher redundancy in host defence. I will also present some examples of molecules (e.g. TLR1, type III IFNs, etc.) that display geographically-restricted signatures of positive selection in specific human populations, indicating that genetic variation at these genes has conferred a selective advantage to the host. Finally, I will discuss our most recent findings about population variation in microRNA transcriptional responses to infection and the extent to which such variation is under genetic control (miR-eQTLs). More generally, I will discuss how adopting an evolutionary, population and cellular genomics approach can provide important insight into immunity genes playing a major role in host survival, and highlight host pathways and mechanisms playing an essential role in pathogen resistance.

L. Quintana-Murci: None.

S08.2
Gene dosage sensitivity and copy-number evolution
A. McLysaght;
Dublin, Ireland.

A significant subset of genes in vertebrate genomes are under dosage constraint. Duplications that disturb their abundance in the cell are deleterious and are the likely cause of pathogenicity of CNVs associated with disease. What factors influence dosage sensitivity, be it haploinsufficiency or dosage-balance? What biological processes are most likely to be dosage-sensitive? How does gene dosage sensitivity influence evolutionary patterns, and how can evolutionary analyses identify these genes?

S08.3
Evolution of vision
D. Arendt;
Heidelberg, Germany.

No abstract received as per date of production. Please check http://www.eshg.org/abstracts2013.0.html for possible updates.

S09.1
Adaptive immunodeficiency and gene therapy treatment
A. Fischer;
INSERM U768 & Unité d'Immunologie Hématologie Pédiatrique, Hôpital Necker-Enfants Malades, Université Paris Descartes ; Imagine Institute, Paris, France.

Over the last 20 years, the genetics and pathophyiology of many primary immunodeficiencies (PID) have been unraveled. This offers the opportunity to treat the most severe of these conditions by gene transfer into hematopoietic stem cells. Primary T cell immunodeficiencies appear as the best therapeutic targets because of the expected selective growth advantage conferred by gene expression into T cell precursors combined with the very long life span of T lymphocytes. Based on this rationale, ex vivo retroviral gene transfer into hematopoietic progenitors has been shown to lead to sustained (over 14 years) correction of severe combined immunodeficiency (SCID). The advent of genotoxicity events in some patients has initiated the development of modified vectors, i.e. "SIN vector" in use over the last 3 years with efficacy and safety to treat SCID as well as other PID such as the Wiskott Aldrich syndrome.

A. Fischer: None.

S09.2
Antisense therapy in SMA
A. Krainer;
Cold Spring Harbor, NY, United States.

No abstract received as per date of production. Please check http://www.eshg.org/abstracts2013.0.html for possible updates.

S09.3
Stem-cell gene therapy for the Wiskott-Aldrich Syndrome
C. Klein;
Munich, Germany.

No abstract received as per date of production. Please check http://www.eshg.org/abstracts2013.0.html for possible updates.

S10.1
Noninvasive whole genome sequencing
S. Quake;
Stanford, CA, United States.

No abstract received as per date of production. Please check http://www.eshg.org/abstracts2013.0.html for possible updates.

S10.2
Advances in embryo selection for optimizing IVF outcome
D. Wells;
Oxford, United Kingdom.

No abstract received as per date of production. Please check http://www.eshg.org/abstracts2013.0.html for possible updates.

S10.3
Newborn screening; what is possible, what do we want?
B. Wilcken;
The Children's Hospital at Westmead and University of Sydney, Sydney, Australia.

The first trial of Guthrie's test for phenylketonuria was completed in 1963; this year we celebrate 50 years of newborn screening. Mass screening of newborns has been found economically feasible for a wide variety of conditions where there is a marker compound detectable in blood. In most

developed and some less-developed countries all newborns are tested for congenital hypothyroidism, plus phenylketonuria and several other conditions. With the advent of tandem mass spectrometry in the mid-1990s over 50 disorders are now sought in many jurisdictions.
Problems encountered and not yet completely solved include: developing rational systems and criteria for selecting disorders for screening; adequate long-term follow-up to assess benefit, with cessation of programmes where benefit cannot be shown; development of national data-bases; the detection of mild phenotypes, and development of definitions to enable recognition of which cases need treatment. Poor availability of confirmatory tests and treatments are limiting factors in less-well-off countries.
The pressure to include new disorders is driven by new treatments for previously untreatable conditions, and advances in technology making screening feasible. Some of the disorders newly being screened for include several lysosomal storage disorders, severe combined immunodeficiencies and recently a trial of screening for X-linked adrenoleukodystrophy. In the future, perhaps quite soon, primary DNA mass testing may become affordable, which will pose many problems. Already we are poised for a second major expansion of screening due partly to advances in mutation-specific treatment of various kinds. This applies particularly to Duchenne muscular dystrophy. Disorders being actively considered include Rett Syndrome, Fragile X, long QT syndrome, and spinal muscular atrophy.
A major problem for the future will be re-defining the aims of newborn screening. There will be pressure to screen for adult-onset disorders and for prediction of risk. Development of economical systems to enable effective mass screening at later ages would be welcome.

B. Wilcken: None.

S11.1
Mousemodels of Down syndrome

Y. Hérault[1,2,3];
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More than 50 years after the discovery of Trisomy 21, the underlying basic genetic of the Down syndrome (DS), is still not completely understood. The current working hypothesis is based on interactions between "dosage sensitive" genes located along the HSA21 that are responsible for the complex features of the pathology.In order to identify the genes involved in Down syndrome features, we created new partial trisomies in the mouse for regions homologous to HSA21 contributing to the Aneuploid zoo of DS models. Here we will report the characterization of new mouse models for various regions of interest with a specific standardized behavioral pipeline and the identification of the cystathionine beta-synthase (Cbs) in learning and memory deficits observed in Down syndrome mouse models. Using two genetic experiments we evaluated the role of Cbs in the trisomy. First we reduced the copy number of Cbs using a knock-out allele in the Abcg1-U2af1 trisomic model. We found that specific learning and memory phenotypes were rescued in compound mice showing that Cbs is required for these phenotypes. Then we generated a conditional transgenic mouse line to direct the sole overexpression of the human CBS gene in the hippocampus and the cortex. These new transgenic mice displayed similar phenotypes to the trisomic mouse lines. Thus we conclude that Cbs gain of function in the brain is sufficient to induce learning and memory phenotypes in DS. The data generated are challenging our current knowledge on the role of Cbs in the physiopathology of the disease, highlighting cross-talks in drug targets and showing the interaction of several loci in DS cognitive traits. We will discuss how this new knowledge would reinforce the development of new therapeutic approaches to facilitate the life of DS people and might help to evaluate promising strategies.

Y. Hérault: None.

S11.2
Protein Degradation in Health and Disease

T. Hoppe;
CECAD at the Institute for Genetics, University of Cologne, Cologne, Germany.

Cellular differentiation, developmental processes, and environmental factors challenge the integrity of the proteome in every eukaryotic cell. The maintenance of protein homeostasis involves the degradation of misfolded and damaged proteins, and is essential for cellular function, organismal growth, and viability. The UPS is one major proteolytic component of the cellular proteostasis network regulating the degradation of damaged proteins that accumulate upon stress and during aging. Selective turnover of substrate proteins is initiated by covalent attachment of the conserved protein ubiquitin predominantly to internal lysine residues. This ubiquitylation step is mediated by an enzymatic cascade that involves ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin protein ligases (E3). Deubiquitylation enzymes (DUBs) modulate the size and topology of polyubiquitin chains, formed by isopeptide bonds between ubiquitin monomers. A chain of four to six ubiquitin moieties efficiently targets a substrate for degradation by the 26S proteasome. The 26S proteasome is a multi-catalytic protease complex composed of a barrel-shaped 20S proteolytic core particle (CP) and a 19S regulatory particle (RP) that translocates substrates into the 20S CP where they are degraded into short peptides.
It is commonly thought that an age-related impairment of protein degradation affects general proteostasis networks, causing enhanced accumulation of damaged proteins that can be cytotoxic and shortens lifespan (Hoppe, 2010). The activity of the 26S proteasome progressively declines during the aging process although the exact molecular aspects of this regulation have not been addressed. Beside the requirement of proteasomal integrity for normal lifespan, the identification of ubiquitin-dependent degradation pathways that specifically control the stability of lifespan regulators further reflects a key role of the UPS in the aging process. For example, different E3 ubiquitin ligases regulate the activity of the FOXO transcription factor (TF) DAF-16, which is central for insulin/insulin-like growth factor 1 (IGF-1) signaling (IIS). A novel mechanistic link between protein degradation and longevity based on deubiquitylation of polyubiquitylated substrate proteins will be discussed (Kuhlbrodt et al., 2011). Interestingly, ubiquitin-mediated degradation pathways are also involved in muscle myosin assembly and age-related muscle-degeneration (Hoppe et al., 2004; Janiesch et al., 2007; Gazda et al., 2013).
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Gazda, L., Pokrzywa, W., Hellerschmied, D., Löwe, T., Forné, I., Mueller-Planitz, F., Hoppe, T.*, and Clausen, T. (2013). The myosin chaperone UNC-45 is organized in tandem modules to support myofilament formation in C. elegans. Cell 152, 183-95.
Kuhlbrodt K., Janiesch P.C., Kevei E., Segref A., Barikbin R., and Hoppe T. (2011). The Machado-Joseph disease deubiquitylase ATX-3 couples longevity and proteostasis. Nat. Cell Biol. 13, 273-81.
Hoppe T. (2010) Life and destruction: ubiquitin-mediated proteolysis in aging and longevity. F1000 Biol Rep 2: 79
Janiesch P.C., Kim J., Mouysset J., Barikbin R., Lochmüller H., Cassata G., Krause S., and Hoppe T. (2007). The ubiquitin-selective chaperone CDC-48/p97 links myosin assembly to human myopathy. Nat. Cell Biol. 9, 379-90.
Hoppe T., Cassata G., Barral J.M., Springer W., Hutagalung A.H., Epstein H.F., and Baumeister R. (2004). Regulation of the Myosin-Directed Chaperone UNC-45 by a Novel E3/E4-Multiubiquitylation Complex in C. elegans. Cell 118, 337-49.

T. Hoppe: None.

S11.3
From bedside to bench: a Fragile X patient mutation yields insights into the functional specialization of the FXR protein family.

Z. Okray, ***B. Hassan***;
VIB and University of Leuven Medical School, Leuven, Belgium.

The most prevalent basis for FMR-1 deficiency in Fragile X (FXS) patients is epigenetic silencing mediated by promoter-proximal CGG repeat expansions. Other instances of the disease phenotype have been associated with mutations in the coding regions of the FMR-1 gene, however, only one of these mutations has been experimentally characterized.
To further investigate how coding mutations in FMR-1 can contribute to FXS etiology, we screened a small group of patients who display typical FXS symptoms, yet do not harbor CGG repeat expansions in the FMR1 locus. In one patient, we recovered a mutation - a single base insertion - in exon 14 of FMR1. The mutation alters the open reading frame, creating a novel amino acid sequence in the C-terminus, followed by a premature stop codon. We find that the novel C-terminus peptide encodes a functional, bipartite nuclear localization signal (NLS), which bears a striking resemblance to the nucleolar localization signals identified in FMR1 paralogs, referred to as the FXR1 and FXR2 proteins. This signal targets the patient FMR1 protein to the nucleolus in cultured human and rat cells. Using the D.melanogaster model, we demonstrate that the presence of the patient NLS motif on FMRP can alter its function in neurons and lead to neomorphic phenotypes in vivo. Taken together, our results provide evidence for novel changes in FMRP function conferred by this clinically relevant point mutation

Z. Okray: None. **B. Hassan:** None.

S12.1
The geneticist's perspective
A. Read;
Genetic Medicine, University of Manchester, Manchester, United Kingdom.

Genetics has two points of contact with the criminal justice system: DNA identification, and the possible role of genetic predisposition in antisocial behaviour. DNA profiling has brought much-needed objectivity into criminal investigation - but it is important to remember that the mere presence of a person's DNA at a crime scene tells us nothing about how or when that DNA got there or what, if anything, its owner was doing at the time. Scientific questions can arise when techniques are pushed to their limits to analyse small, mixed and degraded samples, while some feel that investigative techniques like familial searching raise specific ethical issues.
Once a case comes to trial geneticists have sometimes been asked to testify that a genetic variant possessed by the accused predisposes them to criminal behaviour. Might this make them less responsible for their actions? The popular press loves the idea of a 'criminal gene' - but how convincing is the evidence that XYY men or men with monoamine oxidase A deficiency are 'born criminals'? Are genetic predispositions any different from the innumerable other factors that can influence a person's behaviour? Might it be that the main reason genetic arguments are introduced into criminal trials is not to help the court judge criminals appropriately, but to to bamboozle juries with impressive-looking science?

A. Read: None.

S12.2
Narrowing down the free-will compass? A social and ethical perspective
M. Levitt;
Lancaster, United Kingdom.

Assumptions and outcomes of behavioural genetics have consequences for attributing responsibility and blame. These are not only central elements in the justice system but are also rooted in social and moral reasoning. In most legal cases so far genetic evidence on levels of MAOA expression has been assumed by defence lawyers to be of potential benefit to their clients, however, it could also be used against them. Notions of human agency, free-will and determinism, choice and control are discussed in relation to genetic and environmental defences for violent and antisocial behaviour. The implications of determinist accounts of violent crime for victims,offenders, the justice system and society as a whole are considered. The lecture will draw on both public and expert understandings. The public's understandings of the causes of criminal behaviour and attachment of blame, fairness and responsibility are critical to their acceptance of policy and practice in this area and to their role as lay jurors.

S12.3
Narrowing down the free-will compass? A legal perspective
A. Santosuosso;
Pavia, Italy.

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S13.1
Human pluripotent stem cells for modeling genetic diseases
O. Brüstle;
Bonn, Germany.

No abstract received as per date of production. Please check http://www.eshg.org/abstracts2013.0.html for possible updates.

S13.2
iPS for modelling diseases and drug screening : the example of Steinertmyotonic dystrophy
C. Martinat*, S. Nédelec, Y. Maury, J. Come, M. Peschanski;*
INSERM/UEVE UMR 861, I-STEM, AFM, Evry, France.

The lack of existing models of pathologic tissues has rendered many important questions in disease pathogenesis inaccessible. Human embryonic stem cells derived from affected embryos during a pre-implantation diagnostic (PGD), as well as the technical development to obtain human induced pluripotent stem cells generated from patients, offer the unique opportunity to have access to a large spectrum of disease-specific cell models. Disease-specific pluripotent stem cells capable of differentiation into the various tissues affected in each condition could undoubtedly provide new insights into the pathological mechanisms by permitting analysis in a human system. These new disease-specific cell models are applicable for a wide systemic mechanistic analysis ranging from functional studies at the cellular level to a large-scale functional genomics screening.
As a proof of principle, we demonstrated that PGD-derived hES cells and derivatives which, express the causal mutation implicated in the Myotonic Dystrophy type 1 (DM1), may mimic molecular defects associated to the pathology, such as the nuclear aggregation of mutant RNA.
By taking advantage of this pertinent cellular model, we identified, through a genome-wide analysis, two early developmental defects in genes involved both in myogenesis as well as in neurite formation and establishment of neuromuscular connections. These neuropathological mechanisms may bear clinical significance as related to the functional alteration of neuromuscular connections associated with DM1.
In parallel to these functional pathological studies, we developed two different approaches to identify new therapeutic strategies. The first one was based on a high content screening approach. A pilot drug screening experiment has been successfully conducted in order to identify new molecules which, due to their ability to disrupt the nuclear mutant RNA aggregation, might represent new therapeutic strategies. The second strategy used a genomics screening based on gene knockdown approach. This analysis allowed the identification of a potentially druggable target protein, inhibition of which tends to normalize molecular defects associated to DM1.
All this study demonstrated that these PGD hES–derived cell models could be relevant for wide analysis ranging from the study of the dysfunction of an inter-cellular nerve-muscle system to high-throughput functional genomics screening. Altogether, our results indicate that disease-specific hES cell lines could be used for resources driven large-scale analysis, in complement to classical approach based on the analysis of candidate gene, that could highlight the development of new therapeutic strategies.

C. Martinat: None. **S. Nédelec:** None. **Y. Maury:** None. **J. Come:** None. **M. Peschanski:** None.

S13.3
IPS cells to model genetic diseases and individual variability.
M. Brimpari[1], F. Rouhani[2], N. Kumasaka[2], a. Bradley[2], D. Gaffney[2], ***L. Vallier[3];***
[1]Cambridge University, Cambridge, United Kingdom, [2]Wellcome Trust Institute, Cambridge, United Kingdom, [3]Cambridge University and Wellcome Trust Institute, Cambridge, United Kingdom.

Human induced pluripotent stem cells are generated from somatic cells reprogrammed by overexressing transcription factors. Their pluripotency status confers upon them the capacity to proliferate indefinitely in vitro while maintaining their capacity to differentiate into a broad number of cell types. hIPSCs represent an unique opportunity for regenerative medicine since they could enable the production of patient specific cell types which are fully immuno-compatible with the original donor thereby avoiding the use of immune suppressive treatment during personal cell based therapy. In addition, hIPSCs can be generated from somatic cells isolated from patients suffering from diverse diseases. Then, the resulting "diseased" hIPSCs can be differentiated into the cell type targeted by the disease and thus provide in vitro models to perform large scale studies impossible with primary cell culture or with biopsy material. However, hIPSC lines appear to strongly vary in their capacity of differentiation and the origin of this variability remains unclear. Here we derived a panel of hIPSC lines from a diversity of healthy individuals and then defined their capacity to differentiate into the three germ layers endoderm, mesoderm and ectoderm from which all the adult organs are derived. This analysis revealed that most of hIPSC lines can differentiate efficiently into these primary tissues. However, a limited number of lines were resistant to specific type of differentiation. We then performed functional experiments associated with genome wide analysed to uncover the mechanisms involved. We observed that several aspect influence the capacity of individual hIPSC line to differentiate into specific tissues including the method of reprogramming, epigenetic memory but more importantly genetic variability. Therefore, these results suggest that hIPSCs could represent an unique system to model individual phenotypic variability in vitro.

M. Brimpari: None. **F. Rouhani:** None. **N. Kumasaka:** None. **A. Bradley:** None. **D. Gaffney:** None. **L. Vallier:** None.

S14.1
DNA repair and microcephaly

M. O'Driscoll;
Brighton, United Kingdom.

Mammalian embryonic neurogenesis is exquisitely sensitive to perturbations in cell cycle dynamics. The origins of these perturbations are multifactorial and often interconnected. Severe microcephaly can occur as a consequence of reduced cell division and/or elevated apoptosis in the developing neuroepithelium. Interestingly, defects in genes encoding centrosome, centrosomal-associated and spindle pole proteins are currently the most frequent cause of Primary Microcephaly (PM) and Microcephalic Primordial Dwarfism (MPD) syndromes in humans. The centrosome is an important microtubule-organizing centre essential for coordinating G2-M progression and normal mitotic cell division as well as the DNA damage response. Mitotic progression and segregation defects, microtubule spindle abnormalities and impaired DNA damage-induced G2-M cell cycle checkpoint proficiency have all been reported in these genetically heterogeneous patient-derived cell lines. Interestingly, congenital defects in the ATR-dependent DNA damage response (*ATR-ATRIP Seckel syndrome*) and in DNA replication licensing machinery (*Meier-Gorlin syndrome*) are also associated with centrosome abnormalities and *vice versa*, suggestive of a multifactorial but interconnected underlying pathobiology of MPD. I will present a brief overview of these defects, specifically focusing on mechanisms of genomic instability but also highlighting some unanticipated impacts of some of these defects upon signal transduction. Collectively, these findings help contribute to our evolving understanding of genotype-phenotype relationships.

S14.2
Apparently balanced chromosome rearrangements in human development

C. C. Morton[1], *J. Rosenfeld*[2], *A. M. Lindgren*[3], *S. Pereira*[3], *I. Blumenthal*[4], *C. Chiang*[4], *L. G. Shaffer*[5], *J. F. Gusella*[6], *M. E. Talkowski*[6];
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Apparently balanced chromosomal rearrangements are an invaluable biological resource for annotation of the human genome and the foundation of the Developmental Genome Anatomy Project (DGAP, dgap.harvard.edu). Implementation of next-generation sequencing of rearrangements has facilitated rapid gene discovery for structural and neurodevelopmental (NDD) disorders. Structural disorders elucidated include those from subjects with various craniofacial abnormalities and brain, ocular, auditory, kidney, and bone aberrations, with validation in subjects with overlapping phenotypes or in animal models. Validation of genes in subjects with NDD has been difficult historically but is now feasible taking advantage of convergent genomic evidence. Rearrangement breakpoints were sequenced in 38 subjects with NDD and validation performed using copy number variants (CNVs) in 19,556 cases and 13,991 controls. Genes disrupted within microdeletion syndromes included *SATB2* (2q33.1), *MBD5* (2q23.1) and *EHMT1* (9q34.3), all of which are transcription and epigenetic regulators. In a follow-up study among an international network of investigators, 65 independent 2q23.1 microdeletions were ascertained, defining the role of *MBD5* as necessary and sufficient in the 2q23.1 microdeletion syndrome. Disrupted loci in some subjects associate with a spectrum of developmental and psychiatric disorders, including mental retardation, autism, language delay, schizophrenia, psychosis, cognitive impairment, and bipolar disorder, bridging phenotypes with genetic etiologies. Genes at breakpoints novel for correlation with NDD have diverse functions from methylation and transcriptional regulation to cell signaling, cell adhesion, and nonsense-mediated decay. In addition, a number of observations were made from the sequence analysis concerning genomic architecture of constitutional rearrangements including complex events akin to chromothripsis in neoplasms, nonhomologous end joining as a predominant mechanism and the presence of inversions in derivative chromosomes at sites of rearrangement. Nucleotide level resolution of apparently balanced rearrangements through next-generation sequencing can contribute to diagnostic precision in abnormal human phenotypes leading to improved genetic counseling, and can illuminate underlying genomic molecular mechanisms.

C.C. Morton: None. **J. Rosenfeld:** A. Employment (full or part-time); Significant; Signature Genomic Laboratories. **A.M. Lindgren:** None. **S. Pereira:** None. **I. Blumenthal:** None. **C. Chiang:** None. **L.G. Shaffer:** A. Employment (full or part-time); Significant; Genetic Veterinary Services. **J.F. Gusella:** None. **M.E. Talkowski:** None.

S14.3
Post-zygotic mosaicism for large scale chromosomal aberrations among elderly people; What does it mean?

J. P. Dumanski;
Dept. of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden.

Post-zygotic, acquired with age mutations in normal cells represent understudied aspect of genome biology. Human post-zygotic mosaicism has been studied in embryos, aborted fetuses, children with developmental defects and in cancer. However, little is still known about the type and frequency of acquired genetic aberrations in normal cells from subjects in the general population, especially from cohorts that are well stratified by age. Our recent analyses suggest that such mosaicism is surprisingly common, with the highest estimate of 3.5% for elderly/old subjects being affected by various large-scale aberrations. We have now extended the above analyses and studied 1153 men using blood collected at the ages of 70.7-83.6 years from the Swedish ULSAM population-based cohort that was clinically followed for more than 20 years. We applied 2.5M-Omni Illumina chip with strict selection by genotyping quality and these 1153 samples were selected out 1217 samples originally submitted as a single batch to the genotyping facility. We used the following size of cut-offs for scoring of aberrations: gains - $\geq$ 250 kb; deletions and CNNLOH - $\geq$ 10 kb. Overall, we observed a very high frequency of post-zygotic mosaicim; 42.7% of people displayed at least one aberration with gains as the most common type of aberration. Our results illustrate the high frequency and importance of post-zygotic mosaicism in normal cells, which should be studied further for associations with various diseases.

J.P. Dumanski: None.

S15.1
Drugs and genes - an overview

A. K. Daly;
Newcastle University, Newcastle upon Tyne, United Kingdom.

Individual genetic profiles affect both efficacy of prescribed drugs and susceptibility to adverse drug reactions. Serious adverse drug reactions are generally rare but occur with a range of different prescribed drugs. These reactions can affect a number of different physiological systems and tissues in the body. Progress has been made recently in understanding both their genetic basis and underlying mechanisms. During drug development, idiosyncratic adverse reactions affecting the liver are a major reason for drug attrition during the later stages of development. They are also a rare but serious complication of treatment with a number of commonly prescribed drugs, especially antimicrobial agents. Highly significant associations with particular HLA class I and class II genotypes have been reported for drug-induced liver injury with several specific drugs, for example flucloxacillin and co-amoxiclav. Examples of these associations and possible underlying mechanisms, including how this may relate to reported HLA associations for other types of adverse drug reactions, will be considered. Not all forms of idiosyncratic drug-induced liver injury reactions show HLA gene associations. Genes relevant to the innate immune system and to drug disposition appear to represent additional risk factors for drug-induced liver injury but, as with the adaptive immune system associations involving HLA, risk factors will be drug or, in some cases, drug class dependent. Overall understanding of the genetic basis of drug-induced liver injury remains limited and our ongoing studies, which are concerned with increasing case numbers and performing further genome-wide association studies combined with exome sequencing, will be discussed.

A.K. Daly: None.

S15.2
An immunological basis for pharmacogenetic associations in the MHC

J. McCluskey*, Patricia T. Illing, Julian P. Vivian, NadineL. Dudek, Lyudmila Kostenko, Zhenjun Chen,, Mandvi Bharadwaj, John J. Miles, Lars Kjer-Nielsen, Stephanie Gras, Nicholas A. Williamson,, Scott R. Burrows, Anthony W. Purcell, Jamie Rossjohn,, Monash University and QIMR, QLD;*
The University of Melbourne, Melbourne, Australia.

Human Leukocyte Antigens (HLA) are highly polymorphic proteins that initiate immunity by presenting pathogen-derived peptides to T cells. HLA polymorphisms mostly map to the antigen (Ag)-binding cleft, thereby diversifying the repertoire of self- and pathogen-derived peptide Ags selected by different HLA allotypes. A growing number of immunologically-based drug reactions, including abacavir hypersensitivity syndrome (AHS) and carbamazepine-induced Steven-Johnson's syndrome (SJS), are associated with specific HLA alleles. However, little is known about the underlying mechanisms of these associations, including AHS, a prototypical HLA-associated drug

reaction occurring exclusively in individuals with the common histocompatibility molecule, *HLA-B*57:01,* and with a relative risk of >1000. We show that unmodified abacavir binds non-covalently to HLA-B*57:01, lying across the bottom of the Ag-binding cleft and reaching into the F-pocket where a C-terminal tryptophan typically anchors peptides bound to HLA-B*57:01. Abacavir binds with exquisite specificity to HLA-B*57:01, changing the shape and chemistry of the Ag-binding cleft, thereby altering the repertoire of endogenous peptides that can bind HLA-B*57:01. In this way, abacavir guides selection of new endogenous peptides, inducing a dramatic alteration in 'immunological self'. The resultant peptide-centric 'altered self' activates abacavir-specific T-cells, thereby driving polyclonal $CD8^+$ T cell activation and a systemic reaction manifesting as AHS. We also show that carbamazepine, a widely used anti-epileptic drug associated with hypersensitivity reactions in *HLA-B*15:02* individuals, binds to this allotype, producing alterations in the repertoire of presented self-peptides. These findings simultaneously highlight the importance of HLA polymorphism in the evolution of pharmacogenomics, perhaps providing a general mechanism for some of the growing number of HLA-linked hypersensitivities that involve small molecule drugs, but also suggesting novel pathway for induction of autoimmunity.

J. McCluskey: None.

S15.3
Genetic risk mirrors outcome of anti-TNF therapy in Multiple Sclerosis

L. Fugger;
Oxford, United Kingdom.

No abstract received as per date of production. Please check http://www.eshg.org/abstracts2013.0.html for possible updates.

S16.1
Extreme ciliary phenotypes

T. Attie;
Paris, France.

No abstract received as per date of production. Please check http://www.eshg.org/abstracts2013.0.html for possible updates.

S16.2
Primary cilia -kidney disease - DNA Repair

T. Benzing;
Cologne, Germany.

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S16.3
Moving into and inside cilia: the awesome power of diffusion

D. K. Breslow[1], F. Ye[1,2], E. F. Koslover[3], A. J. Spakowitz[3], W. Nelson[1,2], ***M. V. Nachury[1];***
[1]Department of Molecular and Cellular Physiology, Stanford University, Stanford, CA, United States, [2]Department of Biology, Stanford University, Stanford, CA, United States, [3]Department of Chemical Engineering, Stanford University, Stanford, CA, United States.

The primary cilium is a signaling organelle with a distinct complement of membrane and soluble proteins. How specific proteins are concentrated within cilia while others remain excluded is a major unanswered question. We have now established a method for selective permeabilization of the plasma membrane that leaves the ciliary membrane intact, thus enabling a quantitative and mechanistic analysis of protein entry into primary cilia. Using a diffusion-to-capture assay, we find that soluble proteins larger than 75 kDa fail to enter cilia and that the ciliary permeability barrier is mechanistically distinct from the nuclear pore complex. Applying a mass transport model to this system reveals diffusion coefficients for soluble proteins that are compatible with a rapid exploration of the ciliary space in the absence of active transport.
We extend our conclusions to membrane protein by conducting single molecule imaging of signaling receptors inside cilia. Here, we find that membrane proteins move along primary cilia by a combination of diffusion and motor-driven transport. Perturbation of either diffusion or active transport shows that cargo movements can be uncoupled from movements of the intraflagellar transport (IFT) machinery, and that diffusion is sufficient for membrane proteins to explore the ciliary surface. Taken together, our results indicate that signaling within cilia need not be entirely reliant on active transport and poses the question of the true function of IFT.

D.K. Breslow: None. **F. Ye:** None. **E.F. Koslover:** None. **A.J. Spakowitz:** None. **W. Nelson:** None. **M.V. Nachury:** None.

Educational Sessions

ES1.1
Sequencing at The Wellcome Trust Sanger Institute in the year 2013

M. Quail;
Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

The Wellcome Trust Sanger Institute is powered by sequencing. What are we using all this sequencing for? How is sequencing changing? How do we manage and process the many thousands of samples that we sequence? What technologies are we employing for different applications? What are we developing in our R&D group? In my talk I will describe our sequencing pipelines, optimisations and new protocols for Illumina library preparation and describe our progress, experiences and optimisation of semiconductor and third generation sequencing platforms.

M. Quail: None.

ES1.2
Performance and Improvements in Analyzing Next Generation Sequencing Technologies

H. Tilgner[1], D. Sharon[1], D. Xie[1], V. Kuleshov[2,3], R. Chen[1], D. Pushkarev[3], K. Karczewski[1], A. P. Boyle[1], T. Blauwkamp[3], M. Kertesz[3], R. Chen[4], H. Lam[4], M. Pratt[4], G. Bartha[4], J. Harris[4], J. West[4], ***M. Snyder[1];***
[1]Department of Genetics, Stanford University School of Medicine, Stanford, CA, United States, [2]Department of Computer Science, Stanford University, Stanford, CA, United States, [3]Illumina, Inc., 5200 Illumina Way, San Diego, CA, United States, [4]Personalis Inc., Menlo Park, CA, United States.

We have explored different high throughput sequencing technologies for analyzing human genomes, exomes and transcriptomes. For genomes and exomes we have compared different sequencing technologies and capture methods respectively and evaluated their performance. Limitations of the different platforms were discovered and managed to improve sequence accuracy and coverage, particularly in regions of high clinical relevance. We have also utilized and evaluated new long sequence technologies (e.g. Moleculo/Illumina) for accurate phasing of genome variants and calling of structural variants.
In addition to genome and exome analyses, we have explored different long reads technologies (PacBio, 454) for accurate mapping of transcriptomes. These studies have revealed that many full length transcripts can be revealed using long read sequencing and resulting in accurate and novel transcript definition.
Together the different technologies produce more accurate and comprehensive genomes, exome and transcriptome sequences. An example of using an accurate phased genome for analyzing methylomes will be presented.

H. Tilgner: None. **D. Sharon:** None. **D. Xie:** None. **V. Kuleshov:** None. **R. Chen:** None. **D. Pushkarev:** None. **K. Karczewski:** None. **A.P. Boyle:** None. **T. Blauwkamp:** None. **M. Kertesz:** None. **R. Chen:** None. **H. Lam:** None. **M. Pratt:** None. **G. Bartha:** None. **J. Harris:** None. **J. West:** None. **M. Snyder:** Other; Modest; founder and member of the SAB for Personalis.

ES2.1
Integration of Microarray Technology into Prenatal Diagnosis

R. Wapner;
Columbia University, New York, NY, United States.

New genetic technologies have quickly entered obstetrical care providing an ever-increasing amount of information about the fetus. Less than 5 years ago, karyotyping was the predominant tool for genetic evaluation of the fetus and was used predominately for aneuploidy detection and the identification of relatively large genomic changes of 7-10 million base pairs or larger. More recently, microarray technology has been introduced into prenatal evaluation and has the ability to identify much smaller genomic changes (microdeletions and duplications). With this increased resolution has come the knowledge that smaller findings also have significant clinical implications and are not infrequent in routine prenatal testing.
This genetic knowledge has allowed improved counseling for pregnancies identified with fetal structural anomalies, in which approximately 6% of such cases with a normal karyotype will have a genomic cause identified by microarray testing. Approximately 1% or more of structurally normal pregnancies also have clinically relevant genomic findings, suggesting that all pregnant couples should consider invasive testing with microarray analysis.
This increasing ability to evaluate the fetal genome comes with significant responsibility for clinicians. These technologic advances have rapidly out-

paced our ability to thoughtfully and appropriately use the information. Pretest and post-test counseling must be adapted so they are informative, prepare patients for the reproductive decisions they must make, and still be able to be accomplished within a feasible and timely period.

R. Wapner: None.

ES2.2
New approaches in preimplantation screening

S. Munné;
Livingston, NJ, United States.

No abstract received as per date of production. Please check http://www.eshg.org/abstracts2013.0.html for possible updates.

ES3.1
Understanding Genomics. Trends in Science Communication

A. M. Dijkstra;
University of Twente, Science Communication, Enschede, Netherlands.

Nowadays, publics are becoming more critical towards new technologies, as has been shown with biotechnology and genomics. The relationship between science, technology and society is changing. Society changes and publics are articulating their opinions more often and are expressing more critical thoughts about new developments. At the same time, science and technology are changing as well. As a result, due to related uncertainties and unknown risks, not all of these new developments are welcomed anymore. In this changing society, both politicians and researchers agree that for developing (new) technologies public acceptance and trust is vital. Current insights in literature, therefore, emphasize public engagement processes, involving publics in two-way communication and dialogue instead of educating and persuading publics. However, is more engagement really the key? How should publics understand, for example, genomics? And, what role is there for social media? Based on insights from research, trends in science communication are discussed.

A.M. Dijkstra: None.

ES3.2
Science in the media. Lost in translation

D. M. Secko;
Department of Journalism, Concordia University, Montréal, Québec, QC, Canada.

A recurring and recalcitrant theme in the discussion of science in the media concerns what is "lost" when a scientific paper becomes a news article. While some critics acknowledge the existence of great science communication, they also fiercely point to a number of broader issues, including: uncritical and over-simplistic science reporting, narrow frames of scientific progress and economic prospect, a limited range of expert opinion, preferences toward positive messages, unrealistic time lines, and media professionals engaging in cycles of hype. Some media professionals acknowledge various critiques as well, but also question whether the goals and norms of their profession in choosing what to include and what to exclude is sufficiently understood. In the communication of a complex and rapidly evolving target such as genomics, some things will inevitably be lost. This presentation will explore these inclusions and exclusions, attempting to highlight what remains in examples of inspiring science communication.

D.M. Secko: None.

ES4.1
Cancer risk in overgrowth syndromes

A. Riccio[1,2];
[1]DiSTABiF, University of Naples 2, Naples, Italy, [2]IGB-CNR, Naples, Italy.

Overgrowth syndromes (OGS) are a heterogenous group of disorders characterized by global or regional excess of growth. Many of them show increased risk of tumors requiring specific protocols for tumor surveillance. This lecture will focus on recent advances in disease gene identification which resulted in improvement of our understanding in the molecular mechanisms of childhood overgrowth. Molecular defects include both constitutional and somatic single gene defects as well as genomic imprinting abnormalities. These in turn may result from either mutations of imprinting control regions (ICR) acting in cis or arise de novo as epigenetic mutations possibly caused by stochastic events, environmental factors or mutations of regulatory factors acting in trans. Recurrence risks, clinical phenotype and tumor location and frequencies associated with different type of molecular defect will be described. New advances are expected soon from the wide application of next generation sequencing technologies to the analysis of the genome and epigenome of OGS patients. Likely, these will turn out in even larger impact in clinical medicine through the development of gene-specific follow-up protocols and therapies.

A. Riccio: None.

ES4.2
Cancer risk in RASopathies

K. Gripp;
Wilmington, DE, United States.

Rasopathies are syndromic conditions resulting from germline mutations affecting the RAS/MAPK signaling pathway. Dysregulation of this pathway's key role in cell growth and differentiation during embryogenesis gives rise to a recognizable phenotype affecting multiple organ systems, also referred to as "neuro-cardio-facio-cutaneous syndromes". Postnatal pathway dysregulation through somatic mutations similarly affects cell growth and differentiation and occurs during carcinogenesis. Given this transforming activity of RAS/MAPK pathway mutations, it is not surprising that syndromes resulting from germline mutations entail an increased malignancy risk. The relative risk, cancer type and age are syndrome specific. Neurofibromatosis type 1 is a common rasopathy, and its increased risk for optic gliomata and malignant peripheral nerve sheath tumors is addressed in surveillance recommendations. The heterogeneous Noonan syndrome is also common, and its associated increased risk for juvenile myelomonocytic leukemia (JMML) varies by specific *PTPN11* or other gene mutation. A JMML-like myelodysplastic syndrome in early childhood can have a benign course. Cardio-facio-cutaneous (CFC) syndrome may be associated with an increase in acute lymphoblastic leukemia. For Noonan and CFC syndrome, with a low absolute cancer risk, and malignancies which are not likely to show improved outcome after early detection, few screening recommendations exist. In contrast, Costello syndrome, caused by *HRAS* missense mutations, is associated with a higher tumor risk, approaching 15%. Embryonal rhabdomyosarcoma accounts for about 60% of these malignancies, followed by neuroblastoma and transitional cell carcinoma of the bladder. While screening suggestions for Costello syndrome were published, their benefit remains unknown. In conclusion, while the cancer risk is increased overall in rasopathies, the specific risk varies greatly by syndrome, and often by specific causal mutation. Awareness for the risk increase, education of patients and families, prompt evaluation and standard treatment for malignancies constitute best medical practice.

ES5.1
Spectrum of monogenic forms and clinical importance of de novo mutations

S. Baulac;
ICM, Hôpital Pitié-Salpetrière, Paris, France.

Epilepsy is one of the most common neurological disorder. It encompasses a large group of syndromes, diverse in terms of age, etiology, clinical types of seizures, disabilities and prognosis. Genetic factors play a predominant role in about 40% of all epilepsies. Rare Mendelian forms of epilepsy are now well recognized. Since 1995, family-based linkage studies have revealed numerous causative epilepsy genes (*CHRNA4*, *CHRNA2*, *CHRNB2*, *KCNQ2*, *KCNQ3*, *KCNT1*, *SCN1A*, *SCN2A*, *SCN1B*, *GABRG2*, *GABRA1*, *PRRT2*, *PCDH19*, *LGI1*, *DEPDC5*···). To date, the majority of known epilepsy genes encode neuronal ion channel subunits or neurotransmitter receptor subunits leading many of the genetic epilepsies to be considered as channelopathies. Molecular approaches have revealed great genetic heterogeneity, with the vast majority of genes remaining to be identified. This presentation will review recent findings on the progress in gene discovery in monogenic epilepsies, with particular focus on genes harboring inherited variants involved in large multiplex families and *de novo* mutations responsible for sporadic cases. The gene encoding the α1 subunit of neuronal voltage-gated sodium channels (*SCN1A*) is the most frequently mutated gene in epilepsy so far. Inherited *SCN1A* mutations are found in autosomal dominant families with Genetic Epilepsy with Febrile Seizures Plus (GEFS+), while *de novo* mutations cause a severe epileptic encephalopathy named the Dravet syndrome. Genetic testing of *SCN1A* may prevent the need for unnecessary clinical investigations and provide genetic counseling for families. Mutations in the sodium-gated potassium channel gene *KCNT1* were found in two epileptic disorders: inherited mutations are responsible for severe autosomal dominant nocturnal frontal lobe epilepsy and *de novo* mutations for malignant migrating partial

seizures of infancy, an early infantile epileptic encephalopathy. One of the most recent advances in the field of epilepsy genetics is the discovery that loss-of-function mutations of *DEPDC5* (DEP domain containing protein 5) are a frequent cause for a broad spectrum of familial focal epilepsies. The implication of a DEP domain (Dishevelled, Egl-10 and Pleckstrin domain)-containing protein that may be involved in membrane trafficking and/or G-protein signaling, opens new avenues for research.

S. Baulac: None.

ES5.2
The role of genetic susceptibility factors in epilepsy and their clinical relevance

I. Helbig;
Kiel, Germany.

While there has been exceptional progress in the identification of monogenic forms of epilepsy, the discovery of genetic susceptibility factors for seizure disorders has lagged behind, even in comparison to other neurodevelopmental disorders. Genes identified in familial epilepsies only play a minor role in common, non-familial epilepsies, a finding that has long puzzled scientists and has held back progress in understanding the genetic basis of common epilepsies. Further burdened with the phenotypic heterogeneity of the epilepsies, the field was reluctant to adapt to large-scale association studies. Epilepsy genetics is currently emerging from the "dark ages" of small and underpowered association studies with few sufficiently powered association studies published. Finally, in 2012, a large-scale genome-wide association study identified the first common risk factors for epilepsies including variants at the VRK, PNPO and SCN1A loci. In addition, the unexpected discovery of copy number variants including microdeletions at 15q13.3, 16p13.11 and 15q11.2 has pioneered the research into the role of rare genetic variants. Investigations of these risk factors both in case-control studies and family studies have emphasized relevant issues including incomplete penetrance, phenotypic heterogeneity and incomplete segregration in families. Equipped with the early experiences relating to the role of common and rare susceptibility factors, the field of epilepsy genetics is currently embarking on a journey to discover risk factors with massive parallel sequencing technologies. While there is some emerging evidence for de novo mutations particularly in epileptic encephalopathies, the full impact of de novo versus inherited risk factors remains to be determined. In parallel, the field of epilepsy pharmacogenomics is evolving rapidly and variants such as HLA B*1502 are established risk factors for adverse reactions to certain antiepileptic drugs that may already have clinical consequences.
In this presentation, we will discuss the emerging role of common and rare genetic susceptibility factors in the epilepsies, highlight current controversies and discuss the clinical implications of these findings.

ES6.1
Inherited retinal dystrophies: insights & challenges behind the rainbow

F. P. M. Cremers;
Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.

In the last 25 years ~100 genes have been implicated in inherited retinal dystrophies (IRDs). X-linked RD genes were identified using linkage and deletion mapping. Linkage and homozygosity mapping facilitated IRD gene identification on autosomes. Many IRD genes encode proteins of the visual cycle and phototransduction cascade, or are orthologs of genes mutated in animal models. One-third of the IRD-associated genes encode ciliary proteins. Several IRD genes are ubiquitously expressed (e.g. *CHM*, *SNRNP200*). Many genes mutated in syndromes, are also involved in non-syndromic IRDs. Using IRDs associated with mutations in *BBS1*, *CEP290*, or *MVK* (encoding mevalonate kinase), I will illustrate our (lack of) understanding regarding genotype-phenotype correlations. To shed further light on this matter, we need additional insights in the transcriptional variation of IRD-associated genes due to *cis*- and *trans*-acting factors, but also must further explore the interplay between retinal proteins.
Based on genetic analyses of consanguineous Pakistani IRD families, >80% of the underlying genes are known. Massive parallel sequencing in recent years identified rarely mutated IRD genes such as *C8orf37* and *RAB28*, and enabled comprehensive diagnostics. Surprisingly, we identified *de novo* variants in 14% of isolated cases with retinitis pigmentosa. A shortcoming in current diagnostics is the absence of a cheap sequencing method to analyse individual patients suffering from genetically heterogeneous diseases. Challenges for the future will also be to explore non-coding regions through transcriptome and whole genome sequencing, to make sense of rare missense and synonymous variants, and to register all IRD variants in well-curated databases, such as the Leiden Open Variation Databases. As the most frequently mutated IRD genes have already been identified, there is an increasing need for large cohorts of patients. To this end, the European Retinal Disease Consortium was established, which encompasses 12 research groups in 11 countries, and has ascertained >8.000 IRD families.

F.P.M. Cremers: None.

ES6.2
Inherited retinal disease - management and therapies

A. R. Webster[1,2];
[1]Moorfields Eye Hospital, London, United Kingdom, [2]UCL Institute of Ophthalmology, London, United Kingdom.

Inherited retinal diseases comprise a large and varied group of disorders that adversely affect vision due to the perturbation of genes expressed in the specialised cells of the retina. Vast genetic and allelic heterogeneity confound accurate molecular diagnosis, a challenge now, to a degree, surmountable using high-throughput nucleotide sequencing technology. The identification of causative genes, and the molecular diagnosis in patients and families has an impact on clinical management in a number of ways. Some disorders are phenotypically distinct and immediately suggest a causative gene, and genetic segregation. However, others, such as retinitis pigmentosa, are heterogeneous, and require a molecular diagnosis before segregation and accurate counselling can be offered to patients and relatives. Once a group of patients with retinal degeneration has been molecularly defined, then this allows the acquisition of data delineating the age-related changes in retinal function and structure, important to determine effective treatment strategies, and for chosing metrics to measure the efficacy of treatment in a short a time as possible. Preliminary data on gene-replacement for disease due to mutation of REP1, causative of choroideraemia, will be presented, this being a paradigm for treatments aimed at preserving function of the fovea. This retinal region, 400-500µm in diameter, is packed exclusively with cone photoreceptors, designed specifically to allow fine visual discrimination. Data from our group on the effect on this structure, in vivo, of mutations in the ABCA4 gene, shows significant differences in degeneration, which are important in the consideration of therapies. Examples of diseases in which simple modifications might help sustain vision, based on knowledge of molecular pathology, include disorders due to mutation in ABCA4, GUCA1A and EFEMP1. Finally, the accessibility of the retina makes it tractable to approaches that attempt to restore vision. Data from our group and others show that retinal prostheses can deliver useful visual percepts in those with disease causing none, or vague, light perception. Furthermore, strategies involving cell transplantation and optogenetics show promise in the future.

A.R. Webster: None.

ES7.1
Introducing next generation technology to clinical scientists

C. Wright;
Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

High resolution genomic technologies are increasingly being used in clinical research to identify the genetic causes of a variety of human diseases. The UK-wide Deciphering Developmental Disorders (DDD) project uses exon-enriched aCGH coupled with trio exome sequencing to identify the underlying genetic aetiology in children with severe undiagnosed developmental disorders. Where likely causal variants are identified, they are fed-back to Regional Genetics Services for validation and discussion with the family. At the half-way point of this 5-year project, we have recruited over 5000 families to the study, processed over 1000 trio exomes, and fed-back ~200 potential diagnoses.
In this talk I will outline the interaction with clinical teams in DDD through DECIPHER, creation of a scalable automated analysis and variant filtering pipeline for prioritising likely causal variants, and the use of multidisciplinary clinical reporting meetings for final sign-off. I will also discuss the potential for DDD to act as a prototype for clinical translation of whole genome technology in rare diseases in the NHS, and some of the issues around incidental findings and opportunistic genome screening.

C. Wright: None.

ES7.2
Bioinformatic challenges of applying NGS in the clinic

C. Gilissen;
UMC St Radboud, Nijmegen, Netherlands.

The advent of massive parallel sequencing is rapidly changing the strategies employed for the genetic diagnosis and research of diseases that involve a large number of genes. The implementation of NGS in routine diagnostics is a challenging process in which bioinformatics plays an increasingly important role. These challenges vary from data management issues, and automation to the choice and implementation of basic analysis steps such as alignment of reads and variant calling. Specifically important considering diagnostic guidelines are bioinformatic methods for quality control, interpretation of variants and the prevention of incidental findings.
We implemented exome sequencing as a diagnostic test for five genetically heterogeneous diseases and have tried to resolve these specific challenges: we are able to automatically detect sample mix-up, poor sample quality, and limit the number of variants that require manual follow-up. Thorough comparison of the performance of our traditional Sanger based sequencing approach to our exome sequencing approach for these 5 different conditions showed that exome sequencing has a higher diagnostic yield than Sanger sequencing. Even if all genes that could have been ordered by physicians had been tested, the larger number of genes captured by the exome would still have led to an improved diagnostic yield.

C. Gilissen: None.

ES8.1
How to get published in the European Journal of Human Genetics

G. van Ommen;
Leiden, Netherlands.

The practice of publishing research in peer-reviewed journals is crucial to the advance of science and medicine. Often researchers receive no direct instruction on how to go about this, but instead are expected to pick up the correct procedures from supervisors and colleagues. We propose bringing together a panel of experts made up of *GertJan B. van Ommen*, the long term Editor of*European Journal of Human Genetics* , Joerg Schmidtke, Director of the Institute of Human Genetics at Hannover Medical School, past president of ESHG and member of the **„Genetic testing as part of health care**" unit of EuroGentest and Magdalena Skipper, Senior Editor from *Nature*. This panel will describe the whole process of publishing, from drafting a manuscript to the peer review process through to publication. As such, it will be of interest to the range of participants in the publishing process: from less experienced authors to those undertaking peer review.

ES9.1
A genetics history of our species

E. Heyer;
National Museum of Natural History, Paris, France.

I will start by presenting recent works on the history of our species based on genetic data. I will present our recent origin out of Africa, the admixture with Neandertal and Denisova. Then I will focus my talk on different evolutionary forces that have shaped our genetic diversity: natural selection, population expansions, migration. In particular, I will present some examples on the impact of cultural behaviors and their transmission on genetic diversity. This second part includes ecological inheritance through Niche construction: there are now several documented cases where, by changing our environment, we create new selective pressure. Transmission of cultural traits can also impact our genetic diversity by limiting gene flow among populations, or shaping intra-population genetic diversity. In conclusion, cultural transmission interacts with biological evolution in shaping our genetic diversity.

E. Heyer: None.

ES9.2
Tracing back geographic origine and phenotypes using genetic data

M. Kayser;
Erasmus University Medical Center Rotterdam, Rotterdam, Netherlands.

Human migration history out of Africa into (almost) all worldwide regions left various genetic footprints that can be detected in modern populations and individuals around the world. For instance, genomic signatures allow tracing back the bio-geographic origin of a population and a person either via large number of genetic markers or via specifically ascertained ancestry-informative DNA markers, which can be relevant in medical, anthropological and forensic studies and is important for mapping disease and other genes. The geographic resolution available with DNA ancestry testing varies between worldwide regions but in general larger geographic regions such as continents can be inferred from genetic data more reliably than subregions within continents. For some geographic regions the use of Y-chromosomal or mitochondrial DNA markers allows subregional paternal or maternal ancestry inference not easily provided with autosomal DNA markers. Moreover, genomic signatures shaped by evolutionary forces in combination with migration history allow a better understanding how phenotypic diversity such as appearance observed in humans around the world arose. Recent progress in the genetics of human appearance phenotypes not only delivered numerous genes, some of which show clear signatures of positive selection, but also allows to predict certain appearance traits solely from genetic data, which can be relevant in anthropological, historical and forensic studies. This talk will provide an overview on genetic population substructure around the world and how this knowledge can be used to infer the bio-geographic ancestry from a person's DNA. Furthermore, this talk will highlight the genetic basis of human appearance traits, their evolutionary history and how this knowledge can be used to predict appearance from a person's DNA.

M. Kayser: None.

Concurrent Sessions

C01.1

De novo mutations in the Genome of the Netherlands

L. C. Francioli[1], *P. Polak*[2,3], *W. Kloosterman*[1], *S. Sunyaev*[2,3], *P. I. W. de Bakker*[1,2,3];
[1]*Departments of Medical Genetics and Epidemiology, University Medical Center Utrecht, Utrecht, Netherlands,* [2]*Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States,* [3]*Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA, United States.*

Genome of the Netherlands Consortium

Background: The Genome of the Netherlands (GoNL) is a national effort to characterize genomic variation in the Dutch population through whole-genome sequencing of 250 families (231 trios, 19 twin quartets) at 12x using Illumina HiSeq. Sequencing was performed by BGI (China). The design of our study allowed us to investigate the role of *de novo* mutations in random individuals at an unprecedented scale.

Method: We developed a Bayesian algorithm to detect *de novo* mutations from pedigree data and implemented it as the PhaseByTransmission module in the Genome Analysis Toolkit (GATK). We validated >550 *de novo* mutation calls (Sanger, MiSeq) to estimate the false positive rate. We simulated *de novo* mutations by random injection in our sequence data to assess our false negative rate while preserving the characteristics and intrinsic biases of our data. We used machine-learning techniques to correct for these effects.

Results: We called more than 19,600 *de novo* mutations, of which 44% were called with high confidence. Validation results showed that >60% of all calls and >92% of high-confidence calls were true positives. Through simulations, we estimated that we were able to call 70% of all *de novo* mutations. We observed a strong correlation between the father's age at conception and the *de novo* mutation load in the offspring (r > 0.4). We report that *de novo* mutation properties vary throughout the genome. These results illustrate that *de novo* mutation detection and analysis of their functional relevance can be performed on medium-coverage sequencing.

L.C. Francioli: None. **P. Polak:** None. **W. Kloosterman:** None. **S. Sunyaev:** None. **P.I.W. de Bakker:** None.

C01.2

Type 2 Diabetes strongly increases risk for the pre-cancerous state of clonal mosaicism

A. Bonnefond[1], *B. Skrobek*[1], *S. Lobbens*[1], *E. Eury*[1], *S. Cauchi*[1], *O. Lantieri*[2], *B. Balkau*[3], *E. Riboli*[4], *M. Marre*[5], *G. Charpentier*[6], *L. Yengo*[1], *P. Froguel*[1];
[1]*CNRS, Lille, France,* [2]*IRSA, La Riche, France,* [3]*Inserm, Villejuif, France,* [4]*Imperial College London, London, United Kingdom,* [5]*Inserm, Paris, France,* [6]*Corbeil-Essonnes Hospital, Corbeil-Essonnes, France.*

Large chromosomal clonal mosaic events (CMEs), i.e. the co-existence of cells with two or more distinct karyotypes within an individual, have recently been suggested to be linked with aging and to predict the subsequent development of cancer. Type 2 diabetes (T2D) has been conceptualized as an accelerated ageing disease, and is associated with a higher prevalence (and mortality) of cancers. Here, we aimed to assess the impact of T2D on CME occurrence in blood.

We assessed the presence of large CME (≥5Mb) in 7,437 individuals (2,208 T2D patients; 5,229 controls) using Metabochip DNA arrays (Illumina). The effect of T2D on the occurrence of detectable CME was assessed using logistic regression adjusted for age, gender, body mass index and population stratification.

We found a strong effect of T2D on the risk of detectable CMEs (OR=5.4; P<5×10-5). The association was even stronger when we analysed non-obese T2D patients (OR=5.9; P<5×10-5). Importantly, T2D patients carrying CME had a higher prevalence of vascular complications than T2D non-carriers (71.4% versus 37.1%; P=5.0×10-4). The size of CMEs in blood and the percentage of abnormal cells were stable over a 6-year follow-up period.

We propose that the most severe forms of T2D can favor the development of CMEs which may contribute to the higher risk for cancer in the T2D population. The present study may have profound clinical implications: given the modest cost of Metabochip and the medical interest of detecting pre-cancerous states, a CME genetic testing may be proposed in diabetic patients with early-onset complications.

A. Bonnefond: None. **B. Skrobek:** None. **S. Lobbens:** None. **E. Eury:** None. **S. Cauchi:** None. **O. Lantieri:** None. **B. Balkau:** None. **E. Riboli:** None. **M. Marre:** None. **G. Charpentier:** None. **L. Yengo:** None. **P. Froguel:** None.

C01.3

De novo mutations in the autophagy gene encoding WDR45 (WIPI4) cause static encephalopathy of childhood with neurodegeneration in adulthood

N. Matsumoto[1], *T. Nishimura*[2], *K. Muramatsu*[3], *H. Kodera*[1], *S. Kumada*[4], *K. Sugai*[5], *E. Kasai-Yoshida*[4], *N. Sawaura*[3], *H. Nishida*[6], *A. Hoshino*[6], *F. Ryujin*[7], *S. Yoshioka*[7], *H. Arakawa*[3], *M. Kato*[8], *N. Mizushima*[9], *H. Saitsu*[1];
[1]*Yokohama City University Graduate School of Medicine, Yokohama, Japan,* [2]*Department of Biochemistry and Molecular Biology, Graduate School and Faculty of Medicine, The University of Tokyo, Tokyo, Japan,* [3]*Department of Pediatrics, Gunma University Graduate School of Medicine, Gunma, Japan,* [4]*Department of Neuropediatrics, Tokyo Metropolitan Neurological Hospital, Tokyo, Japan,* [5]*Department of Child Neurology, National Center of Neurology and Psychiatry, Tokyo, Japan,* [6]*Department of Pediatrics, National Rehabilitation Center for Children with Disabilities, Tokyo, Japan,* [7]*Department of Pediatrics, Shiga University of Medical Science, Shiga, Japan,* [8]*Department of Pediatrics, Yamagata University Faculty of Medicine, Yamagata, Japan,* [9]*Department of Physiology and Cell Biology, Graduate School and Faculty of Medicine, Tokyo Medical and Dental University, Tokyo, Japan.*

Static encephalopathy of childhood with neurodegeneration in adulthood (SENDA) is a recently established subtype of neurodegeneration with brain iron accumulation (NBIA)1-3. By exome sequencing, we found de novo heterozygous mutations in WDR45 at Xp11.23 in two individuals with SENDA, and three additional WDR45 mutations in three other subjects by Sanger sequencing. Using lymphoblastoid cell lines (LCLs) derived from the subjects, aberrant splicing was confirmed in two subjects, and protein expression was observed to be severely impaired in all five subjects. WDR45 encodes WD repeat protein interacting with phosphoinoside 4 (WIPI4), one of the four mammalian homologs of yeast Atg18, which plays an important role in autophagy4,5. Decreased autophagic activity and accumulation of aberrant early autophagic structures were demonstrated in the LCLs of the subjects. These findings provide the direct evidence that an autophagy defect is indeed associated with a neurodegenerative disorder in humans.

N. Matsumoto: None. **T. Nishimura:** None. **K. Muramatsu:** None. **H. Kodera:** None. **S. Kumada:** None. **K. Sugai:** None. **E. Kasai-Yoshida:** None. **N. Sawaura:** None. **H. Nishida:** None. **A. Hoshino:** None. **F. Ryujin:** None. **S. Yoshioka:** None. **H. Arakawa:** None. **M. Kato:** None. **N. Mizushima:** None. **H. Saitsu:** None.

C01.4

Mapping of two human genomes with a single molecule nanochannel array platform for genome-wide structural variation analysis and de novo sequence assembly

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Despite recent advances in base-calling accuracy and read length, de novo genome assembly and structural variant analysis using 'short read' shotgun sequencing remain challenging. We have developed a new approach that utilizes highly parallel nanochannel arrays in which many thousands of very long single DNA molecules are linearized and imaged. This novel approach is automated on the Irys System and can scan the entire genome rapidly to generate physical maps that provide a more comprehensive view of the genome. Here we describe the genome maps of the first two diploid human genomes constructed using this approach. Two members of a CEPH-CEU trio (father and daughter, NA12891 and NA12878, respectively) genotyped and sequenced extensively as part of the International HapMap and 1000 Genomes Projects were mapped to 50X coverage with long (150 kb to >500 kb) DNA fragments fluorescently labeled at Nt.BspQI (GCTCTTCN/) sites. The resultant sequence motif maps are used to resolve haplotypes, identify structural variations, and assist in de novo sequence assembly of these two individuals. Particularly complex genomic loci, such as the major histocompatibility (MHC) region are well characterized with these maps. Our results show that the DNA sequence of these two individuals differ significantly from the reference human genome sequence and confirm the majority of the structural variants identified previously. In addition, new structural variants not detected by next-generation sequencing are easily identified. The genome mapping approach is simple and can be performed in any modern molecular genetic laboratory.

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C01.5

Exome sequencing in sporadic cases of schizophrenia identifies de novo protein-altering mutation in candidate genes

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Schizophrenia has a strong genetic component, yet in most cases the underlying causes are unknown. To identify additional genetic risk factors, we have assessed the contribution of de novo mutations by sequencing the exomes of sporadic cases of schizophrenia and their healthy parents (n=54 trios). Whole exomes were captured and sequenced using the Agilent and Illumina technologies, respectively and genetic variants were called using our analytical pipeline. We identified 51 validated de novo mutations, 36 of which were predicted to alter protein structure/function including 31 missense, 2 conserved splice site, 2 nonsense mutations and 1 frameshift deletion. The observed point mutation rate of 0.94 events per trio or 1.8 x 10-8 per base per generation and the nonsynonymous to synonymous ratio of 2.19 did not differ significantly from neutral expectations. A gene ontology analysis on the genes with protein-altering de novo mutations revealed an enrichment for biological processes involved in chromatin regulation and organization, although not statistically significant probably owing to the limited number of genes analyzed. Remarkably, we found a damaging de novo mutation in RGS12 (R54L), a gene already reported as severely mutated in a similar study (Xu et al. 2012) suggesting strong candidacy as a risk factor. We currently investigate the burden and disequilibrium transmission test of damaging mutations in the candidate genes for schizophrenia, defined here. Our and other studies indicate extensive genetic heterogeneity in schizophrenia and provide a list of genes potentially involved in disease susceptibility.

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C01.6

The genome structure of the Dutch population

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On behalf of GONL consortium

The Genome of the Netherlands project (GoNL, http://nlgenome.nl) aims to create a map of genetic variants through whole genome-sequencing of 250 families representing 11 Dutch provinces. Here we report results from the analysis of structural variation employing several complementary approaches. We detected a wide spectrum of genetic variants including short indels, deletions, insertions, tandem duplications, inversions, mobile element insertions and translocations.

Short indels and structural variants (SVs). Using the consensus of 4 callers (GATK_UG, PINDEL, CLEVER and SOAPdenovo assembly) we predicted 1,196,767 small indels of which 1,413 are causing premature stop codons and frameshifts in 1,285 genes. On average, we observed 134 frameshift and 19 splice site mutations per individual genome. By combining different strategies: read depth (CNVnator, DWAC-Seq, Facade), read pair (123SV, BreakDancer), combined approaches (PINDEL, GenomeSTRiP, CLEVER), de novo assembly (SOAPdenovo) we predicted over 10 thousand variants representing different SV types and size ranges.

Validation of structural variant sets. We performed targeted resequencing to: i) determine the false-positive rate for different approaches/tools; ii) validate loss-of-function variants; iii) confirm novel genomic segments, absent in the current genome reference; iv) verify de novo structural events

Significance. In combination with GoNL SNP set (reported separately) these results comprehensively describe common genetic variants in individuals of Dutch origin. This variation catalogue is essential for understanding mechanisms of SV formation, their population dynamics and history. It provides an essential resource for the interpretation of GWAS results and the genomes of other West-European individuals with constitutional or acquired disorders.

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C02.1

From acrodysotosis to acroscyphodysplasia : phenotypic spectrum of PDE4D and PRKAR1A mutations through the study of 26 cases.

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Acrodysostosis (MIM101800) is a dominantly inherited condition associating 1) a skeletal dysplasia characterized by short stature, facial dysostosis and severe brachydactyly with cone-shaped epiphyses 2) resistance to mutiple hormones 3) moderate to mild intellectual disability. Acroscyphodysplasia (MIM250215) is rare and characterized by lower femoral and upper tibial epiphyses embedded in cup-shaped, large metaphyses, associated to severe growth retardation and brachydactyly.

Studying 22 unrelated acrodysostosis cases, we identified heterozygous de novo PRKAR1A mutations in 8 cases and heterozygous de novo PDE4D mutations in 6. Neither PDE4D nor PRKAR1A mutations were found in four patients with characteristic clinical features. The molecular screening is in progress for the four remaining cases. In 4 cases of acroscyphodysplasia, we identified PDE4D mutations.

Splitting our series based on the disease causing gene confirmed genotype-phenotype correlations. Hormone resistance was consistently observed in the patients carrying PRKAR1A mutations while no hormone resistance was observed in 5/6 patients with PDE4D mutations. Moreover, all patients with PDE4D mutations shared characteristic facial features (midface hypoplasia with the canonical nasal hypoplasia) and some degree of intellectual disability. Finally, our findings of PDE4D mutations in 4 cases of acroscyphodysplasia support also that PDE4D is responsible for a severe skeletal dysplasia, with major epiphyseo-metaphyseal changes.

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C02.2

Broadening the clinical spectrum to be ascribed to EFTUD2 haploinsufficiency

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Mandibulofacial dysostosis (MFD) is the consequence of an abnormal development of the first and second branchial arches. The core phenotype is represented by malar and mandibular hypoplasia and dysplastic ears. Conductive hearing loss, lower lid anomalies and/or cleft palate are frequent associated features. Recently, EFTUD2 heterozygous loss-of-function mutations have been identified in patients with MFD and microcephaly (MFDM, Guion-Almeida type, MIM610536), EFTUD2 being a protein of the spliceosome complex.
Here we report 27 patients harbouring a deletion encompassing EFTUD2 or a heterozygous mutation. This series allows us to broaden the spectrum of features ascribed to EFTUD2 loss of function and includes esophageal atresia, semi circular canal anomalies, olfactory bulb agenesis, toe syndactyly and vertebral anomalies. Until now, no syndrome featuring both MFD and EA has been clearly delineated although EA is reported in about 5% of patients diagnosed with oculoauriculovertebral spectrum (OAVS, MIM 164210). Thus, we define a novel syndromic EA entity, for which a locus at 17q had previously been suggested, and emphasize that MFDM syndrome should be considered as a differential diagnosis for phenotypic variants of CHARGE, Feingold, VACTERL syndromes, and Oculo-Auriculo-Vertebral spectrum.
Nager (MIM154400), Taybi-Linder (MIM210710) and MFDM syndromes emphasize the necessity of mRNA maturation through the spliceosome complex not only for global growth but also within specific developmental fields.

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C02.3

Delineation of the clinical spectrum of *RNU4ATAC*-related microcephalic osteodysplastic primordial dwarfism type I syndrome: an international cohort

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Microcephalic osteodysplastic primordial dwarfism type I (MOPD I, OMIM 210710) was initially described in 1967 by Taybi and Linder in two siblings with intrauterine and postnatal growth retardation, dwarfism, a skeletal dysplasia, severe microcephaly with developmental brain malformations and early death. Our team and others have identified mutations in a small nuclear non-coding RNA, named U4atac snRNA, a critical component of the minor spliceosome (Edery P et al, Science 2011 and He H et al, Science 2011). We have also provided evidences that MOPD I syndrome is associated with abnormal splicing of a restricted subgroup of introns of the genome, named U12 introns, which are recognized by the minor spliceosomal machinery through non-consensus U12-intron recognition sites. We are currently perfoming whole transcriptome analysis in fibroblasts derived from MOPD I patients to identify deregulated genes/signalling pathways responsible for the phenotype. Here, we provide further delineation of the clinical spectrum of MOPD I syndrome and *RNU4ATAC* molecular data in 14 patients from our series (article in preparation) and we review data on 10 other patients and a large Amish family reported since the discovery of the causative gene. To our knowledge, this is the largest and most comprehensive clinical and molecular study available in MOPD I syndrome.

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C02.4

Baraitser-Winter syndrome due to ACTB/G1 mutations: delineation of the spectrum in 34 cases

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Baraitser-Winter (BRWS), a dominant MCA disorder, was shown to result from heterozygous gain of function mutations in one of the two ubiquitous cytoplasmic actin-encoding genes ACTB and ACTG1 (Rivière, Nat Genet 2012, 44: 440) which are the structural components of filamentous actin (F-actin), which, together with myosin motors and actin-binding proteins, forms a thin, cross-linked network lying immediately beneath the plasma membrane. ACTB and ACTG1 are important for basic cellular processes such as cytokinesis, cell migration, and embryogenesis.. We present detailed phenotypic description and neuroimaging of 34 patients with molecularly proven BRWS, emphasizing the clinical variability of the syndrome, which also encompass Fryns-Aftimos syndrome. The major clinical anomalies are striking facial dysmorphism (present in all cases) with hypertelorism, broad nose with large tip, congenital ptosis, ridged metopic suture, and highly arched eyebrows. Microcephaly may develop with time. Iris or retinal coloboma is present in many cases, as does sensorineural deafness. Cleft lip and palate, and hallux duplex may be present. Pachygyria with antero-posterior gradient is present in almost all cases. Progressive joint stiffness is observed in elder patients, although early muscular involvement with even congenital arthrogryposis may be present in some. Intellectual disability and epilepsy are variable and correlate with CNS anomalies. Facial dysmorphism appears to be the most reliable criteria to evoke BRWS.

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C02.5

A comprehensive analysis of a cohort of Cornelia de Lange syndrome cases.

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Cornelia de Lange syndrome (CdLS) is a multiple congenital malformation syndrome characterised by mild to severe developmental delay, growth retardation, limb abnormalities and prominent facial features. Approximately 65% of typical cases carry *de novo* heterozygous loss-of-function mutations in genes involved in sister chromatid cohesion; *NIPBL*, *SMC1A*, *HDAC8*, *RAD21* and *SMC3*. We developed an AmpliSeq PCR assay providing a template for IonTorrent sequencing to enable rapid parallel sequencing of all known CdLS genes. Of the 158 CdLS and CdLS-like cases in the MRC HGU CdLS Expanded Phenotype Cohort, 148 have been screened revealing 34, 6, 3 and 1 with mutations in *NIPBL*, *SMC1A*, *HDAC8* and *SMC3* respectively. In one male case a mosaic *SMC1A* mutation was detected at significantly different levels in two separate DNA samples, 53% and 10%. Array-CGH was performed on 104/148 cases and in two cases this identified plausible novel candidate genes for CdLS, *ESPL1* and *CDK11A/B*. DNA from six mutation-negative cases was then used for exome sequencing. In one case a *NIPBL* mosaic nonsense mutation was identified at 15% of reads in a patient with typical CdLS. In a second case a heterozygous frameshift in *ANKRD11* was identified in a patient with a CdLS-like phenotype. These data confirm *NIPBL* as the most commonly detectable cause of CdLS even in a cohort with broad ascertainment criteria. Genetic heterogeneity is marked within CdLS and CdLS-like cases and molecular analysis is showing significant phenotypic overlap with other dysmorphic syndromes such as KBG.

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C02.6

Treacher Collins Syndrome: clinical and molecular study based on a series of 135 patients

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Treacher-Collins/Franceschetti syndrome (TCFS, MIM 154500) is a disorder of craniofacial development belonging to the heterogeneous group of mandibulofacial dysostosis. TCFS is characterized by bilateral mandibular and malar hypoplasia, down slanted palpebral fissures, and microtia. To date 3 genes have been identified in TCFS namely TCOF1, POLR1D and POLR1C. Here we report a clinical and extensive molecular study in a series of 135 TCFS patients. All the patients were screened for TCOF1. In absence of mutation, POLR1D and POLR1C were studied and array-CGH as well as EFTUD2 screening was performed in the remaining negative patients. We have analysed the molecular results from this series in two subsets, namely typical and atypical TCFS, based on clinical features. In typical TCFS, we identified 79% of patients with a molecular anomaly in TCOF1, 6% in POLR1D and 0% in POLR1C. Deletion, nonsense, duplication/insertion, splice site, missense, indels mutations were found respectively in 43 (51%), 17 (20%), 10 (12%), 6 (7%), 3 (3.5%) and 2 (3%) patients. Among the atypical patients (with intellectual disability or microcephaly), we identified 2 patients with a mutation in EFTUD2, and 2 patients with a 5q32 deletion encompassing TCOF1 and CAMK2A, but no mutation in TCOF1, POLR1D or POLR1C.
Phenotype/genotype correlations were investigated for 20 clinical features including choanal atresia, cleft palate and hearing loss. We also compared the type of mutation in TCOF1 and its localization in the gene, in an attempt to associate degrees of severity or peculiar clinical features to a specific part of TCOF1.

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C03.1

Highly multiplexed targeted single-nucleotide polymorphism (SNP) amplification and sequencing as a method for identifying fetal chromosomal disorders from maternal cell-free DNA

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Most multiplex PCR reports describe at most 100-200 assays in a single reaction, as increasing probe number increases probe-probe interactions. This is problematic when sequencing amplified DNA, as resulting uninformative reads are costly. We previously reported multiplexing 11,000 single-nucleotide polymorphisms (SNPs); because increasing analyzed heterozygous SNPs increases test power, we expanded this to 19,488 SNPs, with >98% of sequence reads mapping to targeted regions. This massively multiplexed PCR assay targets SNPs to accurately determine fetal chromosomal copy number from cell-free DNA (cfDNA). This massively multiplexed PCR assay targets single-nucleotide polymorphisms (SNPs) to non-invasively detect fetal chromosomal copy number from cfDNA. As part of an institutional review board-approved study to validate our Next-generation Aneuploidy Test Using SNPs (NATUS) algorithm, cfDNA was isolated from maternal plasma and subjected to a multi-stage, multiplex PCR amplification at Natera's laboratory. Specifically, primers were designed using an informatics-based approach, minimizing primer-primer interactions. Targets included highly heterozygous SNPs from chromosomes 13, 18, 21, X, and Y. Cell-free and genomic DNA samples were pre-amplified for 15 cycles using PCR with 19,488 target-specific assays in a single reaction. An aliquot was transferred to a second 15-cycle PCR reaction. Finally, a 12-cycle PCR reaction added barcoded tags, and amplicons were sequenced using an Illumina HiSeq 2000. This approach accurately detected fetal chromosomal copy number at all five targeted chromosomes from cfDNA isolated from maternal plasma. By targeting SNPs, this approach identifies parental origin of fetal chromosomes and uniparental disomy, and will allow detection of triploidy, microdeletions/microduplications, and haplotype reconstruction.

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C03.2

Diagnostic accuracy for the non-invasive prenatal detection of common autosomal aneuploidies

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Recent advances in non-invasive prenatal diagnosis show that massively parallel sequencing (MPS) of maternal plasma DNA allows accurate detection of common foetal aneuploidies. Here, we describe the results of a collaborative clinical study with the aim to validate the diagnostic accuracy of our non-invasive protocol based on MPS for detecting common autosomal aneuploidies and first clinical application experiences.

In the study maternal blood samples were collected from 522 pregnant women with risk for aneuploidies. Extracted cell-free plasma DNA was analysed using Illumina sequencing platform HiSeq2000 in a multiplexed fashion. Fetal aneuploidies were identified using a Median Absolut Deviation based z-score equation (DAP.21). After unblinding study data, a new bioinformatics algorithm based on GC normalization (DAP.plus) was applied. Results of MPS based technique were compared with those from invasive procedures. 40/41 samples were correctly classified as trisomy 21-positive (sensitivity: 97,6 %; one-sided confidence interval: 88.9 %) and 427/427 samples were correctly classified as trisomy 21-negative (specificity: 100%; one-sided confidence interval: 99.3%). Furthermore, 5/5 T13 cases and 8/8 T18 cases were correctly identified using DAP.plus. The overall detection rate of trisomies 13, 18 and 21 is 98.1% (53/54).

Due to the high accuracy, our DAP.plus algorithm allows detection of common autosomal trisomies and has the potential to decrease the use of invasive procedures. The test is especially suitable for women at risk in addition to first trimester screening to reduce the false-postive-rate. However, future clinical studies are required to validate MPS for the detection further fetal chromosomal abnormalities and fetal genomic imbalances.

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C03.3

Clinical Performance Comparison of the Harmony(TM) Prenatal Test and First Trimester Combined Screening in a General Pregnancy Population

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Objective

Compare clinical accuracy of Harmony™ Prenatal Test, a NIPT based on cfDNA analysis, to first- trimester combined screening using serum markers and nuchal-translucency (NT) measurement.

Methods

A general screening population of pregnant women presenting in their first trimester (11 weeks 0 days to 13 weeks 6 days) underwent screening for fetal trisomy 21 and 18 using free β-hCG, PAPP-A, and NT. Pregnancy outcome was determined by invasive testing or phenotypic evaluation of the newborn. An archived aliquot of plasma at the same time of serum screening was available which underwent blinded analysis with Harmony. Risk score results at various risk cut-offs from 1 in 100 (1%) to 1 in 1,000 (0.1%) from both test modalities were compared.

Results

Study population consisted of 1,949 pregnant women. Median maternal age was 31.8 years (IQR range: (27.7–35.4) Median gestational age was 12.6 weeks (IQR range: 12.3-13.0). 80 (4.1%) underwent invasive testing(CVS or amniocentesis). All eight fetal trisomy 21 and two fetal trisomy 18 cases were detected by both Harmony and first-trimester combined screening and confirmed by invasive testing. False-positive rate for Harmony was 0.1% (2 of 1,939) over all risk cut-off ranges whereas false-positive rate for first-trimester screening at risk cut-offs of 1/100, 1/300, and 1/1,000 was 2.8%, 6.7%, and 18.4% respectively.

Conclusions

Harmony Prenatal Test demonstrates a reduction in false-positive test results compared to first- trimester combined screening in a general pregnancy population. Use of NIPT as a first-line test for aneuploidy screening could reduce maternal anxiety and invasive procedures.

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C03.4

Comprehensive Chromosome Screening in PGD and PGS - Ethical Challenges

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Microarray technology allows for comprehensive screening of chromosomes of in vitro embryos. Such screening can give information about the health status of the potential future child revealing chromosomal abnormalities leading to conditions with variable prognosis, such as sex chromosome aneuploidies.

Ethical questions regarding selection and transfer of embryos are often tackled using different arguments that do not necessarily yield the same answer. Arguments related to the welfare of the potential child try to set a minimum threshold, such as the requirement to avoid serious harm. The principle of procreative beneficence states that couples have the duty to select the embryo with the best chances to lead a healthy life. In assisted reproduction, professionals are actively involved in the creation of the embryo, and are considered by some to be co-responsible for the outcome of the procedure and the welfare of the potential child. A parental duty may be interpreted as a duty to select the best embryo, but also as a duty (or a right) to accept children as they are. The principle of procreative liberty as stated by John Robertson dictates that prospective parents have the right to choose the offspring they want, even if this includes having a child with a chromosomal abnormality. Conflicts between professionals and couples can become apparent when a decision whether to transfer an embryo with a chromosomal abnormality needs to be taken. This paper tackles these potential conflicts assessing the different normative frameworks and suggests how and which decisions are ethically sound.

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C03.5

The challenge of preconceptional, preimplantation, and prenatal genetic diagnoses of mitochondrial DNA disorders

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Mitochondrial DNA (mtDNA) mutations cause a wide range of serious genetic diseases with no efficient therapy and high transmission risk, due to their maternal inheritance. Thus, "at risk" couples often ask for preconceptional (PCD), preimplantation (PGD) or prenatal diagnosis (PND). All these procedures are based on mutant load assessment in polar bodies (PCD), blastomeres (PGD), or fetal tissues (PND).

Here we report our 10-year experience in PCD, PGD and PND for mtDNA mutations in MT-ATP6 (m.8993T>G, m.9185T>C, NARP/ Leigh), MT-ND3 (m.10197G>A), MT-ND4 (m.14459G>A), MT-TL1 (m.3243A>G, MELAS), and MT-TK (m.8344A>G, MERRF). Mutant loads were quantified in 46 polar bodies, 19 oocytes, 52 preimplantation embryos, 35 fetuses, and 10 whole placentas from carrier females.

PCD was not a relevant approach, due to common mutant load discrepancies between first polar bodies and their counterparts. Conversely, mutant loads

were constantly stable among blastomeres from 42 embryos, making PGD an appropriate procedure. Mutant loads were stable across various tissues including amniocytes in 9 fetuses. However, intra-placental mutant load variations up to 55% were found. Such results preclude the use of chorionic villous sampling for PND. No temporal variation of mutant load was observed throughout pregnancy (21 fetuses). These data support analysis of a single amniocyte sample as the best PND approach.
Finally, 22 children were born from PGD/PND procedures and are healthy with a 4 months - 12 years follow-up. Preimplantation and prenatal heteroplasmy assessments are thus valuable tools to prevent the recurrence of the disease in families where a mtDNA mutation segregates.

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C03.6

Exome sequencing of 27 trios to identify genetic causes of fetal abnormalities

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Fetal abnormalities, as revealed by ultrasound, are a relatively common occurrence and the cause is often genetic. The method used to investigate these cases is usually a combination of karyotyping and aCGH, which in many cases does not yield a diagnosis. We propose that exome sequencing of trios in such cases may substantially increase the diagnostic rate, which will improve understanding of the biological basis of abnormal development, and inform the reproductive decisions of affected families.
Therefore, we have exome sequenced a cohort of 27 fetuses with diverse structural abnormalities including cardiac, musculoskeletal and nervous system defects, along with their parents. Additionally, we have performed aCGH on a subset of this cohort. We have identified rare functional SNPs, indels and CNVs, a subset of which are highly likely to be causal, on the basis of prior knowledge of candidate gene function. These include a de novo SNP in FGFR3 in a fetus with features of thanatophoric dysplasia, and a de novo 16.8 kb deletion that includes most of the gene OFD1 in a female fetus with ventriculomegaly and agenesis of the corpus callosum.
Thus far, our data suggest that exome sequencing trios may increase the diagnostic rate in fetuses with structural abnormalities over that achieved by aCGH alone. Further work will include expanding this cohort and functionally validating a subset of our candidate genes using animal models. This is, to our knowledge, the largest such cohort of structurally abnormal fetuses to have been exome sequenced.

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C04.1

Mutations in SMARCE1 cause a novel disorder of multiple spinal meningiomas

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Meningiomas comprise one third of all primary central nervous system tumours in adults. When they occur at multiple sites or in multiple family members, they are usually associated with neurofibromatosis type 2 (NF2) disease or familial schwannomatosis. In a subset of individuals, multiple meningioma disease occurs as an autosomal dominant manner without NF2 or SMARCB1 mutations. We sought to identify novel genes associated with meningioma development.
Method
We sequenced the exomes of three unrelated individuals with familial multiple spinal meningiomas without NF2 or SMARCB1 mutations.
Results
We identified two individuals with heterozygous novel loss of function mutations in the SWI/SNF chromatin remodelling complex subunit, SMARCE1. Sequencing of SMARCE1 in six additional individuals with spinal meningiomas identified two further novel heterozygous loss of function mutations. Tumours from individuals with SMARCE1 mutations were of clear cell histological subtype and all demonstrated loss of SMARCE1 protein consistent with a tumour suppressor mechanism.
Conclusion
Our findings define multiple spinal meningioma disease as a novel discrete entity and establish the key role of the SWI/SNF complex in the pathogenesis of both meningiomas and tumours with clear cell histology.

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C04.2

Mosaic *PPM1D* mutations are associated with predisposition to breast and ovarian cancer

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Breast and ovarian cancer are exemplars of the rare variant - common disease hypothesis, with the identification of rare mutations in the DNA repair genes *BRCA1, BRCA2, ATM, BRIP1, CHEK2, PALB2, RAD51C* and *RAD51D* conferring susceptibility to one, or both, diseases. By undertaking targeted resequencing of 507 genes implicated in DNA repair pathways in 1150 women with breast +/- ovarian cancer, and a large scale replication case-control experiment in 13,642 individuals, we have shown that rare protein truncating variants (PTVs) in the p53-inducible protein phosphatase *PPM1D* are associated with both breast and ovarian cancer. We identified *PPM1D* mutations in 25/7781 cases vs 1/5861 controls; (P=1.12x10-5), including 18 mutations in 6,912 individuals with breast cancer; (P = 2.42x10-4) and 12 mutations in 1,121 individuals with ovarian cancer; (P = 3.10x10-9). The *PPM1D* PTVs cluster within a 370bp region of the final exon of the gene, and are predicted to avoid nonsense mediated decay, resulting in a truncated protein product. Functional studies show that mutant PPM1D isoforms demonstrate enhanced suppression of p53 in response to ionising radiation, suggesting a gain-of-function mechanism of action. Deep resequencing of a subset of samples confirmed that the PTVs were mosaic in lymphocyte DNA and intriguingly absent from tumour tissue, suggesting a novel mechanism of cancer association, which is genetic but not hereditary. This data has clinical implications for the detection and management of women with susceptibility to breast and ovarian cancer, and provides new insights into the role of rare and mosaic variants in common disease.

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C04.3

Germline mutations of inhibin in early-onset ovarian cancer

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The development of sporadic early-onset cancers, in patients without detectable germline mutations within known genes involved in Mendelian cancer predisposition, might be explained by rare de novo mutations under strong negative selection. We considered, as a paradigm, early-onset epithelial ovarian cancer and performed comparative index case-parents exome sequencing in a patient who presented, at 21 years of age, large bilateral serous ovarian adenocarcinoma. We identified a heterozygous de novo mutation (c.1157A>G p.Asn386Ser) within the INHBA gene encoding the betaA subunit of inhibins/activins, which are members of the TGF-beta superfa-

mily secreted by granulosa cells and play a key role in ovarian development. Functional analyses performed in vitro showed that the INHBA p.Asn386Ser mutation results in an increase of inhibin A and a concomitant decrease of activin A and, therefore, favours dimerisation of the inhibin betaA-subunit with the inhibin alpha-subunit. Screening for inhibin mutations in 19 other cases of early-onset ovarian epithelial tumours led us to identify within the INHA gene encoding the inhibin alpha-subunit, a germline mutation (c.179G>T, p.Arg60Leu) affecting the secondary furin cleavage site of the inhibin alpha -prodomain and functional analysis showed that this mutation is disruptive for both inhibin A and B biosynthesis. These results suggest that germline mutations of inhibins might be oncogenic in ovaries by altering the inhibin/activin ratio and, therefore, the crosstalk between granulosa and epithelial cells.

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C04.4

Parkinson's disease and melanoma: a common genetic pathway linked to *PARKIN* inactivation

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Background: Parkinson's disease (PD), a neurodegenerative disease of losing melanin-positive dopaminergic neurons, is epidemiologically linked to cutaneous melanoma (CM). *PARKIN* (*PARK2*), an important genetic predisposition to PD encoding an E3 ubiquitin ligase involved in cyclin E degradation, is also a tumor suppressor gene. We investigated the role of *PARK2* on melanoma susceptibility and oncogenesis.

Patients and methods: We recruited 500 CM patients (familial, multiple, or <25 year), 320 healthy controls, 31 tumors, and 24 melanoma cell lines. Point mutations and copy number variations (CNVs) were investigated by sequencing and MLPA, qPCR, and CGH, respectively. *PARK2* abnormalities were collected for 2060 additional controls from ten publications. PARKIN expression and cell proliferation in melanoma cell lines were also investigated.

Results: We identified 15 inactivated *PARK2* alleles leading to a truncated protein (2 splicing, 1 frameshift, and 12 exonic CNVs) in 16 CM patients (3.2%) and in 0.6% of controls (P<0.0001;OR=5.56[2.49-12.55]). *PARK2* CNVs were present in 60% of cell lines and in 50% of tumors, and LOH was detected in 50% of cell lines. PARKIN was absent in 90% of cell lines whereas present in melanocytes. Transfection of wild type *PARK2* cDNA markedly inhibits cell proliferation in three cell lines.

Conclusion: We point out a common genetic pathway that could explain the epidemiological association between PD and CM, by showing that *PARK2* inactivation plays an important role in melanoma predisposition and oncogenesis. This provides new insights in CM oncogenesis that could help targeted therapy design, and may have dermatological clinical implications in PD.

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C04.5

Loss of a Regulatory Element May Determine Endometrial Cancer Risk in *EPCAM* Deletion Carriers

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Lynch syndrome is caused by germline mutations in the mismatch-repair genes *MLH1*, *MHS2*, *MSH6* and *PMS2*. Mutation carriers exhibit a high risk of developing colorectal cancer (CRC) and endometrial cancer (EC). We recently found that constitutional 3'end deletions of *EPCAM* cause epigenetic silencing of *MSH2* in *EPCAM*-expressing tissues, thus providing a new cause of Lynch syndrome[1]. *EPCAM* deletion carriers have a similar CRC risk as *MSH2* mutation carriers, but ECs were only observed in patients with deletions extending close to the *MSH2* promoter[2]. Therefore, we hypothesized that in endometrial cancer precursor cells *MSH2* inactivation is not caused by read through-mediated silencing, but by loss of a regulatory element. Indeed, a 5.5-kb region upstream of *MSH2*, shows a site with H3K4Me1 and H3K4Me2 in the hepatocellular carcinoma cell line HepG2, suggesting the presence of a regulatory element. We generated and tested luciferase reporter constructs containing segments of the 5.5-kb region in a panel of cancer cell lines. A fragment of 1.5-kb, located 4.8-kb upstream of *MSH2*, revealed enhancer activity in the CRC cell line HCT116. Currently we are studying this region in a zebrafish model for determining its cell type-specific regulatory role *in vivo*. Our data suggest that through *EPCAM* deletions that extend close to the *MSH2* promoter an enhancer element is lost. This loss may cause an increased risk for endometrial cancer in carriers of *EPCAM* deletions that extend close to the *MSH2* promoter.

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C04.6

Suppressor-tRNA Restores Functional E-Cadherin Expression in *Cdh1* Mutant Cancer Cells: A Potential Approach to Treat Hereditary Diffuse Gastric Cancer

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Introduction: Hereditary Diffuse Gastric Cancer (HDGC) is a highly penetrant autosomal dominant disease caused by *CDH1* (E-cadherin encoding gene) germline mutations, often originating premature termination codons (PTC). HDGC arises at early age, with multifocal tumors frequently missdetected by endoscopy. Prophylactic gastrectomy is the only therapeutic approach for asymptomatic mutation carriers. We hypothesize that suppressor-tRNAs could replace PTCs by the correct aminoacid in *CDH1* nonsense-mutation carriers and delay cancer development.

Material and Methods: We established a cell line model expressing wild-type and nonsense mutant *CDH1* mini-genes (full-length cDNA enclosing a minimal intron before the last exon - CDH1_WT and CDH1_1003) to study *CDH1* truncating mutations and its reversion. A suppressor-tRNA was designed to recognize the nonsense codon in AGS, MDA-MB-231 and CHO cells line models. CDH1 mRNA expression was analysed by RT-PCR and protein expression by western blot, immunocytochemistry and flow citometry.

Results: CDH1_WT originates correctly spliced mRNA and full-length E-cadherin, correctly localized at the membrane, rescuing cell-adhesion and suppressing invasion, unlike CDH1_1003. Expression of a suppressor-tRNA rescued membranous and potentially functional E-cadherin expression in CDH1_1003 mutant cells to levels resembling those of CDH1_WT cells.

Conclusions: The *in vitro* recovery of membranous E-cadherin expression in cells expressing a nonsense mutation, through tRNA-nonsense suppression, may constitute the basis for a novel gene therapy aiming at reverting truncating mutations in cancer-associated syndromes.

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C05.1

Post GWAS analysis of a BCL11A intronic region to define its role in regulating HbF levels.

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Association studies identified BCL11A transcription factor as the master regulator of fetal hemoglobin (HbF) expression as well as a key modifier of both β-thalassemia and sickle cell anemia (SCA) phenotypes. Recently, we refined the association signal at this locus by GWAS on 5903 individuals from the SardiNIA project, integrating, with imputation procedure, DNA microarray data and low pass whole-genome sequencing of 2120 Sardinians. Our results revealed two independent SNPs within intron 2 of the BCL11A gene, leading the strongest haplotypic signals and fully accounting for the association observed at this locus. Given that intron 2 shows chromatin signatures of genomic elements with a potential regulatory activity, we carried out functional and expression analysis to asses the specific impact of associated variants in gene action. Preliminary luciferase reporter assays on erythroleukemia cell lines (K562, HEL) have shown a promoter/enhancer activity embedded in the regions encompassing two of them. Notably for one SNP, EMSA on human primary erythroid cells (BFUe) also detected a differential binding pattern showing developmental stage and allele specificity. Furthermore, RT-qPCR and allelic specific expression assays (ASE) conducted on BFUe cells derived from β-thalassemia patients suggest that the associated SNPs, might function as cis-acting regulatory variants, affecting its expression in a temporal and tissue specific manner. Overall our results support that genetic variants at intron 2 of BCL11A play a crucial role in modulating its function controlling HbF levels with consequent amelioration of β-thalassemia severity.

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C05.2

Transcriptome and genome sequencing uncovers functional variation in human populations

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Understanding functional effects of genetic variants is one of the biggest challenges in human genomics. Towards this goal, we sequenced mRNA and small RNA from lymphoblastoid cell lines of 465 individuals from 5 populations of the 1000 Genomes Project. Population comparisons showed splicing rather than expression dominating continental differences (75-85% differentially splices genes EUR-ARF vs 6-40% EUR-EUR), putatively suggesting a special role of splicing in human adaptation. We found extremely widespread regulatory variation, with genetic variants associating to gene expression levels of 7825 genes, transcript structure of 639 genes, as well as expression of 60 miRNAs. Allele-specific transcription analysis allowed us to characterize rare regulatory effects and underlying genetic variants. Importantly, genome sequencing data combined with functional annotation of the genome allowed us to infer putative causal regulatory variants for 57-75% of eQTL genes. We apply this to predict causal variants for 91 disease-associated loci, and also characterize transcriptome effects of loss-of-function variants, thus linking our discoveries to genetic associations to human disease. Altogether, the integration of genome sequencing and functional genomics data in this study takes us towards understanding and predicting molecular genomic effects of causal functional variants in the human genome.

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C05.3

Genetic regulation of lincRNA and protein-coding genes expression variation - similarities and differences

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We have investigated by RNA-seq the gene expression variation in the GenCord collection: three cell types (fibroblasts - F, lymphoblastoid cell lines - L and T-cells - T) from umbilical cord of 195 unrelated European individuals. We have observed expression of 392, 553 and 546 large intergenic non-coding RNA genes (lincRNAs) in F,L and T respectively. LincRNAs are lowly expressed, highly-tissue specific and located close to protein-coding genes involved in regulation of transcription. LincRNAs' gene size negatively correlates with expression level, as it is known for protein-coding genes, thus demonstrating similar evolutionary constraints between lincRNA and protein-coding genes. Among expressed lincRNAs we have found 54, 102 and 65 genes from F,L and T correspondingly with cis expression Quantitative Trait Loci (cis-eQTL). Comparison of regulation patterns of lincRNA and protein-coding genes has revealed two characteristic features of lincRNAs: (i) they have excess of cis-eQTLs and (ii) lincRNAs' eQTLs are located closer to transcription start sites (TSS). Our analysis of protein-coding genes has shown that both excess of cis-eQTLs and close location of cis-eQTLs to TSS are specific also for evolutionary young (primate-specific) protein-coding genes. We conclude that lincRNAs and young protein-coding genes have similar evolutionary landscape of regulation.

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C05.4

Coordinated effects of sequence variation on DNA binding, chromatin structure, and transcription

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Recent advances in genome-wide profiling of transcription factor (TF) binding and histone modifications have identified specific chromatin signatures related to various classes of functional elements in different cell types.

However, their genetic basis and degree of variability across individuals remain largely unknown. We generated genome-wide enrichment profiles of TF binding, chromatin marks, and different measures of transcription in lymphoblastoid cell lines from two trios and 8 unrelated individuals sequenced in high depth as part of the 1000 Genomes project. Inter-individual variability of these phenotypes was quantified to understand both DNA sequence dependent and independent variation on transcription, TF binding, chromatin state, and their interplay in an allele-specific framework. Different organizational layers of the genome show abundant allelic effects and strong allelic coordination between layers, with the genetic control of this coordination acting primarily through transcription factor binding. Our findings support the notion of transcription factors being the primary determinants of gene expression programs, with the overall chromatin state reflecting, but not necessarily driving gene expression activity. We extended our analysis to 54 unrelated individuals for most assays to identify genetic effects affecting chromatin properties on a population level, and continue exploring the combinatorial patterns of all assays to further dissect the components of the general transcriptional state of the cells. This study will significantly improve our understanding of the biological landscape around regulatory and other functional elements of the genome, and provide better means to interpret the heritability and molecular basis of phenotypic diversity, such as disease susceptibility, in humans.

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C05.5

Deciphering vertebrate regulatory grammar using high-throughput in vivo functional assays

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Despite continual progress in the cataloging of vertebrate regulatory elements, little is known about the grammatical rules that govern their intensity and spatio-temporal extent. Deciphering these rules will enable high-resolution mapping of regulatory elements, accurate interpretation of nucleotide variation within them, and the design of sequences that can deliver molecules for therapeutic purposes in a spatio-temporal manner. Here, we describe two novel approaches that we have developed to systematically improve our understanding of regulatory grammar. In the first approach, mathematical theory and computational methods were developed to construct an ultra-compact collection of DNA oligomers encompassing all possible 6bp sequences, which were screened in 15 tissues and two time points using zebrafish transgenesis. Twenty-seven (from 184) of these constructs produced consistent expression patterns, many of which were tissue-specific and were further used to design robust, tissue-specific enhancers. Using a second approach, we systematically decoded the rules governing TFBS arrangement and combination by testing ~5,000 synthetically designed sequences using a massively parallel reporter assay in the mouse liver. We find that certain transcription factors act as direct drivers of gene expression in homotypic clusters, independent of spacing, whereas others function only synergistically. Heterotypic enhancers are stronger than their homotypic analogs, and favor specific TFBS combinations, mimicking putative native enhancers. Finally, exhaustive testing of binding site permutations supported a model with flexibility in binding site order. This work provides a unique catalog of tissue-specific synthetic enhancers as well as a massively parallel view of the basic principles of regulatory function in vivo.

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C05.6

Novel genetic variants associated with alternative polyadenylation and expression of noncoding transcripts

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Many disease-associated variants affect gene expression levels (expression quantitative trait loci, eQTLs). Expression profiling using next generation sequencing (NGS) technology is very powerful to detect these eQTLs.
Here we analyzed 94 total blood samples from healthy volunteers with DeepSAGE to gain specific insight how genetic variants affect the expression of genes and and lengths of 3'-untranslated regions (3'-UTRs). We detected previously unknown cis-eQTL effects for GWAS hits in disease- and physiology-associated traits. Apart from cis-eQTLs that are typically well identifiable using microarrays or RNA-seq, DeepSAGE revealed many cis-eQTLs for antisense and other non-coding transcripts, often in genomic regions containing retrotransposon-derived elements. Furthermore, we identified and confirmed SNPs that affect the usage of alternative polyadenylation sites, thereby potentially influencing the stability of mRNAs.
We subsequently combined the power of RNA-seq with DeepSAGE by performing a meta-analysis of three datasets, resulting in the identification of a substantially increased number of cis-eQTLs.
Our results indicate that DeepSAGE data is useful for eQTL mapping of known and unknown transcripts, and identification of SNPs that affect alternative polyadenylation. Because of the inherent differences between DeepSAGE and RNA-seq, the complementary integrative approach that we describe here helps to gain better insight in the molecular consequences of many disease-associated variants.

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C06.1

The disease mechanisms of FSHD1 and FSHD2 converge at the level of somatic expression of DUX4

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Facioscapulohumeral dystrophy (FSHD) is caused by decreased epigenetic suppression of the D4Z4 repeat array on chromosome 4 resulting in expression of the D4Z4-encoded *DUX4* gene in skeletal muscle.
Two variants of chromosome 4 exist of which only one is permissive to *DUX4* expression due to the presence of a polymorphic *DUX4* polyadenylation signal. In most patients, loss of epigenetic suppression of *DUX4* is caused by contraction of the D4Z4 array (FSHD1) on a permissive allele and is inherited as a dominant trait. However, in some patients the decrease in epigenetic suppression occurs on normal-sized arrays (FSHD2) and shows a complex inheritance pattern. We discovered that mutations in the chromatin modifier SMCHD1 on chromosome 18 segregate with genome-wide D4Z4 CpG hypomethylation in FSHD2 families. In FSHD2 patients the disease is thus caused by digenic inheritance of a SMCHD1 mutation and a normal-sized D4Z4 array permissive for *DUX4* expression. Currently, we have identified >50 *SMCHD1* mutations *SMCHD1* explaining 80% of FSHD2. The mutation spectrum provides new insights in the disease mechanism and we observed that *SMCHD1* mutations can also aggravate disease severity in FSHD1 families.
SMCHD1 is a chromatin modifier that binds directly to the D4Z4 repeat. In FSHD2 patients there is reduced binding of SMCHD1 to D4Z4 and knock down of SMCHD1 in normal myoblasts containing a permissive chromosome 4 leads to the activation of *DUX4*. Our data thus suggest FSHD1 that the disease mechanisms of FSHD1 and FSHD2 converge at the level of D4Z4 chromatin decondensation and somatic *DUX4* expression.

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C06.2

Plastin 3 ameliorates spinal muscular atrophy via delayed axon pruning and improves neuromuscular junction functionality

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F-actin bundling plastin 3 (PLS3) is a fully protective modifier of the neuromuscular disease spinal muscular atrophy (SMA), the most common genetic cause of infant death. The generation of a conditional PLS3 over-expressing mouse and its breeding into an SMA background allowed us to decipher the exact biological mechanism underlying PLS3-mediated SMA protection. We show that PLS3 is a key regulator that restores main processes depending on actin dynamics in SMA motor neurons (MN). MN soma size significantly increased and a higher number of afferent proprioceptive inputs were counted in SMAPLS3 compared to SMA mice. PLS3 increased presynaptic F-actin amount, rescued synaptic vesicle and active zones content, restored the organization of readily releasable pool vesicles and increased quantal content at the neuromuscular junctions (NMJs). Most remarkably, stabilized axons by PLS3 over-expression delayed axon pruning, counteracting poor axonal connectivity at SMA NMJs. These findings together with the observation of increased endplate and muscle fiber size upon MN-specific PLS3 overexpression suggest that PLS3 significantly improves neurotransmission. Indeed, ubiquitous over-expression improves survival and motor function in SMA mice. As PLS3 seems to act independently of Smn, PLS3 might be a potential therapeutic target not only in SMA but also other MN diseases.

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C06.3

The neuronal endopeptidase ECEL1 is associated with autosomal recessive distal arthrogryposis

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Distal arthrogryposis (DA) is a subgroup of arthrogryposis multiplex congenita (AMC) characterized by multiple congenital joint limitations. Using a pangenomic approach in 2 consanguineous families with DA, we identified a common morbid locus that mapped to chromosome 2q37 and harbored the Endothelin Converting Enzyme Like 1 (*ECEL1*) gene. We screened a panel of 20 DA families and identified 7 different mutations in six families. All mutations resulted either in the absence of the protein or in the synthesis of a non-functional protein, suggesting a loss of function mechanism. *ECEL1* encodes a neuronal endopeptidase expressed in the peripheral (PNS) and central nervous system during fetal life and plays a major role in intramuscular branching of motoneurons in skeletal muscle.Patients presented with a homogeneous phenotype characterized by limited knee flexion, flexed third to fifth fingers and relatively spared index finger, severe muscle atrophy in lower limbs and tongue. Muscle imaging showed a recognizable pattern of the thigh muscles. No dysfunction of the neuromuscular transmission was evidenced.On the whole, clinical features point towards a disorder involving the PNS. A developmental dysfunction is suggested by the predominantly prenatal expression of *ECEL1*, the non-progressive course of the disease and K.O. mouse data.

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C06.4

Constitutive activation of STIM1 causes tubular aggregate myopathy

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In skeletal muscle, Ca2+ is mainly stored in the sarcoplasmic reticulum (SR). Ca2+ is released to the cytoplasm upon stimulation, where it triggers muscle contraction and acts as a second messenger controlling growth and differentiation. Ca2+ stores are refilled through a process called store-operated Ca2+ entry (SOCE). Stromal interaction molecule 1 (STIM1) is the main Ca2+ sensor in the endoplasmic reticulum. We identified STIM1 mutations as the genetic cause of tubular aggregate myopathy (TAM), characterized by regular arrays of membrane tubules on muscle biopsies. All nine heterozygous mutations (including 5 novel) were found in the highly conserved intraluminal EF-hands, sensing and binding Ca2+. Upon Ca2+ store depletion, wild-type STIM1 oligomerizes and thereby triggers extracellular Ca2+ entry. In contrast, myoblasts transfected with the mutant constructs displayed constitutive STIM1 clustering, indicating that Ca2+ sensing was lost. We investigated the pathological mechanism underlying the disease and monitored the calcium response of patient myoblasts to SOCE. We found a significantly higher basal Ca2+ level in patient cells as compared to the control. Addition of high [Ca2+] medium induced a sudden and massive Ca2+ influx in the patient myoblasts, as compared to low gradual increase in control cell lines. These data demonstrate that dominant STIM1 mutations induce a constitutive activation of the Ca2+ entry channels in muscle cells. Recessive loss-of-function mutations in STIM1 have been associated with severe immune deficiency, demonstrating that a tight regulation of STIM1-dependent SOCE plays an essential role in T-cell activation as well as in normal skeletal muscle structure and function.

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C06.5

Myotonic dystrophy CTG expansion affects synaptic vesicle proteins, neurotransmission and mouse behavior

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Myotonic dystrophy type 1 (DM1) is a complex multisystemic disorder, which displays multiple debilitating neurological manifestations. Despite recent progress in the understanding of the molecular pathogenesis of DM1 in skeletal muscle and heart, the pathways affected in the central nervous system (CNS) require further investigation.

In our studies we have used DMSXL transgenic mice expressing large CTG trinucleotide repeats within the human DM1 locus, notably in the CNS. These mice recreate molecular features of RNA toxicity in brain, such as RNA foci accumulation and dysregulation of alternative splicing, in association with the sequestration of MBNL splicing regulators and upregulation of CELF proteins.

Detailed phenotyping revealed relevant cognitive deficits and behavioural abnormalities, altered short-term synaptic plasticity, as well as changes in neurochemical levels. To decipher the molecular bases behind these phenotypes, a global proteomics approach revealed RAB3A upregulation and abnormal synapsin 1 hyperphosphorylation in the CNS of transgenic mice. These abnormalities were confirmed in transfected cells and validated in post-mortem DM1 brains. Using complimentary DM1 mouse models, we showed that RAB3A upregulation results from the sequestration of MBNL1 in RNA foci, while synapsin 1 hyperphosphorylation is mediated by the upregulation of CELF proteins. Interestingly, synaptic protein defects were associated with altered spontaneous neurosecretion in cell culture and are likely to contribute to the electrophysiological and behavioural deficits of DM1 transgenic mice.

The novel connection between physiological phenotypes and synaptic protein deregulation reveals synaptic dysfunction in DM1 brain pathology.

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C06.6

Nanoparticles as delivery systems for antisense oligoribonucleotides: biodistribution studies and definition of the release kinetic in intraperitoneally and orally treated mdx mice

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Antisense-mediated exon skipping is a very promising therapeutic option for Duchenne muscular dystrophy and other diseases susceptible of splicing correction. We have tested different types of polymeric cationic core-shell nanoparticles (NPs) for delivering 2-O-methyl-phosphorothioate antisense oligoribonucleotides (AONs), in mdx mice. Both T1 and ZM2 NP bind and convey AONs: intraperitoneal (IP) injections of low doses (52.5mg/kg) of NP-AON complex restored dystrophin protein synthesis in skeletal and cardiac muscles, allowing protein localization in up to 40% of muscle fibers with skipping level up to 20%. We have administered NP-AON complexes orally and tested in vivo (mdx) the tissue biodistribution and elimination timing of NPs by Odyssey, using an infrared dye conjugated ZM2 NP. The NP-AON formulations passes the gastric barrier and induce a limited dystrophin rescue in the intestinal smooth muscles as well as in the diaphragm. WE have also identified a novel NP (ZM5) able to pass the intestinal barrier. In order to get insight about the release kinetic of the AON from NPs, we have performed ELISA assay to dose the antisense in the all mice muscles , both IP and oral treated. We demonstrated that AON are rapidly released from NPs (with no depot effect) but those AON reaching tissues and exerting the functional effect are well protected from degradation. Defining the kinetic of NP-AON complexes is crucial in order to further proceed with other NP studies following non invasive administration routes, as oral and skin adsorption.

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C07.1

New diagnostic paradigms for mitochondriopathies

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Mitochondrial disorders present as a genetically and clinically extremely heterogeneous disease. Up to now 250 mitochondrial genes have been identified, whereas more than 1000 genes are predicted to encode for mitochondrial proteins. To identify disease causing mutations, we applied exome sequencing in combination with stepwise filtering of gene variants and functional complementation for 120 unrelated individuals with juvenile-onset mitochondrial disorders without pathogenic variants in the mtDNA. We were able to detect mutations in known disease genes in 40% of cases, whereas in 10% novel disease causing genes were identified. The list of such newly identified genes increases steadily: most recently we identified MGME1 to be the first exonuclease involved in mitochondrial replication (Kornblum et al., Nat. Genet. 2013). Most notably, it is also possible to detect mutations in genes which are not predicted candidates for being a mitochondrial protein but which cause a clear mitochondriopathy phenotype e.g. mutations in the riboflavin transporter encoding gene SLC52A2. The challenge now is, apart from improving sequencing technology, to annotate variants in non-coding regions, to identify indels and copy number variants, as well as considering how to tackle diseases caused by di- or oligogenic mutations exerting a synergistic effect. Till then, each gene newly identified by exome sequencing holds the promise for new treatment options, as for example riboflavin supplementation in the case of mutations in SLC52A2, and helps to shed more light on mitochondrial physiology.

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C07.2

ER Mannosidase I deficiency: An unexpected CDG-II with intellectual disability and dysmorphic features

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Congenital Disorders of Glycosylation (CDG) are a group of rare metabolic diseases, caused by impaired protein or lipid glycosylation. To date, over 50 distinct disorders have been described. In the last two years, the discovery of new CDG genes has occurred faster than ever thanks to massive parallel sequencing. We identified MAN1B1 as the culprit gene in a patient with CDG-II by means of exome sequencing. Subsequently, six additional cases were identified from a cohort of unsolved CDG-II. All patients presented intellectual disability, facial dysmorphism and truncal obesity. Thus, we describe MAN1B1 deficiency as a relatively frequent cause of CDG.
MAN1B1 is believed to be an ER resident α(1,2)-mannosidase acting as a key enzyme in the glycoprotein quality control system. It targets misfolded glycoproteins for ER associated degradation (ERAD) by cleaving a terminal mannose residue from the asparagine-linked Man9GlcNAc2, forming Man8GlcNAc2 isomer B. Biochemical analysis indeed confirmed a delay in the trimming of Man9GlcNAc2 to Man8GlcNAc2 in patients' cells. Interestingly, Golgi morphology was altered in all patients, with marked dilation and fragmentation. We infer from this observation that part of the phenotype is linked to Golgi disruption. Moreover, we showed that the endogenous MAN1B1 is localized to the Golgi apparatus, instead of the ER where quality control is supposed to occur. Hence, our results challenge the current view on glycoprotein quality control, even though more work is needed to propose a new role for MAN1B1 and to explain the pathophysiology.

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C07.3

Mutations in nuclear-encoded components of mitochondrial respiratory chain complex III and IV cause apoptosis-driven developmental defects, a new mitochondrial phenotype in vertebrates

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Mitochondrial-dependent programmed cell death (PCD), plays an essential homoeostatic role, by selecting bioenergetically proficient cells suitable for normal tissue and organ development. Despite intensive investigation, the impact of this crucial process in human diseases remains poorly understood. In particular, the link between apoptosis and mitochondrial disorders, i.e. primary defects of oxidative phosphorylation, has not been persuasively demonstrated.
We demonstrated that activation of non-canonical mitochondrial-depen-

dent PCD causes the Microphthalmia with Linear Skin lesions (MLS), a developmental disorder associated to mutations in HCCS, encoding the Holo-Cytochrome c-type synthase, which incorporates the heme-c moieties in the mitochondrial respiratory chain (MRC). By generating a Medakafish model that recapitulates the MLS phenotype, we demonstrate that hccs downregulation, induced PCD in the central nervous system (CNS) via an apoptosome-independent caspase-9 activation triggered by MRC impairment and overproduction of reactive oxygen species.

We then screened MRC-related genes in HCCS-negative MLS patients, and found deleterious de novo mutations in two simplex cases and a nonsense mutation, which segregates with the disease in a familial case, in COX7B encoding a poorly characterized subunit of Cytochrome c oxidase (COX), the MRC complex IV. We demonstrated that COX7B is indispensable for COX assembly, COX activity and mitochondrial respiration. Finally downregulation of cox7B in medaka resulted in microcephaly and microphthalmia that recapitulated the MLS phenotype and demonstrated an essential function of complex IV activity in vertebrate CNS development.

These data indicate an evolutionary conserved role for MRC in organogenesis and uncover a group of mitochondrial diseases hallmarked by an apoptosis-driven abnormal development.

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C07.4

Mutation of the iron-sulfur cluster assembly IBA57 gene causes lethal myopathy and encephalopathy.

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The iron sulphur [Fe-S] proteins play an important role in redox reactions of the mitochondrial electron transport chain. In this study, we have identified two siblings from consanguineous parents, who died perinatally from a condition characterised by generalised hypotonia, respiratory insufficiency, anthrogryposis, microcephaly, congenital brain malformations and hyperglycinemia. Analysis of the catalytic activities of the mitochondrial respiratory complexes I and II indicated deficiency in skeletal muscle, suggestive of an inborn error in the mitochondrial iron-sulfur cluster (ISC) biosynthesis pathway. Homozygosity mapping revealed the *IBA57* gene, which is known to be involved in the biosynthesis of mitochondrial [4Fe-4S] proteins and present in the largest homozygous region on chromosome 1, as a candidate gene. Mutation analysis of *IBA57* identified a c.941 A>C transversion causing the aminoacid change p.Gln314Pro. Biochemical analysis of skeletal muscle and skin fibroblasts of affected individuals indicated severely decreased amounts of IBA57 and a decrease in various 4Fe-4S proteins and in proteins covalently linked to lipoic acid. IBA57 depleted HeLa cells reflected biochemical defects consistent with observations in patient derived cells. Defects could be rescued by the introduction of wildtype *IBA57* and partially by mutant *IBA57*. Further functional analysis revealed an increased sensitivity of mutant IBA57 to degradation via proteolysis. Our findings suggest that the mutation leads to functional impairment and degradation below physiologically critical levels, resulting in the condition observed in the patients. In conclusion, we have identified a novel metabolic disorder presenting with a lethal complex biochemical phenotype caused by defective assembly of the ISC protein, IBA57.

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C07.5

Exome sequencing Reveals Mutated NUBPL in Patients with Complex I Deficiency and a Distinct MRI Pattern

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There are many rare childhood leukoencephalopathies and till this day a high percentage of cases remain without a specific diagnosis. We used MRI-pattern recognition analysis to select a group of patients with a similar, novel MRI-pattern and subsequently performed whole-exome-sequencing to identify the mutated gene. Patients' fibroblasts were examined for biochemical consequences of the mutant protein. Six patients from five unrelated families were identified with a similar MRI-pattern showing predominant abnormalities of the cerebellar cortex, deep cerebral white matter and corpus callosum. The four tested patients had a complex I deficiency. Exome-sequencing performed in two patients revealed mutations in *NUBPL*, encoding an iron-sulfur cluster assembly factor for complex I. In one of the two patients only one heterozygous missense mutation was detected. Sanger sequencing revealed the second mutation, and *NUBPL* mutations in all other patients. Upon identification of the mutated gene, the MRI of the previously published case with *NUBPL* mutations was analyzed, which showed exactly the same pattern. A decreased amount of NUBPL protein and fully assembled complex I was found in patients' fibroblasts. Analysis of the effect of mutated NUBPL on the assembly of the peripheral arm of complex I indicated that NUBPL is involved in assembly of iron-sulfur clusters early in the complex I assembly pathway. Our data show that *NUBPL* mutations are associated with a unique, consistent MRI-pattern, which facilitates fast diagnosis and obviates the need for other tests. Whole-exome sequencing can provide a quick diagnosis, however, caution is warranted because certain mutations can be missed.

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C07.6

Pioglitazone prevents mitochondria dysfunction and halts axonal degeneration in a mouse model of X-adrenoleukodystrophy

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Accumulating evidence shows that oxidative stress and mitochondria dysfunction play a major role in the pathogenesis of axonal degeneration in multifactorial conditions such as Alzheimer's disease, Parkinson disease or amyotrophic lateral sclerosis. We have addressed the interplay of both noxious factors in adrenoleukodystrophy (X-ALD, OMIM: 300100), a peroxisomal disorder caused by loss of function of the ABCD1 transporter, leading to accumulation of very long-chain fatty acids (VLCFA) in organs and plasma. The mouse model for X-ALD exhibits a late-onset neurological phenotype with locomotor disability and axonopathy in spinal cords resembling the most common phenotype of adrenomyeloneuropathy patients. Recently, we identified oxidative damage and energetic failure as an early event in life, and the excess of VLCFA as a generator of radical oxygen species (ROS) and oxidative damage in X-ALD. Here, we uncover a mitochondrial impairment and depletion due to an impairment of mitochondria biogenesis via the PPARγ/PGC-1α pathway, which is also observed in brain white matter of X-ALD patients. We thus investigated the therapeutic effect of pioglitazone, an antidiabetic drug and a PPARγ agonist, in a preclinical test. Oral administration of pioglitazone restored mitochondria DNA and protein contents and expression of master regulators of biogenesis, neutralized oxidative damage to proteins and DNA, and reversed bioenergetic failure in terms of ATP levels, NAD^+/NADH ratios, and pyruvate kinase activities. Most importantly, the treatment halted locomotor disability and axonal damage in X-ALD mice. These results constitute a strong rationale for a phase II multicentric clinical trial for adrenomyeloneuropathy patients.

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C08.1

Mutations in the microtubule-associated protein EML1/Eml1 lead to ectopic progenitors during cortical development and heterotopia in mouse and human

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Cortical malformations, such as lissencephaly and subcortical band heterotopia (SBH), are associated with intellectual disability and pharmacoresistant epilepsy. Mouse mutant models for three genes mutated in SBH, *Doublecortin* (*DCX*), *LIS*1 and *alpha1-tubulin* (*TUBA1A*), do not show abnormally positioned heterotopic neurons in the neocortical white matter. On the other hand, the spontaneously arisen *HeCo* mouse mutant displays this phenotype. By whole genome genotyping and gene expression studies, we identified *Eml*1 as the mutated gene in *HeCo* mice. A retrotransposon insertion disrupts the gene, creating aberrant transcripts. Compound heterozygous mutations were identified in human *EML*1 in one family showing an atypical form of giant heterotopia. *Eml*1 codes for a microtubule-associated protein, not previously studied in cortical development. We show its expression in proliferation zones and the cortical plate, and *HeCo* mice have ectopic proliferation, which seems to be the primary cause of heterotopia in this model. Eml1 shows a punctate labeling, partially associated with microtubules in both progenitors and post-mitotic neurons. A patient missense mutation affects the microtubule association. In neuronal progenitors, Eml1 shows a cell cycle- dependent localization, enriched in the midbody region during late mitosis. We thus identify a new corticogenesis gene, and study of Eml1 and the *HeCo* phenotype reveal novel insights into heterotopia formation. Eml1 plays a role in neuronal progenitors and our data makes a link between ectopic progenitors in the developing cortical wall and severe heterotopia in human.

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C08.2

Mutations in *DEAF1* cause intellectual disability with severe speech impairment

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Intellectual disability (ID) is a genetically highly heterogeneous disorder that is frequently caused by *de novo* mutations. Recently, we showed that in about 30-50% of patients *de novo* mutations in known or candidate ID genes can be identified.[1,2,3] For candidate ID genes, it is often difficult to ascertain whether the *de novo* mutation is causative for the patients phenotype. To objectively determine the association of a novel candidate gene with the patients phenotype, the most important criterion is the identification of recurrent mutations in patients with an overlapping phenotype. Therefore, we tested an additional cohort of 765 patients with unexplained ID for mutations in 8 of these novel candidate ID genes, including *DEAF1*. We identified *de novo* mutations in *DEAF1* in three patients. All three patients showed severe ID with a disproportionally severely affected speech. We used functional assays to determine the impact of the mutations on the protein function. In addition, we performed functional studies of *DEAF1* in the fruit fly and zebrafish to further implicate this gene in the phenotype of the patient. In conclusion, we show that transcription factor *DEAF1* is a recurrently mutated gene in three patients with non-syndromic ID with severe speech impairment and we provide supportive functional evidence that links *DEAF1* to the patients phenotype.

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C08.3

Mutations in TTI2 reveal a role for Triple T complex in human brain development

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Tel2-interacting proteins 1 and 2 (TTI1 and TTI2) physically interact with TEL2 (telomere maintenance 2) to form a conserved trimeric complex called the Triple T complex. This complex is a master regulator of phosphoinositide-3-kinase-related protein kinase (PIKKs) abundance and DNA damage response signaling. Using a combination of autozygosity mapping and high-throughput sequencing in a large Algerian consanguineous multiplex family, we found that a missense p.I436N mutation in *TTI2* causes a human autosomal recessive condition characterized by severe cognitive impairment, microcephaly, behavioral troubles, short stature, skeletal anomalies and facial dysmorphic features in three affected sibs. Immunoblotting experiment showed a drastically impaired stability of each subunit of the Triple T complex in patient skin fibroblasts. Consistently, a significantly reduced steady-state level of the two PIKKs tested, ATM and DNA-PKcs were observed in patient cells. Likewise, testing the serum dependant p70-S6Kinase phosphorylation we also found a defective mTOR signaling in patient cells. Combined with previous observations, these findings assess the role of the TTI2 gene in the aetiology of intellectual disability and further support the hypothesis of a crucial role of PIKK signaling in brain development and functioning.

Key words: Intellectual disability, autozygosity mapping, exome sequencing, DNA damage repair

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C08.4

Involvement of kinesin family members KIF4A and KIF5C in intellectual disability and synaptic function

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KIF genes encode different motor proteins that are supposed to have fundamental roles in brain functioning, development, survival and plasticity, by regulating transport of cargoes along microtubules within the axons, dendrites and synapses of neurons. Studies in mouse models support the important functions of KIF genes in the nervous system. Reports that relate KIF genes to human Intellectual disability (ID) phenotypes are rare.
We report the clinical characteristics of individuals with mutations in the kinesin superfamily (KIF) genes KIF4A and KIF5C identified by next generation sequencing approaches. Four males from an X-linked family with a mutation in KIF4A (c.1489-8_1490delins10; p.?- exon skipping) showed mild to moderate intellectual disability (ID) and epilepsy. Furthermore, we identified one familial form and one sporadic patient with de novo dominant missense mutations in KIF5C who presented with severe ID, epilepsy and microcephaly. Interestingly, cerebral MRIs in these cases revealed cortical malformation indicative for a role of KIF5C in neuronal migration. To find

further supportive evidence of the involvement of KIF4A and KIF5C in observed ID phenotypes, we studied the effects on synaptic function of knockdown of these genes in rat primary neurons. At the functional level we found that both, Kif4a and Kif5c altered the balance between excitatory and inhibitory synaptic efficacy leading to changed neuronal excitability. Therefore, we believe that distortion of the balance between excitatory and inhibitory excitability at the synapse by KIF4A and KIF5C is a major mechanism in pathophysiology of the phenotype observed in our cases.

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C08.5

Abnormal expression of sex biased genes in PCDH19-female limited epilepsy and intellectual disability (PCDH19-FLE) suggests a role for neurosteroid hormones.

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PCDH19-Female-Limited-Epilepsy (PCDH19-FLE) is an unusual X-linked disorder that primarily affects females. PCDH19-FLE encompasses a broad clinical spectrum from early infantile epileptic encephalopathy resembling Dravet syndrome to epilepsy with or without intellectual disability and behavioural problems, including autism. PCDH19-FLE is highly but not fully penetrant. We have tackled the questions of molecular pathogenesis of PCDH19-FLE by examining the transcriptomes of primary skin fibroblasts of PCDH19-FLE females (n=12 and n=3 age and passage matched normal controls) and unaffected transmitting males (n=3 and n=3 age and passage matched control males). We found that the expression of genes, which normally show sex (male/female) biased expression in this cell type, was significantly altered (observed = 43/94 vs expected = 223/19223, p=1.09 x 10-55, two-tail Fisher's exact test). Followup studies (including additional skin fibroblast cell lines) validated at least ~60% of selected genes. From among several plausible biological candidates we focused our attention on the aldo-keto reductase family 1, member C1-3 (AKR1C1-3) genes, which play crucial role in neurosteroid hormone metabolism (which skin is endowed with). Additional support for steroid and neurosteroid hormone role in the pathology of PCDH19-FLE came from the age of onset (mean ~10 months) and offset (mean ~12.5 years) of epilepsy (n=100 patients), both of which coincide with dramatically varying sex hormone levels (onset - after 'minipuberty' and offset - with the advent of puberty). This led us to postulate the neurosteroid hypothesis, which may explain PCDH19-FLE and opens realistic opportunities for targeted therapeutic interventions.

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C08.6

Identification of Single-minded 2 (Sim2) binding sites by ChIP-Seq; understanding of the regulatory network of chromosome 21 transcription factors

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Down syndrome (DS) results from trisomy of chromosome 21 (HSA21). Some DS phenotypes may be directly or indirectly related to the increased expression of specific HSA21 genes, in particular transcription factors. The HSA21 Single-minded 2 (SIM2) transcription factor has key neurological functions and appears therefore as a good candidate for some DS features, in particular mental retardation. In order to identify the DNA binding sites and downstream targets of SIM2, we performed three independent chromatin immunoprecipitations and high-throughput sequencing (ChIP-Seq) on a mouse embryonic stem cell (mESC) line that stably overexpresses a Flag-tagged mouse Sim2 under the control of a Tet-off system. ChIP and input DNAs were sequenced on the Illumina HiSeq 2000. Reads uniquely mapped with BWA were submitted to HOMER for the identification of Sim2 targets and the discovery of binding site motifs. We obtained a list of 2373 putative binding sites for Sim2. Importantly, as opposed to other regions, the promoters were significantly over-represented in the list of Sim2 binding sites (p-value 9.3E-12). A gene ontology analysis on the nearby genes revealed enrichment for biological processes potentially related to Sim2 functions such as transmission of nerve impulse or ectoderm development. The RNA-Seq transcriptome of the Sim2 expressing and non-expressing mESC is currently under investigation in order to correlate the Sim2 binding sites with putative target genes. All together, those results suggest the role of a Sim2-related gene network in DS neuronal features and emphasize the importance of the functional characterization of HSA21 transcription factors.

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C09.1

Exome sequencing of 2,000 Danish individuals and the role of rare coding variants in type 2 diabetes

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It has been hypothesized that low-frequency genetic variants in coding regions of genes, in aggregate, explain a substantial fraction of the heritability of common diseases. We sequenced the exomes of 1,000 Danish cases with type 2 diabetes, BMI > 27.5 kg/m^2, and hypertension and 1,000 controls to an average depth of 56x. Simulations suggest our study has substantial statistical power to detect at least one causal gene if the heritability of these common diseases is explained by rare variants in the coding regions of a limited number of genes. We applied a series of gene-based tests to detect such susceptibility genes. However, no gene showed a significant association to disease risk after correcting for the number of genes analyzed. Thus, we can reject a model for the genetic architecture of type 2 diabetes, obesity and hypertension, where much of the missing heritability is explained by rare non-synonymous variants clustered in a small number of genes.

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C09.2

Haplotype sharing reveals fine-scale demographic history

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Identical-by-descent (IBD) chromosomal segments shared by pairs of individuals can now be reliably detected in large genomic datasets of purportedly unrelated samples, and summary statistics of IBD sharing were recently shown to convey information about population-level features such as demography, natural selection and heritability of common traits. We present analytical results for the relationship between haplotype sharing and demography, and show how IBD sharing can be used to infer the demographic history of the recent millennia, where classical methods are typically underpowered. We analyzed 500 Ashkenazi Jewish samples, finding evidence for two periods of expansion, separated by a strong bottleneck. In the reconstructed model, an effective population of tens of thousands of individuals is first drastically reduced to ~300 founders in the 12th century A.D, then ra-

pidly expands (exponential rate ≈ .28) to a modern population of millions of individuals. Analyzing IBD sharing in 56 Kenyan Masai, high levels of cryptic relatedness are pervasive. While this may suggest a strong recent bottleneck (exponential rate ≈ -.14), we show that a model of multiple small-sized demes interacting through high migration rates, consistent with the social structure of this population, results in a compatible pattern of haplotype sharing. We finally analyzed 498 individuals from several Dutch provinces (the Genome of Netherlands dataset) finding evidence for population growth and substantial migration across these groups in the recent millennia.

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C09.3

Signatures of selection in the Genome of the Netherlands Project

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The Genome of the Netherlands Project (GoNL) sequenced 250 pedigrees of Dutch ancestry at 12x coverage in collaboration with BGI (Shenzhen, China).
Using all unrelated samples (N=498), we sought to identify regions in the Dutch genome that show unusual allele frequency spectra, which could indicate evidence of selection. Therefore, we calculated Tajima's D values in 100-kb windows across the genome, stratifying variants by function.
Our results show that the mean Tajima's D value is lower for exonic regions, compared to non-exonic regions ($p=3.6x10^{-19}$). This observation reflects allele frequency distributions that are shifted toward rare variants in these exonic regions, a pattern that can be explained by purifying selection. Furthermore, we studied the regions showing the 5% lowest and highest Tajima's D values by looking for significant overrepresentation of sequence features, pathways and diseases genes. 1603 genes map to the 5% of regions with the lowest Tajima's D. This group of genes is enriched for genes with alternative splicing products ($p=4.1x10^{-13}$) and height genes ($p=5.6x10^{-6}$). 853 genes map to the 5% regions with the highest Tajima's D and here we found an overrepresentation of genes involved in olfactory transduction ($p=3.3x10^{-25}$) and various immune traits (P<0.001). Unsurprisingly, the MHC region mainly drove results in immune traits.
Overall, we conclude that our analysis demonstrates evidence for purifying selection in exonic regions of the Dutch genome. Furthermore, our results suggest signatures of selection for regions containing height, smell and immunity genes. We are currently pursuing alternative methods for detecting genomic regions under evolutionary selection.

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C09.4

The impact of genetic variation on lipid traits from whole exome sequences of 10,000 individuals: the T2D-GENES Consortium

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The T2D-GENES consortium aims to identify cosmopolitan and population-specific variants associated with type 2 diabetes (T2D) and related traits. To do this, we undertook whole-exome sequencing of >10,000 individuals from five ethnic backgrounds, including African-Americans, Mexican-Americans, Europeans (Finnish and Ashkenazim), South Asians (Indian Asians from London and Singapore), and East Asians (Chinese in Singapore and Koreans), half of whom were diagnosed with T2D. We present here initial results from 5,334 individuals (African-American, East and South Asian) for lipids. We tested the hypothesis that low frequency (LF, MAF<5%) variants contribute to variation in lipid levels. We identified 1.8 million exome variants, 91% rare (MAF<0.5%), 5% LF, and 4% common. In covariate-adjusted, single-variant fixed-effects meta-analyses, we have detected genome-wide significant associations ($p<5x10^{-8}$) within known GWAS signals: *CETP* ($p=1.03x10^{-10}$) and *LIPC* ($p=1.9x10^{-8}$) for HDL, *TOMM40* ($p=6.6x10^{-14}$) and *PVRL2* ($p=1.7x10^{-8}$) for LDL, and *APOA5* ($p=7.8x10^{-19}$) for triglycerides. There were suggestive associations at *APOA5* ($p=9.9x10^{-8}$) and *LPL* ($p=4.9x10^{-7}$) for HDL, and *ADAMTSL3* ($p=1.6x10^{-6}$) for LDL. LF variant analysis employing aggregate tests with SKAT across all individuals identified associations with the several previously-implicated genes including *CETP* ($p=1.4x10^{-7}$) and *APOA5* ($p=2.7\ x10^{-6}$) for HDL, and *PCSK9* ($p=3.6x10^{-6}$) for LDL. Novel signals were seen at *ACPP* ($p=3.1x10^{-6}$) for HDL, *ZNF626* ($p=1.47x10^{-4}$) for cholesterol, *MYBPC1* ($p=3.7x10^{-5}$) for LDL, and *c2orf19* ($p=5.9x10^{-5}$) and *TMEM171* ($p=6.5x10^{-5}$) for triglycerides. Whole exome sequencing of a multiethnic sample allows evaluation of effects across the full allele frequency spectrum in coding regions and identification of novel variants contribution to lipid trait variability.

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C09.5

Exome sequence analysis of type 2 diabetes in over 10,000 samples from five ancestry groups: the T2D-GENES Consortium.

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We have undertaken whole-exome sequencing of >10,000 type 2 diabetes (T2D) cases and controls from five major ancestry groups: African American (AA), East Asian (EA), European (EU), Hispanic American, and South Asian (SA). The unique study design will yield a catalogue of coding variation across diverse populations, enabling us to: 1) identify novel T2D association signals; 2) assess evidence for heterogeneity in genetic effects between ancestries; and 3) localize causal variants for T2D.
Sequencing of the first 5,334 individuals (50% cases) of AA (N=1069), EA (N=2163), EU (N=962), and SA (N=1140) ancestry has identified ~1.8M single nucleotide variants (SNVs). Only 81k (5%) are present in all four ancestry groups, 81% of which have minor allele frequency (MAF) >5%. Conversely, the 28%, 31%, 4% and 21% of variants unique to AA, EA, EU and SA ancestries are largely rare (MAF <1%).
Using fixed-effects meta-analysis of single variant association results we identified rs2233580 (p.R192H) to be associated with T2D at genome-wide significance. The association was specific to the EA samples: Korean (p=1.4x10-4, 8% MAF) and Singapore Chinese (p=7.4x10-6, 13% MAF). The variant was monomorphic in AA and EU samples. It was recently shown that p.R192H impairs PAX4's ability to repress transcription of insulin and glucagon. In 5,334 samples, no low-frequency or rare causal variants have been identified using single marker or gene-level tests.
We anticipate that analysis of all 10,000 exomes (early 2013) may identify additional association signals for T2D and provide functional insights into the pathogenesis and genetic architecture of T2D.

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C09.6

Lessons learned from the NHLBI-Exome Sequencing Project (ESP)

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The NHLBI-Exome Sequencing Project (ESP) was conceived to identify rare, putatively functional coding variants associated with heart, lung, and blood related complex traits. To this end, exome sequence data was generated on 4,420 European Americans and 2,312 African Americans who are participants in several large cohort studies. Sequenced individuals included a deeply phenotyped random sample, quantitative trait extremes of interesting phenotypes (e.g., low-density lipoprotein and blood pressure) and disease cases (e.g., early onset myocardial infarction or stroke). Over 80 heart, lung, and blood phenotypes were available for analysis. Given that this is one of the largest medical sequencing studies ever undertaken, it was necessary to develop efficient methods and pipelines for variant cal-

ling, data quality control, analysis, and interpretation of millions of single nucleotide variants from thousands of samples across multiple phenotypes. These methods allowed us to identify novel associations. For instance, we discovered and validated an association between variants in the DCTN4 gene and risk of pseudomonas infection in individuals with cystic fibrosis (n=91, p<2.5x10-6). We also replicated several known associations across a number of traits [e.g., variants in the APOB gene are associated with LDL (p<2.5 x 10-6, n=3342) and the LEPR gene is associated with C-reactive protein (p<2.5x10-6, n=1791)]. The ability to detect associations was very dependent on the genetic architecture. NHLBI-ESP not only provides novel information on the genetic etiology of several heart, lung, and blood related traits, but also offers guidance on using exome sequencing to identify rare variants associated with complex traits.

S.M. Leal: None.

C10.1

From personal genetic counseling to public health screening: The BRCA Opportunity

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BRCA1/2 carriers have increased breast/ovarian cancer risks and are identified based on personal/familial history, through Cancer Genetics clinics providing both pre- and post-test counseling. Among Ashkenazi Jews (AJ) there is a high frequency (2.5%) of three *BRCA1/2* mutations, and ~40% of carriers have no significant family history. These exceptional characteristics suggest that in AJ, BRCA screening fulfills WHO screening criteria. However, the traditional counseling process is difficult to implement on a large scale. We aimed to examine the feasibility of BRCA screening in Israel, using an alternate counseling model.

Screening was offered to healthy AJ, age ≥30, in various medical settings, without pre-test counseling. Risk assessment was based on a self-reported family history questionnaire. Only carriers and high risk participants received post-test counseling. Feelings, knowledge and attitudes toward testing were examined using questionnaires at 2 weeks and 6 months.

1,561/2600 (60%) individuals agreed to be screened, and 28 carriers (1.8%) were identified. Over 90% were satisfied with participation. Mean knowledge score was 70%. Post-test stress was reported by 1.5% at time 2 weeks but resolved in all by 6 months. Only 1% opposed BRCA population screening. Screening led to counseling in 423 (27%) high risk participants who had not been previously referred. 39% of carriers had non-suggestive family history and would not have been identified without screening.

Lack of pre-test counseling is unorthodox, but our results suggest high levels of satisfaction and coping with this process. These results will inform future implementation of genetic advances into the public health arena.

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C10.2

Drivers, barriers and opportunities for genetic testing services in emerging economies: the GenTEE (Genetic Testing in Emerging Economies) project

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Background: Due to the epidemiological transition, the emerging economies of China, East Asia, India, Latin America, the Middle East and South Africa are facing an increasing proportion of morbidity and mortality due to congenital and genetic conditions, a rising need for genetic services to improve patient outcomes and overall population health and the challenge how: (i) to ensure the successful translation of genetic/genomics laboratory and academic research into quality assured pathways, (ii) to develop a service delivery infrastructure that leads to equitable and affordable access to high quality genetic/genomic testing services.

Objectives: To document and compare current practices and the state of genetic service provision in: Argentina, Brazil, China, Egypt, India, Oman, Philippines and South Africa and to identify current knowledge gaps and unmet service needs.

Methods: A standardized survey that is the first of its kind worldwide that allows comparison of services across a number of key dimensions by using a core set of indicators selected by the GenTEE consortium for their relevance.

Results: Underfunded fragmented public services, increasing out-of-pocket expenses for genetic testing services, concentration of services in main cities and skill gaps result in inequitable services or delayed access. The development of services in the private sector is opportunistic and mostly technology and market driven. There is a marked lack of standard operating procedures and agreed quality assessment processes for new technologies.

Discussion: International collaborative networks can provide support for capacity building and help to strengthen the provision of quality genetic/genomic services in emerging economies.

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C10.3

Incidental findings in research: National Health Service Research Ethics Committee member perspectives.

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Genetic healthcare is in the midst of a transition towards genome-wide technologies, driven by the increasing availability of affordable sequencing. The possibility of discovering genetic and genomic incidental findings, outside the scope of the initial objective but with potential health implications, is significant when utilising such an approach.

Issues concerning the obligations to search for and report these findings are extensively discussed in opinion articles, commentaries and ethical discussions. However there is a paucity of primary research data available for analysis as evidenced by a recent systematic review of empirical research that included only four relevant studies.

Following on from our work with members of the public and genetic counsellors, in this phase of the study we sought the opinions of United Kingdom National Health Service Research Ethics Committee members (n=30) who are responsible for approving protocols for research studies. Semi-structured telephone interviews were conducted and transcripts were analysed using thematic analysis to identify common themes.

Participants discussed the requirement for extensive information and appropriate consent forms to facilitate informed decision making, support for return of clinically actionable findings and the need for the researcher to take responsibility for disclosure decisions, although there was no consensus on any one issue. Participants were divided on whether patient autonomy or patient wellbeing should take precedence.

This study provided a useful insight into the opinions of the ethics committee members. However it is clear that guidelines on the issue are essential to ensure equitable research ethics decisions across the UK.

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C10.4

Preferences for priority setting criteria in genetic testing: a discrete choice experiment

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Clinical Center, Munich, Germany.

Given the need of many European health care systems to contain costs of public health care, decisions about prioritizing genetic tests may have to be made. Different criteria have been proposed to guide the process of priority setting, but little is known about the relative weights that should be assigned to them in decision making. This study assesses stakeholder preferences for weighting these criteria in order to support priority setting decision making throughout Europe. In a discrete choice experiment respondents made binary choices between testing options that were described by medical and non-medical criteria. Attributes considered included severity of the disease, risk for the disease, aim of the test, medical benefit of the test, and costs of the test. Responses from 594 participants (including 171 clinical geneticists, 151 laboratory scientists, 83 academic researchers, and 98 patient representatives) were analysed. The most valued attribute levels were a proven medical benefit of the test, high risk for having the disease, and low costs of the test. Some preferences differed between clinicians, patients, and other genetic experts. While clinicians attached greater value on attribute levels referring to risk for having the disease and proven medical benefit of the tests, patients and other stakeholders had increased preferences for testing highly severe conditions. Priority weights determined by scientific methods may help to improve the consistency of priority setting in genetics. Limitations include that the weights might vary by context, and that evidence for operationalizing the criteria is limited for most genetic tests.

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C10.5

Effects of multifaceted oncogenetics training for general practitioners

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Context
Identification of patients at risk for hereditary cancers is essential to inform decisions about early screening, genetic testing, and pre-symptomatic risk reducing options. However, if non-genetic specialists (e.g. general practitioners (GPs)) are to make an effective contribution in this area, competencies need to be upgraded.
Objective
To investigate whether multifaceted oncogenetics training for GPs sustainably improves knowledge and consultation skills, and to evaluate participants' satisfaction with the training and perceived applicability learned.
Interventions
1) Genetics e-learning Continuing Professional Development (G-eCPD) module aimed at improving GPs' oncogenetics knowledge
2) Four-hour interactive live training module covering oncogenetic clinical skills (family history, risk assessment, and efficient referral).
Evaluation Methods
Two parallel-group pre-post-retention (6-month follow-up for G-eCPD, 3-month follow-up for live module) controlled group intervention trials (standardized patients, checklists and validated questionnaires) were conducted. 168 GPs working in the Dutch primary care setting responded to an email invitation and were randomly assigned to intervention or control groups, evaluating the G-eCPD module (n=80, 44 GPs completed all measurements) or the live module (n=88, 56 GPs completed all measurements).
Results
There was a significant follow-up improvement in oncogenetic knowledge (G-eCPD) and consultation skills (live module) after the intervention. Satisfaction and self-perceived applicability was high.
Conclusions
The training proved to be a feasible and satisfactory method to achieve sustained
improvement of oncogenetic knowledge and consultation skills. This educational framework can inform future training activities for GPs and potentially other medical professionals to enhance genetics-related consultation and decision making with the ultimate aim of improving medical care.

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C10.6

The psychological impact of cryptic chromosomal abnormalities diagnosis announcement.

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The aim of this qualitative study was to describe the psychological impact of the diagnosis announcement of pathogenic Copy Number Variations (pCNVs). We performed semi-structured interviews of 60 parents of 41 affected children and 5 clinical geneticists who announced the diagnoses.
Medical doctors emphasized their difficulties in announcing diagnoses made by Chromosomal Microarray (CMA). The diagnosis of the best characterized microdeletion syndromes, defined by patronymic names, is generally clinically suspected and confirmed by Fluorescence In Situ Hybridization analysis. The pCNVs diagnosed by CMA, often named after cytogenetic formulas, are overall rarer and their phenotype and prognosis are less characterized: this can make doctors feel less confident in the diagnosis announcement.
In the presence of a patronymic name, 83% of parents were able to define their child's disease. The announcement of pCNVs named after cytogenetic formulas, on the contrary, did not appear to facilitate a mental representation of the disease, and the majority of parents found it difficult to provide a clear description of the disease. For 13% of them, the genetic diagnosis had "no meaning"; in some cases, the genotype-phenotype correlation was called into question.
The announcement of inherited pCNVs can increase the feeling of parental guilt. The disclosure of *de novo* pCNVs can induce a feeling of "breakage" in the mental representation of the parent-child vertical transmission.
In conclusion, our study shows that the disclosure of pCNVs has a significant psychological impact: a multidisciplinary approach to the diagnosis announcement, including psychological support, should be systematically warranted.

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C11.1

The 3D topographic mapping of genetic variations in treatment naïve advanced ovarian cancer

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Ovarian cancer (OC) is a leading cause of death from gynaecological malignancies, with approximately 225,000 new cases and 140,000 deaths reported worldwide yearly. OC has a unique intra-abdominal metastatic spread and is associated with high morbidity, over 70% of patients present with advanced disease at diagnosis. Advances in genome sequencing have expanded our understanding of the complexity of carcinogenesis and have given insight into inter- and intra-individual heterogeneity of cancer genomes. Here, we present the first systematic and comprehensive study on 3D topography of structural variations, point mutations and RNA expression within tumours. We obtained tissue from 8-20 primary and metastatic sites per patient from three treatment-naïve stage III/IV OC patients. Structural- and copy number variations and their effect on gene expression are detected with high sensitivity by a combination of next-generation sequencing techniques. Bioinformatic cross-sample analysis reveals extensive heterogeneity on multiple levels between primary and metastatic lesions within one patient. Phylogenetic analysis further revealed two very distinct evolutionary

paths: a more linear evolution in one patient and a parallel evolution pattern in the other two. In the latter, a branching event occurring very early in tumour development has led to highly diverge metastatic clones differing both in structural variation and point mutation content. This finding was surprising, as these genetic differences were not expected based upon the histological profiles. Our study provides a foundation of experimental methods able to generate evolutionary models for cancer, leading to the identification of genetic drivers for tumour formation and metastatic spread.

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C11.2

The Genomic Landscape of Somatic Mutations in Subtypes of Germinal-Center derived B-cell Lymphomas

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Germinal-center B-cell derived lymphomas (GCB-lymphomas) are the most common B-cell lymphomas. They include follicular (FL), diffuse large B-cell (DLBCL) and Burkitt lymphomas (BL), as well as intermediate DLBCL/BL (IL). In the framework of International Cancer Genome Consortium (ICGC) the ICGC MMML-Seq-Project funded by the German Federal Ministry of Education and Research (01KU1002A-J) explores whole genome sequencing data of currently 29 GCB-lymphomas and paired normal controls. Genomic sequencing is complemented by transcriptome, miRNAome and whole methylome sequencing. In the 23 yet finished whole genomes (>30x) we identified a median of 35 potentially protein-changing mutations in BL, 58 in DLBCL/IL, and 40 in FL. Recurrently mutated genes include TP53, MYC, SMARCA4, CCND3, MLL2 and ID3, the latter being a novel hallmark of BL (Richter et al., Nat Genet 2012). On the genome-wide level, we identified a median of 4221 small variants in BL, 10782 in DLBCL/IL, and 6705 in FL. Regional clustering of single nucleotide variants (SNVs) was observed which particularly affected regions undergoing somatic hypermutation in B-cells. Investigation of SNV mutation types in the context of the neighboring bases provided evidence for different mutational mechanisms. Structural variants point to novel fusion genes which in part were supported by fusion transcripts. Patterns of SNVs, structural variants and transcriptional profiles differed between BL and the other GCB-lymphomas. Our ongoing complete genomic and transcriptomic sequencing efforts within the ICGC, thus, provide insights into the different mutational mechanisms and driving genes active in the various subtypes of GCB-lymphomas.

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C11.3

FAS/FASL pathways is impaired in chordoma and is involved in notochord development and regression

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Chordoma, a malignant bone tumor arising from notochord remnants, is characterized by unforeseeable prognosis and chemoresistance. Since the apoptotic pathway mediated by FAS-FASL was found to be involved in notochord regression, we studied their expression in 31 chordomas and in the U-CH1 cell line. The lack of FASL expression in most of the chordomas and the detection of FAS anti-apoptotic, besides the pro-apoptotic isoform, in both tumors and U-CH1 cells, are consistent with Fas/Fasl pathway inactivation, also supported by the prevalent detection of the Caspase 3 and Caspase 8 inactive forms. Moreover, we observed that apoptosis was induced in U-CH1 cells following treatments with soluble Fasl, besides the increasing of active Caspase 8. To elucidate the role of FAS/FASL pathway in chordoma development, we started in vivo studies on zebrafish. fas and fasl homologue genes were found to be maternally expressed during development and fas in all zygotic stages, while fasl from 48 hours post fertilization. We then detected fasl expression in notochord, muscles and central nervous system at 5 days post fertilization by immunohistochemistry. Following fas and fasl knock down by morpholino oligos, we observed notochord anomalies such as packed notochordal cells, curved bodies and bent tail. Moreover, the notochord morphology was characterized by bigger notochordal cells compared to the control and the perinotochordal sheath showed an anomalous wavy strand. The obtained results strongly suggest the implication of FAS/FASL in chordoma tumorigenesis, providing new insights on notochord development/regression and helping to address the identification of chordoma pharmacological targets.

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C11.4

SDHB mutations link pheochromocytoma/paraganglioma mailgnancy to epithelial to mesenchymal transition, both in human tumors and in SDHB-/- chromaffin cells

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Pheochromocytomas (PCC) and paragangliomas (PGL) are neuroendocrine tumors derived from chromaffin cells, genetically determined in 35% of cases. It is well established that SDHB gene mutations are associated with increased invasiveness and poor prognosis. The link between mutations in this subunit of a mitochondrial enzyme (succinate dehydrogenase) and PCC/PGL malignancy is still missing. The aim of our study was to address the role of epithelial to mesenchymal transition (EMT) in SDHB-mediated oncogenesis.
The unsupervised classification of transcriptome data of 188 PCC/PGL, based on 94 EMT-associated genes revealed that SDHB-malignant samples displayed the same pattern of gene expression, reflecting EMT activation: up regulation of LOXL2, TWIST1, TCF3, MMP1 and MMP2; down regulation of CDH2 (N-cadherin) and KRT19. At protein level, we demonstrated that all SDHB-malignant samples presented a nuclear translocation of SNAIL (a key EMT inducer transcription factor).
Using a Cre-Lox strategy, we generated an immortalized mouse chromaffin cell (imCC) line harboring an Sdhb gene knock-out that recapitulates most hallmarks of SDH-related PCC/PGL. Immunofluorescence and/or RTqPCR found the same type of variations in Sdhb-/- cells than in SDHB-mutated tumors. We studied cell migration by single cell tracking, wound scratch and

invasion assays, which revealed that Sdhb-/- cells had higher individual cell speed, global migration and invasiveness abilities. The functional validation of two EMT associated genes (KRT19 and SNAIL) with these processes is currently being investigated. We will further investigate the tumoral potential of Sdhb-/- cellular models using a renal capsule xenograft strategy, in order to develop preclinical studies testing innovative therapies.

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C11.5

MicroRNAs as possible initiators and drivers for microsatellite unstable colorectal tumours

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Mismatch repair (MMR)-deficient human tumours develop through a particular molecular pathway characterized by the genetic instability of numerous microsatellite-type DNA repeats dispersed throughout the genome. To date, their pathogenesis is mainly attributed to truncating mutations occurring at coding repeats in cancer-related genes. Meanwhile, altered expression of microRNAs is being increasingly considered as a key event in carcinogenesis. This work sums up the studies we recently performed to investigate the possibility that 1. Deregulation of some microRNA targeting MMR proteins might constitute a pre-neoplastic event that promotes the emergence of MMR-deficient tumours and 2. Somatic mutations in MSI-targeted microRNA genes might help the MSI-driven tumourigenic process. MSI tumour initiation: miR-155 and miR-21 are two inflammation-related microRNAs that target core MMR proteins. Their overexpression was observed in the non-neoplastic mucosa (field-defect) of patients suffering from inflammatory bowel disease (IBD). Interestingly, the emergence of MSI in these rare clinical entities doesn't seem to be dependent on germline inactivation or promoter methylation of MMR proteins but is likely to be a microRNA-driven process (Svrcek *et al.*, 2013). MSI tumour progression: Our results provide evidence that DNA repeats contained in precursor or mature sequence of microRNAs are relatively rare and generally preserved from mutations, but can sometimes be highly mutated in MMR-deficient cancer cells (e.g. miR-1303, miR-567, miR-1273c) (El-Murr *et al.*, 2012). Based on both reports, we propose a model in which microRNA field-defects and MSI-targeted microRNA genes may act first, as initiators and second as drivers for MSI-driven transformation of the colonic mucosa.

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C11.6

Western diet and *Mlh1* mutation predispose colonic mucosa to early inactivation of the Wnt signaling antagonist *Dickkopf-1*

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Colorectal cancer (CRC) is the second most common cause of cancer-related deaths in the Western world and interactions between genetic and environmental factors, including diet, are suggested to play a critical role in its etiology. We conducted a long-term feeding experiment in the mouse to address epigenetic changes arising in normal colonic mucosa and their functional consequences as putative cancer-predisposing events available for early detection. The expression of 94 growth-regulatory genes previously linked to human colorectal cancer and aberrant hypermethylation was studied at two time points (5 weeks and 12 months of age) in the heterozygote *Mlh1*$^{+/-}$ mice analogous to human Lynch syndrome (LS), and wild type *Mlh1*$^{+/+}$ littermates, fed by either Western-style (WD) or AIN-93G control diet. In mice carrying *Mlh1* mutation and/or fed with WD, histologically normal proximal colonic mucosa, the predominant site of cancer formation in LS, exhibited a significant expression decrease in tumor suppressor genes, *Dkk1, Slc5a8, Hoxd1*, and *Socs1*. the reduced expression was associated with age-related promoter hypermethylation in these genes. As evidence of early epigenetic inactivation of *Dkk1* in particular, most mice showing high methylation no longer expressed *Dkk1* mRNA, while *Mlh1* showing only modest methylation was still expressed in both *Mlh1*$^{+/-}$ and *Mlh1*$^{+/+}$ mice. Furthermore, changes in *Dkk1* seem to predispose to neoplasia/hyperplasia in the proximal colon. This together with the methylation and expression data indicates that the inactivation of *Dkk1*, a secreted antagonist of the Wnt/β-catenin signaling pathway, is a remarkably prominent and early marker for colon oncogenesis.

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C12.1

Congenital poikiloderma, fatty infiltration of muscles and pulmonary fibrosis: a new syndrome caused by a new gene

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Congenital poikiloderma associated with tendon contracture and pulmonary fibrosis was described in a South-African family with autosomal dominant inheritance by Khumalo *et al.* in 2006. Here, we report four sporadic cases affected with this syndrome in two males and two females (ages 6-30). Key features consist of: (i) congenital poikiloderma, hypotrichosis, hypohidrosis; (ii) progressive muscular weakness with feet varus deformation and (iii) pulmonary fibrosis. Muscle MRI shows diffuse fatty infiltration confirmed by muscle biopsy. Microscopy of the skin reveals elastic tissue degeneration with alterations of the elastic network.
Whole exome sequencing performed in the South African family and in the trio of one sporadic case led to the identification of a very strong candidate gene, unreported to date. The involvement of this gene has been confirmed by the detection of variations in the other cases. Taken together, three unrelated patients share exactly the same missense mutation; one other patient and the South-African family members carry two very close mutations. We suggest that these three mutations are involved in the same putative functional domain of the protein.
In conclusion, we report a phenotypically recognisable syndrome linked to a new gene. Functional studies are ongoing to better understand the pathophysiology of this entity.

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C12.2

TBX4 mutations (small patella syndrome) are associated with childhood-onset pulmonary arterial hypertension

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Background
Childhood-onset pulmonary arterial hypertension (PAH) is rare and differs

from adult-onset disease in clinical presentation, with often unexplained developmental retardation and dysmorphic features (DR/DF). Mutations in the major PAH gene, *BMPR2,* was reported to cause PAH in only 10-16% of childhood-onset patients. We aimed to identify other genes associated with childhood-onset PAH.

Methods

We studied 20 children with PAH. Six patients with unexplained accompanying DR/DF were selected for array-comparative genomic hybridization analysis, with the aim of finding deletion regions containing candidate genes for PAH. Three patients had overlapping deletions of 17q23.2. *TBX2* and *TBX4* were selected from this area as candidate genes and sequenced in all 20 children. After identifying *TBX4* mutations in these children, we subsequently sequenced *TBX4* in a cohort of 49 adults with PAH. Because *TBX4* mutations are known to cause small patella syndrome (SPS), a lower limb malformation syndrome, all patients with newly detected *TBX4* mutations were screened for features of SPS. We also screened a third cohort of 23 patients with SPS for PAH.

Results

TBX4 mutations (n=3) or *TBX4*-containing deletions (n=3) were detected in 6 out of 20 children with PAH (30%). All living patients and two parents with *TBX4* mutations appeared to have previously unrecognized SPS. In the adult PAH-cohort, one *TBX4* mutation (2%) was detected. Screening in the cohort of (predominantly adult) SPS patients revealed no PAH.

Conclusions

These data indicate that *TBX4* mutations are associated with childhood-onset PAH, although the expressivity of PAH in adult *TBX4* mutation carriers is low.

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C12.3

Mutations in SNRPE, which encodes a core protein of the spliceosome, cause autosomal-dominant hypotrichosis simplex

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Hypotrichosis simplex (HS) comprises a group of hereditary isolated alopecias that are characterized by a diffuse and progressive loss of hair starting in childhood and shows a wide phenotypic variability. We mapped an autosomal-dominant form of HS to chromosome 1q31.3-1q41 in a Spanish family. By direct sequencing, we identified the heterozygous mutation c.1A>G (p.Met1?) in SNRPE that results in loss of the start codon of the transcript. We identified the same mutation in a simplex HS case from the UK and an additional mutation (c.133G>A [p.Gly45Ser]) in a simplex HS case originating from Tunisia. SNRPE encodes a core protein of U snRNPs, the key factors of the pre-mRNA processing spliceosome. The missense mutation c.133G>A leads to a glycine to serine substitution and is predicted to disrupt the structure of SNRPE. Western blot analyses of HEK293T cells expressing SNRPE c.1A>G revealed an N-terminally truncated protein, and therefore the mutation might result in use of an alternative in-frame downstream start codon. Subcellular localization of mutant SNRPE by immunofluorescence analyses as well as incorporation of mutant SNRPE proteins into U snRNPs was found to be normal, suggesting that the function of U snRNPs in splicing, rather than their biogenesis, is affected. In this report we link a core component of the spliceosome to hair loss, thus adding another specific factor in the complexity of hair growth. Furthermore, our findings extend the range of human phenotypes that are linked to the splicing machinery.

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C12.4

Gain-of-function mutations in the mechanically activated ion channel PIEZO2 cause distal arthrogryposis type 5

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Mechanotransduction, the pathway by which mechanical forces are translated to biological signals, plays important but poorly characterized roles in physiology. PIEZOs are recently identified widely-expressed mechanically-activated ion channels that are hypothesized to play a role in mechanotransduction in mammals.

Here, we describe two distinct PIEZO2 mutations in patients with Distal Arthrogryposis Type 5 (DA5), a generalized autosomal dominant contracture syndrome with limited eye movements, restrictive lung disease and variable absence of cruciate knee ligaments. Electrophysiological studies reveal that the two PIEZO2 mutations affect biophysical properties related to channel inactivation: both del2727E and I802F mutations cause the PIEZO2-dependent mechanically-activated currents to recover faster from inactivation, while del2727E also causes a slowing of inactivation. Both types of changes in kinetics result in increased channel activity in response to a given mechanical stimulus, indicating that DA5 is caused by gain-of-function mutations in PIEZO2. We further show that overexpression of mutated PIEZO2 cDNAs does not cause constitutive activity or toxicity to cells, arguing that the observed phenotype is likely to be a mechanotransduction defect.

Our studies identify a new type of channelopathy and link the dysfunction of mechanically activated ion channels to developmental malformations and joint contractures. We are presently sequencing PIEZO2 in other patients with sporadic or familiar arthrogryposis of a compatible phenotype.

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C12.5

Defective initiation of glycosaminoglycan synthesis due to mutations in B3GALT6 causes a pleiotropic connective tissue disorder with severe alterations in proteoglycan assembly and collagen fibrillogenesis

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Proteoglycans are very important components of cell plasma membranes and extracellular matrices of connective tissues. They consist of glycosaminoglycan chains attached to a core protein via a tetrasaccharide linkage, whereby the addition of the third residue is catalyzed by galactosyltransferase II (β3GalT6), encoded by B3GALT6. We identified biallelic B3GALT6 mutations in three families presenting a severe autosomal recessive connective tissue disorder, characterized by skin fragility, delayed wound healing, joint hyperlaxity and contractures, muscle hypotonia, mental retardation and a spondyloepimetaphyseal dysplasia with bone fragility and severe kyphoscoliosis. The phenotype strongly overlaps with several recessive Ehlers-Danlos variants and spondyloepimetaphyseal dysplasia with joint hyperlaxity. Homozygosity mapping and candidate gene sequence analysis identified homozygous B3GALT6 missense mutations in Family 1 (p.(Asp207His) and Family 3 (p.(Gly217Ser)), and compound heterozygous mutations in Family 2 (p.(Ala108Glyfs*163) and p.(Asp207His)). A strong decrease in ability to prime glycosaminoglycan synthesis was observed in patient's fibroblasts, confirming β3GalT6 loss-of-function together with impaired synthesis and glycanation of the small chondroitin/dermatan sulfate proteoglycan decorin. Dermal electron microcopy disclosed abnormal collagen fibril organization, in line with the important regulatory role of decorin in this process. A strong reduction in heparan sulfate was also observed, indicating that β3GalT6 deficiency impairs synthesis of both types of glycosaminoglycans. In vitro wound healing assay revealed significant delay, pointing to a role for glycosaminoglycan defect in impaired wound repair in vivo. Our findings uncover a novel genetic deficiency affecting glycosaminoglycan synthesis and emphasize a crucial role for β3GalT6 in a wide range of developmental and biological processes.

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C12.6

Identification of INPPL1 mutations in Opsismodysplasia

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Opsismodysplasia (OPS) is a severe autosomal-recessive chondrodysplasia characterized by pre- and postnatal micromelia and extremely short hands and feet. The main radiological features are severe platyspondyly, squared metacarpals, delayed skeletal ossification, and metaphyseal cupping. In order to identify mutations causing OPS, a total of 19 cases from 12 unrelated families were included in this study. We performed exome sequencing in three cases from three unrelated families and only one gene was found to harbor mutations in all three cases, namely INPPL1 (inositol polyphosphate phosphatase-like 1). Screening INPPL1 in the remaining cases identified a total of 16 distinct mutations. Among the 19 cases, prenatal findings led to early termination of pregnancies (especially in recurrent sibs) in 10/19 cases and hygroma, short long bones, short extremities and narrow thorax were consistently observed. Four children died early (stillborn 30 WG- 15 months of age). The five remaining cases (3 to 19 years old), had normal cognitive development, severe short stature (below- 4 SDS), lower limb deformity and severe scoliosis with atlanto axial instability. Most mutations (8/16) resulted in premature stop codons, located in the catalytic domain, 5-phosphatase. INPPL1 belongs to the inositol-1,4,5-trisphosphate 5-phosphatase family, that govern a plethora of cellular functions by regulating the levels of specific phosphoinositides.
Our finding of INPPL1 mutations in OPS, a severe spondylodysplastic dysplasia with major growth plate disorganization, supports a key and specific role of this enzyme in endochondral ossification.

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C13.1

Alteration of lipid metabolism in hereditary spastic paraplegia 26

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Hereditary spastic paraplegias are heterogeneous neurological disorders. At least 50 loci and 30 genes can account for these diseases. Whole genome linkage mapping in 2 HSP families originating from Spain and Tunisia allowed us to restrict the candidate interval of the SPG26 locus from 34 to 9 Mb. Capture and whole exome sequencing were then performed in patients from the two linked kindreds and from 3 other autosomal recessive HSP families with German, Portuguese and Brazilian origins. Focused analysis to the SPG26 candidate region, identified 3 truncating and 2 missense mutations segregating at the homozygous state in patients from all families in a gene encoding an enzyme of the lipid metabolism. Three additional truncating mutations were then found in two French isolated cases by direct Sanger sequencing of all coding exons of this novel HSP-related gene. All mutations were absent in large series of healthy unrelated controls. Patients (n=18) presented with an early onset spastic paraplegia preceded by mental impairment in most cases and complicated by the association of cerebellar ataxia and peripheral neuropathy with cortical atrophy at brain MRI. These findings highlight lipid metabolism in HSP, paving the way for a better understanding of the mechanisms involved in these diseases.

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C13.2

WDR45 de novo mutations cause a clinically distinct, X-linked subtype of NBIA

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Neurodegeneration with brain iron accumulation (NBIA) comprises a group of monogenic disorders characterized by extensive iron deposition in the basal ganglia. Several subtypes can be distinguished based on clinical findings and neuroimaging features.
An exome sequencing approach applied to a clinically preselected group of 14 index cases identified *de novo* mutations in X-chromosomal *WDR45* in 13 of them. The associated phenotype includes early-onset global developmental delay and neurological deterioration in adulthood with parkinsonism, dystonia, and dementia. Brain MRI demonstrated marked iron deposition in the substantia nigra and globus pallidus. Of note, the phenotype of males and females is similar, an observation not yet fully understood but potentially explained by somatic mosaicism in males or patterns of skewed X chromosome inactivation in females.
WDR45 is a beta-propeller scaffold protein and plays a putative role in autophagy, possibly via interaction with ATG2A and ATG2B.
Histopathological and cellular studies are ongoing to further substantiate this hypothesis.

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C13.3

A stop mutation in WDR81 causes microcephaly with variable penetrance.

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Severe congenital primary microcephaly is a clinically and genetically heterogeneous disorder. Currently, 10 disease causing genes have been described. These genes alter three main pathways leading to microcephaly: centrosome function, DNA repair and cell cycle regulation. Whole exome sequencing identified a novel co-segregating truncating mutation c.5335 C->T, p.R1779* towards the C-terminus of WDR81 in 5 affected siblings of a large consanguineous Turkish family with primary microcephaly. The same stop mutation in WDR81 was identified in another Turkish case with microcephaly. A missense mutation in WDR81 was described by (Gulsuner et al., 2011) to cause cerebellar hypoplasia only. We observed marked variability of the phenotype. All four females had extreme microcephaly with enlarged extra-axial spaces and ponto-cerebellar hypoplasia (MICaxPCH). One male had a simplified gyral pattern and cerebellar hypoplasia and another male fetus with hydrops fetalis had not yet developed late microcephaly (at 21 weeks of gestation). We created a zebrafish morpholino knock-out model of WDR81 which resembled the observed human microcephaly phenotype. Moreover, we observed in the zebrafish morpholino enlarged ventricles which are in analogy to the enlarged ventricles in the patients. WDR81 was highly expressed in the neocortex of human and mouse embryos by in situ hybridization and immunofluorescence. WDR81 was localised to the nucleus as shown by immunofluorescence and confirmed by western-blot of subcellular fractions. In summary, WDR81 mutations can cause microcephaly with variable penetrance. We propose that WDR81, as a nucleoplasmic protein, would be the first primary microcephaly protein not directly associated with the centrosome.

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C13.4

Chromatin structure, transcription and CAG instability in Huntington's disease

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Expansion of CAG/CTG repeats is the causative mutation of many diseases, including Huntington's disease (HD) and myotonic dystrophy 1. These mutations are unstable in selective somatic tissues, which accelerates disease progression. The mechanisms underlying CAG/CTG repeat instability remain elusive. Here we investigated the relationship between chromatin structure, transcription and somatic CAG/CTG instability. We examined this hypothesis using the R6/1 and R6/2 HD mouse lines, which express a similar HD transgene. These mice present a similar HD-like pathology, though progression of the disease is fastest in R6/2 mice as compared to R6/1 mice. In the two mice, as in HD patients, somatic instability is high in the striatum, the tissue that preferentially degenerates, and low in the cerebellum, a tissue spared by the disease. However, the rate of CAG instability is increased in R6/2 tissues when compared to R6/1 matched-tissues. Our data indicate that an accessible chromatin structure and high transgene transcription underlie the high instability rates seen in R6/2 mice in comparison to R6/1 mice, but are not sufficient to explain tissue specificity of instability. Interestingly, the levels of RNA Pol II and histone mark associated with transcription elongation correlate with tissue-selective somatic CAG instability. Our data suggest that the dynamics of transcription at the HTT gene is differently regulated between the striatum and cerebellum, despite similar bulk levels of transgenic transcripts in the two tissues. We propose that the regulation of the transition from transcription initiation to transcription elongation is a mechanism contributing to tissue-selective CAG instability in HD.

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C13.5

A large genomic deletion upstream of the lamin B1 gene (LMNB1) likely causes adult-onset autosomal dominant leukodystrophy due to alteration of the regulatory landscape of LMNB1.

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We report a position effect mutation in the lamin B1 locus mimicking Adult-onset Autosomal Dominant LeukoDystrophy (ADLD).
ADLD is a rare central nervous system demyelinating disease presently associated to *LMNB1* gene duplication. We collected a large Italian pedigree segregating an autosomal dominant form of leukodystrophy clinically overlapping ADLD. Linkage analysis mapped the disease to the LMNB1 locus, although *LMNB1* was not duplicated or mutated. However, *LMNB1* was overexpressed in fibroblasts both at mRNA and protein level as in reported ADLD patients. Using array-CGH analysis, we identified a 660 kb deletion located approximately 66 kb centromeric to the *LMNB1* gene and spanned three genes: *GRAMD3*, *ALDH7A1* and *PHAX*. shRNA silencing assays of these genes did not affect *LMNB1* expression. To search for non-coding regulatory elements, we performed circular chromosome conformation capture (4C) on fibroblasts from a patient and a control, using lamin B1 promoter as a bait. We identified four potential regulatory regions: one (region A) located within the deletion and shared by case and control, and three patient specific interactors (regions B, C and D) located upstream of the deletion. A dual-luciferase assay showed that regions A and B acted as enhancers (luciferase signal changes from 2.8 to 3.4 fold vs. control, respectively). Therefore, the presence of the deletion takes away region A and moves the cryptic enhancer B closer to the *LMNB1* gene in one allele, resulting in its overexpression and mimicking ADLD. Our results confirms that mutations in regulatory elements may be important determinants of Mendelian phenotypes.

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C13.6

Interferon-beta induces clearance of mutant ataxin-7 and improves locomotion in SCA7 knock-in mice

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We showed previously, in a cell model of spinocerebellar ataxia SCA7 that interferon-beta induces the expression of promyelocytic leukemia protein (PML) and the formation of PML nuclear bodies that degrade mutant ataxin-7, suggesting that the cytokine, used to treat multiple sclerosis, might have therapeutic value in SCA7. We now show that interferon-beta also induces PML-dependent clearance of ataxin-7 in a preclinical model, SCA7266Q/5Q knock-in mice, and improves motor function. Interferon-beta, administered intraperitoneally three times a week, was internalized with its receptor in Purkinje and other cells and translocated to the nucleus. The treatment induced PML expression and the formation of PML nuclear bodies and decreased mutant ataxin-7 in neuronal intranuclear inclusions, the hallmark of the disease. No reactive gliosis or other signs of toxicity were observed in the brain or internal organs. The treatment also significantly improved the performance of the SCA7266Q/5Q knock-in mice on two behavioral tests sensitive to cerebellar function: the Locotronic test of locomotor function and the beam-walking test of balance, motor coordination and fine movements, which are affected in patients with SCA7. Finally, since neuronal death does not occur in the cerebellum of SCA7266Q/5Q mice, we showed in primary cell cultures expressing mutant ataxin-7 that interferon-beta treatment improves Purkinje cell survival. This treatment might apply to all polyglutamine diseases in which PML associates, in cell nuclei, with the mutant proteins responsible for the disease.

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C14.1

ALDH1A3 mutations cause recessive anophthalmia and microphthalmia

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Purpose: Anophthalmia and microphthalmia (A/M) are early eye development anomalies resulting in absent or small ocular globes, respectively. A/Ms occur as syndromic or nonsyndromic forms. They are genetically heterogenous with some mutations in some genes responsible for both anophthalmia and microphthalmia. The purpose of this study was to identify the disease gene involved in A/M in an inbreed Pakistani Family.

Methods: A combination of homozygosity mapping, exome sequencing and Sanger sequencing, was used to identify the disease mutation in the Pakistani family and to screen the A3 isoform of the aldehyde dehydrogenase 1 (*ALDH1A3*) encoding gene for mutations in additional unrelated A/M patients.

Results: We identified homozygosity for a missense mutation in *ALDH1A3* in the Pakistani family. The screening of the gene is a cohort of A/M patients excluding known A/M genes allowed identifying two additional homozygote mutations including another missense change and a splice-site mutation in two consanguineous families. Patients with *ALDH1A3* mutations had A/M with occasional orbital cystic, neurological and cardiac anomalies.

Discussion and conclusion: ALDH1A3 is a key enzyme in the formation of a retinoic acid gradient along the dorso-ventral axis during the early eye development. Transitory expression of mutant *ALDH1A3* cDNAs showed that both missense mutations reduce the accumulation of the enzyme, potentially leading to altered retinoic acid synthesis. While the role of retinoic acid signaling in eye development is well established, our findings provide genetic evidence of a direct link between retinoic acid synthesis dysfunction and early eye development in human.

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C14.2

Mutations in the Nuclear NAD+ synthesising enzyme *NMNAT1* cause Leber congenital amaurosis with early-onset severe macular atrophy and optic atrophy

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Introduction: Leber congenital amaurosis (LCA) is the earliest and most severe retinal dystrophy, responsible for neonatal blindness. In an effort to identify the yet unknown molecular bases of the disease, we subjected to whole exome resequencing (WER) the DNA of five unrelated families excluding known genes.

Methods and Results: Through WER combined with Sanger sequencing, we identified *NMNAT1* mutations in 22 unrelated LCA cases. *NMNAT1* codes for the ubiquitously expressed nuclear isoform of nicotinamide mononucleotide adenyltransferases. In addition to its NAD+ synthesizing activity, NMNAT1 acts as a chaperone that protects against neuronal activity-induced degeneration.

The review of ophthalmological data indicated that NMNAT1 mutations consistently caused LCA with a particular phenotype characterized by neonatal severe atrophy of the central retina leading to a posterior pole coloboma aspect and early-onset optic nerve atrophy.

These findings add further complexity to the physiopathological bases of the most common cause of blindness in childhood by giving support to the view that neuroprotection against light-induced stress is required to allow maintaining photoreceptor cells.

Conclusion: In summary, we report NMNAT1 mutations in 22 LCA families clinically recognizable by the existence of a neonatal macular atrophy and early-onset optic atrophy.

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C14.3

Identification of new genes for Hereditary Hearing Loss (HHL) using linkage studies and Whole Exome Sequencing analysis

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Despite the presence of some major player genes for HHL, still there is a need to search for new causative mutations/genes. Whole Exome Sequencing (WES) coupled with linkage data (even if only suggestive loci) proved to be very useful in identifying 3 new HHL genes in a series of Italian and Qatari families. WES nucleotide variants were called by Samtools V0.1.18 and filtered comparing with in-house, dbSNP and 1000G databases. "*In silico*" functional prediction analysis of variants was done. Gene 1: in a recessive family from Qatar, linkage analysis identified 1 locus with a LOD of 3.8 on chromosome 5q13 subsequently confirmed by NGS with the presence of a mutation elongating the protein and segregating within the family (p.*2625Gluext*11) in BDP1 gene. This gene was also found as expressed in the mouse cochlea. Gene 2: linkage analysis in an Italian dominant family showed a LOD of 3.3 on chromosome 12q24. WES data of the region confirmed the presence of a missense mutation (c.1057G>C; pG353R) in P2X2 gene. A three-dimensional model of this protein, has also suggested that the substitution would destabilize the fold. Gene 3: an Italian Y-linked family was enrolled. Linkage analysis excluded autosomal loci while WES led to the identification of a missense mutation in a gene within DFNY1 locus. Functional *in vitro* studies are now in progress. Additional new HHL genes are under final validation steps in the remaining families. These findings increase our knowledge on molecular bases of HHL suggesting new targets for treatment and prevention

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C14.4

A founder mutation in ULFIN, a new gene on chromosome 16q22.1, in patients with spinocerebellar ataxia type 4 (SCA4)

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SCA4 is an adult-onset neurodegenerative disorder with autosomal dominant inheritance, progressive ataxia, dysarthria and peripheral sensory neuropathy. The candidate region was mapped to an 8 Mb interval on chromosome 16q22.1 in two large families from Northern Germany. Since classical sequencing approaches of about 140 protein coding genes within this region could not reveal any obvious disease causing mutation, we performed whole genome sequencing analyses of one patient from each family.

By this, about 17500 variants were detected. After exclusion of known SNPs and selection based on conservation and putative regulatory function by using the public ENCODE-database, one variant was located within a transcription unit. This variant segregates with disease in both pedigrees and was found in another five unrelated patients with ataxia but excluded in >2500 controls from Northern Germany.

Interestingly, detailed *in silico* analyses as well as RNA analyses identified a hitherto unknown cDNA of 1712 bp encoding a 346 aa protein we named ULFIN. *ULFIN* codes for a WD40-repeat protein and is preferentially expressed in fetal and adult brain. The SCA4-variant identified causes the exchange of a highly conserved amino acid within a WD40 repeat domain. Protein modeling analysis demonstrated a significant conformational change caused by this missense mutation within this conserved structure.
In summary we identified a missense mutation in a so far unknown gene in two families and five unrelated patients with ataxia from Northern Germany. Our results indicate a founder effect as genetic cause of SCA4, a likewise frequent ataxia with ancestry in Northern Europe.

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C14.5

U1 snRNP interference with polyadenylation - a new pathomechanism for unclassified 3'UTR mutations

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While most point mutations occurring in coding regions affect protein function or stability, mutations in regulatory regions are poorly understood. A point mutation in the 3' untranslated region (UTR) of the *p14/robld3* gene leads to a complex immunodeficiency syndrome characterized by albinism, short stature and a severe congenital neutropenia due to decreased protein and mRNA levels of the lysosomal scaffold protein p14. Investigation of the molecular pathomechanism revealed that the point mutation creates a functional 5' splice site (SS). Recognition by the spliceosomal component U1 snRNP, suppresses p14 mRNA expression in the absence of splicing. The extent of suppression correlates with the complementarity to U1 snRNA. P14 expression can be rescued by blocking U1 snRNP with modified antisense oligonucleotides.Although the precise mechanism of U1 snRNP-mediated suppression is not identified, we showed that U1 affects poly(A) site recognition. In our tandem poly(A) site (PAS) reporter system the 3' end processing machinery switches to the downstream PAS in the presence of the p14 mutation and in addition a substantial amount of read-through is produced indicating that U1 snRNP can inhibit two consecutive PAS. A similar 3'UTR mutation is also found in the factor IX gene resulting in hemophila B. We are currently investigating if this mutation found in 8 patients acts via the same pathomechanism as p14. Thus, 5'SS created by point mutations within 3' UTRs illustrate not only a novel mechanism for a primary immunodeficiency, but may also be at the base for other disorders characterized by defective mRNA biogenesis.

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C14.6

Ion transporter deficiency predisposes to pyogenic bacterial infection by partial oxidative burst defect in granulocytes

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Staphylococcal infections are the cause of various diseases associated with a wide spectrum of symptoms and could range from benign to poor prognosis. S.epidermidis is an opportunist pathogen essentially responsible of nosocomial infections and has rarely been described as implicated in primary immunodeficiencies (PID).
We studied a patient from a consanguineous Indian family who suffered from upper respiratory tract infection and recurrent cellulitis to S. epidermidis, suggesting immune susceptibility. To identify the genetic defect, we associated genetic linkage analysis, exome sequencing, and a candidate gene approach. We identified an unreported homozygous variation in the patient. The gene encodes for a divalent cation/proton (Fe2+, Zn2+ and Mn2+) transporter. This mutation impairs protein expression in patient's granulocytes and induces a loss of function in terms of oxidative burst and Staphylococcus killing ability. Identical results were obtained using the PLB-985 granulocytes knock out for this protein. Thus, we identified a new genetic defect responsible of susceptibility to pyogenic bacterial infection, this help to delineate the important role of the NADPH pathway in bacterial immunity responses in granulocytes.

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C15.1

FGFR related anomalies of foramen magnum : phenotypic-genotypic correlation

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Fibroblast Growth Factor Receptor (FGFR) mutations are associated either to chondrodysplasia and craniosynostosis. In both conditions the growth of the skull base is altered. We analysed the extent of such alterations by CT scan in infants with a genetically confirmed mutation in FGFR and correlated them to the clinical phenotype.
The area of the Foramen Magnum (FM) was measured on CT scan obtained prior to any surgery in infants with FGFR mutation: Crouzon syndrome (FGFR2) n: 7, (mean age: 4.3m, 1 to 9), Pfeiffer syndrome (FGFR2) n:6 (mean age: 5.3 m; 3 to 11), Apert syndrome (FGFR2) n:9 (mean age: 5.8m, 3 to 7), Muenke-Lajeunie (FGFR3)n:16 (mean age: 5.6m, 3 to 8) , and achondroplasia (FGFR3) n: 4 (mean age 4.4m, 1-8) and compared to 30 controls (mean age: 4.7m, 0-12).
The FM size was reduced in all FGFR-related anomalies compared to controls (489.77 mm2) except for Apert syndrome (Crouzon: 346.39 mm2, $p<0.001$, Muenke-Lajeunie: 426.13mm2, $p<0.04$, Achondroplasia: 125.27 mm2, $p<0.001$, Pfeiffer: 383.78 mm2, $p<0.02$, Apert: 422.02 mm2, $p=0.07$). However, the extent of such alterations was extremely variable. The higher degree of reduction was found in achondroplasia whereas the milder degree was that of Apert patients. Smaller FM were associated to cerebrospinal fluid disorders (Subarachnoid or ventricular dilatations).
In conclusion, the large spectrum of FM alterations associated to FGFR mutations is associated to the type of gene mutation. The size of the FM seems to play a role in the occurrence of cerebrospinal fluid disorders disorders.

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C15.2

New genes in epilepsy and its co-morbidities: a linkage and whole exome sequencing approach.

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Purpose: The majority of epilepsies are focal in origin, with seizures arising in one region of the brain. The aetiology of most cases of focal epilepsy remains unknown and has not generally been thought to be genetic. We studied large pedigrees with forms of focal epilepsy (NFLE and FFEVF) which showed autosomal dominant (AD) inheritance to identify the genes responsible and then studied their broader contribution to the focal epilepsies.
Method: We used a strategy of whole genome linkage analysis followed by whole exome sequencing in two families with FFEVF mapping to Chromosome 22q12 and one large family with ADNFLE mapping to 9q34.3.
Results: We detected mutations in DEPDC5 in the two families and subsequently in 5/6 additional families with FFEVF. Furthermore, we found DEPDC5 mutations in approximately 12% (10/82) of families with non-lesional focal epilepsy. A mutation in the potassium channel gene KCNT1 was identified in the family with NFLE and subsequently in three other families with NFLE. Mutation-positive family members were also affected with psychiatric features, intellectual disability or autism spectrum disorders.
Conclusions: Mutations in DEPDC5 account for approximately 12% of cases of non-lesional familial focal epilepsy, becoming the most common known cause of familial focal epilepsy. KCNT1 mutations contribute to nocturnal frontal lobe epilepsy (NFLE) as well as to psychiatric features. Genetic factors therefore play an increasing role in the focal epilepsies. These findings enable improved patient diagnoses and reveal new pathways involved in the

pathogenesis of the focal epilepsies and related disorders.

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C15.3

Targeted sequencing of GPI anchor synthesis pathway genes identifies mutations in PGAP2 as a new cause of hyperphosphatasia with mental retardation

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Recently, mutations in genes involved in the biosynthesis of the glycosylphosphatidylinositol (GPI) anchor have been identified in a new subclass of congenital disorders of glycosylation with a distinct spectrum of clinical features. To date mutations have been identified in six genes of the GPI-anchor synthesis pathway, PIGA, PIGL, PIGM, PIGN, PIGO, and PIGV in patients with severe neurological features including seizures, muscular hypotonia, and intellectual disability. We developed a diagnostic gene panel for targeting all known genes of the GPI-anchor synthesis pathway to screen patients matching these features, and detected three missense mutations in PGAP2, c.46C>T, c.380T>C, and c.479C>T, in two unrelated patients with hyperphosphatasia mental retardation syndrome (HPMRS). The mutations cosegregated in the investigated families. PGAP2 is a gene involved in fatty acid remodeling of the GPI-anchor, which occurs in the Golgi apparatus and is required for stable association of GPI-anchored proteins with the cell surface membrane rafts. Transfection of the mutant constructs p.Arg16Trp, p.Leu127Ser, and p.Thr160Ile into PGAP2-null cells showed only partial restoration of GPI-anchored marker proteins, CD55 and CD59, on the cell surface. In this work we show that also an impairment of GPI-anchor remodeling causes HPMRS and conclude that targeted sequencing of the GPI-anchor pathway genes is an effective diagnostic approach for this subclass of congenital disorders of glycosylation.

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C15.4

Diagnostic exome sequencing to elucidate the genetic basis of likely recessive disorders in consanguineous families

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Consanguinity increases the risk for autosomal recessive (AR) disorders. Exome sequencing provides an opportunity to arrive at a molecular diagnosis in a proportion of unresolved phenotypes in these families, thereby identifying novel candidate genes for AR phenotypes. Samples were collected from 32 consanguineous families characterized by a wide spectrum of phenotypes suggestive of AR inheritance. DNA was taken from the patient(s), all unaffected siblings and parents. All samples were genotyped with 720K SNP array to identify Runs of Homozygosity, allowing the definition of identical-by-descent chromosomal regions likely to contain the responsible pathogenic variants. Exome sequencing was performed on one affected individual per family. Variants within the identified target areas were called and filtered for various criteria for pathogenicity. Samples from one affected individual per family were also examined by aCGH to detect homozygous deletions and duplications. On average, 21,719 variants were identified per patient. The putative pathogenic variant was found in known disease-causing genes (VLDLR, DMP1, FKTN, SEPSECS, GUCY2D, BBS4, SYNE1 and POMGNT1) in 8 families. In two families, strong candidate mutations were identified and in a further 16 families, variants of likely pathogenicity were found. In 6 families, no plausible candidates were identified. Consanguineous families provide a unique opportunity to identify pathogenic variants in both known and candidate genes responsible for recessive phenotypes in order to populate the missing space of genotype-to-phenotype links. We identified a putative causative variant in 25% of families tested. High-throughput sequencing represents a substantial improvement in our ability to diagnose recessively inherited disorders.

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C15.5

Exome Sequencing in the Diagnostics of non-motile Ciliopathies (113 cases)

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Background: Non-motile cilia are hair like structures extending from the surface of most cells in mammalian organisms. Mutations in genes encoding for cilia associated proteins lead to complex developmental defects and although individual ciliopathy diseases are very rare, together they represent a significant disease burden. Due to extensive genetic heterogeneity and phenotypic as well as genetic overlap, molecular diagnosis using Sanger sequencing has been proven very difficult in the past. However, the development of Next Generation Sequencing techniques such as Whole Exome Sequencing (WES) offers new diagnostic tools. **Results:** We have investigated 61 Jeune Asphyxiating Thoracic Dysplasia (JATD), 37 Bardet-Biedl-Syndrome (BBS), 7 Joubert and 9 other ciliopathy cases (total of 113 cases) using WES and were able to identify the disease causing gene in approximately two thirds of all cases with about 50% of JATD and Joubert cases but up to 90% of BBS cases being caused by known disease causing genes. Our findings lead to revision of the clinical diagnosis in some cases and revealed new phenotype-genotype associations, especially in JATD. Functional studies for several new genes identified are currently under investigation. However, the proportion of solved cases was much lower among less well defined phenotypes. **Summary:** Our findings in this large non-motile ciliopathy cohort demonstrate that WES is a very efficient tool in genetics diagnosis of heterogenous recessive disorders facilitated by deep phenotyping. Compared to NGS gene panel sequencing, WES offers additional opportunities to identify new genes previously not associated with the condition investigated.

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C15.6

Detection of clinically relevant copy number variants with whole exome sequencing

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Copy number variation (CNV) is a common source of genetic variation that has been implicated in many genomic disorders. This has resulted in the widespread application of genomic microarrays as a first tier diagnostic tool. More recently whole exome sequencing (WES) has proven highly successful for unbiased detection of clinically relevant point mutations and small insertion-deletions. Here, we evaluated the clinical utility of WES to detect CNVs in a set of DNAs from 10 patients with intellectual disability in which diagnostic SNP arrays previously detected 12 pathogenic CNVs. WES read depth approaches were compared to CNV detection provided by a range of (ultra) high-resolution microarrays (250K, 2.7M and 4.2M (exon targeted)), to assess WES performance. Using WES read depth data, we detected 11 of the 12 clinically relevant CNVs; a heterozygous single-exon deletion remained undetected. WES identified an average of five CNVs per exome, encompassing on average 8 genes (1-118). Breakpoint and gene content analysis showed that even though WES lacks breakpoint accuracy compared to the high-resolution microarrays, the gene content of clinically relevant CNVs, encompassing 3 or more exons, can be determined reliably. The detection power of WES for small and single-exon CNVs currently does not match the detection power of high-resolution microarray platforms, and should be optimized to detect these. The majority (76%) of all 33 rare coding CNVs (≥3 exons), identified by two independent microarray platforms, were successfully identified by WES making it even more attractive as a first tier diagnostic tool in genetically heterogeneous disorders.

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C16.1

Patterns and Rates of Exonic *de novo* Mutations in Sporadic Hirschsprung Disease Patients

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Hirschsprung disease (HSCR, aganglionic megacolon; 1 in 5000 live births) is a disorder of the enteric nervous system (ENS) characterized by the absence of enteric neurons along a variable length of the intestine. HSCR most commonly presents sporadically, although it can be familial (5-20% of the patients). The sporadic

form of the disorder is believed to be a genetically complex disease with both *de novo* and/or inherited genetic lesions. To assess the role of *de novo* mutations (DNM) in sporadic HSCR, we performed exome sequencing on 16 HSCR patients and their unaffected parents. Standard BWA/GATK pipeline was used to map the sequence reads to human reference genome 19 and call genomic variants for all 48 samples simultaneously. Exonic DNM mutations were identified using KGGSeq. DNM candidates were scrutinized using Integrative Genomics Viewer (IGV) and those variants deemed plausible were validated by Sanger sequencing. In total we confirmed 20 *DNM* mutations (17 SNVs, 3 Indels) in 16 genes. Five DNM were identified in RET (major HSCR gene). CCR2, COL6A3, MED26, NUP98, HMCN1 and DENND3 had *DNM* mutations and were found mutated in independent HSCR patients. Importantly, some of these genes are members of pathways involved in the development of the ENS and the encoded proteins interact with known key signaling molecules. The overall exonic DNM mutation rate is 1.25 per HSCR trio, with 10 out of 16 (62.5%) patients harboring ≥ 1 DNM mutations. Therefore, DNM mutations, as inherited mutations, contribute to the development of sporadic HSCR.

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C16.2

A novel chromosomal breakage syndrome caused by a missense mutation in a gene from the SMC5/6 complex

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We characterized two Caucasian sisters who died at the age of one year of a sudden progressive pulmonary deterioration with multiple viral infections despite an intact immune system. They showed failure to thrive, hypotonia, mild psychomotor retardation, eczema, sparse hair growth, prominent veins, multiple aspecific cerebral calcifications and allergy. Karyotyping in cultured lymphocytes showed up to two supernumerary marker chromosomes in the first girl and up to eight supernumerary marker chromosomes and missing chromosomal parts in the second girl. Array-CGH at 180K resolution based on DNA from blood of both sisters showed a normal female profile. Cultured skin fibroblasts grow very poorly; survivors show no chromosomal anomalies. All known chromosomal breakage syndromes were excluded by genetic testing.

Homozygosity mapping in the family revealed a large homozygous stretch of more than 8Mb and subsequent genealogical analysis proved consanguinity as far as ten generations back. Whole-exome sequencing in the two affected girls followed by additional segregation analysis in their healthy parents, two sisters and a brother identified a novel missense variant in a gene from the SMC5/6 complex proofing inheritance in an autosomal recessive manner. The missense variant overlaps with the largest homozygous stretch, is located in a conserved region, and predicted to have a damaging effect on the protein function by PolyPhen and SIFT. It is absent in all available databases.

This work suggests the first evidence of a germline mutation in a gene from the structural maintenance of chromosomes complex SMC5/6 linked to a novel chromosomal breakage syndrome.

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C16.3

SPRTN deficiency causes a novel segmental progeroid syndrome with chromosomal instability

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Syndromes with signs of premature aging affecting several tissues are crucial to identify novel genes and pathways involved in the aging process. We have studied a consanguineous patient affected by a segmental progeroid syndrome with chromosomal instability and early-onset hepatocellular carcinoma. Exome sequencing revealed just a single non-annotated homozygous sequence change with a severe impact on protein structure: a homozygous frame-shift mutation in a formerly uncharacterized gene SPRTN. We showed that this protein is recruited to sites of DNA damage after treatment with genotoxic agents and is crucially involved in various DNA damage response pathways. siRNA-mediated depletion in cultured cells resulted in both severe

proliferation defects and chromosomal instability with increased sensitivity towards genotoxic agents comparable to the findings in patients' primary cells. We also observed a siRNA-mediated distortion of the nuclear envelope like seen in other types of segmental progeria, which could be rescued by expression of human wildtype but not by a construct expressing the mutant isoform. In addition, we cloned the zebrafish ortholog and could show that morpholino-based silencing resulted in an increase of γH2AX foci, as a marker of DNA damage, as well as in developmental defects and increased mortality. The latter could be partially rescued by expression of human wildtype but not by a construct expressing the mutant protein. In summary, we identified the cause of a novel segmental progeroid syndrome and a crucial role of this formerly uncharacterized gene in the regulation of aging.

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C16.4

Mutations in HDAC8 cause a clinically recognizable Cornelia de Lange Syndrome (CdLS)-like disorder

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CdLS is a rare multisystem genetic disorder typified by characteristic facies, prenatal onset growth failure, intellectual disability, distal limb anomalies, gastrointestinal and neurological disease. The phenotype shows a broad range of variability. About half of patients have mutations in *NIPBL*, which encodes a cohesin-associated factor. An additional 5% of the patients have mutations in *SMC1A* or *SMC3*, which encode structural components of cohesin. Loose genotype-phenotype correlations exist, with mutations in *NIPBL* resulting in the majority of the "classically" affected patients. However atypical or variant CdLS cases have a much lower mutation detection rate. Here we demonstrate that mutations in the X chromosome gene *HDAC8* are present in about 5% of our patient cohort and cause a recognizable subgroup of CdLS-like phenotypes. Most of these mutations are missense and *de novo*. About two-thirds of affected cases are heterozygous females and marked skewing of X-inactivation in peripheral blood DNA is common. Hemizygous males are more severely affected than females. The craniofacial appearance of *HDAC8*-cases is distinct from classical CdLS, and delayed anterior fontanelle closure may be a useful discriminating feature. Limb malformations appear to be rare and postnatal growth failure is less severe than classical CdLS. Functional analysis of mutant HDAC8 proteins demonstrates partial or complete loss function. These data demonstrate that mutations in *HDAC8* cause a clinically distinct subgroup of CdLS and solidify HDAC8-mediated deacetylation in the molecular mechanism in cohesin function.

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C16.5

A homozygous PDE6D mutation in Joubert syndrome impairs targeting of farnesylated INPP5E protein to the primary cilium

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Primary cilia are microtubule-based organelles whose formation and compartmentalization are ensured by selective import of specific cargoes through a barrier at the ciliary base. Given the structural constraints imposed by this selectivity barrier, how membrane-associated proteins are imported into the primary cilia has remained unclear. In a consanguineous family with Joubert Syndrome (JS), combined exome sequencing and mapping identified a homozygous splice site mutation in PDE6D, encoding a prenyl-binding protein. We found that pde6d depletion in zebrafish leads to renal and retinal developmental anomalies and that wild type but not mutant PDE6D is able to rescue this phenotype. In an effort to understand the pathophysiological mechanisms underlying PDE6D mutation in JS we used tandem affinity purification/mass spectrometry and identified the ciliary farnesylated protein INPP5E involved in two ciliopathies (Joubert and MORM syndromes) as a cargo of PDE6D. We showed that PDE6D binds to INPP5E in a farnesyl-dependent manner and that patient truncated PDE6D, which localizes properly to the basal body, shows reduced binding to INPP5E and to ARL2 and ARL3 that act as cargo-release factors for PDE6D-bound farnesylated proteins. In patients and PDE6D-depleted cells, INPP5E fails to localize to the primary cilia indicating that PDE6D is required for INPP5E ciliary targeting. This study provides the first evidence of prenyl-binding dependent trafficking in ciliopathies and highlights a specific mechanism enabling prenylated protein import within primary cilia.

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C16.6

Integrator Complex Subunit 8 mutations associated with abnormal brain development and spliceosomal defect

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The Integrator Complex (IC) is responsible for the 3'-end processing of the U1/U2 major spliceosome snRNAs. Maturation of these snRNAs presumably takes place in the subnuclear Cajal body and the IC is an essential component for its integrity. There is accumulating evidence that the IC is necessary for normal vertebrate development, however no association with human disease has been reported yet.
Three siblings presented with severe developmental brain abnormalities, cerebellar hypoplasia and periventricular nodular heterotopia (PNH) of unknown cause. Using whole genome sequencing (Complete Genomics), and filtering for recessive inheritance, we discovered compound heterozygous mutations in *Integrator Complex Subunit 8* (*INTS8*) in the three sibs, the parents being heterozygous. QRT-PCR showed 2-fold decreased *INTS8* RNA levels in patients' cells compared to controls. Pathway analysis (Ingenuity) of expression data (Affymetrix U133 expression arrays and Gene Chip 1.0 ST exon arrays) of RNA extracted from patient fibroblasts showed enrichment of deregulated genes involved in mRNA splicing and posttranscriptional modification. Expression data of (developing) human brain structures (Allen Brain Atlas) show that expression of *INTS8* peaks prenatally, especially in the ganglionic eminences, (sub)ventricular zone and hindbrain, similar to mouse embryo (Emage databse). From these areas neuronal precursors migrate to the cortex, and these regions are compatible with the PNH and cerebellar phenotype in the patients. Immuno-staining of Cajal bodies in *INTS8*-depleted and in patients' fibroblasts is ongoing and will be presented.
We propose that dysfunction of the Integrator Complex, through a spliceosomal defect, leads to severely disrupted brain development in humans.

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C17.1

Natural variation in the histone demethylase, KDM4C, influences transcriptional regulation and cell growth

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Histone demethylases are chromatin modifiers; while their biochemical activities have been characterized, very few of their target genes are known.

In this study, by genetic and molecular analyses, we identified and characterized over 300 target genes of KDM4C, a member of the Jumonji family of histone demethylases. KDM4C demethylates tri-methylated H3K9 histone marks, which are associated with transcriptional repression.
We measured gene expression in B-cells from members of 45 large pedigrees, and treated gene expression levels as quantitative traits in linkage and association analyses. We found extensive individual variation in the expression level of *KDM4C*. Genetic analyses showed that *KDM4C* expression is regulated in *cis* by variants near the 3' end of *KDM4C*, and luciferase reporter assays confirmed the presence of enhancer activity in this region. The genetic studies also identified more than 300 genes whose expression levels are regulated by KDM4C. By chromatin immunoprecipitation, we found that KDM4C localized to these genes. Individuals with high *KDM4C* expression had more KDM4C binding to its target genes than individuals with lower *KDM4C* expression. Many of the target genes play key roles in cell proliferation. We found that cells from individuals with high *KDM4C* expression grew faster than those from individuals with lower *KDM4C* expression. In addition, we found *KDM4C* to be expressed at higher levels in cancers than in corresponding normal cells, and that KDM4C depletion attenuates cancer cell growth. In this presentation, I will describe how transcriptional regulation by KDM4C differs among individuals and how this influences cell growth.

B.L. Gregory: None. **V.G. Cheung:** None.

C17.2

Enrichment of uniparental disomy events detected in the Deciphering Developmental Disorders rare disease study

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The Deciphering Developmental Disorders (DDD) project aims to identify the disease-causing mutations for 12,000 children in the UK with rare, presumed genetic, developmental diseases. This study uses a proband-mother-father trio design, which by its nature facilitates the identification of inheritance anomalies. This work focuses on the investigation of uniparental disomy (UPD), an inheritance defect characterised by the inheritance of both chromosome homologs from only one parent. UPD can result in imprinting disorders, or lead to the unmasking of recessive disorders, phenomena that are generally uncommon, but over-represented among individuals with rare diseases. Our newly developed method reads VCF-formatted trio genotype data, identifies genotypes in the proband only compatible with uniparental inheritance, and performs a statistical test to identify chromosomes with a significant enrichment of these informative genotypes. Simulation testing demonstrated 100% sensitivity in detecting whole-chromosome UPD events from both SNP genotyping chip-derived and exome-derived genotypes, and remained completely sensitive for segmental UPD detection down to ten megabases for most types of UPD. We applied the method on 1,075 trios from the DDD project and identified five probands with complete chromosomal uniparental disomy and one proband with segmental uniparental disomy. This is a significant enrichment over the population prevalence of UPD, suggesting that most of these events are pathogenic. One of these events represents a known imprinting disorder and exome analysis has identified several rare homozygous candidate variants, mainly in the isodisomic regions of UPD chromosomes, which provide targets for further functional evaluation.

D.A. King: None. **M.E. Hurles:** None.

C17.3

Exonic splicing mutations in Mendelian disorders: more prevalent than currently estimated

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The identification of a causal mutation is essential for molecular diagnosis and clinical management of Mendelian diseases. Even if new-generation exome sequencing has greatly improved the detection of exonic changes, the biological interpretation of most exonic variants remains challenging. We evaluated the prevalence of RNA splicing mutations among exonic variants identified in *BRCA2* and *MLH1*, two genes implicated in the most frequent Mendelian forms of cancer. *BRCA2* exon 7 and *MLH1* exon 10 were used as model systems. By performing minigene-based *ex vivo* assays, we determined the effect on splicing produced by all single-substitutions listed in public databases, within these exons, initially reported as synonymous, missense or nonsense mutations (n=52). Our results revealed that a significant fraction of these variants have an effect on splicing. Importantly, when patient RNA was available, minigene and *in vivo* data were shown to be concordant. Remarkably, a significant subset of variants induced exon skipping in spite of being located outside splice sites (10 in *BRCA2* and 7 in *MLH1*), an effect that could not be predicted by commonly used bioinformatic approaches. This observation led us to test a newly developed *in silico* tool aiming at detecting exonic splicing regulatory elements. With very few exceptions, we found the predictions produced by this tool to be concordant with our minigene-derived data. This study shows that the fraction of splicing mutations is underestimated in Mendelian diseases and highlights the predictive potential of a newly developed *in silico* tool in pinpointing exonic mutations that affect RNA splicing.

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C17.4

New insights into human germline chromothripsis: underlying mechanisms and definition

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Recently, a new phenomenon of highly complex genomic rearrangements was revealed, which was termed chromothripsis and defined by the accumulation of multiple breakpoints within relatively small chromosomal regions and by extensive rearrangements within a single cell cycle. So far there are only data from 13 reported germline chromothripsis (GC) cases with a maximum number of breakpoints < 25. The lower complexity has been suggested to be due to selection. Herein, we present five new cases of GC, two of which are the most complex cases studied so far, involving 46 and 140 breakpoints, respectively. This supports that viability of these cases is not determined by the number of breakpoints per se, but rather by the functional consequences of the truncated genes and regulatory landscapes. We confirmed the surprisingly balanced state of GC in four cases, but one is associated with larger deletions at all breakpoints involved. The most likely mechanism for these rearrangements would be local shattering of the chromosomal regions generating numerous double-strand DNA breaks, which have subsequently been repaired by non-homologous or microhomology-mediated end-joining mechanisms. However, we do not exclude the involvement of replication based mechanisms in the case with deletions at all breakpoints. Combined with the reported GC cases, we observe a significant overlap of the shattered chromosomal regions suggesting that the shattering may be a non-random process. Finally, we clarify the term chromothripsis and classify chromosome shattering as mild, moderate and severe based on the number of breakpoints accumulated within a cluster.

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C17.5

Whole-genome sequencing analysis of human induced pluripotent stem cells uncovers lineage-manifested CNVs

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We have performed whole-genome sequencing based CNV analysis in 7 fibroblast samples and 20 corresponding induced pluripotent stem cell lines obtained from two families [Abyzov et al., Nature. 2012 Dec 20;492(7429):438-42].
We found that on average an iPSC line has two LM-CNVs (lineage-manifested CNVs). We defined the term LM-CNV to describe CNVs detected by genome-wide analyses in an iPSC line but not in the fibroblast culture from which the given iPSC line was derived but without making a statement as to the nature of the CNV-forming event (i.e. *de novo* formation during reprogramming from fibroblast to iPSC or unmasking of a somatic variant present in mosaic fashion in the fibroblast tissue).
After detecting LM-CNVs by sequencing based analysis in the iPSC lines we investigated the masked, mosaic presence of the same CNVs in the fibroblast tissue of origin. We determined that more than half of the LM-CNVs detected

in iPSC lines were already present as low allele frequency, mosaic somatic CNVs in the fibroblasts and that up to 40% of fibroblast cells carry such medium-sized to large somatic CNVs. Therefore, *de novo* CNVs in iPSCs may not be an obligate consequence of reprogramming. Our analysis unexpectedly revealed extensive somatic copy number variability in fibroblasts carrying over into iPSC and becoming unmasked in the process.
Our results underline the necessity of carrying out high-resolution genome analysis during iPSC-model based studies and demonstrate that whole-genome sequencing allows for detection and managing of the potential confounds caused by genomic variation such as LM-CNVs.

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C17.6

Modelling and rescuing neurodevelopmental defect of Down syndrome using induced pluripotent stem cells from monozygotic twins discordant for trisomy 21

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Down syndrome (DS, trisomy 21), is the most common chromosomal disorder, with an incidence of 1 in 800 live births. Its phenotypic characteristics include intellectual impairment and several other developmental abnormalities, for the majority of which the pathogenetic mechanisms remain unknown. Here, we have generated induced pluripotent stem cells (iPSCs) derived from monozygotic twins discordant for trisomy 21: Twin-N-iPSCs and Twin-DS-iPSCs. Since the rest of the genome is identical between the two samples, we hypothesize that these twins were ideal to study the effect of the supernumerary chromosome 21.
We found that Twin-DS-iPSCs recapitulate the neurodevelopmental features of DS. The alterations observed by genetic analysis at the iPSC level and at first approximation in early development illustrate the developmental disease transcriptional signature of DS. DS-iPSCs exhibited an abnormal neural differentiation *in vivo* and *in vitro*. Twin-DS-iPSC-derived neurospheres showed a reduced number of neuroprogenitor cells (NPCs). When NPCs were further differentiated into neurons, we found structural changes in the architecture and density of neuron, glial cell and oligodendrocyte populations together with misexpression of genes involved in lineage specification, neurogenesis and brain development. Importantly, targeting *DYRK1A* (on chromosome 21) pharmacologically or by shRNA corrected these defects. These improvements were associated with the restoration of *REST/NRSF*, *WNT* and *NOTCH* signaling to near normal levels.
In conclusion, these findings establish iPSCs generated from human monozygotic twins discordant for trisomy 21 as a unique model to recapitulate DS phenotypes, study the detailed mechanisms involved in the pathogenesis of DS and design new therapies.

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C18.1

Meta-analysis of 233,000 individuals identifies sex- and age-dependent genetic associations for obesity traits

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Previous investigations by the Genetic Investigation of ANthropometric Traits (GIANT) consortium have shown that seven of the 14 single nucleotide polymorphisms (SNPs) found to influence body shape have significantly larger effect in women than in men. Adult body composition and body shape change over time (e.g. following menopause), which may be explained by age-and-sex-dependent genetic effects.
We conducted age-and-sex stratified genome-wide association (GWA) meta-analyses of 117 studies (133,012 women, 100,652 men) within the GIANT consortium. Each study tested for association of up to 2.8M SNPs and obesity related traits: body mass index (BMI) and waist-hip ratio (WHR). The effects were estimated separately in four strata divided by age (<50y, >=50y) and sex and subsequently compared against each other via heterogeneity test.
Our analysis identified no sex- and eight age-specific BMI associations at 5% FDR. Three novel BMI SNPs are located in or near PVRL2, TCF7L2 and DDC. The novel TCF7L2 association (observed in >=50y) is also linked to type 2 diabetes, however the effect direction is opposite. An additional five SNPs are located near established BMI loci: SEC16B, ADCY3, TNNI3K, TMEM18, NEGR1. Two out of the 8 SNPs have stronger effect in the older group. For WHR, we identified no age- and 29 sex-specific associations, 20 of which are novel loci. Strikingly, all but three SNPs have a more pronounced effect in women than in men.
Our results highlight the importance of sex- and age-stratified GWAS analyses in revealing the sex- and age-dependent genetic underpinnings of obesity traits.

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C18.2

A causal association between vitamin D status and blood pressure: a Mendelian Randomization study in up to 150,846 individuals

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Low vitamin D status is highly prevalent and associated with high blood pressure (BP) and hypertension in observational studies. We used a Mendelian Randomization approach to investigate the causal relationship between vitamin D status [measured by 25-hydroxyvitamin D, 25(OH)D] and BP/hypertension. We examined associations of four vitamin D-related SNPs with 25(OH)D (for validation) and with BP/hypertension (for causal inference) individually and in combination using separate allele scores for SNPs involved in synthesis (comprising DHCR7 and CYP2R1) and metabolism (comprising GC and CYP24A1) of 25(OH)D. Meta-analysis included up to 108,173 individuals from 35 studies. 25(OH)D was associated with systolic BP (SBP) (p=0.01) and hypertension (p=0.01). All four SNPs were strongly associated with 25(OH)D (p<7.59 x 10-14). None of the SNPs/allele scores was associated with BP/hypertension. However, when meta-analysing D-CarDia results together with results from International Consortium

for Blood Pressure to increase statistical power (n=125,172, overlapping studies excluded), both CYP2R1 (p=0.03) and synthesis score (p=0.01) were associated with DBP. Similarly, CYP2R1 (p=0.02) and synthesis score (p=0.002) showed an association with hypertension after meta-analysing D-CarDia results with results from CHARGE and Global BPgen consortia (n=150,846). Instrumental variable analyses using synthesis score as an instrument suggested a modest causal association, where for every 10% increase in 25(OH)D concentrations, there was a 0.24 mmHg decrease in DBP (95%CI:-0.43,-0.05,p=0.02) and 7% decrease in the odds of hypertension (95%CI:2.4%,11.1%,p=0.003). In conclusion, these data support a causal role of higher 25(OH)D in leading to reductions in blood pressure, providing further support for important non-skeletal effects for vitamin D.

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C18.3

Large-scale genome-wide association meta-analysis using imputation from the dense 1000 Genomes Project identifies novel susceptibility loci for glycemic and obesity traits: ENGAGE Consortium report

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Genome-wide association studies (GWAS) detected numerous associations with quantitative glycemic and obesity traits: only ~5% of phenotypic variance been explained mainly by common variants (minor allele frequency, MAF >5%). Our aim was to detect novel associated loci; low frequency variants influencing trait variability and novel independent variants at known loci. We performed large-scale fixed-effects meta-analysis of GWAS data including up to 38 million SNPs imputed using the 1000 Genomes Project reference panel (06/2011 release). The analysis was performed in up to 18 European GWAS, for fasting glucose (FG, n=46,694), fasting insulin (FI, n=24,245), body-mass-index (BMI, n=87,048) and waist-to-hip ratio adjusted for BMI (WHR, n=54,572).

We identified novel loci at genome-wide significance for FG (1 locus near *RMST*, p=2.0x10^{-10}, MAF=0.10) and BMI (5 loci near *GALNT10*, *DTX2P1*, *GRID1*, *EPYC* and *AKAP6*; p<4.5x10^{-8}, MAF=0.07~0.49). Through conditional analysis, we identified a novel low-frequency variant associated with FG independent of the lead SNP at *G6PC2* (2-169748691, MAF=0.012, p=3.0x10^{-19}, conditional p=1.7x10^{-21}). At a known locus for WHR, *RSPO3*, we detected a novel independent lead signal with stronger association and larger effect size than the previously reported signal (new β=0.11, p=1.7x10^{-13}, MAF=0.08 vs. old β=0.04, p=2.1x10^{-11}, MAF=0.49).

Our results highlight the potential advantages of imputation using the high-density reference panels for the identification of novel associated signals for both common and low frequency variants for quantitative metabolic traits.

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C18.4

The ‚Genome of The Netherlands' outperforms ‚1000 genomes' as a reference set for imputing rare variants in the European population

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Imputation of missing genotypes can be an effective method to improve genome-wide association studies (GWAS) by increasing the number of SNPs to be tested for association. By¬¬¬ matching the study haplotypes to high-resolution reference haplotypes, the imputation process is able to infer genotypes of un-typed variants. We show that the Genome of The Netherlands (GoNL) reference set not only outperforms the 1000 genomes (1000G) reference set for imputing Dutch samples but also for Italian samples. We also assessed the effect of fine-mapping on an eQTL study.

We quantified the difference in imputation quality by using samples genotyped on the Hap550 and the ImmunoChip, which we used as our gold standard. Genotypes from Hap550 were imputed using GoNL and 1000G to produce two datasets. The imputed genotypes were compared to genotypes present on the ImmunoChip. The median concordance in the Dutch samples of 4,192 rare SNPs (MAF <0.5%) increased from 0.37 to 0.54 when the imputing used GoNL compared to 1000G. In the Italian samples the median concordance of rare variants also improved when using GoNL (from 0.27 to 0.37). We found large deviations of the predicted concordance to the observed concordance, validating the use of our gold-standard benchmarking and making it evident that interesting imputed variants should always be validated.

An eQTL dataset was used to assess the effects of a better imputation on GWAS. We found a significant improvement when using imputation, both in signal strength and in fine-mapping. For the fine-mapping we performed an enrichment on ENCODE's regulomeDB.

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C18.5

Genome-wide assocation study identifies common variation associated with congenital heart disease

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We tested the hypothesis that common variants contribute to congenital heart disease by conducting a genome-wide association study for Tetralogy of Fallot (TOF) using a northern European discovery set of 835 cases and 5159 controls. A large region on chromosome 12q24 which includes *PTPN11* was associated (P = 1.4 × 10^{-7}) and replicated in 798 cases and 2931 controls (replication P = 3.9 × 10^{-5}; combined P = 7.7 × 10^{-11}). SNPs in *GPC5* on chromosome 13q32 were also associated (P = 1.7 × 10^{-7}) and replicated convincingly in 789 cases and 2927 controls (replication P = 1.2 × 10^{-5}; combined P = 3.03 × 10^{-11}). We extended the GWAS to a discovery cohort of 1,995 cases with various CHD phenotypes. A region on chromosome 4p16, between *MSX1* and *STX18*, was associated (P=9.5x10^{-7}) with the risk of atrial septal defect (ASD; N=340 cases), and this was replicated in a further 445 ASD cases and 2520 controls (replication P=1.1x10^{-4}; combined P=6.9x10^{-10}). The association between SNPs at 12q24 and TOF was not apparent in any other CHD subgroup; similarly, the association between SNPs at 4p16 and ASD was not apparent for CHD phenotypes other than ASD. We conclude that there is phenotypic specificity for common variants associated with CHD.

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C18.6

Common genetic variants predispose to a rare disease with high risk of sudden cardiac death

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The Brugada Syndrome (BrS) is considered as a rare Mendelian disorder with autosomal dominant transmission. BrS is associated with an increased risk of sudden cardiac death and specific electrocardiographic features consisting of ST-segment elevation in the right precordial leads. Loss-of-function mutations in SCN5A, encoding the pore-forming subunit of the cardiac sodium channel (Nav1.5), are identified in ~20% of patients. However, studies in families harbouring mutations in SCN5A have demonstrated low disease penetrance and in some instances absence of the familial SCN5A mutation in some affected members. These observations suggest a more complex inheritance model.

To identify common genetic factors modulating disease risk, we conducted a genome-wide association study on 312 individuals with BrS and 1115 ancestry-matched controls. Two genomic regions displayed significant association. Replication testing on two independent case/control sets from Europe (598/855) and Japan (208/1016) confirmed both associations and revealed a third one. While two loci displaying association hits had already been shown to influence ECG parameters in the general population, the third one encompasses a transcription factor which had never been related to cardiac arrhythmia We showed that this factor regulates Nav1.5 channel expression in hearts of homozygous knockout embryos and influence cardiac conduction velocity in adult heterozygous mice. At last, we found that the cumulative effect of the 3 loci on disease susceptibility was unexpectedly large, indicating that common genetic variation may have a strong impact on predisposition to rare disease.

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C19.1

Mutations in *PIK3R1* cause syndromic insulin resistance and lipodystrophy

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Insulin resistance (IR) is closely associated with many common, complex and multifactorial conditions. Identifying the genetic basis of rare monogenic forms of IR provides unique opportunities to decipher the molecular mechanisms underlying more prevalent forms. SHORT syndrome (Short stature, Hyperextensibility of joints and/or inguinal hernia, Ocular depression, Rieger abnormality, Teething delay) is characterized by the presence of lipodystrophy with generalized thin habitus despite normal food intake. Onset of IR and/or diabetes mellitus usually occur in adolescence. We studied eight families with SHORT syndrome using a combination of trio-based exome sequencing and capillary sequencing, and identified five *de novo* or inherited mutations of *PIK3R1* in all affected individuals. *PIK3R1* encodes the regulatory subunits of class IA phosphatidylinositol 3-kinases (PI3K), which play a key role in insulin signaling through binding of phosphorylated insulin receptor substrates and subsequent activation of AKT serine/threonine kinase. Multiple lines of evidence indicate that the *PIK3R1* mutations identified in individuals with SHORT syndrome should result in decreased PI3K/AKT signaling. Because growth hormone (GH) treatment is known to induce hyperinsulinemia and to decrease insulin sensitivity, a phenomenon that may aggravate IR and accelerate the onset of diabetes in subjects with SHORT syndrome, GH treatment was ceased in the patients treated. In conclusion, our findings highlight the critical role of *PIK3R1* in insulin action, normal growth and development. They also well illustrate the fact that novel genetic findings can have direct implications for patient care by enabling a more personalized clinical management.

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C19.2

Genome sequencing identifies mutations causing pancreatic agenesis in a novel *PTF1A* enhancer

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Mutations in non-coding regulatory elements are a rare cause of human disease. Genome sequencing promises to uncover disease causing mutations in novel regulatory elements, but as yet there are no reported successes using this approach.

We used a combination of genome sequencing and linkage in consanguineous pedigrees affected with isolated pancreatic agenesis. Homozygosity mapping revealed a shared region encompassing the *PTF1A* gene in which mutations are known to cause a rare syndrome of pancreatic agenesis and cerebellar hypoplasia. Sequencing of *PTF1A* and 31 other genes within the linkage interval failed to identify causative mutations.

Genome sequencing in two probands identified 6380 variants within the 7.6Mb shared homozygous region. The 193 novel variants included a base substitution affecting a highly conserved nucleotide (GERP score = 5.65) located within a ~400bp region of strong evolutionary conservation >20kb from the *PTF1A* gene. Four additional mutations and one large deletion spanning the element were identified in a total of 10 families.

Functional studies showed that the conserved element acts as an enhancer of *PTF1A* in human and mouse pancreatic progenitor cells. The enhancer binds transcription factors known to be critical for pancreatic development and the mutations abolish transcription factor binding.

Mutations in this novel enhancer are the most common cause of non-syndromic pancreatic agenesis in our cohort and these results provide new insights into the regulatory mechanisms underlying pancreatic development. To our knowledge, this is the first study to report the identification of pathogenic mutations in a novel regulatory element by genome sequencing.

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C19.3

Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension

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Primary aldosteronism is present in up to 7% of hypertensive individuals. It is caused by bilateral adrenal hyperplasia or unilateral aldosterone-producing adenomas (APAs) in 50% of cases, each. Recent reports identified mutations in the potassium channel gene KCNJ5 as a cause of 30-40% of all APAs.

We sequenced additional APA-tumors without KCNJ5 mutations and matched control tissue from nine males. We identified recurrent mutations in two members of the P-type ATPase gene family, ATP1A1 (encoding an Na+/K+ ATPase) and ATP2B3 (encoding the plasma membrane Ca2+ ATPase).

All five identified alterations are either located in the transmembraneous α helix M1 or the juxtaposed α helix M4 in both ATP1A1 and ATP2B3. The recurrence of mutations affecting these highly conserved regions involved in interaction with the transported cations in two paralogs is suggestive of a gain-of-function effect.

We then investigated a collection of 309 APAs and found 16 (5.2%) somatic mutations in ATP1A1 and 5 (1.6%) in ATP2B3. Mutation-positive cases showed male dominance, increased plasma aldosterone concentrations and lower potassium concentrations compared with mutation-negative cases.

Functional in vitro studies of ATP1A1 mutants showed loss of pump activity and strongly reduced affinity for potassium. Electrophysiological ex vivo studies on primary adrenal adenoma cells provided evidence for inappropriate depolarization of cells with ATPase alterations.

In summary, dominant somatic alterations in two members of the ATPase gene family result in autonomous aldosterone secretion in roughly 7% of aldosterone-producing adenomas.

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C19.4

Mutation-dependent recessive inheritance in NPHS2-associated steroid-resistant nephrotic syndrome. Beyond Mendel's laws

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NPHS2, encoding the membrane-anchored podocin, is the most frequently mutated gene in steroid-resistant nephrotic syndrome (SRNS). Its mutations are inherited in an autosomal recessive fashion. While mutations on both alleles ([mut];[mut]) lead to early-onset SRNS, the trans-association of the polymorphism c.686G>A (p.Arg229Gln, rs61747728, MAF in Europeans: 3-4%) and a mutation ([p.Arg229Gln];[mut]) is known to cause late-onset SRNS.

As there is an important difference in the observed and the expected frequency of patients with [p.Arg229Gln];[mut], we hypothesized that [p.Arg229Gln];[mut] may not be pathogenic in all individuals. This was confirmed by the identification of [p.Arg229Gln];[mut] in 6/129 unaffected parents of children, who carried *NPHS2* mutations in exons 1-5. Interestingly, the mutations associated to p.Arg229Gln in affected patients are missense mutations of exons 7-8 in the majority of cases (56/71 alleles, 79%), though these mutations are rare in patients with [mut];[mut] (34/498 alleles, 7%, $p=1.4x10^{-39}$). This striking difference points to the pivotal role of the associated mutation in the pathogenicity of [p.Arg229Gln];[mut]. To understand their pathogenic association at a cellular level, we studied the subcellular localization of podocin^{Arg229Gln} in function of the associated mutation in cultured podocytes. Podocin^{Arg229Gln} was mislocalized only when co-expressed with podocins carrying the substitutions encoded by exons 7-8, but not with substitutions encoded by exons 1-6. Accordingly, a structural model of homodimerization supported that significant structural reorganization only appears in dimers of pathoghenic associations.

Here, we described for the first time an autosomal recessive disorder, in which the pathogenicity of the two alleles depends on each other.

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C19.5

A recurrent homozygous missense mutation in TTC21B encoding the ciliary protein IFT139 unexpectedly causes steroid-resistant nephrotic syndrome

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Steroid-resistant nephrotic syndrome (SRNS) is a glomerular disorder with a high genetic heterogeneity. Using exome sequencing in 7 families with SRNS, we found a recurrent homozygous missense mutation (p.P209L) in the *TTC21B* gene encoding the retrograde intraflagellar transport protein IFT139, which was previously reported to cause isolated nephronophthisis (NPH). Interestingly, analysis of biopsies of SRNS patients revealed tubular basement membrane thickening and tubulointerstitial anomalies similar to NPH. Moreover, analysis of 3 unrelated patients carrying this homozygous mutation and initially diagnosed as NPH reveals that they also have proteinuria and glomerular lesions as in SRNS patients.

We demonstrated that IFT139 is expressed in podocytes and distal tubules in fetal and adult human kidneys. Developing podocytes in fetal kidney and undifferentiated podocytes *in vitro* have a cilium, whereas it is absent in mature and differentiated podocytes. IFT139 localizes at the base of cilia in undifferentiated podocytes, whereas it stains the extended microtubule network in non-ciliated differentiated cells. While the P209L mutant and wild-type IFT139 display similar subcellular distribution, over-expression of the P209L mutant in both podocytes and tubular cells leads to migration defects. Our results, showing for the first time a mutation of a gene encoding a ciliary protein in a glomerular disorder, point to a critical function of IFT139 in podocytes in addition to its well established role in tubular epithelial cells, likely via dysregulation of ciliary transport and microtubule network which are essential for cell migration, polarity and proper organization/maintenance of intercellular junctions, including the slit diaphragm.

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C19.6

Characterization of the large patient cohort of the International Inflammatory Bowel Disease Genetics Consortium (IIBDGC)

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Large-scale genetic studies performed by the IIBDGC have concentrated on Crohn's disease (CD) and ulcerative colitis (UC) overall. Their most recent study on ~75,000 individuals expanded the number of known IBD loci to 163. The enormous resource assembled by the IIBDGC provides an opportunity to evaluate clinically important sub-phenotypic variables and their

relation to genetic variation.
Clinical data on 14,569 CD and 10,867 UC have been collected (age at diagnosis, CD location and behaviour, UC extent, duration of follow-up···). QC analyses were done to identify non-random patterns of missing data and potential classification differences across centres. Genotype-phenotype analyses were performed using the Immunochip release 5 dataset, containing QC'd data on 157,020 variants.
The median age at diagnosis for CD was 23y (IQR 16-33), and 30y (21-43) for UC. 32% of CD patients had ileal (n=3252), 23% colonic (n=2376), and 45% ileocolonic (n=4619) disease. 25% of CD patients had stricturing (n=2574) and 31% penetrating (n=3188) disease. 13% of UC patients had proctitis (n=992), 38% left-sided (n=2941), and 49% extensive disease (n=3757). Genotype-phenotype analyses confirmed a genome-wide significant association for the CD-associated NOD2 locus and the MHC/HLA region with ileal versus colonic CD (rs2066843, p=2.99×10-38, OR=1.70[1.57-1.84] and rs6930777, p=1.83×10−19, OR=0.57[0.51-0.65] respectively). The MHC/HLA region was also associated with extensive UC (rs3115674, p=2.82×10−13, OR=1.47[1.32-1.62]). Interestingly the HLA signals for CD and UC overall are different from those for disease location/extent, warranting more detailed analysis.
These findings provide a robust platform for further analyses enhancing our understanding of the underlying pathogenesis of IBD sub-groups.

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C20.1

RNA foci in C9FTD/ALS patients sequester RNA binding proteins and subsequently alter downstream splicing and expression of their RNA targets.

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Background: Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are two devastating neurodegenerative diseases with substantial clinical and neuropathological overlap. This connection was confirmed by recent groundbreaking genetic studies identifying hexanucleotide (GGGGCC)$_n$ repeat expansions in the *C9ORF72* gene as the major cause of both ALS and FTLD-TDP, the most common pathological subtype of FTD. Transcripts containing the expanded GGGGCC repeat accumulate as nuclear RNA foci in the frontal cortex and spinal cord of *C9ORF72* mutation carriers, an RNA mechanism emerging as a common pathogenic process in many neurodegenerative diseases caused by non-coding repeat expansions. *Methods:* Considering the possibility of subsequent sequestration and altered activity of RNA-binding proteins by these foci and consequently altering the splicing and expression of their RNA targets, we attempted to determine using RNA FISH and commercially available antibodies whether RNA binding proteins already known to be sequestered by foci in patients affected with other neurodegenerative diseases also co-localize with C9ORF72 foci. After identifying two sequestered proteins, we sequenced the cDNA of C9FTD/ALS patients and conducted semi-quantitative as well as qRT-PCR for ten of their known RNA targets. Any variation in expression was confirmed by western blot. *Results:* Our findings confirm that RNA foci formation in C9FTD/ALS lead to the sequestration of two RNA binding proteins, and consequently to the aberrant splicing and expression of several targets. *Conclusions:* RNA foci formation in C9FTD/ALS leads to a loss of function of two sequestered RNA proteins, causing aberrant splicing and expression of genes having critical functions in neuronal survival.

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C20.2

Rapid identification of autosomal recessive and X-chromosomal mutations in small sibling families

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Recent studies have shown that severe intellectual disability (ID) is mostly monogenic and highly heterogeneous. Small sibling families with ID have received little attention from the scientific community due to technical limitations to identify the underlying genetic defect. We studied 20 such families. Pathogenic mutations were identified in three genes. In *DDHD2*, compound heterozygous frameshift mutations where identified in a family with complex ID. Follow-up studies revealed recessive mutations in three additional families with a similar phenotype[a]. Further support that DDHD2 plays an essential role in the human CNS was obtained by a reduced number of active zones at synaptic terminals in Ddhd-knockdown Drosophila models. On the X-chromosome, mutations in *SLC9A6* and *SLC6A8*, both previously implicated in X-linked ID, were identified in two brotherpair families.
Six novel candidate genes for ID were identified. In one of these, a homozygous splice donor site mutation segregated in a brotherpair family. This mutation results in aberrant mRNA splicing and subsequent absent enzymatic protein activity. Rare recessive missense variations were identified in *SYNE1*, *ZNF582* and *MCM3AP*. In *PTPRT*, we identified a compound heterozygous deletion and missense variation. Lastly, a hemizygous missense variation in *BCORL1* on the X-chromosome was present in a brotherpair family.
In this study, we identified (potentially) pathogenic mutations in one novel, two known and six candidate ID genes in nine out of 20 (45%) families, thus demonstrating that recessive and X-linked mutations can readily be identified in small sibling families.
[a] Schuurs-Hoeijmakers et al. *AJHG* December 2012

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C20.3

Associations between gene expression and phenotypes in 16p11.2 rearrangements

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The recurrent 600kb-long 16p11.2 deletion and its reciprocal duplication impact carriers' cognition and behavior. These rearrangements are associated with mirror phenotypes such as obesity and underweight, macro- and microcephaly, as well as autism spectrum disorder and schizophrenia. Although these imbalances are among the most frequent causes of neurodevelopmental disorders, the triggered transcriptome alterations and their associations with the aforementioned phenotypes remained unexplored.
We generated transcriptome profiles of lymphoblastoid cell lines from 16p11.2 deletion and duplication carriers, as well as controls. The expression levels of the 29 genes mapping to the imbalanced interval correlate to their gene dosage. We observed a greater correlation of the expression levels of KCTD13, MVP and MAPK3, three genes shown to have an epistatic effect on zebrafish head size. To investigate the functional relationship between gene-expression levels and phenotypes, we transformed anthropometric measurements into Z-scores. We found that Z-scores computed for weight and BMI were significantly associated with MAPK3 and MVP expression after adjustment for copy-number status in a multivariate normal linear model for adults. We also show that transcription modules (subsets of genes that exhibit a coherent expression profile) involved in the regulation of gene expression, chromatin modification, control of apoptosis and cell division are perturbed in cells of 16p11.2 patients, further confirming the emerging association between "cancer-associated" genes/pathways and neurodevelopmental disorders. Of note, genes whose expression levels correlated with the number of copies of the 16p11.2 CNV were enriched for genes associated with intellectual disability and psychiatric disorders.

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C20.4

GATAD2B loss-of-function mutations cause a recognizable syndrome with intellectual disability and are associated with learning deficits and synaptic undergrowth in *Drosophila*

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Introduction: Family based whole exome sequencing (WES) is successful in the elucidation of causative *de novo* gene mutations in sporadic patients with intellectual disability (ID) not presenting with clinically recognizable syndromes. Establishing conclusive molecular diagnoses in patients with disruptive mutations in genes not previously associated with ID however, requires the identification of additional mutations in the same gene in similarly affected individuals.

Patients, Methods, Results: By WES we recently identified *de novo* loss-of-function mutations in *GATA zinc finger domain containing 2B (GATAD2B)* in two unrelated individuals. We identified an additional disruptive mutation in *GATAD2B* in a third individual by targeted Sanger sequencing in a selected cohort with phenotypic overlap. Detailed clinical characterization revealed a distinctive, highly similar phenotype comprising childhood hypotonia, severe ID, limited speech, abnormal shaped nose, sparse hair growth and strabismus, defining a novel clinically recognizable syndrome. Modelling of *GATAD2B* loss-of-function in *Drosophila* further confirmed its involvement in learning processes and synapse development.

Discussion: *GATAD2B* encodes a subunit of the MeCP1-Mi-2/ NuRD complex involved in transcriptional repression. It can be added to the growing list of ID genes implicated in chromatin remodelling. This study shows that exome sequencing is not only successful in the elucidation of responsible genes in established clinical syndromes, but has also the potential to identify and define novel clinically recognizable syndromes. Moreover, we faced the challenge to make conclusive decisions about the pathogenicity of *de novo* mutations in potentially novel ID genes, which ultimately requires the identification of disruptive mutations in similarly affected individuals.

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C20.5

Disruption of Methyl CpG Binding Protein 5 contributes to a spectrum of psychopathology and neurodevelopmental abnormalities

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Microdeletions of chromosomal region 2q23.1 that disrupt MBD5 contribute to a spectrum of neurodevelopmental phenotypes. To characterize the structural variation landscape of MBD5 disruptions and the associated human psychopathology, 22 individuals with genomic disruption of MBD5 (translocation, point mutation, and deletion) were identified through whole-genome sequencing or cytogenomic microarray at 11 molecular diagnostic centers. The genomic impact ranged from a single base pair to 5.4 Mb. Parents were available for 11 cases, all of which confirmed the rearrangement arose de novo. Phenotypes were largely indistinguishable between patients with full-segment 2q23.1 deletions and those with intragenic MBD5 rearrangements, including alterations confined entirely to the 5'UTR, confirming the critical impact of non-coding sequence at this locus. We identified heterogeneous, multi-system pathogenic effects of MBD5 disruption and characterized the associated spectrum of psychopathology, including the novel finding of anxiety and bipolar disorder in multiple patients. Importantly, one of the unique features of the oldest known patient was behavioral regression. Analyses also revealed phenotypes that distinguish MBD5 disruptions from seven well-established syndromes with significant diagnostic overlap. This study demonstrates that haploinsufficiency of MBD5 causes diverse phenotypes, yields insight into the spectrum of resulting neurodevelopmental and behavioral psychopathology, and provides clinical context for interpretation of MBD5 structural variations. Empirical evidence also indicates that disruption of non-coding MBD5 regulatory regions is sufficient for clinical manifestation, highlighting the limitations of exon-focused assessments. These results suggest an ongoing perturbation of neurological function throughout the lifespan, including risks for neurobehavioral regression.

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C20.6

Analysis of copy number variations at 15 schizophrenia-associated loci in a large, independent cohort

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Rare copy number variants (CNVs) at 15 different loci have been suggested as susceptibility factors for schizophrenia (SCZ). However, as some of these CNVs have only been observed in a single study, it is not clear whether they are true risk factors for the disorder. We set out to test the associations between these CNVs and SCZ, and to estimate their combined contribution in a large new sample of 6,882 cases and 6,316 controls genotyped with Illumina arrays. CNVs were called with PennCNV and verified with a Z-score algorithm.

We found higher rates in cases than in controls for 13 of the 15 previously implicated CNVs. Six were nominally significantly associated ($p<0.05$): deletions at 1q21.1, NRXN1, 15q11.2 and 22q11.2, and duplications at 16p11.2 and the Angelman/Prader-Willi Syndrome region. When combined with published data, 11 of the 15 loci showed highly significant evidence for association with SCZ ($p<4.1 \times 10\text{-}4$). Moreover, two CNVs surpassed genome-wide significance that had not done so previously: duplications at the Angelman/Prader-Willi locus and at 16p13.11. All eight Angelman/Prader-Willi duplications in cases were of maternal origin.

Our study strengthens the support for the majority of the previously implicated CNVs in SCZ and brings the evidence for two loci (duplications at the AS/PWS locus and at 16p13.11), into the genome-wide significant level. Overall, about 2.5% of SCZ patients and 0.9% of controls carry a large, detectable CNV at one of the previously implicated loci.

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Posters

P01.001

ACTA2 mutation with childhood cardiovascular, autonomic and brain anomalies and severe outcome

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INTRODUCTION Connective tissue disorders are a common cause of vascular disorders, such as thoracic aortic aneurysm and dissection (TAAD). Cerebrovascular anomalies in connective tissue disorders are less frequently reported, although they can be associated with mutations in *TGFβR1*, *TGFβR2*, *SMAD3*, *ACTA2* and *COL3A1*, most frequently resulting in cerebral aneurysms, or mutations in *COL4A1* and *COL4A2*, resulting in hereditary porencephaly and cerebral hemorrhage. Recently, *ACTA2* mutations have been described in Moyamoya(like) disease and ischemic stroke. In addition, a specific phenotype has been ascribed to the R179H mutation, leading to a childhood presentation of disruption of smooth muscle cell dependent organs, brain developmental defects and abnormal cerebral vasculature.
METHODS We report on a patient presenting with pulmonary hypertension, patent DA, mydriasis, intestinal malrotation, bladder dysfunction and an abnormal, straightened course of the anterior cerebral arteries. Our patient shows previously not described abnormal lobulation of the frontal lobes and position of the gyrus cinguli and rostral dysplasia of the corpus callosum. She died at the age of 3 years during surgery due to vascular fragility and ductus arteriosus rupture.
RESULTS We identified a novel *de novo* c.535C>T in exon 6 leading to p.R179C amino acid substitution in *ACTA2.*
DISCUSSION Altogether these observations support a role of *ACTA2* in brain (vascular) development, especially related to the arginine at position 179. Although all previously reported patients with R179H substitution successfully underwent the same surgery at younger ages, the severe outcome of our patient warns against the devastating effects of the R179C substitution on vasculature.

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P01.002

Cherubism: a case report

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According to the WHO classification, cherubism belongs to a group of non neoplastic bone lesions that affect the jaws. Cherubism was first described in 1950 by Jones who described a condition which was characterized by marked fullness of the cheeks and jaws and a slight upturning of the eyes giving the children a cherubic appearance Cherubism is a hereditary disease with an autosomal dominant pattern of inheritance. Mangion et al. (1999) mapped the gene to 4p16.3. Mutations in the SH3BP2 gene was demonstrated (Ueki et al. 2001). Patients with cherubism should be carefully evaluated for Noonan syndrome. Clinically, in cherubism, the lesions usually start in early childhood, affect both jaws, and have a symmetric distribution. It begins to swell gradually until puberty. Although the condition is known to regress during the third decade of life, radiological changes persist until the fourth. Although the disease is rare and painless, the affected suffer the emotional trauma of disfigurement. cherubism interferes with normal jaw motion and speech. Currently, surgical removal of the fibrous tissue and bone is the treatment available. This treatment has no expected results, when introduced before puberty, results in an increase in bone growth .The use of calcitonin and bisphosphonate with these surgeries was found to enhance the outcome greatly and to prevent fierce recurrences. This report describes the clinical features of a 14-year-old girl with a severe case of cherubism affecting both jaws. We summarize the natural history of the disease over a period of 11 years.

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P01.003

Mutation spectrum of the COL2A1 gene in 112 patients

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Background:
A broad spectrum of 322 unique COL2A1 mutations is currently described in the corresponding LOVD database gene entry that we manage (https://grenada.lumc.nl/LOVD2/mendelian_genes/home.php?select_db=COL2A1). Heterozygous mutations in this gene cause a large panel of skeletal dysplasia classified as Type 2 collagen group and similar disorders.
Objective: To report on 54 unique mutations including 36 novel and to investigate possible phenotype-genotype correlation.
Methods: Molecular analysis of the COL2A1 gene in 112 patients with clinical diagnosis of skeletal dysplasia was performed by Sanger bidirectional sequencing.
Results:
We identified 54 unique pathogenic mutations including 35 (65%) missense, 5 (9%) nonsense, 7 (13%) frame-shifts and 7 (13%) splice-site distributed over the whole COL2A1 gene. Fourty-six of 54 (85%) were substitutions, with 23 of 46 (50%) resulting in glycine substitution within a Gly-X-Y repeat. Fifty-five (49.5%) of the 112 patients carried one COL2A1 mutation. Unexpectedly, 6 patients (3 Stickler, 1 Spondyloepiphyseal Dysplasia Congenita, SEDC, 1 Kniest and 1 Spondyloepimetaphyseal Dysplasia, Strudwick type) were compound heterozygous. Twelve (92%) of the 13 SEDC patients carried a glycine substitution, whereas the Stickler patients displayed a broader spectrum of mutations: 8 missenses, 4 nonsenses, 5 frame-shifts, and 4 splice-sites.
Conclusions:
This series of skeletal dysplasia, one of the largest reported so far, adds 36 (10%) novel mutations to those reported in LOVD and reveals first cases with dual mutations in these classically dominant syndromes. Moreover, we confirm that 51% of the Stickler patients carry COL2A1 mutation, and that this phenotype is associated with a larger mutational spectrum than SEDC.

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P01.004

Craniofrontonasal Syndrome: A family with unusual inheritance pattern of an X-linked Dominant genetic disease.

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Craniofrontonasal syndrome (CFNS [MIM 304110]) is an X-linked dominant disorder of craniofacial and skeletal development. CFNS unexpectedly presents severe phenotype in heterozygous females and no or mild phenotype in hemizygous males. While heterozygous females show coronal craniosynostosis, craniofacial asymmetry, cleft palate, bifid nasal tip, Sprengel deformity, wiry hair and hypertelorism, males generally only show hypertelorism.
EFNB1 gene is located in Xq13.1 region and it is involved in random X-inactivation process in females. In heterozygous females, random X-inactivation process leads to mosaic distrubution of cells carrying mutant and wild type EFNB1 genes, thus the severity of heterozygous females is associated with patchy tissue pattern regarding the EFNB1 functional and non-functional cells. Here we report a Turkish family with CFNS. The parents were 5th degree relatives and they had 5 children. Two female sibs were similarly affected and 2 male sibs were healthy. The father and mother were not presenting any signs or symptoms regarding to CFNS. EFNB1 gene sequencing analysis revealed c.451G>A heterozygous mutation in 2 affected female sibs and unexpectedly c.451G>A hemizygous mutation in their clinically unaffected father. The identification of the clinically unaffected father as hemizygous for EFNB1 mutation, provides additional data for previously reported unusual genotype-phenotype pattern of the disease.

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P01.005

Multiple craniosynostoses with unique digital anomalies: a new craniosynostosis syndrome?

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Syndromic craniosynostosis syndromes are typically caused by single gene autosomal dominant disorders or chromosomal duplications or deletions. We report a 15 month old girl with multiple congenital anomalies. She was born with bilateral coronal synostosis and abnormal occipital bone with premature fusion of the supraoccipital and interparietal portions, resulting in turribrachycephaly, hypertelorism, and downslanting palpebral fissures. Transverse and sigmoid sinuses are congenitally absent, and a large single vein from the superior sagittal sinus drains into large scalp veins through the posterior fontanelle. At 3 months, she developed severe intracranial hypertension requiring a posterior craniectomy and ventriculoperitoneal shunt. Surgical correction of the skull deformities has been challenging. She had redundant nuchal skin, a ventricular septal defect (spontaneously closed), and a persistently patent ductus arteriosus. Her symmetric hand anomalies involve hypoplasia of the proximal phalanges of the thumb and index fingers, and hypoplasia of the middle phalanges of the 3rdand 5th digits, resulting in marked brachydactyly and ulnar deviation of the index fingers, and 5th finger clinodactyly. The 4th digits are spared. Radii appear normal. Family history is negative for any of these features. Array CGH using a 180k oligonucleotide array, as well as full sequencing of FGFR2 and FGFR3, targeted mutation analysis of FGFR1, and gene dosage studies of TWIST1 have not identified any alteration to account for these features. We propose that this disorder represents a previously undescribed craniosynostosis syndrome with multiple congenital anomalies. Next generation sequencing may potentially aid in the identification of the causative gene.

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P01.006

Expression of the Bardet-Biedl Syndrome gene 9 (BBS9) in calvarial suture of nonsyndromic craniosynostosis: pathophysiology of primary cilium in suture morphogenesis.

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Nonsyndromic craniosynostosis (NCS) is a relatively common craniofacial malformation, with a strong genetic background. A recent GWAS found a significant association for sagittal NCS (sNCS) within the BBS9 gene locus (*Justice et al, Nat Genet 2012*). BBS9 is a component of the BBBSome involved in the primary cilium-related signalling. BBS9 knockdown leads to cilia defect in the zebrafish, displaying severe developmental aberrations (*Veleri et al, PlosOne 2012*). Interestingly, genes causing at least two craniosynostosis syndromes (Carpenter and Sensenbrenner syndrome) are involved in the cilia formation and function (*Huber and Cormier-Daire, Am J Med Genet C Semin Med Genet.2012*). This study was aimed at clarifying the role of BBS9 and related cilium-associated genes in suture ossification. We have performed an in-depth in silico analysis of our previous microarray data and identified a relevant number of ciliome-associated genes differentially expressed in fused-versus-patent sutures of NCS patients. BBS9 was among the most significantly up-regulated genes in the list; these data were confirmed in tissues and cells from an independent set of NCS patients. Confocal microscopy showed that fused suture-derived cells has a decreased tendency to form primary cilia. Moreover we have analyzed the expression of BBS9 and related cilium-associated genes in the suture mesenchyme of the rat skull. The expression of BBS9 increased progressively over time. Overall, these data seemed to confirm an active role of BBS9 in osteogenesis and may suggest that BBS9 sequence variants associated to a dysregulation of BBS9 expression could lead to premature suture ossification in NCS.

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P01.007

FGFR2 mutations in syndromic craniosynostosis patients

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Craniosynostosis which is defined as the premature fusion of one or more sutures of the skull is caused by mutations in FGFR1, FGFR2, or FGFR3. Among all craniosynostosis, syndromic craniosynostosis account for 15% of the cases. The most common forms of syndromic craniosynostosis are Crouzon, Apert, Pfeiffer and Muncke syndrome that are mostly caused by mutations in FGFR2 gene in which more than 60 mutations have been identified up to date. In this study we screened for FGR2 mutations in 8 patients having Crouzon, Apert and Pfieffer syndromes. We found heterozygous FGFR2 mutations (p.P253R in 2 patients and p.S252W in one patient) in 3 Apert syndrome patients which were also reported to be the most common mutations in Apert syndrome. In 3 Crouzon syndrome patients heterozygous p.W290R, p.S351C and p.S252P mutations were detected. Heterozygous p.W290C mutation was found in one patient diagnosed clinically as Pfeiffer syndrome. One novel heterozygous mutation p.F334S has been detected in a patient with isolated nonsyndromic craniosynostosis. SIFT and PolyPhen analysis demonstrated that this aminoacid change may be tolerated, but functional analysis is needed. Revealing the phenotype-genotype correlation of particular syndromic forms of craniosynostosis leads to accurate diagnosis, efficient management, and long-term prognosis of the patients.

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P01.008

Rapid translation of research findings into clinical service for two new craniofacial genes identified in Oxford.

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Craniosynostosis (premature fusion of at least one cranial suture) results in skull and facial deformity, and is the second most common craniofacial malformation. Currently, single gene mutations/chromosomal abnormalities can only be identified in about one-fifth of cases, with mutations in the FGFR2, TWIST1, FGFR3, and EFNB1 genes accounting for the majority (~16%). Identifying the genetic cause in infants is important for genetic counselling, risk assessment, surgical management and outcome.

Close partnership between the diagnostic Oxford Regional Genetics Laboratory and Professor Wilkie's research group in Oxford has enabled two new craniofacial genes; ERF and TCF12, to be rapidly translated into the diagnostic service. Mutations in these two genes have been shown to account for a further ~2% of cases.

To date we have screened seventeen patients with multi-suture synostosis in whom no mutation was identified in previous screens. An ERF mutation was detected in one patient with pansynostosis.

Of fifteen patients referred with uni- or bi-coronal synostosis, in whom no mutation was identified in previous screens; two patients were shown to carry a mutation in TCF12.

Confirmation of other mutations in ERF and TCF12 identified in the research setting is currently in progress, and we have also undertaken cascade screening in two of these families. This is important as variable penetrance has been observed.

Incorporation of these new genes into our diagnostic service has identified the genetic cause in three new cases in our cohort, which will enable cascade screening and accurate prognostic advice to be given.

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P01.009

Crouzon Syndrome and bent bone dysplasia associated to mutations at the same TYR-381 residue in FGFR2 gene

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Crouzon syndrome has an high penetrance and an extreme clinical variability. The majority of the mutations responsible for his syndrome are located in the exon 8 (IIIa) and 10 (IIIc) of the fibr oblast growth factor receptor 2 gene (FGFR2) corresponding to an immunoglobulin domain. We report here a familial Crouzon syndrome associated to a new p.Tyr381Asn mutation in exon 11 of FGFR2 gene. The proband was suspected of this syndrome at the age of 3 years. At that time he presented with adolichocephaly with bilateral moderate exophtalmia, parrot-beaked nose and frontal bossing. A skull CT examination was performed at age 5 confirming the closure of the sagittal suture associated a facial retrusion and inverted bite. His father and his sister displayed a milder phenotype. The Crouzon syndrome was confirmed after detection of novel heterozygous p.Tyr381Asn(c.1141T>C) mutation in FGFR2 gene located in the transmembrane domain for the 3 affected members of the family, segregating with the craniosynostosis phenotype. This mutation was located at the same position (p.Tyr381Asp(c.1141T>G)) that the recently described perinatal lethal bent bone dysplasia which is characterized by co ronal craniosynostosis associated with hypoplastic clavicle, bent long bones in the lower extremities, hypoplastic pubis and abnormal phalanges. Ours cases did not show any appendicular bony abnormality on X-rays. This observation highlights the need of further researches to explain such major clinical difference between those two syndromes associated with a FGFR2 mutation affecting the same Tyr-381 residue.

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P01.010

1150 T>C change in exon 10 of the *FGFR3* is preferentially associated with pathogenic mutations

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Few pathogenic mutations in *FGFR3* are known to be associated with the achondroplasia phenotypic spectrum, leading to a good genotype-phenotype correlation. Uncommonly, however, phenotype variations have been observed. The speculation about a possible modulating effect of the 1150 T>C transition in the *FGFR3*, and the identification of a newborn with a severe form of hypochondroplasia with positive mutation (1620 C>A) and also presenting the 1150 T>C substitution in the exon 10 prompted us to better investigate the role of this polymorphism. We evaluated the frequency of the 1150 T>C polymorphism in the *FGFR3* in both - a cohort of patients of the FGFR3 phenotypic spectrum with known mutations, and in normal controls of the general population. Hitherto, using direct sequencing of the exon 10 of the *FGFR3*, we have studied 39 patients and 265 normal controls. We have found the 1150 T>C polymorphism in two patients (2 alleles in 78 chromosomes or 2.6% of the C allele). One patient had a typical achondroplasia (1138 G>A) and the second one was a hypochondroplastic patient (1620 C>A) with a severe phenotype. Among the 265 studied controls, the 1150 T>C polymorphism was found in only three individuals (3 alleles in 530 or 0.6% of the C allele). In conclusion, the 1150 T>C polymorphism was found nearly five times more frequent in patients of the FGFR3 family than in normal controls, suggesting a possible role in pathogenic mutations of the *FGFR3*.

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P01.011

A new insight into structural and functional impact of FGFR3 mutations at the same position in three FGFR3-related chondrodysplasia.

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Three missense mutations in the tyrosine kinase domain of fibroblast growth factor receptor 3 (FGFR3) affecting a lysine residue at position 650 lead to dwarfism with a spectrum of severity, hypochondroplasia (p. Lys650Asn), severe achondroplasia with developmental delay and acanthosis nigricans (p. Lys650Met), and thanatophoric dysplasia (p. Lys650Glu). Fgfr3 mutations induce a constitutive activation of the receptor characterized by a sustained phosphorylation. To understand the severity of the clinical phenotype, we developed computational and biological studies. Computational studies were conducted to get an atomic description of the p. Lys650Met, p. Lys650Glu and p. Lys650Asn built using a validated structural model of the FGFR3 kinase domain. Structural analyses indicate that a salt bridge between R655 and E686 is the cornerstone of the Tyr647 solvent exposition. This salt bridge is, significantly, disturbed with p. Lys650Glu mutation and is destroyed with p. Lys650Met and p. Lys650Asn mutants. There is a qualitative correlation with the severity of the clinical phenotype. We evaluated also the impact of the FGFR3 mutants on signaling pathways. Transient transfections of DNA mutants in human chondrocytes show both a gradient of phosphorylation levels and an increased proliferation correlated with the severity of the disease. In addition, a higher activation of the signalling pathways (ERK1/2, AKT, STAT, b-catenin) was also observed.
In conclusion, the three lysine 650 substitutions alter differently the conformation of the kinase domain thus leading to various activations of signalling pathways. Different mechanisms seem to be responsible for mild and lethal dwarfism.

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P01.012

The STARD3NL locus is strongly associated with pediatric spine bone mineral density in a regression-based multi-ethnic meta-analysis of known adult loci.

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Osteoporosis has its origins in childhood, where bone mineral accretion during development is a critical determinant of future bone health. We elected to leverage data from an ongoing GWAS of pediatric bone accretion to investigate the association of previously reported adult BMD loci with pediatric BMD of the spine using a linear regression based meta-analysis of three ethnicities. Our cohort is derived from the multi-center NICHD Bone Mineral Density in Childhood (BMDC) Study, which was initiated in 2001 to establish national reference standards for BMD and bone accrual for children ages 6 years and older. In order to maximize the statistical power in our cohort, we carried out a meta-analysis leveraging the three main ethnicities in the collection. This led to 753 Caucasian, 308 African American and 205 Hispanic samples being included in the analysis. All samples were genotyped on the Illumina OMNI-Express-Exome BeadChip platform, enabling us to query 59 of the 64 adult BMD. A linear regression in plink was used to test for association with pediatric spine BMD followed by a meta-analysis. No association was found when the three groups were analyzed together, but when investigating each ethnicity individually, we found that SNP rs940347 at the STARD3NL locus yielded a P-value of 1.74x10-4 in the Caucasian cohort and survived Bonferroni correction; indeed, this is the same ethnicity where the original adult discovery was made. In summary, we conclude that there is evidence that the STARD3NL locus contributes to adult spine BMD by exer-

ting its effect early in life.

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P01.013

Correlation between platelet phenotype and NBEAL2 genotype in patients with congenital thrombocytopenia and α-granule deficiency

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The gray platelet syndrome (GPS) is a rare inherited bleeding disorder characterized by thrombocytopenia, increased volume of platelets and defects of α-granules formation which gives a characteristic gray appearance to platelets.
After the recent identification of mutations in NBEAL2 gene as the genetic defect responsible for GPS, we hypothesized that this gene was responsible for low platelet count and α-granule deficiency in 11 unrelated probands with inherited thrombocytopenia and α-granules defects.
DNA sequencing of NBEAL2 led to identification of 9 novel alteration of the gene including 2 missense, 3 nonsense, 2 frameshift mutations, as well as 2 nucleotide substitutions that altered the splicing mechanisms, in 4 families. In addiction, evaluation of platelet phenotype revealed that all patients with NBEAL2 biallelic mutation showed thrombocytopenia, large platelets and severe reduction of α-granules. Of note, although they were not thrombocytopenic, also individuals carrying only one mutated allele had platelet macrocytosis and significant reduction of the α-granules in contrast with an autosomal recessive transmission. In the remaining 7 probands, we did not identify any NBEAL2 alterations, suggesting that other genetic defects are responsible for their platelet phenotype.
Our data extend the spectrum of mutations responsible for GPS and demonstrate that macrothrombocytopenia with α-granule deficiency is a genetic heterogeneous trait.

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P01.014

Analysis of the high bone mass phenotype using genomic and transcriptomic tools: evidences of genetic heterogeneity and of additive effects of TWIST1, IL6R, DLX3 and PPARG

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Osteoporosis is characterized by very low bone mineral density (BMD). The high bone mass (HBM) phenotype is a non-pathogenic condition characterized by BMD values at the other extreme of the spectrum. The aims of the study were to establish the prevalence of HBM in a cohort of Spanish postmenopausal women (BARCOS); to determine the contribution of LRP5 and DKK1 mutations to this phenotype; to test the hypothesis of an inverse correlation between the number of common variant risk alleles and HBM; and to characterize the expression of 88 osteoblast-specific and Wnt pathway genes in primary osteoblasts from two HBM cases.
A 0.6% of individuals (10/1600) displayed bone mineral density Z-scores in the HBM range [sum (lumbar spine + femoral neck) Z-score >4]. No mutations in relevant exons of LRP5 were found and one woman had a rare missense change in DKK1 (p.Y74F). Fifty-five BMD SNPs from Estrada et al [NatGenet 44:491-501,2012] were genotyped in the HBM cases to obtain risk-scores for each individual. Z-scores were found to be negatively correlated with these risk-scores, with a single exception, which may be explained by a rare penetrant genetic variant. The expression analysis in primary osteoblasts from two HBM cases and five controls, showed that IL6R, DLX3, TWIST1 and PPARG were negatively related to Z-score. One HBM case presented with high levels of RUNX2 while the other displayed very low SOX6. In conclusion, we provide evidences of heterogeneity and of additive effects of several genes for the HBM phenotype.

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P01.015

A homozygous missense mutation in *HSPA9* causes epiphyseal-vertebral-ear (EVE) dysplasia

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Epiphyseal-vertebral-ear (EVE) dysplasia is an extremely rare disorder with distinctive dysmorphic features and ossification defects. A single family has been reported, with two affected sisters born to healthy, consanguineous parents. Key features were midface hypoplasia, depressed nasal bridge, anteverted nares, symmetrical dysplasia of the helices, epiphyseal dysplasia, mid-coronal clefting of vertebral bodies, odontoid hypoplasia, elbow dislocation and intrauterine growth restriction with post-natal catch-up growth. Similar craniofacial and skeletal features have been noted in cerebral, ocular, dental, auricular, skeletal anomalies (CODAS) syndrome. The genetic causes of EVE and CODAS syndromes have not been reported.We performed exome sequencing of the two affected EVE dysplasia patients, and after filtering for published and in-house SNPs and selecting for deleterious variants, the only homozygous variant shared between both sisters was the missense mutation p.Thr362Ile in heat shock 70kDa protein 9 (HSPA9). The catalytic activity of HSPA9 is encoded by the ATPase domain, a structure conserved in chaperones from vertebrates to bacteria. Thr362 is absolutely conserved in known orthologues of HSPA9 and falls within the ATPase domain. Structural modelling of the mutation Thr362Ile indicates disruption of hydrogen bonding, predicted to lead to protein instability. Published transcriptomic data indicates that *HSPA9* is highly expressed in human fetal cartilage, and we have explored this expression pattern in greater detail by in situ hybridisation.This work identifies *HSPA9* as a novel regulator of craniofacial and skeletal development, with its mutation causing EVE dysplasia. CODAS syndrome may be caused by mutations in a related gene or pathway.

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P01.017

The genetic study of intracranial aneurysm in the Japanese population

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Intracranial aneurysms (IA) are balloon like dilation of arterial walls in the brain; rupture of IA leads to aneurysmal subarachnoid hemorrhage (aSAH), which is the most serious subtype of stroke. Genetic factors have been known to play an important role in the development of IA. Here, we conducted a genome-wide association study (GWAS) to identify common genetic variants that are associated with IA by using 1383 aSAH subjects and 5484 controls from the Japanese population. The variants that showed suggestive association in the GWAS were validated in an independent set of 1048 IA cases and 7212 controls. We identified, rs6842241, near *EDNRA* at chromosome 4q31.22 (combined P-value = 9.58×10^{-9}; odds ratio = 1.25), to be significantly associated with IA. Owing to the functional importance of *EDNRA* to IA, we performed functional analysis of the associated variants on *EDNRA* and identified rs6841581 as a functional variant that might affect the expression of EDNRA and subsequently result in the IA susceptibility. To further understand the etiology of the disease, we performed pathway analysis utilizing the GWAS dataset and identified Notch signaling pathway to be significantly associated with IA ($P_{GSEA}=3.0 \times 10^{-6}$). In addition, we also participated in a collaborative study that performed meta-analysis, which successfully identified six genetic loci, 8q12.1, 9p21.3, 10q24.32, 13q13.1, 18q11.2 and 4q31.22, as IA common susceptible loci of the European and

Japanese population. Our findings have reveal a better understanding of the contribution of genetic factors to IA.

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P01.018

A novel missense mutation in ANO5 is causative for gnathodiaphyseal dysplasia in a large Italian pedigree

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Gnathodiaphyseal dysplasia (GDD) is an autosomal dominant syndrome characterized by frequent bone fractures at a young age, bowing of tubular bones and cemento-osseus lesions of the jawbones. To date, two mutations on the same cysteine residue at position 356 of Anoctamin 5 (ANO5) have been described in GDD families. Sequencing the entire ANO5 coding and untranslated regions in 21 individuals (7 affected, 14 not affected) of a large Italian GDD family, we found a novel missense mutation causing the p.Thr513Ile substitution. The mutation perfectly segregates in the family and has never been described in any database as a polymorphism. Clinical features of the patients are completely superimposable to those of previously described families . Thus this is the third mutation described to be causative for GDD. ANO5 belongs to the anoctamin protein family that includes calcium-activated chloride channels. However, our experiments show that ANO5 may not function as calcium-activated chloride channel. Mutations in ANO5 have also been found in many cases of rare forms of muscular dystrophy (MMD3 and LGMD2L). The mutations described until now are spread throughout the gene, with no apparent trend in their position with regard to specific motifs, transmembrane domains or cytoplasmic versus extracellular regions of the protein. This heterogeneity and the still-unknown function of ANO5 make it difficult to unravel the molecular pathophysiology of GDD. The findings of this third mutation may help in this unraveling process and in the understanding of the anoctamins' function and role in these different diseases.

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P01.019

A Clinical and Molecular Genetic Study of 6 Arab families with Microcephalic Osteodysplastic Primordial Dwarfism type I

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Backgroud: Microcephalic osteodysplastic primordial dwarfism type I (MOPD I) is a rare recessive disorder characterized by severe microcephaly, intrauterine growth retardation, developmental brain malformations, skeletal changes and early lethality. It is caused by mutations in the RNU4ATAC gene encoding U4atac, a small nuclear RNA that is a crucial component of the minor spliceosome. Objective: To determine the spectrum of clinical and neuroradiologic features in MOPD I patients due to RNU4ATAC gene mutations. Methods: RNU4ATAC was sequenced in 10 patients from 6 unrelated Arab families with MOPD I. In addition, we evaluated the clinical phenotype, cognition, behavior and brain MRI.
Results: All patients were homozygous or compound heterozygous of RNU4-ATAC mutations. The probands harbored 4 different mutations (g.55G>A, g.51G>A, g.66G>C and g.124G>A) of which 2 were novel. The severity of brain and skeletal anomalies, degree of motor and speech delay, and survival varied considerably among patients even with the same mutation. Mild motor delay was noted in 3/10 patients. Typical skeletal changes were found in all patients but metopic suture synostosis were additional finding in 5 patients. All patients showed hypogenesis of corpus callosum but 2 patients had pachygyria and interhemispheric cyst in addition. Interestingly, 4 sibs from showed oculocutaneous albinism. One patient showed long survival (more than 5 years) Conclusions: We provide a detailed description of features associated with RNU4ATAC mutations. Our results expand the phenotypic and mutational spectrum of this malformation syndrome and further investigations of the prevalence of RNU4ATAC mutations in correlation with clinical phenotype are recommended.

L.K. Effat: None.

P01.020

Multicentric carpo-tarsal osteolysis: clinical and molecular review of 8 cases

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Multicentric carpo-tarsal osteolysis (MCTO) with or without nephropathy is a rare osteolysis disorder with autosomal dominant inheritance beginning in early childhood and involving mainly carpal and tarsal bones. In the majority of cases, renal disease appears later in life and evolves quickly to end stage renal disease. Recently, mutations in the amino-terminal transcriptional activation domain of the MAFB gene (v-maf musculoaponeurotic fibrosarcoma oncogene ortholog B) have been identified in MCTO patients by exome sequencing. MAFB, known as a regulator of various developmental processes, is essential for osteoclastogenesis and podocyte differentiation and renal tubule survival. We collected the data of eight patients with MCTO issued from six different families to review the clinical features and perform the molecular analysis of *MAFB*. Among the 8 cases, renal disease was present in the six older. We identified five missense mutations in all screened cases confirming the genetic homogeneity of MCTO. Three among the five mutations were novel clustered within the hot spot mutation region of the gene. Disease variability and associated features were observed , concerning especially the extension and consequences of osteolysis and age of onset of renal manifestations. Since the intensity of rheumatologic and nephrologic symptoms varied even within the same family, we conclude that MCTO is a genetically homogeneous but clinically variable condition; the molecular screening of *MAFB* will allow early diagnosis especially in isolated cases.

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P01.021

Nail-Patella Syndrome: evidence for genetic heterogeneity

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Nail patella syndrome (NPS) is a hereditary osteo-onychodysplasia with autosomal dominant inheritance. Prevalence is estimated at 1/50 000. This affection comprises characteristic skeletal anomalies (nail dysplasia, hypoplastic or absent patella, iliac exostoses···) frequently associated with ocular or renal involvement. NPS results from mutations in the *LMX1B* gene, localized in 9q34 and spanning approximately 95 kb. This gene encodes a transcription factor belonging to the LIM homeodomain protein family. This protein plays a crucial role in the dorso-ventral polarisation of the limbs. Mutations responsible for haploinsuffficiency of *LMX1B* are identified in 85-90% of patients.
In about 10-15% of patients, no mutation is identified in *LMX1B*. To our knowledge, the hypothesis of a genetic heterogeneity has never been studied in this disease. We study 5 families affected with typical NPS for whom routine screening has not identified a mutation in *LMX1B* (exons and flanking introns sequencing, MLPA). We performed NGS for the whole *LMX1B* gene in 4 families, revealing no mutation in coding or non-coding sequence. In one family, results provide clues for non-linkage at 9q34, confirming the likelihood of a genetic heterogeneity.

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P01.022

Genetic variants in SKI, FGFR1, WNT3, IRF6 and BMP4 genes are associated with risk for non-syndromic CL/CLP and/or CP in Latvian population

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The identification of susceptibility genes for non-syndromic cleft lip with or without cleft palate and isolated cleft palate (CL/CLP/CP) has been the subject of extensive research. To localize candidate genes and loci of non-syndromic clefts, several genome-wide linkage screens, association studies and fine mapping have been published. However, only the *IRF6* gene has shown a convincing degree of consistency across studies and was considered to be responsible for less than 20% of non-syndromic CL/CLP and/or CP.
The aim of the present study was the identification of candidate genes involved in the etiology of non-syndromic CL/CLP and CP in Latvian population. Selection of the candidate genes were based on recent publications regarding confirmed linkage and association studies with non-syndromic CL/CLP or CP. Genotyping of 672 SNPs in 45 genes were done using APEX-2 technology, MALDI-TOFF technology and TaqMan assays. For case-control study 178 non-syndromic CL/CLP/CP patients (135 CL/CLP patients and 43 CP patients) and 483 unaffected individuals as controls were analyzed, in addition TDT was carried out in 122 trios.
Our results showed very strong association between *FGFR1*, *WNT3*, *SKI*, *BMP4* and *IRF6* genes and non-syndromic CL/CLP and CP, and possible interaction between 19q13 locus and non-syndromic CL/CLP. Genetic variants in *SKI*, *FGFR1, WNT3* and *IRF6* genes may contribute susceptibility to both non-syndromic CL/CLP and CP, whereas *BMP4* gene could have protective effect in the susceptibility for non-syndromic CL/CLP in Latvian population.
Obtained results continue to support the involvement of these genes in the development of non-syndromic CL/CLP/CP in Caucasians.

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P01.023

Genome-wide analysis of parent-of-origin effects in non-syndromic orofacial clefts

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Non-syndromic orofacial clefts (NSOFC), one of the most common congenital malformations, have a genetically complex background. Although recent GWAS detected a number of NSOFC susceptibility loci, these conventional association studies have the inherent presumption that the effect of a variant is independent of parental origin. They are thus underpowered to detect possible parent-of-origin (PofO) effects, a phenomenon where the effect of an allele on phenotype depends on whether it was transmitted by the mother or the father. In this study we hypothesized that PofO effects play a role in NSOFC etiology.
We applied a number of statistical tests on published genome-wide SNP data from ~2500 trios with NSOFC, mainly of European and Asian ethnicity. These tests included a modification of the family-based Transmission Disequilibrium Test, biased ratios of maternal versus paternal transmissions, and biased frequencies of reciprocal classes of heterozygotes in the offspring. Sixty-four candidate SNPs were followed-up in a replication cohort of ~1200 NSOFC trios of European origin.
In the combined analysis, we did not identify any SNPs that achieved genome-wide significance. However, we observed an overall excess of maternal versus paternal signals (P=0.013) and identified several loci that showed nominally significant effects in the same direction in both discovery and replication cohorts, suggesting variants with potential PofO effects which escaped the genome-wide threshold due to the lack of power. Those latter include a possible maternal effect associated with rs12543318 at 8q21.3, a locus also identified in a recent meta-analysis of non-syndromic cleft lip with/without cleft palate (Pmat=1.5x10-7, Ppat=0.17).

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P01.024

Genome-wide association study reveals new candidate loci for knee osteoarthritis

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Objective. Osteoarthritis is a complex disease common in the elderly. Our aim was to study single-nucleotide polymorphisms (SNPs) in genome-wide association study (GWAS) of knee OA.
Methods. The study subjects were part of Finnish Health2000 study sample (Genmets) consisting of 2118 individuals genotyped using Illumina HumanHap 610k. Presence of osteoarthritis in the knee joints were evaluated by a clinician based on physical status, symptoms, and medical history. Individuals suffering from rheumatoid arthritis or below the age of 50 were excluded. Seventy-five individuals had OA in at least one knee and 895 were healthy controls without knee or hip OA based on the clinical evaluation. Association was monitored using the Plink program, age, sex and body mass index as covariates.
Results. In the knee OA GWAS analysis the most significant results were for SNPs in or next to MARK1 gene on chromosome 1q41 (OR=2.44, 95%CI 1.68-3.54, p=2.67x10-6), MYO10 gene on chromosome 5p15.1 (OR=2.61, 95%CI 1.71-4.00, p=9.19x10-6), and on chromosome 13q21.1 (OR=2.29, 95%CI 1.59-3.28, p=7.12x10-6).
Conclusion. Variants in/near MARK1 and MYO10 showed suggestive evidence for association with knee OA. MARK1 plays a role in cell polarity and microtubule dynamics. MYO10 has a role in integration of F-actin and microtubule cytoskeletons during meiosis. Results did not reach genome-wide significance, but a follow-up study in an independent study set is ongoing: The three SNPs are being genotyped in the remaining part of the Health2000 with 302 knee OA cases and 1700 controls based on the same criterion as in the initial screening.

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P01.025

Phenotype-genotype correlation and role of ancillary investigations in atypical and rare forms of Osteogenesis Imperfecta

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Background: Osteogenesis imperfecta (OI) is a heterogeneous group of inherited disorders of bone formation. About 90% of patients have mutations in *COL1A1/ COL1A2*, with an autosomal dominant pattern of inheritance. Other genes are associated with autosomal recessive forms of OI. Other, rare phenotypes have been described.
For this study, patients with phenotypic similarities to Russell-Silver syndrome (RSS) [Parker et al., 2011], OI syndromes (not commonly fitting the Sillence classification) such as Cole-Carpenter and Bruck syndrome and Type V OI were considered. All these conditions were collectively referred to as Atypical OI.
Aims: To investigate individuals with atypical forms of OI, with a view to proposing sub-classifications and identify genotype-phenotype correlations.
Methods: Patients who fulfilled the inclusion criteria were recruited from the Sheffield Clinical Genetics and OI Services. Detailed phenotyping, skin biopsy for histology, including electron microscopy (EM) and collagen species analysis (CSA), skeletal survey, and sequencing of OI genes and aCGH were performed.
Results: Recruited patients (n=14) were phenotypically divided into three sub-groups: Group 1) predominant features of RSS (n=5); Group 2) OI with additional features (n=6); and Group 3) Type V OI (n=3). Common features included poor growth, feeding difficulties, facial dysmorphism +/- fractures. Pathogenic oligogenic mutations were identified in seven patients; chromosomal imbalances in three patients. Skeletal surveys and skin biopsies have revealed common findings. CSA has correlated with molecular findings.
Conclusions: This study has enabled us to sub-classify patients with Atypical OI and established the need for aCGH, skin EM and CSA, routinely in investigating these patients.

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P01.026

Identification of novel COL1A1 gene mutations in Brazilian patients with Osteogenesis Imperfecta

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Osteogenesis Imperfecta (OI) is a type I collagenopathy that has received great attention in the last decades because of the relevant number of new genes recently related to this disorder. The main symptoms of OI patients are bone fragility and recurrent fractures. Although several new genes have been described for the disease, *COL1A1* and *COL1A2* genes mutations are present in the majority of OI cases. The characterization of mutation pattern among OI genes in patients from different ethnic profile will help us to improve the development of strategies for molecular diagnosis in OI. The goal of this work was to detect the pattern of *COL1A1* gene mutations in OI patients from Espírito Santo, southeast Brazil. A total of 33 unrelated patients were analyzed through a PCR-SSCP screening mutation method and sequencing of abnormal fragments. Patients showed mild, moderate and severe clinical symptoms in 45%, 25% and 30% of the sample, respectively. Two novel frameshift mutations were found in a mild OI case [c.2750delG (p.Gly917AspfsX191)], and a sporadic severe OI [c.3239delC (p.Pro1080LeufsX28)]. We hypothesize that these frameshifts mutations are pathogenics since they can result in abnormal sized protein products with an incorrect amino acid sequence. Furthermore, two missense mutations [c.1138G>T (p.Gly380Cys); c.3235G>A (p.Gly1079Ser)], and three splice site mutations [c.1056+1G>A; c.1875+1G>C; c.2559+1G>A] previously reported were also identified. In conclusion, due to genetic heterogeneity, phenotypic variability and limitations in SSCP technique sensitivity, we strongly support the importance of molecular studies in OI. Financial support: FAPES, FACITEC, and MCTI/CNPq/MEC/CAPES.

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P01.027

FKBP10 gene and recessive Osteogenesis Imperfecta

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Osteogenesis Imperfecta (OI) is a very heterogenic genetic disorder with phenotypes that varies from mild to neonatal lethal forms. Most cases of OI are caused by dominant mutations in *COL1A1* and *COL1A2* genes, that codes for the procollagen type I chains. In the last few years, the number of genes related with recessive OI has risen significantly. One of the most recent among them is *FKBP10* gene that codifies a chaperone that interacts with the collagen protein. Our objective was to determine the mutations pattern in *FKBP10* gene from Brazilian patients diagnosed with OI. A total of 26 patients from Espírito Santo (southeast of Brazil) without mutations in *COL1A1* or *COL1A2* genes were analyzed. DNA mutations in *FKBP10* gene were screened using SSCP method and sequencing of abnormal fragments. We detected the c.1546G>A missense change in the exon 9 of *FKBP10* in one of the OI patients. This mutation was not previously described and results in an amino acid change (p.Leu516Phe) into the protein. This mutation was not observed in 90 control samples from the same population. Additional studies are necessary, but we suggest that the p.Leu516Phe mutation may be related with OI in this particularly case. Molecular studies are strongly required because of the great genetic and phenotypic heterogeneity in OI. Thus, these results increase our knowledge about the genetic characteristics in OI and may contribute to the development of more efficient methods to diagnose and treat OI patients. Financial support: FAPES, FACITEC, and MCTI/CNPq/MEC/CAPES.

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P01.028

Osteogenesis Imperfecta Type V with Hyperplastic Callus, a clinical and molecular review of 9 cases

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The Osteogenesis Imperfecta (OI) group includes various heritable diseases of the extracellular bone matrix, characterized by low bone mass, variable deformities and high fractures incidence. Among them, OI type V is characterized by propensity to hyperplasic callus formation, calcification of the forearm interosseous membrane and radial head dislocation. This dominant inherited OI subtype is associated with a specific histopathologic pattern. Recently, the recurrent c.14 C>T mutation was identified in IFITM5 gene as the unique molecular cause of OI type V in all patients.
We describe here 9 patients from 7 families, regularly followed in Necker-Enfants malades Hospital with OI type V (age: 6 months-38 years). In all cases, OI was caused by the c.-14C>T mutation in *IFITM5*. 6/ 9 patients presented with a severe form of OI, with a progressive evolution leading to significant mobility impairment and wheelchair in 4/6 older cases. The age of first fracture was early in all cases (between birth and 2.5years). By contrast, the number of fractures (from 6 to > 60) and age of the first hyperplastic callus varied widely (between 3 months and 13 years). The adult height ranged from 1.30 to 1.50 meter. Scoliosis was noted in 4 cases. Surprisingly, persistent alkaline phosphatase without D vitamin defect was noted in 3 cases. The study confirms the clinical variability, and further describes the clinical and radiological evolution under medical (bisphosphonates, anti-inflammatory) and surgical (intramedullary rods, vertebral arthrodesis) treatments.

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P01.030

A deletion mutation in TMEM38B associated with autosomal recessive osteogenesis imperfecta

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Autosomal recessive osteogensis imperfecta (OI) was diagnosed in three unrelated Israeli Bedouin consanguineous families. Fractures were evident in all cases in infancy. Genome wide linkage analysis ruled out association with any of the known OI genes, and identified a single homozygosity locus of ~2 Mb on chromosome 9 common to all affected individuals (maximum multipoint lod score 6.5). Whole exome sequencing identified only a single mutation within this locus that was shared by all affected individuals: a homozygous deletion mutation of exon 4 of TMEM38B, leading to an early stop codon and a truncated protein, as well as low TMEM38B mRNA levels. TMEM38B encodes TRIC-B, a ubiquitous component of TRIC, a monovalent cation-specific channel involved in Ca2+ release from intracellular stores that has been shown to act in cell differentiation. Molecular mechanisms through which a TMEM38B mutation might lead to an OI phenotype are yet to be explored.

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P01.031

TAPT1 and CREB3L1 interfere with normal type I collagen secretion and further expand the genetic spectrum of recessive Osteogenesis Imperfecta

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Osteogenesis imperfecta (OI) is a heritable brittle bone disorder with high clinical variability. Dominant forms are mostly due to heterozygous mutati-

ons in a type I procollagen gene (*COL1A1/COL1A2*). In recessive OI the underlying mutations often affect type I collagen processing or chaperoning. A small proportion of OI cases still remains molecularly unexplained.
We identified mutations in two novel genes for human OI in two separate families. In family 1, candidate gene analysis revealed a homozygous genomic deletion of CREB3L1 in the affected siblings. *CREB3L1* encodes OASIS, an endoplasmatic reticulum (ER)-stress transducer that intranuclearly regulates procollagen type I expression upon processing by regulated intramembrane proteolysis during murine bone formation. In family 2, with three affected foetuses and six healthy siblings, genome-wide linkage analysis and exome sequencing revealed a homozygous acceptor-splice site mutation in *TAPT1* (*Transmembrane Anterior Posterior Transformation-1*), that causes an in-frame deletion of the highly conserved exon 10. *TAPT1* has been reported in the context of murine skeletal patterning.
In both defects, type I (pro)collagen posttranslational modification was grossly preserved, but secretion was delayed in *TAPT1* mutated fibroblasts. Similarly to OASIS, immunocytochemistry localized TAPT1 to the ER, and *TAPT1* expression was upregulated upon ER stress. Moreover, both genetic defects demonstrate alterations in the ER stress response pathways with nearly absent expression of CHOP and XBP1.
In conclusion, *CREB3L1* and *TAPT1* are two novel genes for human recessive OI. Mutations in both genes similarly affect the normal ER stress response that links to type I collagen production.

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P01.032
Osteomalacia as a key feature of Raine syndrome: changing the paradigm.

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INTRODUCTION: Raine syndrome is a skeletal dysplasia characterised mainly by osteosclerosis of bones, facial dysmorphism, and a course frequently lethal. However, we present the first alive case molecularly confirmed whose radiographs showed osteomalacia. **CASE REPORT:** The patient was born at 26 weeks of gestation to non-consanguineous and healthy parents, due to intrauterine growth retardation and worsening of Doppler examination. His birth parameters were all p<2, and albeit delivery and Apgar scores were normal, he subsequently suffered from different premature birth complications. Furthermore, at 70 days old bilateral forearm spontaneous fractures were suspected and confirmed, whereas follow-up radiographs revealed broad epiphyses and thin diaphyses of long bones, accompanied of generalised osteomalacia. At physical examination, the patient had some dysmorphic characteristics which suggest Raine syndrome such as ocular proptosis, hypertelorism, slightly downslanted palpebral fissures, broad nasal bridge, midface hypoplasia, micrognathia and short limbs and fingers. Karyotype was normal, and although his cerebral ultrasounds were normal, renal echography revealed nephrocalcinosis. One year later, his health status has improved substantially, specially his osteomalacia and nephrocalcinosis. Parallelly, *FAM20C* sequencing analysis was performed, which demonstrated the heterozygous duplication g.286470_286503dup34 and heterozygous SNP rs192542992, which is present just in 0.7% of the population. *In silico* analysis of these findings showed rupture of splicing site of exon 4, and the creation of a new splicing regulatory element, respectively. **CONCLUSION:** Although these results should be confirmed experimentally, it seems that osteomalacia is also a key feature of Raine syndrome, in concordance with the recently published murine model.

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P01.033
Abnormal Type I Collagen Glycosylation Pattern and Cross-linking in a Cyclophilin B KO Mouse Model of Recessive Osteogenesis Imperfecta

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Recessive osteogenesis imperfecta (OI) is caused by mutations in genes encoding proteins involved in post-translational interactions with type I collagen. Types VII-IX OI involve defects in the collagen proly 3-hydroxylation complex, which modifies α1(I)Pro986. Cyclophilin B, encoded by *PPIB*, is a complex component with PPIase activity that facilitates collagen folding. To investigate the role of CyPB in collagen post-translational modifications and crosslinking, we generated a *Ppib* KO mouse using gene-trap technology. *Ppib* expression is absent in skin, fibroblasts, femora, and calvarial osteoblasts; only residual (<11%) α1(I)P986 3-hydroxylation is detectable in cell cultures, skin and bone tissues. Collagen from KO cells has delayed electrophoretic mobility, consistent with delayed intracellular folding in cell culture assays. However, total collagen 5-lysyl and prolyl 4-hydroxylation was normal, suggesting altered glycosylation in KO. We demonstrated that, except for lysyl residues involved in crosslinking, most helical residues of KO collagen have increased diglycosylation. Although total bone collagen crosslinks (HP+LP) in KO were double WT (p=0.003), abnormal modification is associated with a 70-80% reduction of collagen deposited into KO matrix. We detected a 4-fold increase in trivalent LP crosslinks (p=0.001) and corresponding decrease in the HP/LP ratio, due to reduced hydroxylation of helical crosslink residues in KO bone. This *Ppib* KO mouse recapitulates the OI type IX phenotype including delayed collagen folding, and smaller long bones with significantly reduced BMD, BV and TbN. The finding that collagen crosslink patterns are shifted to trivalent forms lacking helical Hyl may contribute to decreased collagen matrix deposition and bone strength.

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P01.034
Russell-Silver syndrome and preaxial polydactyly

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Russell-Silver syndrome is a growth disorder characterized by pre and postnatal growth restriction, conserved head circumference, distinctive facies and body asymmetry. Its molecular mechanisms are complex including maternal disomy of chromosome 7 for less than 10% of cases and methylation defect of the 11p15 chromosomal region for the majority of them. Among skeletal anomalies, short stature, short arms, fifth fingers clinodactyly, short and stubby fingers and toes are usual. We report here a case of Russell-Silver syndrome associated with unilateral preaxial polydactyly.

This male patient was born at 37 weeks of amenorrhea, after a pregnancy characterized by IUGR and unilateral kidney agenesis. Prenatal karyotype on amniotic cells was unremarkable and array-cgh without imbalance. At birth, weight was 1400g, height was 36.5cm and OFC 33.5cm. Facies was triangular with broad forehead, naevus flammeus, small pointed chin, small philtrum angioma. He had bilateral fifth fingers clinodactyly, moderate hypospadias and right thumb duplication. Renal ultrasound examination showed a horseshoe kidney. The presence of demethylation of the H19 locus confirmed the diagnosis of Russell-Silver syndrome.

The association of Russell-Silver syndrome and preaxial polydactyly is unusual. To our knowledge, it was reported only one time in a 4-year-old boy presenting with marked language delay and uniparental maternal disomy of chromosome 7 (Potgieter 2000). With a frequency of preaxial polydactyly in the general population of 1 in 3000 births, a simple coincidence cannot be rule out. However, a real association is possible with Russell-Silver syndrome, particularly with these 2 different molecular mechanisms.

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P01.035

Results of longstanding clinical observation of siblings with Sensenbrenner syndrome

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Sensenbrenner syndrome (cranioectodermal dysplasia, CED) is a very rare autosomal recessive ciliopathy, characterized by craniofacial, skeletal, and ectodermal abnormalities. About 40 cases have been described to date. CED is a heterogeneous genetic disorder and recently four genes: *IFT122(WDR10)*, *IFT121(WDR35)*, *IFT43(C14ORF179)* and *IFT144(WDR19)*, have been identified (all encoding proteins that are part of the intraflagellar transport complex A, IFTA). IFTA is involved in retrograde ciliary transport, which returns proteins from the tip of the cilia to the cell body and pays an import role in the assembly and maintenance of cilia structure.
Here we report on a 11-year-old girl and her 7-year-old brother with CED. Their healthy parents are distantly consanguineous. Molecular analysis revealed a homozygous missense mutation p.V553G (c.T1658G) in the *IFT122* gene. Clinical features included short stature with rhizomelic shortening of limbs, brachydactyly, narrow chest, craniosynostosis, dolichocephaly, high forehead, full cheeks, telecanthus, broad nasal bridge, low-set prominent ears, small and widely spaced teeth, dysplastic auricles, fine sparse hair, abnormal nails, bilateral inguinal hernia, skin laxity. Mental development is normal. Both children suffer from tubulointerstitial nephropathy with more severe features in the brother (kidney transplantation at the age of 7 was necessary). We present changing phenotype of the sibs during long clinical observation.

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P01.036

A distinctive autosomal recessive osteocutaneous syndrome with unusual face caused by a mutation in *POC1A*

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We describe multiple affected individuals from two families of Arab Moslem origin with a distinctive syndrome of severe short stature, peculiar facial and other clinical features, with autosomal recessive mode of inheritance. The affected individuals have pre and post- natal growth retardation, which manifests probably as early as the second trimester of pregnancy. Typical skeletal changes included short long bones especially of the femurs, humerii and hands, with mild metaphyseal changes and very short femoral necks.
Homozygosity mapping located the disease gene to 3p21.1-3p21.31. Using whole exome sequencing analysis complemented with Sanger direct sequencing, we identified a homozygous point mutation (p.Leu171Pro) in *POC1A* (Centriolar Protein Homolog A), that co-segregate with the disease phenotype in two families. The mutation affects a highly conserved amino acid residue and is predicted to interfere with protein function. Poc1, POC1A homolog, had previously been found to have a role in centrosome stability in unicellular organisms. Accordingly, although centrosome structure was preserved, the number of centrosomes and their distribution was abnormal in affected cells. In addition, the Golgi apparatus presented a dispersed morphology and cholera toxin trafficking from the plasma membrane to the Golgi was aberrant, with accumulation in large vesicles in the cytosol. Our data underscore the importance of POC1A for proper bone, hair and nail formation as well as the importance of normal centrosome in Golgi assembly and trafficking from the plasma membrane to the Golgi apparatus.

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P01.037

Collagen deregulation in the glenohumeral capsule of patients with anterior shoulder instability

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Anterior shoulder instability is a common orthopedic problem among young athletes. After a traumatic shoulder dislocation, patients present capsular laxity. Molecular modifications in the glenohumeral capsule may be associated with this injury. Here, we aimed to evaluate the *COL1A1*, *COL1A2*, *COL3A1*, and *COL5A1* expression in traumatic anterior shoulder instability. Collagen gene expression was analyzed by real-time quantitative PCR in the antero-inferior, antero-superior, and posterior portions of the shoulder capsule from 33 patients with traumatic anterior shoulder instability and 5 individuals with acromioclavicular injury (controls). We observed an increased expression of *COL1A1* and *COL3A1* in the antero-superior region (P=0.038 and P=0.034, respectively) and of *COL1A2* in the posterior portion (P=0.043) of glenohumeral capsule of shoulder instability patients compared with the controls. Concerning the clinical data, patients who had experienced more than 1 year of shoulder instability demonstrated reduced expression of *COL1A1* in the antero-inferior and in the posterior regions compared with patients with less than 1 year of symptoms (P=0.014 and P<0.001, respectively). In the antero-inferior portion, reduced expression of *COL5A1* was associated with recurrent shoulder dislocation compared with a single dislocation episode (P=0.049). Reduced *COL1A1* was also associated with recurrent shoulder dislocation in the posterior region (P=0.006). Thus, collagen expression was deregulated in the three evaluated portions of the glenohumeral capsule of shoulder instability patients which may be related to the capsular laxity. Since there is a reciprocal load-sharing relationship in the capsule, these modifications in distinct portions of the capsule may reflect a compensatory mechanism after a dislocation episode.

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P01.038

Severe brachydactyly and absent ossification of several tubular bones in hands and feet as distinctive features of a rare skeletal dysplasia

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We report on a boy with a generalized skeletal dysplasia characterized by severe brachydactyly and pronounced growth failure. The proband is the first child of healthy parents originating from the same village in Macedonia. A non-lethal skeletal dysplasia was suspected on prenatal ultrasound. The boy was born at 36 weeks with a weight of 1990 g, length of 36.5 cm and head circumference of 30.5 cm. He did not require assisted ventilation in the neonatal period. On follow-up a severe growth failure became apparent. The last clinical evaluation at the age of 9 months revealed a weight of 2510 g and a length of 52.4 cm. He has a relatively large head with frontal bossing, bluish sclerae, redundant and soft skin and very tiny fingers and toes. In the literature only 5 other cases with a similar phenotype have been described. All patients died during the first year of life. They share a similar and unusual pattern of ossification defects in hands and feet. Two pairs of affected sibs suggest an autosomal recessive inheritance. Whole exome sequencing in the proband revealed a list of 37 homozygous, non-synonymous variants not annotated in databases of common variation. Eleven variants were predicted to have a deleterious impact on the protein. Although none of these variants involve genes that have been associated with a known skeletal dysplasia, some are interesting to further explore since they are either associated with limb development or play a role in growth and cell cycle regulation.

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P01.039

Rare cases with skeletal dysplasia - Romanian experience. Diagnosis based on clinical and radiographic correlations

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Skeletal dysplasias represent a group of more than 400 conditions which affect cartilage and bone growth, defined by molecular, biochemical and ra-

diographic criteria, included in 40 different groups, after the last Revision of Nosology and Classification of Genetic Skeletal Disorders. Although individually rare or very rare, collectively there are many patients with these congenital disorders.
Clinical evaluation is the fundation upon which any suspicion of skeletal dysplasia is built. X-ray films are all that are needed to identify a particular diagnosis in most cases. Suspected cases of skeletal dysplasias involves systematic imaging of the long bones, thorax, hands and feets, skull and spine.
Our Medical Genetics Department provides evaluation from clinical and radiological point of view, diagnosis and counselling for children with skeletal dysplasia and their families. In this work we present selected cases with bone dysplasias, some of them very rare, from our clinic day experience: achondroplasia, osteogenesis imperfecta, lysosomal storage disorders with skeletal involvement, geleophysic dysplasia, Camurati-Engelmann disorder, Conradi-Hünermann syndrome, Ellis-van Creveld syndrome, short rib-polydactyly, nail-patella syndrome, metaphyseal dysplasias, multiple epiphyseal dysplasia, Langer-Giedon syndrome, Proteus-like syndrome etc. Clinical summary of the patients, including the pedigree, clinical photographs and X-rays are presented, focussing on diagnostic issues for each condition.
Because of their variety, skeletal dysplasias remain a diagnostic challenge. Clinical exam and radiological findings represent the key for the recognition of a skeletal dysplasia condition from the long list of genetic skeletal disorders and for the pre-test counselling.

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P01.040

Small Patella Syndrome : new *TBX4* mutations and literature review

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Small patella syndrome (SPS), also called ischiopatellar dysplasia, coxopodopatellar or ischio-pubic-patellar syndrome (OMIM 147891), is characterised by patellar agenesis or hypoplasia, abnormal ossification of the ischio pubic junction and feet abnormalities, comprising an enlarged space between the 1st and 2nd toes (sandal gap), short 4th and 5th rays and pes planus. This rare autosomal dominant disorder is caused by mutations in *TBX4*, known to be involved in hindlimb development and outgrowth. Here, we describe four families presenting with SPS, and a *TBX4* mutation has been identified in three of them. One mutation had been previously reported: c.1112dupC (p.Pro372SerfsX14), whereas the two others had not been yet identified: c.1062T>G (p.Tyr354X) and c.(?_-45)_(401+?)del (an intragenic deletion of *TBX4*'s first three exons). In addition to SPS's common features, our patients presented with some minor associated findings such as syndactyly of the 2nd and 3rd toes, dental anomalies and/or hip dysplasia. We compare their phenotypes to those of previously described patients, who had been molecularly characterised or not, in order to underline the expression variability of SPS. Furthermore, we discuss if the associated minor features could also be explained by *TBX4* mutations. Since SPS is a rare condition, achieving a better clinical description and establishing correlations with the genotype might help towards an earlier diagnosis and an appropriate medical management.

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P01.041

Split hand/split foot malformation type 6 in multiple consanguineousOmani family with a novel homozygous mutation in *WNT10B* gene providingfurther evidence of recessive inheritance in SHFM

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Split hand/split foot malformation (SHFM) is a rare isolated congenital limb malformation, characterized by a deep median cleft of the hand and/or foot due to the absence of the central rays and aplasia/or hypoplasia of the phalanges, metacarpals and metatarsals. SHFM6 (OMIM # 225300) is the rarest among familial SHFM subtypes and is inherited in an autosomal recessive manner. It has wide clinical variability including isolated cutaneous syndactyly and even non-penetrance and should be excluded in seemingly sporadic cases. Clinical variability also exists between limbs of a single individual. We describe the variable clinical and morphologic features of four affected individuals with SHFM6 in a highly inbred Omani family. The first two female siblings have the classical phenotype with asymmetrical involvement of both hands and feet with ectrodactyly, oligodactyly, syndactyly, absent carpal and tarsal bones. In contrast, their two double first cousins, a boy and a girl, present mildly with only cutaneous syndactyly of the feet. This is the first case report of an Omani family with SHFM6 with a novel homozygous mutation c.290T>C p.Leu97Pro in exon 3 of WNT10B gene. The limb malformations required multiple surgeries for cosmetic and functional corrections and had profound psychosocial impact and stigma in this family such that they opted for preimplantation genetic diagnosis.

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P01.042

A novel role of MARP proteins in intercellular communication

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The MARP family members Ankrd1/CARP and Ankrd2/ARPP are components of the sarcomeric I band mechanosensory complex in both cardiac and skeletal muscles, where they bind N2A region of titin. In response to mechanical stimuli they shuttle to the nucleus, acting as messengers in stress response pathways. Since they are altered in a number of neuromuscular and cardiovascular disorders, as well in some tumors such as rhabdomyosarcoma, renal oncocytoma and ovarian cancer their potential role as diagnostic and/or prognostic markers is under the study. Recently, regulatory role of Ankrd2/Arpp was corroborated demonstrating its ability to bind signaling proteins that contain PDZ motifs. Here, using protein array methodology, we have identified 10 PDZ proteins that directly interact with Ankrd1/CARP. Moreover we have confirmed its interaction with Zonula Occludens 1 (ZO-1) employing in vitro binding assay. Apart from p53, titin and calpain 3, ZO-1 represents a novel common partner for both MARPs. ZO proteins are localized at tight junctions and establish a link between the transmembrane components and the perijunctional cytoskeleton at cell-cell contacts. They are delocalized or down-regulated in several types of carcinomas: breast cancer, primary and metastatic pancreatic cancer and brain microvascular endothelial cells from human brain tumors. Interaction with ZO-1 suggest possible role of Ankrd1/CARP and Ankrd2/Arpp in intercellular communication. The functional interplay between MARPs and tight junction proteins could have potential role in mechanisms involved in triggering malignant transformation.

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P01.043

Split hand/foot malformation with long bone deficiency and *BHLHA9* duplication: report of 13 new families

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Split hand/foot malformation (SHFM) with long bone deficiency (SHFLD, MIM#119100) is a rare condition characterized by SHFM associated with long-bone malformation usually involving the tibia. Previous published data reported several unrelated patients with 17p13.3 duplication and SHFLD. Recently, *BHLHA9* duplication has been identified to be responsible for this limb malformation. No mutation has been reported so far. We described 13 families affected with ectrodactyly harbouring a *BHLHA9* duplication, identified either by array-CGH or quantitative PCR. Affected patients present with isolated limb malformations. Hands defects consisted usually in uni- or bilateral ectrodactyly. Syndactyly or monodactyly were infrequent findings. Long bone deficiency was present in 41% of the affected patients, being a specific finding regarding which BHLHA9 duplication testing should be addressed. This defect concerned the tibia in most cases, and infrequent-

ly the radius or the femur. As reported before, pedigrees show incomplete penetrance of BHLHA9 duplication, and high intrafamilial and intraindividual variability.

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P01.044
Vitamin- D deficiency and Familial Brachydactyly mimicking Pseudohypoparathyroidism (PHP)

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OBJECTIVE:Vitamin D deficiency is not a rare disorder. The Endocrine Society recommends levels >30 ng/mL for good health. Pseudohypoparathyroidism (PHP) is a rare disorder characterized by parathyroid hormone (PTH) resistance with (type 1a) or without (type 1b) the Albright Hereditary Osteodystrophy (AHO) phenotype of short stature, brachydactyly, and mental retardation [2]. Patients with PHP have elevated PTH levels. However, the same laboratory values can also be seen in children or adults with vitamin D deficiency, and diagnostic confusion is common. We report three cases of vitamin D deficiency and familial brachydactyly with presentations suggestive of PHP.
Case Presentation: We report a family with 3 affected members in three generations confirming autosomal dominant inheritance and variable expressivity of short metacarpals and metatarsals (Figure-1). Biochemical results are evaluated. All patients revealed high PTH and low Vitamin D levels (Table-1). Short metacarpals and metatarsals are frequent findings in brachydactyly type E. Poznanski *et al*. [1] concluded that ,brachydactyly E is radiologically indistinguishable from the PHP syndrome. Elevated PTH levels are treated with vitamin- D replacement therapy. Correction of Vitamin D deficiency resulted in a return to normal range of PTH levels in whole patients (Table-2).
*Conclusions:*We report a case of co-existence of brachydactyly and vitamin D deficiency for the first time in the literature. After correction of vitamin D deficiency, Parathormon levels returned to normal range, which in return prevented misdiagnosis. Geneticists should be more careful about diagnosis of PHP syndrome. Otherwise, a chance of therapy and preventing hyperparathyroidism complications will be missed.

M.A. Soylemez: None.

P01.045
Mutations in *ECEL1* cause distal arthrogryposis type 5 with ophthalmoplegia

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Using a combination of homozygosity mapping and whole-exome sequencing, we identified a novel mutation in the endothelin-converting enzyme-like 1 (*ECEL1*) gene in a consanguineous

pedigree of Turkish origin presenting with camptodactyly, scoliosis, limited knee flexion, severe myopia, facial weakness and ophthalmoplegia. The missense c.1819 G>A mutation results

in the substitution of serine for the highly conserved glycine at residue 607 of ECEL1. *ECEL1* mutations were recently reported to cause recessive forms of distal arthrogryposis without ophthalmoplegia. Distal arthrogryposis is most commonly inherited as a dominant trait with reduced penetrance and variable expressivity. This report expands on the molecular basis and the phenotypic spectrum of *ECEL1*-associated congenital contracture syndromes.

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P01.046
A genome wide association study in dogs reveals a new candidate gene for human chondrodysplasias

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Similar to humans, dogs suffer from different types of genetic skeletal disorders affecting the bone and cartilage tissues. We have established pedigrees and sample cohorts of two dog breeds that suffer from an autosomal recessive chondrodysplasia. The affected dogs present with a disproportionate short stature dwarfism of varying severity. Radiographic examination of two affected animals revealed from mild to severe skeletal changes, including curvature of radii and ulnae, metaphyseal flaring and misshapen femoral heads and necks. A genome wide association study in a cohort of nine affected and nine healthy dogs mapped the disease to a 2-Mb region on canine chromosome 17 using a canine 22K SNP chip. The associated locus contained a promising cartilage-specific candidate gene that was screened for possible disease causing mutations. Sequencing of all the coding regions revealed a nonsense mutation that segregated fully with the recessive disease in both breeds. The encoded protein is known to be expressed in cartilage chondrocytes and to be involved in the process of endochondral ossification. As a consequence of the nonsense mutation, the protein is severely truncated and was not detected in the affected cartilage tissue. Our study describes a large animal model to further examine the role of the gene in growth plate function. The mutated gene has not been reported in human chonrodysplasias and thus represents a novel candidate for human patients. Meanwhile, a genetic test enables the eradication of the condition in the affected breeds.

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P01.047
A novel ARSE mutation in a patient with chondrodysplasia punctata 1 and in his mother with low frequent somatic and/or germline mosaicism detected by deep sequencing using NGS

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Chondrodysplasia punctata 1 (CDPX1) is an X-linked recessive disorder characterised by punctate calcifications in radiographs of the feet and other sites, nasomaxillary hypoplasia and brachytelephalangy. It is known that mutations of the *arylsulfatase E* (*ARSE*) gene affect the disorder.
We met a male patient clinically diagnosed with CDPX1. Examination of the *ARSE* gene by Sanger sequencing for all exons in the patient revealed a missense mutation, c.266G>A (p.S89N). The mutation was not found in 250 healthy controls. Prediction of functional effects of the substitution by PolyPhen-2 and SIFT displayed that the mutation affects the protein function.
In order to confirm whether his mother is carrier, the molecular test was done as well. However, the Sanger sequencing could not detect the mutation in the mother, suggesting that the mutation was de novo. However, since there is no report of de novo mutation in CDPX1 patients, there was a possibility that the mother had a germline mosaicism and/or somatic mosaicism.
Thus, we performed deep sequencing of the *ARSE* gene in the mother using GS Junior, next generation sequencer, resulted that the mother had a somatic mosaicism of the mutation in the blood cells at a ratio of 11%.
We concluded that the deep sequencing using NGS could detect low frequent mosaicism, which might be a part of germline mosaicism, and should be performed in patient's parents with such 'de novo' mutation to make reliable genetic counselling.

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P01.048
Founder effects in Galician (NW Spain) lamellar ichthyosis families

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Mutations in transglutaminase 1 gene (TGM1) are a major cause of la-

mellar ichthyosis. Three mutations, c.2278C>T, c.1223_1227delACAC and c.984+1G>A, were observed at high frequency (~46%, ~21% and ~13% of all TGM1 gene mutations, respectively) in 11 non-consanguineous Galician (NW Spain) ichthyosis families.
We aimed to determine whether these mutations were inherited from a common ancestor, and to estimate the number of generations since their initial appearance. We carried out a haplotype-based analysis of a region 12 Mb. encompassing TGM1. Haplotype reconstruction from unphased genotypes of the members of the affected families showed that all carriers for each of the mutations harbored the same haplotypes, indicating common ancestry. Two linkage disequilibrium based methods were used to estimate the time to the most recent common ancestor (TMRCA), while a Bayesian-based procedure was used to estimate the age of the two most frequent mutations.
In good agreement with the documentation record and the census, both mutations arose between 2,800-2,900 years ago (y.a.), but their TMRCA was in the range 600-1,290 y.a., pointing to the existence of historical bottlenecks in the region followed by population growth. This demographic scenario finds further support on a Bayesian Coalescent Analysis based on TGM1 haplotypes that allowed to estimate the occurrence of a dramatic reduction of effective population size occurring around 900-4,500 y.a. (95% highest posterior density) followed by exponential population growth.
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P01.049

Myhre syndrome-causing SMAD4 mutations result in disorganization of extracellular matrix that is corrected by losartan treatment.

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Myrhe syndrome (MS, MIM 139210) is an autosomal dominant disorder presenting with short stature, laryngotracheal stenosis, brachydactyly, generalized muscle hypertrophy, joint stiffness, thick skin, and a distinctive facies. Cognitive disability and cardiac involvement are also frequently reported. Recently, recurrent mutations affecting codon Ile500 of *SMAD4* gene have been found in patients with MS. Mutations in this residue result in perturbation of expression in both TGF-β and BMP target genes that are involved in extra-cellular matrix (ECM) homeostasis.
In this study, we investigated the functional consequences of *SMAD4* mutations and the efficacy of TGF-β signaling antagonist losartan for correction of ECM defect in four MS fibroblast cell lines harboring the p.Ile500Val mutation and the p.Arg496Cys mutation. Western blot analysis showed increased SMAD4 levels in MS fibroblasts. Compared to control cells, MS fibroblasts showed upregulation of *MMP2* and downregulation of *SERPINE1* genes, suggesting an imbalance in protease/inhibitors ratio. Based on these findings, we hypothesized an impaired ECM deposition that was confirmed by reduced length and thickness of fibrillin microfibrils in MS fibroblasts compared to controls. Because disruption of microfibril network results in increased bioavailability of TGF-β, we investigated whether losartan is effective at improving ECM deposition in MS cells. We found that losartan treatment improved microfibril thickness and networking compared to vehicle-treated fibroblasts, normalized phosphorylated SMAD2 protein levels, and *MMP2* and *SERPINE1* mRNA levels. In conclusion, the results of these studies suggest that losartan might be a good drug candidate for treatment of connective tissue manifestations in MS.

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P01.050

Nager syndrome: report on 11 families and confirmation of SF3B4 haploinsufficiency as the major cause

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Nager syndrome belongs to the acrofacial dysostoses group, which are characterized by the association of craniofacial and limb malformations. Recently, exome sequencing studies identified the *SF3B4* gene as the cause of this autosomal dominant condition in most patients. *SF3B4* encodes a highly conserved protein implicated in mRNA splicing and bone morphogenic protein (BMP) signaling. We performed *SF3B4* sequencing in 11 families (15 patients) suggestive of Nager syndrome and found 7 mutations predicted to result in haploinsufficiency. *SF3B4* is the major gene responsible for autosomal dominant Nager syndrome. All mutations reported were null mutations thus no genotype-phenotype correlation could be delineated. Mutation negative patients were phenotypically indistinguishable from mutated patients, thus genetic heterogeneity is likely.

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P01.051

Contribution of MSX1, PAX9, AXIN2 and WNT10A genes in four families with non syndromic tooth agenesis

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Tooth agenesis is the most common developmental abnormality of human dentition. Oligodontia is a subgroup of this developmental absence, where six or more permanent teeth are congenitally missing, excluding third molars. Oligodontia or hypodontia, both may occur as a single clinical entity or as a part of a syndrome. Investigations on model organisms and similarity analysis subjecting sequence/structure/functional relationships revealed more than hundreds of genes either directly or indirectly involved in the regulation of tooth development. Nevertheless, previously reported *MSX1*, *PAX9* and recently addressed *AXIN2* and *WNT10A* are the only genes currently associated with human non-syndromic tooth agenesis.

Four families,with isolated oligodontia are ascertained by detailed oral examination and panoramic radiographs. We aimed to investigate the role of *MSX1*, *PAX9*, *AXIN2* and *WNT10A* genes in the oligodontia in our cases, by sequencing the genes primarily only on index cases. If an alteration is identified, the concordance with oligodontia will be expanded to convey the point by analyzing other affected and unaffected individuals in the family.

PAX9 screening identified known heterozygous c.G718C in two of the families, one with further known heterozygous c.C119G, and other with further heterozygous novel c.-18G>A in *MSX1*, both segregating with oligodontia. Our investigation is continuing for screening of *AXIN2* and *WNT10A* genes.

We hope our findings will further delineate the contribution of *PAX9*, *MSX1*, *AXIN1* and *WNT10A* genes human tooth development during embryogenesis.

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P01.052

Copy number changes underlying syndromic and isolated bilateral radial ray aplasia: screening of a cohort of 20 probands

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Radial ray aplasia is characterized by the absence of radial bone(s) often including thumb(s) and can either be isolated or syndromic. Although several causative genetic alterations resulting in radial ray aplasia have been identified, many patients have normal result of appropriate molecular screening. In this report we studied a cohort of 20 unrelated individuals affected by bilateral radial ray deficiency by means of conventional GTG-banding, MLPA for 1q21.1 locus, sequencing and MLPA for all exons of both TBX5 and SALL4, as well as array CGH. In six unrelated patients we found a microdeletion on 1q21.1, which was typical of TAR syndrome. Two probands carried point mutations in genes known to be involved in radial ray deficiency syndromes: one in TBX5, and the other in SALL4. Two additional probands had deletions encompassing either TBX5 or SALL4 detected upon MLPA and subsequently confirmed by array CGH. Finally, one additional case carried a pure terminal microduplication of 5q35.2-5q35.3 encompassing distal 5.4-5.6 Mb. To conclude, we detected copy number changes in nine patients, whereas a point mutation only in two out of the 20 probands from the initial

cohort. Although based on a small sample, our study shows that submicroscopic genomic rearrangements may account for a significant proportion of radial ray aplasia causative mutations. Therefore, we propose that either a combination of MLPA or qPCR for specific genes/loci or array CGH should be included into routine molecular screening of patients affected by syndromic or isolated radial ray deficiency.

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P01.053

Clinical and molecular review of 8 new cases of spondyloepiphyseal dysplasia congenital-Strudwick type

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Mutations in *COL2A1* cause a spectrum of clinically distinct conditions, including Stickler Syndrome, achondrogenesis and spondyloepimetaphyseal dysplasia (SEMD). SEMD is characterised by significantly short long bones, epi-metaphyseal irregularities and platyspondyly. The rare clinical variant, SEMD type Strudwick (OMIM reference 184250) is distinguished by congenital metaphyseal spurs and by early radiographic development of distinctive irregular 'dappled' metaphyses. Other features are chest deformities, secondary to rib involvement, cleft palate, laryngobronchomalacia, vitreoretinal anomalies and hearing loss. Previously only 10 individuals with six different *COL2A1* mutations have been described. Here eight new cases of SEMD type Strudwick are presented, the largest cohort to be reported so far. The clinical and radiological findings are described in detail to characterise the key manifestations further and highlight variability of this skeletal dysplasia condition. Analysis of the entire *COL2A1* gene revealed mutations in seven of these individuals. The majority of these are glycine missense mutations clustered together in a small number of exons in the triple helix domain, near similar mutations known to cause Kniest dysplasia and SEDC, though one mutation is located in exon 45. Mutations at glycine residues disrupt the tightly bound type II collagen helix, qualitatively affecting this key growth plate cartilage extracellular matrix protein, a molecular mechanism also of aetiological significance in a number of other conditions affecting fibrillar collagen. This increases to 13 the number of reported cases, expands the clinical spectrum of *COL2A1* mutations associated with SEMD type Strudwick and highlights the clinical utility of of characterising *COL2A1* mutations.

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P01.054

Clinical reappraisal of Shprintzen-Goldberg syndrome at the light of the gene discovery

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Shprintzen-Goldberg syndrome (SGS) is characterized by severe marfanoid habitus, intellectual disability, camptodactyly, facial dysmorphism and craniosynostosis. Recently, using family-based exome sequencing, we identified *SKI* as the gene responsible for SGS, with a cluster of mutations in exon 1 in 18 patients. Taking advantage of this work, we further clinically delineate SGS from the original cohort as well as new patients (mean age 20 years). All cases were sporadic except one family with somatic mosaicism and another following dominant inheritance. All mutations were found in exon 1, inframe. All patients display a remarkable phenotype associating a recognizable facial dysmorphism (scapho/dolichocephaly 94%, hypertelorism 89%, down slanting palpebral fissures 84%, malar hypoplasia 84%, proptosis 79%, micrognathia 79%) and severe skeletal manifestations (arachnodactyly 94%, feet malposition 89%, scoliosis 84%, pectus deformation 79%, joint contractures 79%, camptodactyly 47%). Craniosynostosis is not mandatory for the diagnosis (63%). Other common manifestations include hernia, loss of subcutaneous fat and myopia. The gene identification permitted to confirm the risk of aortic dilatation in affected patients (16% of the cohort, even in childhood), besides to the presence of valvular abnormalities (31%), justifying long-term regular cardiac follow-up from infancy. One young adult died suddenly without aortic dilatation, and another female died in her forties of respiratory insufficiency. Even if all genotyped cases reported to date had intellectual disabilities, we identified a case of SGS and a recurrent *SKI* mutation with normal intelligence but typical morphology, showing the importance of clinical delineation of a disease in light with molecular results.

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P01.055

The SMAD-binding domain of SKI: a hotspot for de novo mutations causing Shprintzen-Goldberg syndrome.

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Shprintzen-Goldberg syndrome (SGS) is a rare systemic connective tissue disorder characterized by craniofacial, cardiovascular, skeletal and skin manifestations that show significant overlap with Marfan (MFS) and Loeys-Dietz syndrome (LDS) features. A distinguishing finding in SGS patients is the presence of intellectual disability. Several lines of evidence confirm the key role of the transforming growth factor beta (TGFβ) signaling in the pathogenesis of MFS and LDS. Based on exome sequencing experiments and the hypothesis that the TGFβ pathway was highly likely to be involved in SGS, we identified de novo heterozygous mutations in the proto-oncogene SKI, a known repressor of TGFβ activity, in ten SGS patients. All mutations cluster in two distinct N-terminal regions of the protein: the SMAD2/3-binding domain and the Dachshund-homology domain (DHD) which mediates binding to SNW1 and N-Cor proteins, both essential for the transforming activity of SKI and for the recruitment of transcriptional corepressors.
Here we report on five additional de novo heterozygous missense SKI mutations in SGS patients, four recurrent and one novel. Adding our new findings to the existing data clearly reveals a pattern of hotspot mutations, with 48% (13/27) of the hitherto described mutations affecting the SKI-residues p.Gly34 and p.Pro35, both located in the SMAD2/3-binding domain. The importance of TGFβ in the SGS pathogenesis is further confirmed by enhanced activation of TGFβ signaling cascades and higher expression of TGFβ-responsive genes in cultured dermal fibroblasts from SGS patients.

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Novel mutations in a family with spondylocostal dysostosis

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Spondylocostal dysostosis (SCDO) comprises a heterogeneous group of vertebral malsegmentation disorders characterized by hemivertebrae, rib fusions and deletions. A SCDO family with two affected children inherited as an autosomal recessive mode was identified in Taiwan. Four known genes, DLL3, MESP2, LFNG, and HES7, causing autosomal recessive forms of SCDO were excluded to be disease-causing gene for this family by direct Sanger sequencing. Exome sequencing of this family using Illumina Hiseq2000 was subjected to identify novel homozygous and compound heterozygous variants. Four novel homozygous variants were identified and were not present in 376 Taiwanese controls. One of the variants is a homozygous insertion variant that results in a frameshift in amino acid sequence of a gene that has caused other disorders with vertebral malsegmentation and much severe clinical manifestations. Functional analysis revealed that the mutant protein presented different cytoplasmic pattern compared to the normal protein and may be functional inactive. This finding expends the phenotypic spectrum resulting from novel mutation of the gene.

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Ift140/IFT140 mutations shown to cause *cauli* mouse phenotype and Jeune syndrome: a new biologic model for the study of ciliopathies

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Defects in cilia formation and function result in diverse ranges of human skeletal and visceral abnormalities, termed *ciliopathies*. As part of an ENU mouse mutation screen, we identified a mutant, *cauli*, with a phenotype consistent with an underling ciliopathy.
Mutant *cauli* embryos are characterized by mid-gestation lethality, craniofacial dysmorphism, neural tube defects, short, disorganised ribs, polydactyly, syndactyly, and internal organ defects. Linkage and candidate gene sequencing revealed homozygous mutations in the cilial gene, *Ift140,* as the cause of the *cauli* phenotype.
In mutant embryos; skeletal preparations revealed defects of the craniofacial skeleton, vertebrae, and thoracic rib cage; *in situ* hybridisation with *myogenin* showed disordered somite patterning, and with *Msx1* and *Sox9* abnormalities of the axial neural tube; and examination of primary cilia showed abnormal morphology, consistent with a defect of retrograde cilial transport. Components of the Shh/Grem1/Fgf signalling systems involved in limb development were also analysed by *in situ* hybridisation, showing consistent disruption of expression patterns between controls and *cauli* mutants.
The *cauli* phenotype is reminiscent of human short-rib polydactylies (SRP). Mutations in *IFT140* have also been shown to underlie a subset of patients with Jeune and Mainzer-Saldino syndromes. We screened patients with SRP phenotypes and found a novel, homozygous *IFT140* mutation in a patient diagnosed with Jeune syndrome.
These data confirm the utility of this mouse model to further understand the pleiotropic anomalies that arise in Jeune syndrome and other skeletal ciliopathies. It provides a platform for further analysis of the developmental signalling systems regulated via the primary cilium.

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Deafness, myopia and slight bones changes caused by autosomal recessive mutations of COL9A3 gene: a new clinical entity?

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Type IX collagen is expressed mainly in cartilage together with collagens II and XI. Collagen IX has been shown to be present also in the human intervertebral disc, vitreous humor, in the tectorial membrane of the inner ear. Also, there is temporal and spatial regulation of collagen IX gene expression, which seems to be more tightly regulated in cartilaginous than non-cartilaginous tissues. It is a heterotrimeric molecule formed by three genetically distinct chains: α1, α2, and α3 encoded by the COL9A1, COL9A2, and COL9A3 genes. Proteins are characterized by three collagenous (COL1, COL2, and COL3, numbered from the C terminus) that are joined by four small non-collagenous domains (NC1 to NC4). Although dominant and recessive mutations have been described in α1 and α2 chains, only heterozygous mutations of COL9A3 gene have been reported in human and related to: 1) multiple epiphyseal dysplasia 3, 2) susceptibility to an intervertebral disc disease, and 3) hearing loss. Here, we describe the first autosomal recessive family presenting with deafness, myopia and slight bones changes due to loss of functional mutations (c.1176_1198del, p.Gln393Cysfs*25) of COL9A3 gene. These findings further extend the knowledge of the role of type IX collagen in generating disease.

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Cosegregation of Freiberg's infraction with a familial translocation t(5;7)(p13.3;p22.2) with breakpoints mapping at the *ADAMTS12* gene and at the *SDK1* gene

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We observed a 16 year -old girl with features of Cri du Chat syndrome (CDC) connected with unusual changes of skeletal system due to unbalanced rearrangement der(5) t(5;7)(p13.3;p22.2). Interestingly epiphysial aseptic necrosis of the head of the second metatarsal bone well known as Freiberg's infraction (FI) were present in her mother and her grandmother, both carriers of t(5;7)(p13.3;p22.2). The mapping both chromosome breakpoints using RP11-666A5 and G248P8216C9 clones from 5p as well and RP11-261N10 and RP11-348A21 clones from 7p have been performed. We found that the breakpoint position mapped on 5p13.3 were in coding sequence of *ADAMTS12* gene and the breakpoint on 7p22.2 involved *SDK1* gene. Both genes probably were interrupted as a result of this rearrangement. Unknown genetic predisposition to occurrence of FI from one side and the observation of the cosegregation of FI together with t(5;7)(p13.3;p22.2) are suggestive as causal relationship with *ADAMTS12* gene disruption.

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Fryns mesomelic dysplasia is caused by duplication of the HOXD cluster on chromosome 2q31

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Mesomelic dysplasias are a heterogeneous group of inherited skeletal dysplasias among which Leri-Weill dyschondrosteosis caused by SHOX haploinsufficiency and its homozygous form, Langer dysplasia, are the most frequent and best known. Kantaputra type dysplasia is characterized by marked mesomelic shortening of the upper and lower limbs. A complex ~1 Mb genomic imbalance composed of two microduplications of 481 and 507 kb, separated by a segment of normal copy number, on chromosome 2 (2q31.1-q31.2) has been identified in the original Thai family described by Kantaputra et al.
In 2011, at the ESHG meeting we reported a female patient and her similarly affected father who had the same phenotype as a family published in 1988 by Fryns et *al.* In both families the patients had severe bilateral shortening and bowing of the forearms with ulnar deviation of the hands resulting from severe developmental abnormality of the ulna, without any other features particularly with no lower limbs involvement. A 60K oligoarray CGH performed in our patient identified in the 2q31.1 region a 200 Kb microduplication which was confirmed by qPCR. No other imbalance was identified. The genomic rearrangement resulted in the duplication of the entire

HOXD cluster and of the neighbouring gene EVX2. Parental analysis showed that it was present in her affected father. Molecular karyotyping was: arr 2q31.1(176,883,603-177,090,474)x3 pat (hg19). Our results confirm that duplication to the entire HOXD cluster leads to mesomelic dysplasia and significantly reduce the minimum size of the duplication responsible for this phenotype.

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Retrospective study of 16 prenatal forms of hypophosphatasia with fetal examination.

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Hypophosphatasia (HP) is a rare inherited disorder characterized by bone defective mineralization, caused by mutations within the gene that encodes the tissue-nonspecific enzyme of alkaline phosphatase (TNSALP). Two of the 6 recognized forms can be detected by prenatal ultrasounds, showing poor skeletal mineralization and fetal crowding. If most prenatal forms are lethal (LP-HP), a few benign forms are reported with a third trimester and a postnatal improvement (BP-HP).
Sixteen fetal cases of P-HP were collected from members of the Société Française de Foetopathlogie (SoFFoet) between 1983 and 2012 in order to precise the fetal phenotype. This retrospective study included X-Rays, fetal examination and TNSALP mutations.
Termination of pregnancy was made after molecular prenatal diagnosis in 2 cases at 15 and 16 weeks of gestation (WG), and after prenatal ultrasounds in 13 cases between 15 and 33 WG. One fetus was dead in utero at 35 WG. Dysmorphy was reported in 10/16 cases. The skull was soft on the touch with absence of ossification on X-Ray in any case. Micromelia and short bowing legs were present in all the fetus except one. Spurs were present in 9/16 cases. The 16 fetuses showed a severe hypomineralization without any fracture. Absence ossification was constant but heterogeneous along the vertebral column and the extremities. The metaphyses appeared bifid with a thickened hypertrophic cartilage on microscopic sections. X The fetus were composite heterozygous in 10/16 genetic studies with different family genotypes.
The 2 forms of LP-HP and BP-HP will be discussed in this retrospective study.

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P01.062

Three generation family with kyphomelic dysplasia suggests autosomal dominant inheritance

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Kyphomelic dysplasia (KD) is a rare skeletal dysplasia belonging to the group of bent bone dysplasias. It represents a heterogenous group of disorders, with at least three distinct entities: Schwartz-Jampel syndrome (SJS), Cartilage Hair Hypoplasia (CHH) and 'true' KD. For the latter, the genetic background is unknown and autosomal recessive inheritance is presumed based on several sporadic cases with parental consanguinity.
We report a three generation family presenting with a true KD phenotype. The proband was born at term after an uneventful pregnancy. Her length and weight at birth were 37cm and 1578g respectively. She had severe rhizomelic shortening of upper and lower limbs. Radiographs showed stubby femora which were extremely curved. Iliac wings were short and widened; the humeri were also short and dumbbell-shaped.
The mother of the proband has a similar disproportionate short stature phenotype. Radiographs taken at birth revealed severe femoral bowing, though less prominent compared to her daughter. Follow-up radiographies in the mother demonstrated gradual improvement of the bowing over several years. The maternal grandfather of the proband had an identical phenotype. All had a normal neuromotor development. There were no signs of myotonia or ocular problems (SJS) nor evidence for immunodeficiencies or anaemia (CHH).
This family with true KD is to our knowledge the first to demonstrate that at least a subtype of KD has an autosomal dominant mode of inheritance. This observation has important consequences for genetic counselling of patients with KD, and identification of other families may allow clarification of the genetic background.

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P01.063

Identification of new SQSTM1 mutations in familiar Paget's disease

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Paget's disease of bone (PDB) is a focal disorder of bone that affects segmentally the skeleton, presenting a increase in osteoclast number, size and activity that results in a variegated and anarchic bone structure that alters the mechanical properties.
There is a tendency to familial aggregation (15-40%) with an autosomal dominant pattern modified by environmental factors. The most plausible candidate gene is sequestosoma1 gene, encoding the p62 protein, which plays an important role in cellular signal crossroads related with osteoclastogesis such as NF-kB pathway.
We studied in 21 families with proven diagnosis of PDB, exons 6, 7 and 8 of SQSTM1 gene in genomic DNA by PCR and subsequent automatic sequencing Big DyeTerminators.
Our results show that 8 of the 21 families studied (38.09%) were carriers of a mutation in the SQSTM1 gene. Three of the eight families carry the mutation p.P392L (12.5% of all patients studied), often described in the literature. In three families appears the p.E273D mutation and in other appears the p.R321C mutation neither described in the literature.
"In silico" study categorizes both novel mutatations as pathogenic. A population study in 100 healthy individuals alleles by dHPLC (Denaturing High Pressure Liquid Chromatography), failed to detect the mutations.
We report two new Spanish families carrying novel mutations in SQSTM1 gene which reinforce the role of this gene in Paget disease.

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The role of matrix metalloproteinases (MMP2, MMP9 and MMP12) gene polymorphisms in abdominal aortic aneurysm and aortoiliac occlusive disease

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Abdominal aortic aneurysm (AAA) and aortoiliac occlusive disease (AIOD) are common vascular disorders considered to be complex diseases with both genetic and environmental risk factors involved. Destructive remodeling of extracellular matrix and histological signs of chronic inflammation associates both of these pathologies. Matrix metalloproteinases play a major role in the remodeling of the extracellular matrix of aorta, and their contribution to the pathogenesis of AAA is unquestionable. The purpose of this study was to examine the role of MMP2(nt-1306); MMP9(nt-1562); MMP12 (nt-82) gene polymorphisms in the development of AAA or AIOD in Polish patients. The case-control designed study included three selected groups: 308 patients with AAA and 312 patients with AIOD who underwent surgery; 375 individuals from control group free of these vascular diseases. Genotypes were compared with demographic and clinical data of subjects and analyzed in relation to risk factors. We found significant differences between patients with AAA and control group for MMP9 (nt-1562) (p=0,026) and MMP12 (nt-82) (p=0,037) gene polymorphisms. There were also significant differences between patients with AIOD and control group for MMP9 (nt-1562) (p=0,014). No differences were found in case of MMP2 (nt-1306) gene polymorphism. In a multivariable logistic regression analysis adjusted for traditional cardiovascular risk factors MMP12 was an independent risk factor for AAA and MMP9 for AIOD. In conclusion, we suggest that polymorphism in MMP12 may independently contribute to pathogenesis of AAA whereas MMP9 polymorphism is a risk factor of AIOD. Supported by Natio-

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Genetic variants in selenoprotein genes SEPP1, TXNRD1, TXNRD2 and abdominal aortic aneurysms development

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Enhanced oxidative stress in cardiac and vascular myocytes is causative to cardiovascular diseases. Selenium, a trace element, as a component of selenoproteins helps control the intracellular redox state. In the presented study the associations between the four SNPs in genes encoding selenoproteines: selenoprotein P (SEPP1 rs7579G>A, rs3877899G>A), thioredoxin reductase 1 (TXNRD1 rs35009941C>G) and 2 (TXNRD2 rs9605031C>T) and the abdominal aortic aneurysm (AAA) occurrence were examined.
The case-control study encompassed a series of 506 AAA patients and 468 controls. In patients the presence of symptomatic peripheral arterial disease (PAD) was determined. High-throughput SNPs genotyping was performed using the TaqMan pre-designed assays. Associations between alleles/haplotypes and the intermediate traits related to the vascular diseases were also examined.
SEPP1 SNPs were in complete linkage disequilibrium, then only three of rs7579-rs3877899 haplotypes: G-G (0.469), A-G (0.293), G-A (0.238) were observed. None of the studied alleles/haplotypes was associated with AAA. However, the higher frequency of the SEPP1 rs3877899A allele (the G-A haplotype) carriers in AAA subjects with advanced PAD (53.5%) as compared to those without PAD (30.9%, p=0.012) and controls (41.7%, p=0.1) were noted. The association between the SEPP1 rs7579A allele (the A-G haplotype) and obesity in AAAs was also found (p<0.005), but no significant relations between studied variants and age, gender, hypertension, diabetes and HDLC level were noted.
In conclusion, selenoprotein P gene haplotypes are associated with the development of peripheral atherosclerosis and with obesity in abdominal aortic aneurysms. The work was supported by the National Science Centre in Poland under grant No. NN403250440.

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P01.066

The IL23R Non-Synonymous Polymorphism rs11209026 is Associated with Radiographic Sacroiliitis in Spondyloarthritis

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Objective: Spondyloarthritis (SpA) is a group of inflammatory articular disorders sharing genetic background. The non-synonymous single-nucleotide polymorphism rs11209026 (Arg381Gln) in the IL23R gene, has reproducibly been shown as associated with ankylosing spondylitis (AS). Here, we examined the association between rs11209026 and SpA as a whole. Particular attention was devoted to genotype-phenotype correlation.
Methods: rs11209026 was genotyped in a French collection of 415 cases/372 controls and 383 trios. Association analysis was carried out in SpA as a whole group and then separately in AS and non-radiographic SpA (non-AS) patients. Phenotype/genotype correlations were examined using a logistic regression analysis.
Results: A significant association between rs11209026 and SpA was only identified in the familial dataset (P = 0.03; OR = 0.57). Strong association with AS was observed in both case/control and familial datasets (P = 4.5x10-4 and 4x10-3, respectively). In contrast, such association was not detected with the non-AS group. Furthermore, rs11209026 frequency was significantly different between the AS and non-AS patients (P = 2.5x10-3). Phenotype/genotype correlation study revealed that both radiographic sacroiliitis and an early onset were independently associated with a lower frequency of the rare protective rs11209026 allele A in patients (P = 9x10-3 and 8x10-3, respectively).
Conclusion: Our study replicated the robust association between rs11209026 and AS, in the French population. However such association was restricted to AS patients, as compared to non-radiographic SpA. The fact that it was independently conditioned by radiographic sacroiliitis and age at onset suggests that rs11209026 could affect the disease severity rather than susceptibility.

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P01.067

Transcriptomic Analysis Of Derived Dendritic Cells (MD-DCs) Reveals Several Genes Differentially Regulated Between Spondyloarthritis (SpA) Patients And Healthy Controls

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Introduction: As originally shown in an HLA-B27 transgenic rat model, monocytes-derived DCs from SpA patients exhibit a weaker stimulatory efficiency of CD4+ T cells than control DCs.
Aim: To identify genes differentially expressed in MD-DCs from SpA patients, as compared to healthy controls.
Materials and Methods: Monocytes were purified from 9 HLA-B27+ SpA patients and 10 controls PBMCs using anti-CD14-coupled immunomagnetic beads and cells were further cultured with IL-4 and GM-CSF for 7 days. MD-DCs were then stimulated with LPS for 6 and 24 hours. Transcriptomic study was performed with Affymetrix HuGene 1.0 ST microarrays on unstimulated and stimulated MD-DCs. Gene expression levels in patients and controls were compared using a multivariate design under a linear model. Real-time quantitative PCR (RT-PCR) was performed for genes validation.
Results: Transcriptomic analysis revealed 104 genes differentially expressed in MD-DCs from SpA patients compared with controls (p<0.01 and fold-change ±1.5). Five candidate genes have been validated by RT-PCR (ADAMTS15, F13A1, SELL, ERAP1 and CITED2). Interestingly, expression of ADAMTS15, belonging to the metallopeptidase family, was inversely correlated with the expression of the transcription factor CITED2 (R=0.75, p=0.0003). Further, in silico pathway analysis conducted on CITED2 co-expressed genes provided identification of the Wnt signaling pathway.
Discussion: This transcriptomic study reveals striking differences in the gene expression patterns of SpA MD-DCs compared to controls. Up-regulation of ADAMTS15 highlights the potential role of metallopeptidase in SpA pathogeny. Moreover, deregulation in SpA patients of the Wnt signaling pathway may have consequences on the balance between osteoblast and osteoclast functions.

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P01.068

Novel homozygous missense mutations in the SLC2A10 gene in a Turkish pediatric patient with arterial tortuosity syndrome.

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Arterial tortuosity syndrome (ATS) is a rare autosomal recessive connective tissue disorder, mainly characterized by tortuosity and elongation of the large- and medium-sized arteries with predisposition to stenoses and aneurysms. ATS is caused by mutations in the SLC2A10 gene, encoding for the facilitative glucose transporter 10 (GLUT10) and is described typically in pediatric patients. We report a 2 years old boy with ATS who initially presented with a cardiac murmur. Echocardiography at the age of one month demonstrated dilatation of the aortic root (15mm) and tortousity in the descending aorta. The main clinical findings included elongated face, saggy cheeks, micrognathia, malar hypoplasia, joint hypermobility and hyperextensible skin. Follow-up echocardiography showed an increase of the diameter of the aortic root to 32mm and 35 mm at the age of 8 months and 2 years old, respectively. Angiography and computerized tomography angiography showed fusiform aneurysmatic dilatation of ascending aorta, marked tortuosity of both pulmonary arteries and thoracic aorta, stenosis in vena cava inferior and collateral structure associated with right hepatic vein. Sequencing of the SLC2A10 gene in the proband revealed the presence of a novel pathogenic homozygous missense variant (c.727C>A). Heterozygous SLC2A10 mutations were shown in her mother and father, demonstrating true homozygosity. The pathogenic variant leads to a p.Gln243Lys change

in the seventh transmembrane domain of GLUT10. The proband underwent aortic root replacement surgery. ATS resembles Loeys-Dietz and Marfan related disorders, so timely differential diagnosis is extremely important for early diagnosis and intervention of aneurysms to prevent serious vascular complications.

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P01.069

SLC2A10 knockdown in aortic smooth muscle cells underscores mitochondrial dysfunction as a key pathogenetic event in arterial tortuosity syndrome

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Arterial tortuosity syndrome (ATS) is a recessively inherited connective tissue disorder, mainly characterized by tortuosity, stenosis and aneurysm formation of the main arteries. In patient tissue samples, fragmentation of elastic fibers and enhanced TGFβ-signaling was found. ATS is caused by loss-of-function mutations in the *SLC2A10* gene, encoding the facilitative glucose transporter GLUT10. The function of this transporter and the pathogenesis underlying ATS has been a matter of debate. Cellular studies and a recent ATS zebrafish model suggested a role for GLUT10 as a mitochondrial transporter of the antioxidant dehydroascorbic acid (vitamin C). GLUT10 loss-of-function would therefore lead to increased oxidative stress and mitochondrial dysfunction associated with disturbed TGFβ-signaling. In this study we wanted to investigate the initial alterations in gene expression following *SCL2A10* knockdown in human aortic vascular smooth muscle cells (aVSMC). Two different siRNA's, targeting *SLC2A10*, were separately transfected in aVSMC, both resulting in knockdown efficiencies of >80%. RNA was extracted at 24 hours post-transfection and loaded on Agilent 8X60k expression arrays. Differential gene expression was determined as transcript-level changes of ≥1.5 ($p<0.05$) relative to control samples. As a result, 264 different genes and 7 lincRNA's showed differential expression in both siRNA treatments. In-depth data mining indicates disturbed mitochondrial functioning, elevated mitochondria-mediated apoptosis, altered TGFβ-signaling and disturbed production of extracellular matrix components. However, at the cellular level, no apoptosis or pronounced disruption of mitochondrial integrity was detected at this early stage after knockdown. Altogether, these data further evidence a key role for proper mitochondrial functioning in vascular development.

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P01.070

Influence of promoter SNPs on the ABCG1 transporter gene expression level

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Atherosclerosis is characterized by cholesterol deposition in vascular wall. Transporter ABCG1 mediates cholesterol efflux from macrophage foam cells to high density lipoproteins. However the importance of ABCG1 gene expression in human macrophages for atherosclerosis development is still undiscovered. Two SNPs -134T/G (rs1378577) and -204A/C (rs1893590), which can affect ABCG1 gene expression level, were identified in promoter region of ABCG1 gene. The aim of this study was to investigate influence of SNPs -134T/G and -204A/C on ABCG1 gene expression level and association of ABCG1 gene expression in monocytes and macrophages with atherosclerosis. Human peripheral blood monocytes were obtained from 26 patients with angiographically proved atherosclerosis and 20 healthy individuals. To get monocyte-derived macrophages for 11 persons of each group, monocytes were cultured with macrophage colony-stimulating factor (M-CSF) for 5 days. ABCG1 mRNA levels were measured using real time PCR. For identification of -134(T/G) and -204(A/C) genotypes allele-specific PCR and PCR with restriction analysis, respectively, were used. Macrophage ABCG1 mRNA levels were significantly reduced in group of patients when compared with the controls: average levels were 1.33±0.48 and 2.46±0.45 ($p<0.001$). There were no differences in monocyte ABCG1 mRNA levels between patients and controls. And there was no correlation between -134(T/G) and -204(A/C) SNPs and ABCG1 mRNA levels in monocytes and macrophages. These results suggest that reduction of ABCG1 expression in macrophages is associated with atherosclerosis, but ABCG1 genetic variants -134(T/G) and -204(A/C) do not affect this expression.

V. Miroshnikova: None. **E. Demina:** None. **N. Mayorov:** None. **V. Davydenko:** None. **P. Kurjanov:** None. **V. Vavilov:** None. **A. Denisenko:** None. **A. Schwarzman:** None.

P01.071

Fetal presentation of Cartilage Hair Hypoplasia with extensive granulomatous inflammation

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Cartilage-hair-hypoplasia is a rare autosomal recessive metaphyseal dysplasia due to RMRP (the RNA component of the RNase MRP -ribonuclease mitochondrial RNA processing- complex) gene mutations. So far, more than 90 mutations have been reported in the promoter and the transcribed regions. Clinical characteristics include short-limbed short stature, sparse hair and defective cell-mediated immunity. We report herein the antenatal presentation of a female fetus, in whom CHH was suspected from 23 weeks' gestation, leading to a medical termination of the pregnancy at 34 weeks'gestation, and thereafter confirmed by morphological and molecular studies. Post-mortem examination confirmed short stature and limbs, and revealed thymic hypoplasia associated with severe CD4 T-cell immunodeficiency along with extensive non caseating epithelioid granulomas in almost all organs, which to our knowledge has been described only in five cases. Molecular studies evidenced on one allele the most frequently reported founder mutation NR_003051: g.70A>G, which is present in 92% of Finnish patients with Cartilage Hair Hypoplasia. On the second allele, a novel mutation consisting of a 10 nucleotide insertion at position -18 of the promoter region of the RMRP gene (M29916.1:g.726_727insCTCACTACTC) was detected. The founder mutation was inherited from the father, and the novel mutation from the mother. To our knowledge, this case report represents the first detailed fetal analysis described in the literature.

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P01.073

Expanding the phenotypic and mutational spectrum of EDS dermatosparaxis type: a report of five new patients

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Ehlers-Danlos Syndrome (EDS) dermatosparaxis type is a recessively inherited connective tissue disorder, caused by deficient activity of ADAMTS2, which cleaves the amino (N)-propeptide of type I procollagen. Only ten human EDS dermatosparaxis patients have been reported, in six of whom a homozygous p.(Q225*) was identified. Due to the decreased N-proteinase activity, uncleaved pN-collagen molecules are incorporated in the collagen fibrils, resulting in fibrils with a pathognomonic 'hieroglyphic' appearance. All reported patients present a recognizable phenotype with a characteristic dysmorphic facies, extreme skin fragility and -laxity, excessive bruising and sometimes internal complications.
We report clinical, molecular, biochemichal and ultrastructural findings in five new EDS dermatosparaxis patients from four independent families. Three patients display a strikingly milder phenotype than that of previously reported patients, mainly with regard to skin fragility and -laxity and dysmorphic features. Molecular ADAMTS2 analysis revealed three novel homozygous loss-of-function mutations (c.2927_2928delC (p.(P976Rfs*42)), c.669-670dupG (p.Pro224Alafs*41), c.2751-2A>T) and a compound heterozygous mutation (c.2T>C (p.(Met1Thr)) and c.888-891delTGAA (p.(M295Nfs25*))).
SDS-PAGE showed accumulation of bands representing pN alpha chains and decrease of bands representing mature alpha chains of type I procollagen in all patients. Ultrastructural dermal analysis revealed milder collagen fibril

abnormalities than those of previously reported patients. Collagen fibrils showed irregular contours and a somewhat branched appearance, but their general cylindrical appearance was preserved. These observations suggest incomplete N-propeptide cleavage, either due to residual enzyme activity or compensating enzymes.
Together our findings show that milder forms of EDS dermatosparaxis type exist, which may remain undiagnosed (in a non-specialised clinical setting).

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P01.074

Marked intrafamilial variation in Ehlers-Danlos Syndrome type IV is explained by biallelic *COL3A1* mutations.

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Ehlers-Danlos syndrome (EDS) type IV, the vascular type, is an autosomal dominant disorder caused by mutations in *COL3A1*,which encodes the chains of type III procollagen. We identified a family in which there was marked clinical variation with the earliest death due to extensive aortic dissection at age 15 years and other family members in their 80s with no complications. The proband was born with right sided clubfoot but was otherwise healthy until he died unexpectedly at 15 years. He and his sister each had two *COL3A1* mutations (c.1786C>T, p.Arg596Ter, in exon 26 and c.3851G>A, p.Gly1284Glu, in exon 50). The mutations segregated to different alleles. In mRNA in cells from the sister the missense mutation in exon 50 was apparently „homozygous", consistent with nonsense mediated instability of the mRNA that carried the premature termination codon. Cells from the compound heterozygote produced a reduced amount of type III procollagen that was largely retained in the cells, the chains of which all had abnormal electrophoretic mobility. Cells from an individual heterozygous for the missense mutation produced some normal and some abnormal type III procollagen. Cells from an individual heterozygous for the nonsense mutation produced a reduced amount of apparently normal type III procollagen. This is only the second instance of compound heterozygosity for deleterious *COL3A1* mutations and in both instances the individuals with bi-allelic mutations have a significantly worse outcome than the heterozygotes. This is a rare explanation for marked clinical variation in individuals with mutations in *COL3A1*.

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P01.075

Dermal electron microscopy findings in the Ehlers-Danlos Syndromes (EDS).

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There is limited published data describing dermal collagen ultrastructure (Electron Microscopy, EM) across EDS subtypes. We present data from 188 patients, correlated with clinical, molecular and biochemical findings.
We identified normal collagen fibril size in the majority of patients classified as hypermobile EDS (64.6% [n=82]), as well as other patients with arterial aneurysm and dissection and non-specific features of hereditary disorders of connective tissue.
Fibril size irregularity is seen in the majority of patients with confirmed vascular EDS (vEDS) (90% [n= 10]), but also in patients with confirmed collagen (I) and (V) abnormalities, and rarely in other patients with EDS III/BHS. We could not confirm the findings of Ong et al (2012) of abnormal fibroblast shape, lysosomal inclusions, dermo-epidermal junction abnormalities in 10 confirmed vEDS patients.
Occasional small collagen flowers were common in EDS III/BHS (30.7% [n=39]), but also seen across all EDS subtypes. Dilated endoplasmic reticulum, collagen fibril kinking and loose or dense packing were seen across all EDS subtypes and were non-specific findings. Numerous (>5%) collagen cauliflower fibrils were specific to the EDS classical subtype, however we did not identify Collagen (V) or (I) mutations in all of these patients (25% [n=3]).
No EM finding was completely sensitive or specific to a particular EDS subtype. Detailed clinical, genetic, biochemical and structural assessment remains the gold standard for assessment of these patients. There is limited data describing dermal collagen fibril characteristics and fibroblast architecture and dimensions in the normal population.
Ong et al. Virchows Arch (2012) 460:637-649

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P01.076

A second family carrying a mutation of the PLOD3 identified by whole exome sequencing: a new subtype of EDS?

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A male patient aged 10 years was referred with severe S-formed scoliosis, ptosis of the right eyelid, asymmetrical face, midface hypoplasia and shallow orbitae with pseudoexophtalmus. Additionally, bilateral adducted thumbs, non progressive flexion contractures of proximal finger joints, atrophy of thenar muscles and overall poor muscle development were noted. Bilateral sensorineural deafness was diagnosed at the age of two years. He has a thin and soft skin, without scarring, and reduced skin creases on both palms. Motor development was slightly retarded and cognitive function mildly impaired. The patient's sister was similarly affected, but without significant scoliosis. Parents were healthy, non-consanguineous, but originating from an isolated rural region. A so far uncharacterized "complicated form" of Ehlers-Danlos-syndrome was suspected. We performed whole exome sequencing, analyzed the data under the hypothesis of parental consanguinity and identified the Lysyl hydroxylase3 gene (PLOD3) involved in the posttranslational modification of collagen biosynthesis as a strong candidate gene. Both patients were homozygous for the c.1935+1G>A mutation and both parents were carriers. Study of the literature unveiled one single family carrying a mutation in this gene with a strikingly similar phenotype (Salo et al., 2008 Am J Hum Genet 83:495, OMIM: #612394), which suggests a syndromic pattern caused by PLOD3-mutations. Therefore the PLOD3 caused disease may be considered a new form of the vascular-contractural-deafness type of EDS, expanding the group of diseases caused by a dysfunction of the collagen assembly.

E. Steichen-Gersdorf: None. **S. Kircher:** None. **M. Hagl:** None. **J. Behunova:** None. **O. Brandau:** None. **F. Laccone:** None.

P01.077

Development of sporadic dilatative pathology of ascending thoracic aorta (DPATA): impact of Fibrillin-1(FBN1) polymorphysms (rs1036477, rs10519177, rs755251, and rs2118181)

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Dilatation of ascending thoracic aorta in patients with Marfan syndrome is caused by *FBN1* mutations. The role of *FBN1* polymorphisms within sporadic cases of DPATA is less explored.
The aim of our study - to investigate the association of *FBN1* gene *SNPs* (*rs1036477, rs10519177, rs755251* and *rs2118181)* within development of different phenotypes (post-stenotic aortic dilatation (PSAD); aneurysm of thoracic aorta (ATA); and dissection of thoracic aorta (DTA) of sporadic DPATA.
Methods. Study included **238** patients who were undergoing aortic reconstruction due to DPTA at the Department of Cardiovascular Surgery, Lithuanian University of Health Sciences. No patients had phenotypic characteristic of Marfan syndrome. Reference group (**n=445**) was formed from a random sample of Kaunas population. Both groups age and sex matched. DNA was extracted from blood, concentration was measured by spectrophotometry. *FBN1 SNPs* (*rs1036477* and *rs2118181, rs10519177, rs755251)* were analysed by real-time PCR. Results were calculated statistically by approved Fisher's exact test, logistic regression.
Results: The patients with DTA had a significantly higher frequency of *FBN1* G/G genotype than the reference group subjects.

Table 1

FBN1 GG genotype frequency	DTA n=52	PSAD n=66	ATA n=120	Reference group n=445
rs2118181	8.51% *	3.17%	2.63%	1.35%
rs1036477	8.51% *	3.17%	2.63%	1.35%
rs10519177	17.02% *	7.81%	12.39%	6.97%
rs755251	17.02% *	7.81%	12.39%	6.97%

*p<0.05

It was associated with increased odds of DTA (*rs2118181, rs1036477* OR=6.8; 95 % CI 1.849-25.058, p=0.004; rs10519177, rs755251 OR=2.7; 95% CI 1.178-6.369, p=0.019).

Summary. Our data draw attention to the pathogenetic link between sporadic DTA and aortic pathology in Marfan syndrome.

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P01.078

Assessment of the enrichment for rare coding variants in 16 cases of familial fibromuscular dysplasia

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Fibromuscular dysplasia (FMD) is an arterial disease characterized by non-atherosclerotic stenoses reported in renal (70%) and extra-cranial carotid (30%) arteries. FMD predisposes to hypertension, renal ischemia and stroke. The causes of FMD are unknown and it occurs predominantly in females (age<50 years) with a prevalence of ~4/1000. Despite evidence for its inheritance the genetics of FMD is under-investigated. The aim of this study is to assess the role of rare coding and putatively functional variants in the onset of familial FMD.

Patients are five affected sib-pairs and two sib-trios (N=16; mean age=51yrs, min=32; max=61) with confirmed multifocal "string-of-beads" FMD diagnosed by angiography/scanner. Mean age diagnosis =44yrs (min=23; max=57). Exome sequencing and variant calling was performed by IntegraGen® using Agilent's capture Kit (V4) and Hiseq2000 (Illumina®).

We identified 4,311 confident (high calling-score and depth >8X) missense rare (MAF<0.02 in EVS and/or 1000G) variants predicted to be damaging (PolyPhen2) in 3,414 genes. We performed a genomic burden test for highly mutated genes (>= 4 variants, N=45) and used the EVS data from ~4,300 unselected Europeans as controls. Fischer exact test revealed two genes significantly enriched for putatively causative variants at the genomic level (P=4.9x10^{-8} and P=7.1x10^{-7}). We have demonstrated previously important fibrosis and loss of extracellular matrix (ECM) at the media of FMD arteries. Using less stringent significance threshold (P<0.01), we identified several genes involved in ECM structure, cell shape and junction, which are interesting candidates for FMD. Validation of the identified variants both in statistical and candidate genes is underway.

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P01.079

Clinical symptoms in fibromyalgia are associated to catechol-O-methyltransferase (COMT) gene Val158Met polymorphism

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Fibromyalgia syndrome (FMS) is a common chronic widespread pain syndrome mainly affecting women. Genetic risk factors are known to contribute to the etiology of the syndrome. The aim of this study was to explore the frequency and clinical significance of catechol-O-methyltransferase (COMT) gene Val158Met polymorphism in a large cohort of Turkish patients with FMS. The study included 379 patients with FMS and 290 healthy controls. Genomic DNA was isolated and genotyped using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analyses for the COMT gene Val158Met polymorphism. The genotype frequencies of COMT gene Val158Met polymorphism showed a small difference between FMS patients and healthy controls (p = 0.047), however the Met/Met genotype was significantly higher in FMS patients than healthy controls (p=0.016, OR: 1.64, 95% CI: 1.09-2.45). No difference was observed for allele frequencies between two groups (p=0.143). Stratification analysis according to clinical features for this disease reveals that weight, FIQ score, algometry (pressure pain threshold) and Raynaud's syndrome, were detected to have statistically significant associations with Val158Met polymorphism (p=0.037, p=0.042, p=0.039 and p=0.033, respectively). Pain sensitivity, measured by algometry, was statistically higher in patients with Met/Met genotype than the patients with Val/Val and Val/Met genotypes (p=0.017). The results of this study suggest that COMT gene Val158Met polymorphism is positively associated with predisposition to develop FMS and play a relevant role in the clinical symptoms of the disease.

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P01.080

Paternally inherited mutations in *GNAS* exons 1-13 lead to severe intrauterine growth retardation (IUGR)

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Maternally inherited, heterozygous mutations in *GNAS*, the imprinted gene complex encoding the alpha-subunit of the stimulatory G protein (Gs), cause pseudohypoparathyroidism type Ia (PHP-Ia), a disorder characterized by resistance toward parathyroid hormone (PTH) and developmental defects referred to as Albright's Hereditary Osteodystrophy (AHO). When inherited paternally, the same heterozygous *GNAS* mutations lead to pseudo-pseudohypoparathyroidism (PPHP), i.e. AHO features in the absence of hormonal resistance. Subcutaneous ossifications (referred to as progressive osseous heteroplasia (POH)) occur predominantly with paternally inherited mutations. Recent studies suggested that paternal mutations are associated with intrauterine growth retardation (IUGR).

Objectives: To confirm and extend earlier findings, we obtained birth weight (W) length (L) and head circumference (HC) for patients, who had been diagnosed with either PHP-Ia (n=26) or PPHP/POH (n=22), and are carrier of *GNAS* mutations on the maternal or paternal allele, respectively. W, L and HC parameters at birth were compared to the French reference charts for girls and boys; results were expressed as Z-scores for gestational age.

Results: The Z-scores for all three parameters at birth (Table) suggest that mutations on the maternal allele affect intrauterine development to a lesser extent than mutations on the paternal allele.

Conclusion: Our cohort shows that *GNAS* mutations on the paternal allele can be responsible for severe IUGR in humans, indicating that paternal *GNAS* transcripts play a major role in the normal feto-placental development.

Table: Birth parameters (Z-scores)

	Weight (W)	Lenght (L)	Head Circumference (HC)
	Mean ± SEM (n)	Mean ± SEM (n)	Mean ± SEM (n)
PHP-Ia (maternal	-0.65 ± 0.25 (26)	-1.14 ± 0.22 (23)	-0.04 ± 0.29 (19)
PPHP/POH (paternal	-2.80 ± 0.24 (22)	-2.34 ± 0.20 (20)	-1.39 ± 0.23 (19)
p	<0.0001	0.0003	0.0009

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P01.081

Genome-wide association analysis identifies a susceptibility locus for pulmonary arterial hypertension

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Pulmonary arterial hypertension (PAH) is a rare and devastating disease, resulting from progressive obliteration of small caliber pulmonary arteries by proliferating vascular cells, and leading to cardiac failure, with an untreated mean survival of less than three years. PAH can complicate other pathological conditions, or can occur in the context of genetic mutations causing heritable PAH, or can be considered as idiopathic (iPAH), which represents approximately 40% of all PAH. Low penetrance dominant BMPR2 mutations are found in ~80% of familial PAH (fPAH), and in ~15% of iPAH which are thereafter considered as heritable PAH. We conducted a Genome-Wide Association Study (GWAS) based on two independent case-control studies for iPAH and fPAH (without BMPR2 mutations) totaling 625 patients and 1,525 healthy individuals, to identify novel genetic factors associated with iPAH and fPAH (i/fPAH). A genome wide significant association was detected at 18q22.3, the risk allele being associated with an odds ratio for i/fPAH of 1.97 [1.59 - 2.45] (P = 7.47 x 10-10). We have shown by immunochemistry and RT-PCR analysis that the closely mapping gene is expressed in the lung, particularly in pulmonary vascular endothelial cells. Furthermore, its expression is increased in explanted lungs from PAH patients and in endothelial cells cultured from explanted PAH lungs. Altogether, these results strongly suggest a role for this gene as a novel contributor to the physiopathology of i/fPAH.

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P01.082

Clinical expression of Hereditary Haemorrhagic Telangiectasia and digestive lesion characteristics in patients with SMAD4 mutation.

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Backgrounds: Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant vascular disorder characterized by epistaxis, telangiectasia and arteriovenous malformations. Most of HHT cases result from mutations in *ENG* (coding for endoglin) and *ACVRL1* (coding for activin receptorlike kinase 1). In addition, mutations in *MADH4* (encoding SMAD4) which cause a juvenile polyposis /HHT overlap syndrome, have been described. Our aim was to define clinical expression of HHT and digestive lesions in HHT patients with *SMAD 4* mutation followed in the French reference center.

Methods: Restrospective analysis of HHT patients with *SMAD4* mutations. Curaçao criterias were used for HHT diagnosis and Jass criterias for juvenile polyposis.

Results: 14 among 589 patients with identified mutation had *SMAD4* mutation (2.3%). Epistaxis and telangiectasia were present in 13 (93%) and 11 (79%) patients respectively. Nine patients among 13 screened patients had pulmonary arterioveinous malformations (PAVMs) (69%). Among them 4 had diffuse or multiple PAVMs associated with hypoxemia. Three patients among 11 screened patients (27%) had severe hepatic AVM with high cardiac output. Among eleven SMAD4 patients who had digestive endoscopies: 10 (91%) had digestive lesions.

Conclusion: In this study the digestive presentation in patients with SMAD4 mutations is not limited to juvenile polyposis. The HHT phenotype appeared to be more severe for hepatic and pulmonary arteriovenous malformations. This study confirms that any patient with *SMAD4* mutation should be screened for juvenile polyposis or digestive tract cancers as well as for HHT complications.

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P01.083

A Molecular Genetic Study of Congenital Lymphedema

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Scientific Background: Primary lymphedema results from a dysfunction of the lymphatic system. Familial lymphedema usually segregates as an autosomal dominant trait, with only one case described in line with autosomal recessive transmission. Genetic studies of families with hereditary lymphedema have yielded mutations in three lymphangiogentic genes: FLT4 (VEGFR3), FOXC2, SOX18. Our research seeks to map and ultimately hunt the mutation that causes hereditary lymphedema in an extended consanguineous Muslim family consisting of four affected individuals: one female, presenting with isolated edema of the lower extremities, and three males presenting with lymphedema of the lower extremities, lymphocele and mild mental retardation.

Material and Methods: After locating a candidate locus using whole genome linkage analysis (Illumina CytoSNP6000 array), Sanger sequencing of the candidate genes was preformed.

Results: A candidate locus of 2.3 Mb located on chromosome 5q35.3 was identified, showing homozygosity in the affected individuals. This locus has been previously linked to congenital lymphedema. Using SUPERLINK online, linkage analysis generated a two-point LOD score of 3.18. Several mutations in the candidate gene, FLT4, previously described in Muslim-Israeli families, were discarded as culprit using sequence analysis. While sequencing FLT4, a missense mutation has been discovered in exon 28 (Ser1235Cys). Unlike other mutations previously described, this mutation is located outside the tyrosine-kinase domains, and has perfect segregation within the family. A population screen for the mutation was performed, with no carriers found in 100 Muslim Israeli-Arabs. Furthermore, a whole-exome analysis of an affected individual revealed no other mutations in lymphedema-related genes.

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P01.084

Pulmonary venoocclusive disease and interstitial fibrosis in a female with Myhre syndrome due to the common SMAD4 mutation: evidence for pathogenic mechanism.

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Myhre syndrome (MS) is a rare connective tissue disease characterized by short stature, a peculiar body build, hearing loss, joint stiffness, brachydactyly, variable intellectual disability and characteristic skeletal and facial features. A common missense mutation in the MAD homology 2 domain of SMAD4, a gene involved in TGF-β/BMP2 signaling, was recently shown to be causative of the condition. The majority of previously reported individuals are males with only eight females described to date. We report on a female patient with Myhre syndrome identified with the common SMAD4 heterozygous mutation who developed severe pulmonary venoocclusive disease and pulmonary interstitial fibrosis. This respiratory complication has been previously reported only in a single male patient with Myhre syndrome.

We performed in vitro studies on skin fibroblasts from the proband and identified a chain of molecular events triggered by the SMAD4 mutation. The principal defect results in furin deficiency leading to a deficit of a functional form of CD109, which in turn causes a decreased rate of TGF-β receptor degradation. The latter results in an exaggerated response to normal levels of TGF-β1 and higher than normal production of fibrous components of extracellular matrix (elastin, collagen type 1 and fibronectin). We propose that this chain of events contributes to the development of the severe pulmonary, vascular and interstitial disease in Myhre Syndrome.

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P01.085

Spherophakia and megalocornea in a German patient with LTBP2 mutations

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Mutations in LTBP2 have been originally identified as a cause of primary congenital glaucoma; furthermore mutations were reported in megalocornea, ectopia lentis and secondary glaucoma , in one patient with isolated spherophakia, and most recently associated with features of Weill-Marchesani syndrome . So far only homozygous mutations in patients from consanguineous and Gipsy families were reported. We report on a German boy with spherophakia and megalocornea (corneal diameter LE 14.5 mm; RE 14.5 mm) without other signs of connective tissue disease. Mutation analysis of LTBP2 revealed two mutatios c.688delC; p.(Gln230Argfs*50) and c.1610delC; p.(Pro537Leufs*20). Segregation analysis revealed the presence of one mutation in each parent. Thus, mutations in LTBP2 should be considered not only in patients with primary congenital glaucoma, but more importantly in patients with variable ocular phenotypes including megalocornea, ectopia lentis, and (micro-) spherophakia, also, if extraocular features are present and regardless of the ethnic background.

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P01.086

First description of a homozygous whole gene deletion of COL6A1 gene in a patient with Ullrich Congenital Muscular Dystrophy

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Ullrich Congenital Muscular Dystrophy (UCMD; OMIM 254090) is a rare neuromuscular disease which is difficult to diagnose by the clinical phenotype being mainly characterized by congenital muscle weakness as well as wasting and proximal joint contractures associated with severe distal joint laxity. We present a girl with congenital general muscular hypotonia - suspected to be syndromic - failure to thrive due to sucking weakness, joint laxity, and discrete dysmorphic features, who was seen by the genetic counsellor for the first time at the age of 4 months. She suffered from bronchopneumonia at the age of 3 ½ months and showed a twofold elevated CK-MB in the course of the laboratory evaluation. Since it was not possible to identify the cause of her symptoms during the counselling interview, array-CGH was performed revealing three chromosomal imbalances - a heterozygous duplication 5q35.3, a heterozygous duplication 16q12.2 and a homozygous deletion 21q22.3. The deletion spans 56,94 kb of genomic DNA and contains the COL6A1 gene, one of the three causative genes in UCMD. Array-CGH of the parents revealed both as carriers of the deletion 21q22.3 though consanguinity was denied by the parents. This case demonstrates the possibility to detect a recessive monogenic disease by array-CGH. To the best of our knowledge this is the first case of UCMD due to a homozygous deletion of COL6A1. The causality of this deletion is very probable, because nonsense mutations leading to a lack of the COL6A1 gene product have been already described as a reason for UCMD.

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P01.087

NSAIDs-induced acute urticaria/angioedema: Genome-wide association study in Spanish and Han-Chinese populations

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Background: Acute urticaria/angioedema (AUA) induced by cross-intolerance (CI) to NSAIDs is the most frequent clinical entity in hypersensitivity reactions (HRs) to drugs. However, most of genetics studies in NSAIDs HRs have been carried out in patients with aspirin-induced asthma, mainly in Asian populations, and have followed the candidate gene approach.
Objective: We conducted a genome-wide association study (GWAS) in two ethnically different populations of Spanish and Han-Chinese patients suffering from NSAIDs-induced AUA.
Methods: We included a total of 232 cases (112 from Spain and 120 from Taiwan) with NSAIDs-induced AUA, without airway involvement or underlying chronic urticaria, and 225 unrelated controls (124 from Spain and 101 from Taiwan). Whole-genome scan was performed using Affymetrix® Genome-wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA, USA).
Results: We obtained suggestive associations for a total of 3 clusters in the Spanish group (RIMS1, BICC1, and RAD51L1), whereas one main region was identified in the Han-Chinese population (ABI3BP). Five regions showed suggestive associations after meta-analysis: HLF, RAD51L1, COL24A1, GalNAc-T13, and FBXL7. Some of these genes are related with Ca++ channels and cAMP signaling pathways.
Conclusions: The associations described with genetic variants different from those related with the metabolism of the arachidonic acid open up new potential pathways for understanding the mechanisms underlying NSAID-induced AUA.

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P01.088

Copy number variants in patients with abdominal aortic aneurysms

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Abdominal aortic aneurysms (AAA) are a major cause of morbidity and mortality in the elderly and are characterized by extracellular matrix (ECM) degeneration, dysregulation of vascular smooth muscle (SMC) cells, inflammation and atherosclerosis. In approximately 20% of cases abdominal aneurysm are familial (fAAA). The genes involved in fAAA have not been identified sofar. The aim of the current study was to investigate if copy number variants (CNV) may add in discovering pathofysiologic pathways and genes involved in AAA formation.
AAA patients referred for genetic counseling in 2011 and 2012 were included. Patients were classified as familial when at least one first-degree was reported to have an aortic aneurysm. Patients without a positive family history were classified as sporadic (spAAA). Microarray was performed in the index patient and in case of a CNV, also affected relatives were analysed, when possible.
Microarray was performed by using the Illumina HumanCytoSNP-12v2.1 and analysed using Nexus CopyNumber Discovery v6.1 software. Results were evaluated using the UCSC Genome Browser March 2006, the Database of Genomic Variants and an in-house database of controls. Inguinuity pathway (IPA) pathway analysis was performed of genes identified in rare CNV regions.
Results: The study population consisted of 77 index AAA patients; 62 were classified as fAAA and 15 as spAAA. We found 19 rare CNV variants in 14 (23%) of the fAAA patients and in one (7%) spAAA. The CNV's and the genes localized in the affected regions will be presented together with the IPA pathway analysis.

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P01.089

Twenty patients including 7 probands with autosomal dominant cutis laxa confirm clinical and molecular homogeneity

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Elastin gene mutations have been associated with a variety of phenotypes. Autosomal dominant cutis laxa (ADCL) is a rare disorder that presents with typical facial characteristics, inguinal hernias, aortic root dilatation and pulmonary emphysema. In most patients, frameshift mutations are found in the 3' region of the elastin gene (exons 30-34) which result in a C-terminally

extended protein, though exceptions have been reported.
We clinically and molecularly characterized the thus far largest cohort of ADCL patients, consisting of 19 patients from six families and one sporadic patient.
Molecular analysis showed C-terminal frameshift mutations in exon 30, 32, and 34 of the elastin gene and identified a mutational hotspot in exon 32 (c.2262delA). This cohort further confirms the previously reported clinical constellation of skin laxity (100%), inguinal hernia (51%), aortic root dilatation (55%) and emphysema (37%).
In conclusion, ADCL is a clinically and molecularly homogeneous disorder, but intra- and interfamilial variability in the severity of organ involvement needs to be taken into account. Regular cardiovascular and pulmonary evaluations are imperative in the clinical follow-up of these patients.

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P01.090

Severe Congenital Lipodystrophy and Progeroid Appearance: Mutation in the Penultimate Exon of FBN1 Causing a Recognizable Phenotype

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Recently, three Marfanoid patients with congenital lipodystrophy and a neonatal progeroid appearance were reported. Although their phenotype was distinct from that of classic Marfan syndrome, they all had a truncating mutation in the penultimate exon, i.e., exon 64, of FBN1, the causative gene for Marfan syndrome. These cases might represent a new entity, but the exact phenotypic and genotypic spectrum remains unknown. Here, we report a girl born prematurely who exhibited severe congenital lipodystrophy and a neonatal progeroid appearance. The patient exhibited a peculiar growth pattern consisting of an accelerated growth in height with a discrepant poor weight gain. She had a characteristic facial appearance with craniosynostosis. A mutation analysis identified c.8175_8182del8bp p.Arg2726Glufs*9 in exon 64 of the FBN1 gene. A review of similar, recently reported patients revealed that the cardinal features of these patients includes 1) congenital lipodystrophy, 2) premature birth with an accelerated linear growth disproportionate to the weight gain, and 3) a progeroid appearance with distinct facial features. Lines of molecular evidence suggested that this new progeroid syndrome represents a neomorphic phenotype caused by truncated transcripts with an extremely charged protein motif that escapes from nonsense-mediated mRNA decay, altering FBN1-TGF beta signaling, rather than representing the severe end of the hypomorphic phenotype of the FBN1-TGF beta disorder spectrum. We propose that this Marfanoid entity comprised of congenital lipodystrophy, a neonatal progeroid appearance, and a peculiar growth prolife and caused by rare mutations in the penultimate exon of FBN1 be newly referred to as Marfanoid-progeroid syndrome.

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P01.091

Homozygous *LMNA* lipodystrophy

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Background:
Heterozygous *LMNA* mutations cause a broad spectrum of diseases such as progeria, muscular dystrophies, dilated cardiomyopathy and lipodystrophy. Lipodystrophy is characterised by loss of subcutaneous fat from limbs and trunk and a tendency to insulin resistance, dyslipidemia and hypertension. So far homozygous *LMNA* mutations causing lipodystrophy have not been described.
Methods and results:
We report the first two lipodystrophy patients with homozygous *LMNA* mutations. The index patient was referred under the suspicion of mosaic Turner syndrome. She had hypertension and hypercholesterolemia. Her build showed typical partial lipodystrophy and a homozygous mutation was identified in the *LMNA* gene (R582H). Subsequent cardiac and neurologic examinations were normal. The brother of the index patient was diagnosed with diabetes mellitus type I, hypertension and hypercholesterolemia at the age of 27 years. He had the same characteristic build and was also homozygous for the R582H mutation in *LMNA*. Molecular diagnostics identified heterozygous *LMNA* mutations in both parents, who were unaware of consanguinity. The father had a mild lipodystrophy build without other disease characteristics; the mother had hypertension and hypercholesterolemia without the typical build.
Conclusion:Homozygous *LMNA* mutations causing lipodystrophy show slight intrafamilial variability in phenotype and do not seem to cause a more severe phenotype than heterozygous *LMNA* mutations.

Y.M. Hoedemaekers: None. **L.P.V. Berger:** None. **J. Langendonk:** None. **E. Brusse:** None. **M. Michels:** None. **M. van Slegtenhorst:** None. **G. Mancini:** None. **Y. van Bever:** None.

P01.092

Loeys-Dietz syndrome caused by a *TGFB2* mutation: further delineation of the phenotype

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Loeys-Dietz syndrome (LDS) is a rare autosomal dominant connective tissue disorder which has many features in common with Marfan syndrome (MFS). The disease is typically characterized by the triad of arterial tortuosity and aneurysms, hypertelorism, and bifid uvula or cleft palate. In most cases, LDS is caused by heterozygous mutations in genes encoding positive effectors of TGFβ signaling, including the subunits of the TGFβ receptor *TGFBR1* and *TBFBR2* or *SMAD3.* Most recently, we showed that loss-of-function mutations in the gene encoding the TGFβ2 ligand can also cause a phenotype within the LDS spectrum.
Here we describe the phenotypic characteristics of a large family (n=9 affected individuals) with a *TGFB2* mutation (c.1097 C>A; p.Pro366His). Within this family we observe a variable clinical expression with near absent to severe cardiovascular involvement. The main clinical features shared with MFS include aortic aneurysm, pectus deformity, arachnodactyly, scoliosis and skin striae. Clinical features other than in MFS include hypertelorism, bifid uvula, bicuspid aortic valve, arterial tortuosity, club feet and thin skin with easy bruising. Ectopia lentis was not observed. Compared to LDS patients with a *TGFBR1, TGFBR2, SMAD3,* patients with a *TGFB2* mutation do present rather mild aortic disease, more frequent mitral valve disease and increased incidence of club feet.

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P01.094

Coexistence of pathogenic mutations in APC and NF1 in the same patient.

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Neurofibromatosis type I is a rare genetic disorder that causes benign tumors on nerves and other skin and bone manifestations. NF1 gene mutations cause this disease. Familial adenomatous polyposis (FAP) is a rare inherited disease characterized by the appearance of more than 100 colonic polyps. APC mutations are responsible of increased risk of FAP.
We report the case of a 20 years old woman with pink polypoid lesions associated with a possible neurofibromatosis type I. The patient had a preventive total colectomy due to maternal inherited, surgeries due to cysts and epidermal Trichilemmal and a abdominal laparotomy due to a desmoid tumor.
We analyzed DNA extracted from peripheral blood and from neurofibroma. The neurofibroma showed the presence of a pathogenic NF1 mutation (c.5418_5422delGGGC / p.Q1806QfsX) that produces a stop codon, resulting in a truncated protein responsible of the disease. Moreover, analysis of APC showed a second pathogenic mutation (c.3783_3785delTT/p.T1261TfsX) that produces a stop codon in the position 1273. The resulting a truncated protein is believed to cause increased risk of FAP. Neither of the two mutations were described previously.
Thus we present a case of a woman carrying a germline mutation in the APC gene and a somatic mutation in the NF1 gene that could generate a somatic mosaicism and explain the presence of two hereditary diseases in the same patient.
Supported FIS PI 10/00219

Pathogenic mutations		
Sample	APC study	NF1 study
DNA from peripheral blood	c.3783_3785delTT/p.T1261TfsX	No mutations
DNA from tumor	c.3783_3785delTT/p.T1261TfsX	c.5418_5422delGGGC/p.Q1806QfsX

C. Jimenez Criado: None.

P01.095

Neurofibromatosis type 1 gene mutation analysis using sequence capture and high throughput sequencing.

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Background: Neurofibromatosis type 1 syndrome is caused by mutations in the *NF1* gene. More than thousand pathogenic mutations have been published. At present, diagnostic laboratories use traditional sequencing methods, which often require fresh blood, or tissue sample.
Aims: The aim of this study is to develop an *NF1* mutation analysis method which does not require invasive sampling and is performed with lower costs than the traditional methods.
Methods: Genomic DNA (gDNA) of 16 NF1 patients was isolated from saliva using saliva sampling (Oragene, DNA Genotek). *NF1* exome with additional 50 nucleotides of flanking intronic sequences was captured and enriched using SeqCap EZ Choice Library protocol (Roche Nimblegen). Captured DNA was sequenced with Roche/454 GS Junior system in two sample sets of different size (6 and 10 samples).
Results: The average coverage of targeted regions was 41x (10 samples) and 74x (6 samples). A putative mutation was discovered in 10 samples out of the total 16. In addition, evidence of one known microdeletion was observed.
Conclusions: Our study provides proof of principle that the sequence capture methodology combined with high-throughput sequencing is applicable to *NF1* mutation analysis. The advantages include that saliva sampling is noninvasive and the samples can be sent to the laboratory via regular mail. The limitations include that deep intronic mutations may be undetectable and change at the DNA level does not always predict the outcome at the mRNA or protein levels.

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P01.096

A Family With Basex-Dupré-Christol Syndrome with Persistent Milia

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Bazex-Dupré-Christol Syndrome (BDCS) is a rare X-linked dominant inherited genodermatosis with cancer predisposition, characterized by follicular atrophoderma, congenital hypotrichosis, milia and basal cell malformations.
We present a three-generation family without a diagnosis until index case was referred to our center from department of pediatrics. Index case was nine months old boy and third child of the non-consanguineous parent. He has persistent milia and congenital hypotrichosis. He has two healthy brothers. His mother and maternal aunt had milia until age 2. There is a family history of congenital hypotrichosis, follicular atrophoderma and basal cell carcinoma on his mother, maternal grandfather and grandaunt and maternal cousins. The differential diagnosis of BDCS includes other hereditary syndromes associated with the development of BCCs at early age, such as basal cell nevus syndrome, Rombo syndrome, Oley syndrome and Xeroderma pigmentosum.
Based on the clinical findings of index case, family history and inheritance pattern we diagnosed the family as BDCS. To the best of our knowledge, about 20 families referred to as BDCS and this is the third family from Turkey with BDCS.

A. Kuskucu: None. **M. Ugras:** None. **A. Vitrinel:** None.

P01.097

Familial presentation of Cartilage-Hair Hypoplasia (Metyaphyseal chondrodysplasia Mckusick type)

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INTRODUCTION:
Cartilage-hair hypoplasia (CHH) is an autosomal recesive disorder affecting the bone metaphyses, causing small stature from birth and sometimes immune deficiencies. The CHH-AD spectrum includes a range of phenotypes: metaphyseal dysplasia without hypotrichosis (MDWH), CHH and anauxetic dysplasia (AD) with the most pronounced skeletal phenotype.
Mutations in the *RMRP* gene are responsible for the disease.
CASE:
Proband 30 months old with short stature, low weight, blonde, fine silky hair, laxity of ligaments with hypermobility of joints and short thick tubular bones. Father diagnosed of metaphyseal chondrodysplasia Schmid type (MCDS) in childhood. Proband´s mother has normal phenotype, but several maternal relatives with clinical diagnosis of CHH. No evidence of consanguinity. Sequence analysis of the *RMRP* showed the following results:
Proband: *RMRP* c.70A>G (homozygous)
Father: *RMRP* c.70A>G and c.4C>T (compound heterozygous)
Mother: *RMRP* c.70A>G (heterozygous)
Genetic study was extended to the family of the mother. Neither of her two sisters was carrier of the mutation c.70A>G. but both of them were c.4C>T (heterozygous). The molecular study of their parents showed that the mother was compound heterozygous for mutations c.70A>G and c.4C>T and had short stature.
The father´s proband had normal hair and was diagnosed of MCDS. The molecular study showed that the disease was in fact MDWH, which could explain the confusion in the diagnostic.
CHH-AD disorder has a high incidence in certains populations with consaguinity (Amish 1-2:1000). In this family there was no evidence of consanguinity but they come from a small village of aproximately 10000 inhabitants.

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P01.098

RAB33B is mutated in Smith-McCort Dysplasia

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Smith–McCort dysplasia (SMC) is a rare autosomal recessive skeletal dysplasia characterized by a short limbs and trunk dwarfism with a barrel-shaped chest and specific radiological appearance. Skeletal features of SMC are identical to those of Dyggve–Melchior–Clausen syndrome (DMC) but with normal intelligence and no microcephaly. SMC and DMC were shown to be allelic disorders that both result from mutations in the *DYM* gene, which encodes the Golgi protein DYMECLIN. However, a few SMC patients remained negative in *DYM* mutation screening. Recently, autozygosity mapping and exome sequencing in a large consanguineous SMC family have allowed the identification of a missense mutation in the *RAB33B* gene. This gene encodes another Golgi protein which belongs to the large Rab family of small GTP-binding proteins involved in defined steps of vesicular transport including protein secretion and endocytosis. Here, we report a novel missense mutation in a SMC case that affects the GTP-binding domain of *RAB33B* and leads to a marked reduction of the protein. These data confirm the genetic heterogeneity of SMC dysplasia and highlight the role of Golgi transport in the pathogenesis of SMC and DMC syndromes. Our results also raise the question of whether the *RAB33B* gene would account exclusively for SMC, suggesting that mental retardation and microcephaly would be mainly associated with a complete loss-of function of the *DYM* gene. Analyses in additional cases of SMC and DMC syndromes are now required to answer this question.

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P01.099

Mutations in *COL2A1* in a Brazilian cohort of patients with typical phenotypes of the type 2 collagenoapthie spectrum

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Heterozygous mutations in *COL2A1* can produce different phenotypes, but with the same pattern known as type-2 collagenopathies. Although the clinical-radiological pattern is recognizable, the correlation genotype-phenotype still needs additional studies. In order to better understand the molecular basis of type-2 collagenopathies the aim of this investigation was to study a cohort of patients with suggestive phenotype of type-2 collagenopathies. The most phenotypes of the 2 collagen spectrum are represented in the 13 patients here reported. Molecular analysis was performed by direct sequencing of the known hot spots of the *COL2A1* according to the phenotype. So far four new mutations have been found - two point mutations (c.1438G>C - p.G480A, c.1546G>A - p.G516S) associated with SEDC, a T-to-G transversion (p.C1315G) in a patient with Torrance dysplasia (TD), and a deletion (4387_4389delATT - Ile1463del) also associated with TD. These last two mutations are placed in the C-propeptide domain. In addition, six previously described mutations were found, two transitions with a glycine substitution in the triple helix domain [SEDC and ACGII], three identical C-to-T transitions - p.R989C associated with SEMD phenotype, also called Strudwick dysplasia, and a deletion (g.899_924+2del) in a patient with Kniest dysplasia (KD). Finally, for three cases (KD, Hypochondrogenesis, and SEDC) no mutations were found on the respective hot spots and the complete sequencing of *COL2A1* is ongoing. In conclusion, four new mutations in *COL2A1* were found contributing to a better understanding about the type-2 collagenopathies spectrum.

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P01.100

Lysosomal Cathepsin A deficient mouse model shows altered vasoactive peptid Endothelin I (ET-1) level in serum and organs

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Cathepsin A is lysosomal enzyme that makes complex with α-neuraminidase and β-galactosidase. It has also carboxypeptidase activity catalyzing the hydrolysis of C-terminal peptide bond, potentially affects vasoactive peptide actions in normal and pathological conditions. In order to elucidate the biological function of Cathepsin A in vivo, previously we generated knock-in mouse model with catalytically inactive Cathepsin A protein. We showed that Cathepsin A-/- mice have significantly increased arterial blood pressure and higher level of ET-1 secreted from cultured brain cells. In this work we present our further study of ET-1 levels from serum and four organs including lung, kidney, liver and brain of 3 and 6-months old male Cathepsin A-/- mice (n=6-7) and age matched wild-type mice (n=7). Our ELISA data shows that ET-1 level is slightly higher in liver and brain tissues of 3 months as well as in kidney and brain tissues of 6 months old Cathepsin A-/- mice compared to wild-type mice. Interestingly, although there is no difference in the serum ET-1 level between 3 and 6 months old wild type mice, five times lower ET-1 level in serum of 6-months compared to 3-months old Cathepsin A-/- mice may suggest the contribution of other carboxypeptidases such as homologous Scpep1 for the degradation of ET-1 in aging mice. The detailed analysis of other vasoactive peptides including substance P in different organs of CathepsinA-/- mice will be valuable to understand better the physilogical role of Cathepsin A in the modulation of vasoactive peptides and complex processes regulating blood pressure.

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P01.101

Comprehensive characterization of a zebrafish model for pseudoxanthoma elasticum role reveals a role for the abcc6 transporter in cardiovascular development

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Background. Pseudoxanthoma elasticum (PXE) is characterized by elastic fiber calcification and fragmentation, resulting from ABCC6 mutations. Recently, upregulation of the pro-osteogenic BMP2-RUNX2 pathway was demonstrated in patients. Also, recent reports on vascular malformations in patients suggested a possible developmental role for ABCC6.
Methods and results. We used 4 zebrafish (ZF) models, targeting each of 3 abcc6 isoforms and a combined knock-down, to assess the developmental phenotype, mineralization and mineralization-associated pathways. In-situ Hybridisation demonstrated all isoforms to be mainly expressed in the pronephric ducts. Abcc6 knock-down was obtained by injecting translational blocking or splice junction morpholinos in 1-4 cell stage embryos. Morphants exhibited a delay in gastrulation. At 3 days post fertilization (dpf), curving and shortening of the tail, variable in severity, pericardial edema, decreased mobility, total body length and body pigmentation as well as underdevelopment of head and eyes were observed. Cardiovascular evaluation, using MO injection on Fli:gfp embryos demonstrated the development of a rudimentary tubular heart compared to controls, contributing to early demise of the morphants. Further, underdevelopment and delayed sprouting of multiple vessels was noted. Calcein staining of the morphants (5dpf) showed advanced skeletal mineralization compared to controls. QPCR analysis revealed upregulation of BMP2a, RUNX2a and MSXc.
Conclusion. We illustrate a distinct phenotype, affecting longitudinal growth, eye and cardiovascular development. The cardiovascular underdevelopment in the morphants corroborate the hypothesis that ABCC6 may also have an embryological role. Advanced mineralization and confirmation of BMP2-RUNX2 involvement suggest that ZF is a useful model organism for further PXE cell signalling research.

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P01.102

Two unusual manifestations in Sturge-Weber Syndrome: ipsilateral retinal vascular anomalies and cerebellum calcifications

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Introduction. Sturge-Weber syndrome is a rare genetic disorder (frequency 1:50,000 newborns) with unknown etiology. Also called encephalotrigeminal angiomatosis, it is characterized by facial hemangioma (Porto wine stain) extending to the supraorbital region and associated with eye abnormalities and leptomeningeal angiomatosis. **Objective.** To present a case of Sturge-Weber syndrome associated with unusual retinal vascular abnormalities and cerebellum calcifications. **Methods**. Case report. **Results**. A 12-year-old girl who presented at birth with a hemangioma extending from the left hemiface, including over- and periorbital regions, to the neck, ear, cheek, lower lip and chin. Ophthalmologic examination showed anomalies exclusive to the left eye: secondary glaucoma and fundus vascular anomalies: tortuous and dilated veins, arteries „in vortex" around veins and pseudoneovascularisation. CT revealed multiples calcifications in the ipsilateral cerebellum. **Conclusion**. The case presented shows two unusual manifestations of Sturge-Weber syndrome: ipsilateral retinal vascular anomalies and multiple calcifications in the ipsilateral cerebellum.

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P01.103

Association of hereditary thrombocytosis and transverse limb deficiency in three independent families with THPO mutations

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Hereditary thrombocytosis, usually inherited as a dominant trait, can be caused either by heterozygous mutations in the thrombopoietin gene (THPO) or by heterozygous mutations in the THPO receptor MPL. Germline mutations in THPO identified so far affect the 5'-UTR and remove the ORFs upstream of the regular translation start, which have an inhibitory effect on translation. As a consequence, the mutations result in increased translation of the THPO m-RNA and overproduction of platelets.
Vascular disruption is believed to be the most common cause of unilateral limb deficiency, usually a sporadic birth defect. We describe three families where thrombocytosis caused by a dominant THPO mutation is associated

with unilateral transverse limb defects in multiple affected family members. We hypothesize an involvement of THPO signaling in embryo vasculature development, since THPO was identified as a critical factor for the proliferation of the hemangioblast, the embryonic progenitor of hematopoietic and endothelial lineages. THPO excess may thus alter vasculogenesis, conferring predisposition to vascular disruptions.
We speculate that this could be possible on a specific genetic background, since the same pathogenetic mechanism (THPO overproduction) is observed in large families with isolated thrombocytosis. This speaks against a simple direct relationship between the THPO mutation and the limb defects and suggests the necessity of an additional common genetic variant in the determination of limb defects. In order to address this issue, we are undertaking NGS analysis to compare family members with thrombocytosis only and those with thrombocytosis plus limb deficiency.

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P01.104

Some novel *COL3A1* mutations with unexpected clinical phenotypes.

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COL3A1 mutations commonly cause obvious vascular EDS phenotypes; cutaneous thinning, vascular fragility and rupture, especially of large and medium sized arteries. Dermal histology, (light and electron microscopy), shows characteristic changes in collagen morphology, Collagen protein analysis from cultured skin fibroblasts may show procollagen and collagen type III deficiency. COL3A1 gene sequencing identifies helical glycine missense substitutions, exon skips or rare variably sized COL3A1 deletions. Here we describe five patients with unusual clinical phenotypes and novel COL3A1 mutations. Case 1 with a COL3A1 substitution, c.1771G>C, p.(Gly591Arg) had an unexpectedly mild clinical course, having ruptured her aorta at age 42, during a femoral embolectomy; she has survived for 25 years with no further complications. Case 2 with glycine substitution c.3256G>T, p.(Gly1086Cys) had infantile talipes equinovarus and features consistent with BHS/EDS III. A routine colonoscopy perforated his sigmoid colon, subsequent imaging showed an asymptomatic iliac artery aneurysm. Case 3 with an X position mutation c.2044G>A, p.(Glu582Lys) presented with spontaneous oesophageal rupture at age 43 and generalised external features consistent with Classical EDS. Electron microscopy showed no collagen rosettes, collagen V gene sequencing was normal. The COL3A1 mutation segregates in a large autosomal dominant pedigree, with occasional intestinal fragility. Case 4 has a splice donor site mutation c.(1923+2_1923+5del); his childhood clinical features resembled Classical EDS. Later, he perforated a diverticulum at age 19, eventually requiring a total colectomy. Case 5 with an X position mutation c.2513C>T, p.(Pro838Leu) presented with bilateral carotid artery dissection and features consistent with BHS/EDS III.

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P01.105

Mutation analysis of TGFβ pathway genes in Hereditary Hemorrhagic Telangiectasia patients in Japan: Genotype-phenotype correlations in 119 cases

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Hereditary Hemorrhagic Telangiectasia (HHT) is an autosomal dominant disease characterized by recurrent epistaxis, mucocutaneous telangiectasia and visceral arteriovenous malformations. The majority of cases are caused by mutations in either endoglin (*ENG*) or activin receptor-like kinase 1 (*ACVRL1*) gene, while mutations in *SMAD4* are seen in patients with the combined syndrome of juvenile polyposis and HHT. We have screened for mutations in a total of 80 unselected Japanese index cases with the suspected diagnosis of HHT. Mutation analysis was performed using direct sequencing of exonic regions and gene dosage analysis with MLPA probes. *ENG* mutations and *ACVRL1* mutations were identified in 47 cases (59%) and 23 cases (29%), respectively. Two cases were associated with *SMAD4* mutations, and 1 case was caused by a mutation in the other TGFβ signaling molecule. In total, disease causing mutations were identified in 73 index cases (91%). 66% of *ENG* mutations were predicted to cause premature termination with nonsense, frameshift, or out-of -frame deletion mutations, while 65% of *ACVRL1* mutations were missense or in-frame deletion mutations. Families study added another 46 mutation-positive cases and genotype-phenotype correlation analysis was performed in a total of 119 genetically defined HHT patients. Pulmonary arteriovenous malformation (PAVF) and cerebral arteriovenous malformation (CAVF) were observed significantly more frequently with *ENG* mutation carriers than *ACVRL1* mutation carriers (PAVF: 85% vs. 30%; CAVF: 33% vs. 11%), while hepatic arteriovenous malformation was significantly more common among *ACVRL1* mutation carriers (11% vs. 45%). This is the first and largest Japanese national study with genetically defined HHT.

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Development of a novel Col3a1 transgenic mouse model for vascular Ehlers-Danlos Syndrome

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Vascular Ehlers-Danlos syndrome (vEDS) is a dominantly inherited connective tissue disorder, characterized by thin, translucent skin, easy bruising, typical facies and arterial/intestinal/uterine fragility and ruptures. Defects in type III procollagen (encoded by *COL3A1*) underlie vEDS and mostly comprise glycine-substitutions in the collagen triple-helical domain. While currently two *Col3a1*-/- mice models have been generated, no mouse model is available for the typical glycine-substitutions.
We generated a transgenic mouse model harboring the p.Gly183Ser-substitution (*Col3a1*-c.547G>A), which was identified in several vEDS patients. Five *Col3a1*-transgenic mouse lines were produced, each harboring different *Col3a1*-transgene copies, of which line 1 mice carried the highest transgene-copy number. Line 1 mice had a decreased pre-weaning survival in comparison to WT-mice (p-value<0.05) and male mice were smaller at age 12- and 16-weeks when compared to WT-mice (12 and 16.2%, respectively). Additionally, line 1 transgenic males displayed a fragile, thin skin and spontaneously developed severe transdermal wounds progressing from the shoulder area to the abdomen. Tensile strength measurements demonstrated that the mechanical properties (e.g. maximum load, extensibility, stiffness, relative failure energy) of the skin and aorta were significantly decreased, thereby confirming the observed fragility when handling both tissues. Ultrastructural data of the dermis showed a disorganised aspect of the extracellular matrix and an increased variability in collagen fibril diameter, which demonstrates disturbed collagen fibrillogenesis. Dilatation of the endoplasmic reticulum of dermal fibroblasts was also seen, which implies the involvement of ER-stress in the observed phenotype.
In conclusion, the phenotype of this transgenic *Col3a1*-mouse model faithfully recapitulates that of human vEDS.

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Emotional reactions to genetic testing for vascular Ehlers-Danlos Syndrome in young adults

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Introduction and Objectives: Vascular Ehlers-Danlos (vEDS) syndrome is a rare and autosomal dominant arterial disease. The occurrence of organ rup-

tures in young adults reduces life-expectancy. Thus genetic testing for vEDS may have an important psychological impact. Our study aimed to evaluate the emotional consequences of a positive genetic testing and to compare emotional reactions according to sex.
Population and Methods: This study included consecutive patients with a positive COL3A1 mutation diagnosed in our centre between 2003 and 2012. Our multidisciplinary testing procedure included a systematic psychiatric visit after the announcement of the molecular diagnosis. Psychiatric minutes were retrospectively reviewed for type of emotional reaction (adapted, trivialization, depression) and nature of the predominant preoccupation (transmission to offspring; limitation of physical activities; other preoccupation).
Results: Thirty-eight patients aged 18 to 40 years (n=26 females) were included in the study. Predominant reaction to diagnosis was depression, n=17 (45%), especially for women: n=16 (62%) vs. n=1(8.3%) in men. Antidepressive therapy was necessary in n=11 (29%) patients. Trivialization was observed in n=7(19%) patients: 7/12 men vs. 0/26 women (p<0.001). Risk of transmission was the first preoccupation in 19/26 women and 2/12 men, followed by limitation of physical activity, expressed by 5/12 men vs. 0/26 women (p<0.001). Depression and risk of transmission were associated.
Conclusions: Diagnosis of vEDS is associated with a high rate of depression. Emotional reactions significantly differed between genders. These findings emphasize the necessity of multidisciplinary management of vEDs genetic testing, and the adaptation of psychological support to each gender's coping mechanism.

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Natural course of pregnancy and delivery in vascular Ehlers-Danlos syndrome: a retrospective study of 37 patients and 81 pregnancies.

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Background: Pregnancy in vascular Ehlers-Danlos syndrome (vEDS) is at high risk of maternal death. There is still limited data however on the natural course of pregnancy and the peri-partum period in the course of vEDS.
Objective: To assess obstetrical events in genetically proven vEDS patients followed in a single French referral centre.
Methods: vEDS patients with a history of pregnancy were interviewed and standardized data on number and course of pregnancies, type of birth, anaesthesia and on events during pregnancy and the peri-partum period were collected.
Results: A total of 37 patients with at least one pregnancy were included in the study. Eighty pregnancies dividing into 57 life-born infants, 2 stillborn, 12 spontaneous and 1 therapeutic abortions, 7 voluntary terminations and 1 ectopic pregnancy, were identified. Median age at delivery and at molecular diagnosis was 27[24-30] and 38[29-44] years, respectively. Diagnosis was known in 9(24%) patients at the time of delivery. One fatal and 1(1.7%) non-fatal arterial event occurred. Non arterial, vEDS-related complications (colon (1), bladder (1), uterine (2) and mitral papillary muscle (1) ruptures) occurred in 6(10.2%) deliveries. Course of pregnancy was marked by an increase in prevalence of premature rupture of membranes (20%) and preterm labour (37%). Vaginal deliveries (61%) were associated with high-grade perineal tears (50%), and caesarean-sections with severe haemorrhages (29%; p=0.003). Epidural anaesthesia (63%) when performed remained uneventful.
Conclusion: Pregnancy and the post-partum period in vEDS patients are not only marked by maternal death, but also by severe maternal morbidity, regardless of type of delivery.

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Exome sequencing in a family presenting with an unclassified autosomal-recessive childhood vasculitis/stroke syndrome

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We present a family from Southern Germany with an autosomal-recessive vasculitis of unknown aetiology resulting in multiple strokes with onset at childhood as the cardinal symptom. The index case had recurrent ischemias at the age of two and nine years, accompanied by fever and skin alterations. Two brothers and one sister are also affected to varying degrees of severity, whereas one sister as well as both parents don't show any symptoms. Known causes of early-onset stroke/vasculitides were excluded based on extensive clinical evaluation and laboratory tests. We performed exome sequencing in the index case using Agilent SureSelect AllExome-Kit for enrichment and Illumina 100bp-paired-end-reads for sequencing. A total of 10.8Gb of sequence were generated with 92% of the target sequence covered ≥20x. For candidate variant identification, we analysed homozygous and compound heterozygous missense, nonsense, splice-site, stoploss and frameshift variants. We excluded variants present in dbSNP135, the 1000genomes data, the ESP dataset, and an in-house exome-database with MAF>2%. Thereby, we aimed to investigate for both private and combinations of private and rare variants with putative disease-causing compound heterozygous state. We found no private or rare (MAF<2%) variants in known stroke or vasculitis risk genes. Our approach identified 24 genes with variants which were tested for co-segregation in the family. Top candidates are *ZNF259*, possibly affecting Lp-PLA_2-levels and thus vascular inflammation, as well as *CECR1*, encoding an adenosine-deaminase-related growth factor that stimulates macrophage proliferation. Additional cases with a similar phenotype are needed in order to confirm the true causal variants in this family.

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Retinopathy in HbSS and HbSC genotypes from sickle cell disease and relationship with the -308G/A polymorphism in the TNFA gene

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Sickle cell disease (SCD) is among the most common genetic complications in the world. The more frequents genotypes are homozygous (HbSS) and double heterozygous HbSC. Ocular manifestations are long term complications of the SCD and mainly affect the SC individual, due to increased blood viscosity, besides possible inflammatory role in this process. The aim of this study was to determine the occurrence of retinopathy in HbSS and HbSC Brazilians patients and to relate this clinical event with the polymorphism -308G/A in the gene encoding pro-inflammatory protein TNF-α (tumor necrosis factor-alpha). We evaluated 303 SCD patients from Brazilian southeast region. The genotypes for the SCD were confirmed by classical tests and molecular analysis. The -308G/A (*TNFA*) polymorphism was performed by PCR-RFLP. Statistical analysis was conducted by the *Chi*-Square and Odds *Ratio* (OR) tests. 263 samples corresponded to HbSS patients and 40 to HbSC. The retinopathies occurrence was higher in HbSC patients, with 33.3% against 5.2% in HbSS group (p<0.05). The HbSC genotype was associated with retinopathy risk (OR=6.4;2.6≤CI95%≤15.9). The allele A (-308G/A-*TNFA*) was more frequent in patients with retinopathy in both groups: HbSS (10.71% against 3.38%; p=0.02) and HbSC (40.0% against 22.8%; p=0.4). Risk factor of the allele A (-308G/A-*TNFA*) in the retinopathy in HbSS group was OR=3.43 (1.1≤CI95%≤10.6). These results confirm the prevalence of ocular manifestations in HbSC patients and suggest some effect of allele A (-308G/A-*TNFA*) in the retinopathy process, probably due its elevated ability to increase cell adhesion through the recruitment of inflammatory modulators, contributing to vaso-occlusive events.

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P01.111

Maternal mortality in Vascular Ehlers-Danlos Syndrome patients : retrospective analysis of 40 french families

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Background : Vascular Ehlers-Danlos syndrome (vEDS) is a rare genetic disease caused by mutations in the *COL3A1* gene. Affected patients show increased vascular, intestinal and uterine fragility. Pregnancies are considered at high risk for vEDS patients, but there is a paucity of data on the prevalence of mortality in this population.
Objective : To assess maternal mortality (MM) in vEDS patients followed in a single French referral center.
Methods : We retrospectively reviewed medical charts and pedigrees of all vEDS patients referred to our centre. Index-cases and their female ascendants (mothers and grand-mothers) were classified as carriers, likely or unlikely transmitters according to their family pedigree. Live births in pedigrees of carriers and likely transmitters were selected for mortality calculation. Neomutations (both parents negative for *COL3A1* mutation) were excluded from analysis.
Results : A total of 85 families were identified of which 45 were neomutations. The remaining 40 vEDS families were eligible for analysis. Pedigrees of n=35 index-cases and female ascendants in these families showed 6 genetically confirmed vEDS cases and 32 likely transmitters. All these women gave birth to 116 live-born children. Maternal death occurred in n=9 parturients. Causes of death were either intra-abdominal arterial rupture (n=6), either uterine rupture (n=3). Medium age at death was 30.9 years ± 4.0 years. Maternal mortality rate was estimated about 7.3 %.
Conclusion : These findings confirm previous maternal mortality estimations in vEDS. However, due to specific obstetrical protective measures, a possible effect of the latter could not be measured.

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P01.112

The clinical and molecular spectrum of D4ST1-deficient Ehlers-Danlos Syndrome

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Recently, we described Ehlers-Danlos syndrome (EDS), musculocontractural type (former EDS-type-VIB), an autosomal recessive (AR) EDS-subtype caused by loss-of-function mutations in *CHST14*, encoding dermatan-4-sulfotransferase-1 (D4ST1). D4ST1 catalyzes 4-O-sulfation of the glycosaminoglycan dermatan-sulfate (DS), which is covalently linked to proteoglycans. Loss of D4ST1-activity results in the replacement of DS by chondroitin-sulfate on the decorin proteoglycan and leads to impaired collagen fibril assembly.
CHST14-defects have previously been associated with the 'adducted-thumb-clubfoot-syndrome' and the EDS-Kosho-type. Based on their clinical and molecular overlap, these entities likely represent one phenotypic continuum, referred to as 'D4ST1-deficient EDS'. Here, we present a comprehensive phenotypic characterization and novel homozygous *CHST14*-mutations in three new D4ST1-deficient EDS patients to highlight the severe and pleiotropic presentation of the disorder and to further document its molecular basis. Characteristic clinical manifestations are the craniofacial abnormalities, severe skin fragility with dystrophic scarring, and easy bruising, excessive palmar wrinkling, tapered fingers, congenital joint contractures with distal joint hyperlaxity, clubfeet, and early-onset eye abnormalities, including glaucoma, cataract and retinal detachment.
Despite clinical overlap with several other recessive EDS subtypes such as EDS-type-VIA, spondylocheirodysplastic EDS, FKBP14-deficient EDS, and progeroid EDS, the condition can be recognized by its characteristic craniofacial features, congenital joint contractures with adducted thumb and clubfeet, and severe gastrointestinal and urogenital manifestations. The mutation spectrum includes homozygous and/or compound heterozygous nonsense, missense and frameshift mutations, all causing loss of D4ST1 function. By linking EDS to genetic defects in proteoglycan synthesis, this disorder further highlights the broad genetic heterogeneity of the EDS spectrum.

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A complex rearrangement of the OCA2 gene in two unrelated albino patients

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Oculocutaneous albinism (OCA) is an autosomal recessive disease characterized by hypopigmentation of the skin, hair and eyes. Molecular analysis allows to classify patients, depending on the gene that is mutated, into OCA1 (*TYR* gene), OCA2 (formerly called P gene), OCA3 (*TYRP1*) and OCA4 (*SLC45A2*). Deletions and duplications are investigated using ultra-high resolution array-CGH, in which the 4 genes are covered at a density of 1 probe/102bp on average. We report two patients with a complex rearrangement. Array-CGH analysis indicated the presence of two heterozygous deletions restricted to introns 2 and 20 respectively, the intervening segment being present in two copies. Both deletions were inherited from her mother, thus indicating that both deletions were on the same allele. Patient 2 was heterozygous for the same deletions of introns 2 and 20 as patient 1. Remarkably, the 5' breakpoint of the intron 2 deletion and the 3' breakpoint of the intron 20 deletion seemed to be at the same position as the breakpoints of an intron 2-20 deletion that previously identified in three patients of polish origin. The same haplotype as that associated with the intron 2-20 deletion in the polish patients was observed. A PCR-sequence analysis performed with primers located on each side of the intron 2-20 deletion confirmed that patients 1 and 2 indeed harbored the "polish" deletion. FISH analysis showed that the intervening segment was inserted near the *OCA2* gene. High throughput sequencing of the entire 15q11-13 region revealed a complex rearrangement in *OCA2* gene.

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KRT74 mutation associated with autosomal recessive hair-nail ectodermal dysplasia

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Pure hair and nail ectodermal dysplasia (PHNED) comprises a heterogeneous group of rare heritable disorders characterized by thin and brittle hair, hypotrichosis, onychodystrophy and micronychia. PHNED has previously been associated with mutations in *KRTHB5* and *HOXC13* on chromosome 12p11.1-q14.3. We have investigated a consanguineous Pakistani family with autosomal recessive PHNED linked to the keratin gene cluster on 12p11.1 but without detectable mutations in *KRTHB5* and *HOXC13*. Exome sequencing of affected family members revealed three linked genes with missense variants that were excluded in a control population. One of these variants (c.T821C, p.Phe274Ser) is located in the *KRT74* gene expressed in hair follicles and nail matrix. The *KRT74* variant segregates in a homozygous state in all affected family members, whereas all heterozygous carriers have normal hair and nails. Heterozygous *KRT74* mutations have previously been associated with autosomal dominant hypotrichosis simplex and wooly hair (HSWH). Our results suggest for the first time that homozygosity for a *KRT74* missense mutation causes autosomal recessive PHNED. These findings expand the phenotypic spectrum associated with *KRT74* mutations and imply that autosomal recessive PHNED and autosomal dominant HSWH are allelic conditions.

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Novel ZMPSTE24 (FACE1) mutations in patients affected with Restrictive Dermopathy or related progeroid syndromes and mutation update.

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Restrictive dermopathy (RD) is a rare and extremely severe congenital genodermatosis, characterized by a tight rigid skin with erosions at flexure sites, multiple joint contractures, low bone density and pulmonary insufficiency generally leading to death in the perinatal period. RD is caused in most patients by compound heterozygous or homozygous *ZMPSTE24* null mutations. This gene encodes a metalloprotease specifically involved in lamin A post-translational processing. Here, we report 7 novel *ZMPSTE24* mutations, identified in classical RD or Mandibulo-acral Dysplasia (MAD) affected patients. We also report 9 new families with one or two affected children carrying the common, homozygous thymine insertion in exon 9 and demonstrate the lack of a founder effect. Additionally, we describe several new *ZMPSTE24* variants identified in unaffected controls or in patients affected with non-classical progeroid syndromes. This mutation update includes a comprehensive search of the literature on previously described *ZMPSTE24* mutations and associated phenotypes. Moreover, pathophysiological mechanisms underlying RD and other premature aging syndromes linked to *ZMPSTE24* are deeply discussed, confirming a general rule: RD mutations lead to complete loss-of-function of ZMPSTE24, whereas other less severe phenotypes are associated with at least one haploinsufficient allele.

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P01.116

The class II cytokines and psoriasis: a case-control study and meta-analysis

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Background: The molecular basis of pathogenesis of psoriasis remains unclear, but one unifying
hypothesis of disease aetiology is the cytokine network model. The class II cytokines (CF2) and their receptors (CRF2) are all involved in the inflammatory processes and single nucleotide polymorphisms (SNPs) in respective genes have been associated with psoriasis in a previous study of the Estonian population.
Methods: DNA was obtained from 395 psoriasis patients of two ethnic groups from the Volga-Ural region of Russia and 476 ethnically matched controls. 47 SNPs in the loci of the CF2 genes and CRF2 were selected by SNPbrowser version 3.5. Genotyping was performed using the SNPlexTM platform.
Results: The genetic variant rs30461 previously associated in original case-control study in Estonians, was also associated in Russians (Pc = 0.008, OR = 0.44), but did not reach statistical significance in the Bashkir population. Combined meta-analysis of three populations, including 943 psoriasis patients and 812 healthy controls, showed that the IL29 rs30461 C-allele was not associated with decreased risk of psoriasis (P = 0.165, OR = 0.68). Moreover, stratification of studies by ethnicity revealed a significant association in the European cohort (P = 9.506E-006, OR = 0.53).
Conclusion: Therefore, there is no overall evidence of association between psoriasis and SNP rs30461 of the IL29 gene, but there is some evidence to suggest that an association exists in Europeans. However, this current concept should be considered as preliminary and the results need to be confirmed in future independent studies.

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P01.117

Antisense mediated exon skipping gene therapy for dystrophic epidermolysis bullosa

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Dystrophic Epidermolysis Bullosa (DEB) is a group of genodermatoses inherited in a dominant (DDEB) or recessive (RDEB) manner, characterised by severe skin and mucosae blistering after mild trauma. DEB is caused by mutations in *COL7A1* encoding type VII collagen that assembles into anchoring fibrils to form key dermo-epidermal adhesion structures. Exon skipping relies on modulating the splicing of pre-messenger RNA to induce skipping of a mutated exon. Exons 73 and 80 of *COL7A1* are of particular interest since they carry many recurrent mutations and their excision preserves the open reading frame. We first demonstrated the dispensability of these exons in an *in vivo* xenograft model using collagen VII null RDEB cells transduced with retroviral vectors containing *COL7A1* cDNAs deleted of exon 73 or exon 80. We subsequently transfected primary RDEB keratinocytes and fibroblasts with antisense oligoribonucleotides (AONs) targeting key splicing regulatory elements of these exons, and achieved efficient skipping (up to 90%). Western blot and immunocytochemistry analyses demonstrated significant type VII collagen re-expression (up to 24%) in cells from three RDEB patients which were completely deficient in type VII collagen expression before transfection. We have now grafted RDEB skin equivalents generated from patients' cells and have injected several doses of AONs to address *in vivo* efficiency. A humanized transgenic mouse carrying only the human *COL7A1* gene will also be used for pre-clinical studies to evaluate toxicity and routes of administration. If successful, this approach would offer potential for treating RDEB patients by local or systemic delivery of AONs.

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Revertant mosaicism in the first case of recessive Epidermolysis Bullosa Simplex due to *KRT5* loss of function mutations

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Epidermolysis bullosa simplex (EBS) is characterized by intra-epidermal blistering upon mild trauma. Most EBS cases are caused by dominant missense mutations in *KRT5* or *KRT14* encoding keratin 5 and keratin 14, but few cases of recessively inherited EBS due to *KRT14* mutations have been reported. In this study, we report the case of a 14 months-old girl from unrelated healthy parents, presenting with severe and extensive skin blistering at birth with partial improvement during childhood and patches of non-blistering skin. Direct sequencing of *KRT5* from blood leukocyte DNA of the proband and her parents revealed two recessive loss-of-function mutations leading to frameshift and PTC: 1) a two base-pair deletion in exon 3 inherited from the mother; 2) a T>C transition deep within intron 2 inherited from the father. The latter disrupts *KRT5* pre-mRNA splicing and leads to the inclusion of a 41 nucleotides pseudo-exon through imbalanced binding of SRp40 and hnRNPA1 splicing factors. Western blot analysis from patient keratinocytes demonstrated reduced level of full-length keratin 5 indicating that the intronic mutation is leaky, rescuing the synthesis of a small amount of wild-type protein. Splicing modulation using an antisense oligoribonucleotide targeting the mutation restored the synthesis of wild type *KRT5* mRNA and keratin 5 protein. Remarkably, direct sequencing, RT-PCR and immunohistofluorescence analyses of islands of non-blistering skin demonstrated somatic revertant mutations. This is the first reported case of recessive EBS due to *KRT5* mutations, demonstrating gene dosage effect in EBS and presenting with revertant mosaicism.

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P01.119

Filaggrin mutations in Slovenian patients with atopic and contact dermatitis

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Dysfunction of epidermal barrier contributes significantly to the development of common skin diseases, such as atopic dermatitis (AD), allergic contact (ACD) and chronic irritant contact dermatitis (CICD). One of the most important proteins, involved in generation and maintenance of epidermal barrier, is filaggrin, encoded by the filaggrin gene (*FLG*), located within the epidermal differentiation complex on chromosome 1q21.
Four prevalent loss-of-function mutations in the *FLG* gene (R501X, 2282del4, R2447X and S3247X) were genotyped in 241 AD, 100 ACD and 44 CICD patients and in 164 healthy controls from Slovenia.
The most frequent *FLG* mutation 2282del4, but not R501X, R2447X or S3247X, was significantly associated with greater risk for AD (OR=4.33, 95% CI: 1.26-14.96, P=0.012). Furthermore, subgroup analysis among AD patients revealed that carriers of *FLG* loss-of-function mutation were more likely to have earlier onset (<2 years: OR=8.09, 95% CI: 2.19-29.87, P=0.0006) and longer persistence of the disease (>20 years: OR=15.54, 95% CI: 4.13-58.44, P<0.0001) as well as they more often required hospitalisation (OR=12.38, 95% CI: 3.36-45.60, P<0.0001). On the other hand no association between *FLG* mutations and ACD or CICD, were found.
The *FLG* loss-of-function mutations that contribute to the impaired epithelial barrier function, increases the risk for development of AD, but not ACD or CICD, in Slovenian patients. *FLG* mutations are also a predisposing factor for a more severe AD phenotype, leading to earlier onset, longer persistence and hospitalisation. Genotyping of *FLG* mutations is helpful for identification of patients at higher risk for severe and more persistent disease.

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Mutations in *IL36RN* in patients with generalized pustular psoriasis

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GPP is a rare disorder affecting 2 of 1 million individuals in Europe. It is characterized by repeating flare-ups of pustular psoriasis and a potentially life-threatening accompanying multisystemic inflammation. Recently, missense mutations in IL36RN were identified for GPP. We screened IL36RN in 19 GPP patients and identified mutations in 7 patients: 4 homozygous for p.His32Arg, p.Pro76Leu or p.Ser113Leu (twice), and 3 patients compound heterozygous for p.Ser113Leu and either p.Arg48Trp, p.Glu94X or p.Pro76Leu. p.Glu94X as well as the 2 missense mutations p.His32Arg and p.Pro76Leu affecting highly conserved residues were novel. Molecular modeling of missense mutations revealed effects on protein stability or binding to IL-36 receptor. The newly identified mutations were not present in 190 controls. Available parents were tested as heterozygous mutations carriers. 1 further patient was heterozygous for p.S113L, while neither he nor 11 patients without IL36RN mutations had an intragenic deletion. Phenotype genotype correlation was difficult due to different mutations in few carriers. No significant difference in age of onset between noncarriers/ carriers of mutations was observed. The widely varying age of onset (1-51 y.) in 4 homozygous patients for p.Ser113Leu (2 previously published) can currently not be explained. An additional coding variant (p.Ser200Asn) in *CARD14* was identified in one of them (aoo.: 1 year) which might modify disease severity. The rate of 35.2% IL36RN mutation carriers provides further evidence that GPP is heterogeneous. As therapy with anakinra in some GPP patients is effective, revealing the molecular basis of GPP in single patients is important for therapeutic decision-making.

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Novel γ-secretase gene mutations in familial Hidradenitis suppurativa / Acne inversa and evidence for genetic heterogeneity.

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Hidradenitis Suppurativa / acne inversa (AI) is a chronic, inflammatory, recurrent, debilitating skin follicle disease that usually presents after puberty with painful, deep-seated, inflamed lesions in the apocrine gland-bearing areas of the body, most commonly the axillaries, inguinal, and anogenital regions. Recently, mutations of *NCSTN*, *PSEN1* and *PSENEN* genes located at 1q23.3, 19q13.12 and 14q24.2, respectively, have been reported in sporadic and familial AI patients, suggesting that these genes play an important role in AI development. Here, we report two novel mutations of *NCSTN* in two multi-generational Indian families with AI and various associated anomalies including squamous cell carcinoma. Mutation search in exons and splice junctions of above three candidate genes identified a frameshift mutation, c.687insCC (p.Cys230ProfsX31), which is predicted to terminate at codon 261 after the addition of 31 novel amino acids within exon 6 of *NCSTN*. A nonsense mutation due to a 2-nt deletion, resulting in a stop codon (L600X), within exon 16 of *NCSTN* was found in family UR-253. It is known that *NCSTN* is subunit of the γ-secretase complex, an endoprotease complex that catalyzes the intramembrane cleavage of integral membrane proteins such as Notch receptors and APP (β-amyloid precursor protein). We did not detect a pathogenic or predisposing variant in any candidate genes in another three multigenerational AI families, indicating genetic heterogeneity in AI. Identification of present novel mutations expands *NCSTN* gene mutation spectrum associated with AI phenotype. The present data supported the significance of γ-secretase dysfunction in the etiology of AI.

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P01.122

IP-associated NEMO mutations as a tool to unravel the key molecular mechanisms in NF-kappaB activation

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Mutations of the gene coding for NEMO has been shown to cause the X-linked inherited disease Incontinentia Pigmenti (IP). IP presents with skin lesions appearing at birth and evolving in four typical inflammatory stages. In addition, other neuroectodermal tissues can be affected. NEMO is the regulatory subunit of the kinase complex IKK, required for the canonical NF-kappaB activations. Further progress on the comprehension of the IP physiopathology depends also on the detailed understanding of the mechanisms of action of NEMO mutated protein in the context of IP pathology.
Starting from the NEMO screening in a large cohort of IP patients from EU we investigated the IP physiopathology by studying selected mutations in several cell lines.
We described a NUB (NEMO Ubiquitin Binding) domain mutation, A323P that we found in a patient with a severe form of IP. This substitution caused a defect in the non-degradative ubiquitination process and, consequently, drastically decreased NEMO-dependent NF-kappaB activation. Combining these evidences to the molecular study of an N-terminal missense mutation (E57K), we finally characterized the domains participating in NEMO/TRAF6 interaction, providing genetic and molecular evidences supporting the role of this physical interaction in NF-kappaB activation. More important, dissecting specific molecular interactions within the NEMO-related activity, not only as NF-kappaB regulator, could suggest new targets for the development of potentially effective therapeutic strategies.
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P01.123

Naturally occurring keratoderma in dog breeds as relevant models for human palmoplantar keratoderma.

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In humans, inherited palmoplantar keratoderma is a complex heterogeneous group of genodermatoses clinically presenting as hyperkeratosis of the palms and soles. Genetic causes of non syndromic autosomal recessive keratoderma are not well known except for Meleda's disease. In dogs, footpad hyperkeratosis naturally segregates mainly in two breeds, Dogue de Bordeaux and Irish Terrier. The onset usually occurs in puppies of six months old or younger. Clinical signs consist of dermatological lesions affecting footpads and/or nose showing thickening with severe keratinous proliferations and fissures. These two breeds having concentrated deleterious alleles due to the drastic selection of these breeds. Pedigree analyses led us to hypothesize a monogenic recessive inheritance of the disease in each breed. The genetic causes of the disease in the two dog breeds are searched by genetic linkage and genetic association studies. We collected blood samples from 100 Dogues de Bordeaux, including 30 affected dogs and 33 Irish Terrier, including 12 affected dogs. We performed a Genome Wide Association Study in both breeds using the recently available Illumina Canine HD 170,000 SNPs array. To date, most of the genes known to be involved in human Palmoplantar Keratoderma, have been excluded by genetic linkage, allowing to hypothesize that novel genes could be discovered in the two dog breeds. These approaches, in addition to comparative genetics, bring alternative spontaneous models and also open the field for clinical trials for the benefit of dogs and humans.

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Molecular study in a patient with *Incontinentia pigmenti*

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Introduction
Incontinentia pigmenti (IP) is a rare X-linked dominant genodermatosis (Xq28), lethal in the majority of affected males in utero and variably expressed in females due to lyonization phenomenon. The diagnosis of IP is based on clinical findings and molecular genetic testing of *IKBKG* (*NEMO*) gene. It consists of 10 exons and encodes a protein of 419 amino acids. Approximately 65% of affected females present a 11.7 kb deletion that removes exons 4-10 of the *NEMO* gene.
Subjects and Methods
A one and a half year old female patient, with healthy and non-consanguineous parents, was dignosed with IP by skin biopsy. The karyotype was normal, 46XX. Multiplex PCR and electrophoresis were performed to identify the common deletion. In addition, sequencing analysis of all the 10 exons was performed. The X chromosome inactivation (XCI) pattern was determined by PCR amplification at the Androgen receptor locus (HUMARA).
Results
Deletion of exons 4 to 10 was not detected. Moreover, no additional variants were found. The XCI showed a partial skewing: 16% / 84%.
Discussion
IP is a severe systemic pathology that requires multidisciplinary follow-up, particularly during the first year of life. Currently, *NEMO* is the only known gene associated with IP, and it is responsible of 73% of the cases. cDNA sequencing analysis will be analyzed. Due to the etiology is still unknown in the remaining 27%, it suggests that other genes should be further investigated.

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P01.125

Porphyria Cutanea Tarda (PCT): An exploratory focus group study

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PCT is characterized by fragile skin with blistering on sun exposed areas, and exists in both hereditary and sporadic forms. Symptoms typically develop in late adulthood and can be triggered by iron overload, alcohol intake, estrogens and various liver diseases. Treatment consists of phlebotomy to reduce iron, or to increase urinary porphyrin excretion by administering chlorochin. The Norwegian Porphyria Centre (NAPOS) offers predictive genetic testing and counseling to at-risk family members in hereditary cases.
The aim was to explore what experiences persons with PCT have concerning symptoms, treatment, prevention and follow up. Interpretive description was used as a qualitative approach. Three focus groups were held, each consisting of seven participants, 11 women and 10 men aged between 31 and 77. Eleven participants had hereditary PCT, and all had experienced symptoms during the last five years.
Participants' symptoms varied from fragile skin to what was described as a desperate situation, with huge blisters, skin falling off and feeling as if in a "horror movie". Treatment with phlebotomy did cause rapid improvement and made the situation less dramatic. PCT was perceived as being in the blood, rather than a skin disease, and made participants contemplate if other health issues were caused by PCT, and if there were more to this disease than the specialist knew. Although most participants had not experienced relapses, they regarded PCT as a chronic disease, requiring controls and prophylactic phlebotomy for the reminder of their lives.

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Frame shift mutation of the ZMPSTE24 gene in two siblings affected with restrictive dermopathy

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Restrictive dermopathy (RD) is a rare lethal autosomal genodermatosis. We report on 2 siblings from consecutive pregnancies affected with RD. The proband was born at 28 weeks of gestation to a 27-year-old healthy woman whose first child died due to respiratory insufficiency on his 16th day, falsely diagnosed with congenital ichthyosis by dermatologist. There were no further investigations performed for him at that time. Clinical symptoms of the first child showed the same morphology as that of the second baby. Physical examination of the proband showed extensive areas of tight, shiny, translucent skin with multiple fissures on the neck and shoulders and prominent cutaneous vessels. Face showed the characteristic dysmorphic features: hypertelorism, antimongoloid slant, sparsed eyelashes, small pinched nose, micrognathia, mouth O-shaped, multiple joint contractures. The patient died on 23rd day after birth due to pulmocardial insufficiency.
Skin biopsy microscopic examinations revealed evenly thickened epidermis, hypergranulosis with large keratohyalin grains and decrease of the elastic fibers in the dermis, undeveloped sweat glands.
The DNA of the proband and his parents were extracted from the venous peripheral blood samples using phenol-chlorophorm DNA extraction method. The sibling DNA was extracted from dried blood spot sample (Guthrie card) using the InstaGene™ Matrix (BIO-RAD Laboratories, Inc., USA). Amplification using specific primers for 10 exons of the *ZMPSTE24* gene was performed for all samples.
Homozygous frame shift mutation c.50delA (p.[Lys17Serfs*21];[Lys17Serfs *21]) was identified in exon 1 of the *ZMPSTE24* gene for both affected children. The parents genotypes were heterozygous for this mutation.

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Retinitis Pigmentosa, Cutis Laxa and Pseudoxanthoma Elasticum-Like Skin Manifestations Associated with *GGCX* mutations

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We describe the clinical findings and molecular results in 13 affected members of two families who had a remarkably consistent phenotype consisting of pseudoxanthoma elasticum (PXE)- like skin manifestations in neck and trunk, cutis laxa of trunk and upper limbs, and retinitis pigmentosa. They had no coagulation abnormalities.
No mutation was identified in *ABCC6* known to cause PXE. Subsequently, all 13 affected family members were found to be homozygous for the splice-site mutation c373+3G>T in the Gamma-Glutamyl carboxylase (*GGCX*) gene. Parents were heterozygous and healthy siblings were either heterozygous or had the wild-type. The two families were not known to be related, but originated from the same geographic region suggesting a founder effect.
GGCX mutations have been reported in patients with a PXE-like phenotype and cutis laxa before in patients who also had multiple Vitamin K-dependent coagulation factor deficiencies and no retinitis pigmentosa. We suggest that the present affected individuals represent a hitherto unreported phenotype associated with *GGCX* mutations, as the presence of two independent disorders co-segregating in 13 family members and not occurring in an isolated way in other family members us unlikely. Di-genic inheritance has been suggested to explain the variations in phenotype in *GGCX* mutation carriers. Consequently, the present unusual phenotype may not be explained by the GGCX mutations alone but may be influenced by variants in other genes or epigenetic and environmental factors.

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Odonto-Onycho-Dermal Dysplasia/Schopf-Schulz-Passarge Syndrome spectrum: An interesting case.

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We present a case of a 38-year-old female from Newfoundland with a multitude of symptoms pertaining to her hair, nails, teeth and skin. For many years, she has had slow growing, fine, brittle hair on her scalp and scarring alopecia. Since childhood, she has had dystrophic finger and toenails and previously had dental work for peg shaped teeth. Furthermore, she has always had diminished sweating and palmo-plantar keratoderma. Recently, she has been diagnosed with a melanoma in-situ and eyelid cysts. Dysmorphology exam noted a bird-like facies.
This patient's presentation is consistent with features of both Odonto-Onycho-Dermal Dysplasia (OODD) and Schopf-Schulz-Passarge syndrome (SSPS). There is considerable overlap in clinical features between the two conditions. Both types of ectodermal dysplasia are autosomal recessive and have been associated with homozygous and compound heterozygous mutations of the WNT10A gene. Our patient was found to be homozygous for the Cys107X mutation in WNT10A. This particular mutation has been reported in cases of both OODS and SSPS suggesting that features of these two conditions are part of a spectrum connected by the same gene. Basal cell and squamous cell carcinomas have been reported in SSPS. Unique to this case is the occurrence of melanoma in situ. To our knowledge this is the first case of a WNT10A gene mutation reported in the Newfoundland population.

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The transglutaminase 5 mutations are frequent among Polish patients with acral skin blistering

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Localized Simplex Epidermolysis Bullosa (EBS-loc., EBS Weber-Cockayne) is caused by mutations in keratins *5* and *14*. However, in 50% of patients these mutations are not found despite acral skin blistering presence. This symptom is also present in another condition - acral peeling skin syndrome (APSS) caused by mutations in a *TGM5* gene encoding transglutaminase 5. So far only 24 APSS cases have been reported worldwide and no epidemiological data for Slavic population are available.
The aim of the study was to verify the hypothesis that *TGM5* mutations occur in Polish patients with EBS-loc. clinical suspicion, no mutations in *KRT5, KRT14* and no family history of blistering disorders.
DNA from 20 unrelated probands was analyzed by direct sequencing of the *TGM5.*
The Gly113Cys mutation was detected in 55% (22/40) alleles of affected patients (9 homozygous, 4 heterozygous cases) and in 2 (2%) healthy controls (n=103). A novel mutation - c.331delG was found in three proband's alleles. Molecular studies are currently being pursued.
Our results indicate that *TGM5* mutations are common in Polish patients with the clinical suspicion of the EBS-loc., which strongly supports the idea of the *TGM5* molecular analysis incorporation into the first line diagnostic procedure in such cases. We showed for the first time that Gly113Cys mutation is common in Polish patients and we identified previously unreported recurrent mutation - c.331delG. High frequency of Gly113Cys in control population may also suggest that APSS incidence is highly underestimated in Poland.

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P01.130

No mutations in the serotonin related TPH1 and HTR1B genes in patients with monogenic sclerosing bone disorders.

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Since the identification of LRP5 as the causative gene for the osteoporosis pseudoglioma syndrome (OPPG) as well as the high bone mass (HBM) phenotype, LRP5 and the Wnt/β-catenin signaling have been extensively studied for their role in the differentiation, proliferation and apoptosis of osteoblasts and osteocytes. However, more recently the direct effect of LRP5 on osteoblasts and bone formation has been questioned. Gene expression studies showed that mice lacking lrp5 have increased expression of tryptophan hydroxylase 1 (tph1), the rate limiting enzyme for the production of serotonin in the gut. Furthermore, mice lacking either tph1 or htr1B, the receptor for serotonin on the osteoblasts, were reported to have an increased bone mass due to increased bone formation. This led to the hypothesis that LRP5 influences bone formation indirectly by regulating the expression of thp1 and the production of serotonin in the gut. Based on these data we decided to evaluate the role of TPH1 and HTR1B in the development of craniotubular hyperostoses, a group of monogenic sclerosing bone dysplasias. Using Sanger sequencing, we screened the coding regions of both selected genes in 53 patients with a form of craniotubular hyperostosis which lack a mutation in the known causative genes LRP5, LRP4 and SOST. We found several common and rare coding variants in both studied genes. However, we could not identify disease-causing variants in neither of the tested genes. Therefore, we cannot provide support for an important function of serotonin in the pathogenesis of sclerosing bone dysplasias.

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P01.131

ZNF750 is expressed in differentiated keratinocytes and regulates epidermal late differentiation genes

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Disrupted skin barrier due to altered keratinocyte differentiation is common in pathologic conditions such as atopic dermatitis, ichthyosis and psoriasis. However, the molecular cascades governing keratinocyte terminal differentiation are not fully understood. We have previously demonstrated that a

dominant mutation in ZNF750 leads to a clinical phenotype reminiscent of psoriasis and seborrheic dermatitis. Here we show that ZNF750 is a nuclear effector that is strongly activated in and essential for keratinocyte terminal differentiation. ZNF750 was specifically expressed in the epidermal suprabasal layers and its expression was augmented during differentiation, both in human skin and in-vitro, peaking in the granular layer. Silencing of ZNF750 in differentiated HaCaT keratinocytes led to sustained proliferation, as well as diminished apoptosis. Genome-wide expression analysis of keratinocytes in which ZNF750 expression was silenced, showed markedly reduced expression of epidermal late differentiation genes, including gene subsets of epidermal differentiation complex and skin barrier formation such as FLG, LOR, SPINK5, ALOX12B and DSG1, known to be mutated in various human skin diseases. Furthermore, overexpression of ZNF750 in undifferentiated keratinocytes induced terminal differentiation genes. Thus, ZNF750 is a regulator of keratinocyte terminal differentiation and with its downstream targets can serve in future elucidation of therapeutics for common diseases of skin barrier.

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P01.132

Deciphering the genetics of inherited zinc deficiencies

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Background: Acrodermatitis enteropathica (AE; MIM #201100) is a rare and severe autosomal recessive zinc deficiency disorder, characterized by acral and periorificial dermatitis, alopecia and diarrhea. In 2002, we and others identified *SLC39A4* as the AE-causing gene, the mutation of which induced a defective intestinal zinc transport. The routine testing of *SLC39A4* which we proposed in our laboratory since then showed that only 43% of patients with a clinical picture suggestive of AE presented clearly pathogenic anomalies in *SLC39A4*.
Purpose: Our goal was to determine the genetic cause of the cases unlinked to *SLC39A4*.
Method: 76 patients with a severe zinc deficiency were included: 70 with no identified *SLC39A4* mutation, seven heterozygous for a deleterious *SLC39A4* variant, and two not screened yet by the routine testing of *SLC39A4*. A custom next-generation sequencing approach was used which targeted 50 genes directly involved in zinc homeostasis.
Results: A still preliminary analysis showed that ten cases could be related to mutations in *SLC39A4*. At least 12 patients were found heterozygous carriers of a very likely pathogenic variant in other genes of zinc homeostasis, and a differential diagnosis could be established for two patients.
Conclusions: We confirm *SLC39A4* as the main causative gene of inherited zinc deficiency. Furthermore, we highlighted several genes potentially responsible for rarer forms of zinc deficiency, which could contribute to extend our knowledge on zinc physiology. As it turns out, a whole exome sequencing approach could be followed to identify new candidate genes in still unexplained AE-like cases.

S. Küry: None. **S. Schmitt:** None. **E. Blouin:** None. **S. Bezieau:** None.

P01.133

A milder phenotype of acrodysostosis-2 due to a deletion of the proximal part of the PDE4D gene?

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Background: Acrodysostosis-2 (#MIM 614613) is a rare autosomal dominant skeletal dysplasia characterized by short stature, severe brachydactyly with cone-shaped epiphyses, midface and nasal hypoplasia and spinal stenosis. Many patients have (moderate) intellectual disability, some have hormone resistance (e.g. congenital hypothyroidism). In 2012, Lee *et al*. and Michot *et al*. both identified *de novo* heterozygous missense mutations in the phosphodiesterase 4D, *PDE4D* gene (5q12)(#MIM 600129), which is involved in the cyclic AMP metabolism. All mutations were absent in controls and were predicted to be pathogenic. Functional studies were not performed.
Case: a 43 year-old female patient presented with a mild intellectual disability, autism-like behaviour and depression, congenital hypothyroidism, short hands and feet (but not too short, resembling most brachydactyly type D), pectus excavatum, mild scoliosis, short stature (-1.5SD), strabismus and carpal tunnel syndrome. Childhood pictures show underdevelopment of the middle one third of the face (no longer visible now due to facial surgery). She was found to have a *de novo* 5q12.1 deletion of 64 kb (hg18: 59783994-59848643) of which the proximal breakpoint is within *PDE4D* (promoter region and first exons).
Discussion: this is, as far as we know, the first non-missense *PDE4D* mutation identified. The deletion is expected to result in decreased *PDE4D* functioning. No functional studies were performed to explain the milder skeletal phenotype. Possibly, *PDE4D* missense mutations result in a dominant negative effect on protein analysis.
Conclusion: We present a relatively mild skeletal phenotype of acrodysostosis-2 due to a *de novo* deletion of the proximal part of *PDE4D*.

P. Lakeman: None. **L.C. Knegt:** None. **V. Cormier-Daire:** None. **R.C.M. Hennekam:** None.

P01.134

Genetic and functional studies on Hungarian families with Brooke-Spiegler Syndrome

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Brooke-Spiegler syndrome (BSS; OMIM 605041) is an autosomal dominant condition characterized by the development of skin appendage tumors, such as cylindromas, trichoepitheliomas and spiradenomas. BSS develops due to mutations in the tumor suppressor gene, CYLD. In this study we aimed to perform genetic and functional studies on Hungarian BSS families. Genetic screening of the CYLD gene identified a novel heterozygous missense mutation (c.2613C>G; p.His871Gln) in a Hungarian BSS pedigree located in Szeged. Functional studies on the novel missense mutation demonstrated, that the CYLD-interactor NEMO protein immunoprecipitated from fibroblasts carrying the mutation is more ubiquitinated than the one immunoprecipitated from control cells. Since NEMO is a well-known negative regulator of the NF-κB signaling pathway, we hypothesize that this novel missense mutation of the CYLD gene might influence the NF-κB signaling pathway. In another family screening we identified a recurrent heterozygous nonsense mutation (c.2806C>T, p.Arg936X) in a BSS family located in the region of Szekszárd. The same recurrent nonsense mutation was identified in an Anglo-Saxon pedigree in the UK (region of Newcastle). Haplotype analysis of the Hungarian and Anglo-Saxon BSS pedigrees carrying the same nonsense mutation revealed independent mutation events in the background of the disease. Our results suggest that the position of the identified recurrent nonsense mutation is a mutational hotspot on the gene.
The genetic screening of BSS pedigrees may identify the causative mutation on the CYLD gene thus we can offer presymptomatic screening and prenatal diagnosis, which may have a huge impact on family planning.

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P01.135

Kallikrein-related peptidase 5 transgenic mice recapitulate cutaneous and systemic hallmarks of Netherton syndrome, supporting a central role of this protease in the disease

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Netherton syndrome (NS) is a severe, autosomal recessive ichthyosiform dermatosis caused by mutations of the *SPINK5*-encoded lymphoepithelial Kazal-type-related inhibitor (LEKTI). Complementary studies in humans and mice demonstrate the complexity of NS pathophysiology, which involves abnormal desquamation and constitutive activation of pro-inflammatory and pro-allergic pathways. Although several proteases (kallikrein-related peptidase (KLK) 5, KLK7 and elastase 2) are overactive following absence of LEKTI, the known functions of KLK5 suggest it is central to the NS phenotype. To explore the role of KLK5 in NS, we have developed KLK5 transgenic mice (TgKLK5) whereby human KLK5 is expressed under the control of the involucrin promoter. KLK5 immunostaining demonstrated high transgene expression in the granular layer of TgKLK5 epidermis. Increased proteolytic activity was detected by zymography, both for KLK5 and for two proteases it is proposed to activate, KLK7 and elastase 2. Like in NS, TgKLK5 mice show a dramatic skin barrier defect with extensive scaling, accelerated filaggrin

processing and increased transepidermal water loss. The stratum corneum was also detached from the underlying epidermis through degradation of desmosomes. Additionally, TgKLK5 mice displayed hallmarks of cutaneous inflammation and allergy including high expression of IL-1β, TNF-α and the pro-Th2 cytokine TSLP and infiltration of mast cells and eosinophils, overtime leading to development of skin lesions due to persistent scratching. Effects of inflammation extended to the systemic level with high serum IgE and TSLP levels. As KLK5 overexpression is sufficient to replicate NS, these findings establish KLK5 as the key initiator of the biological cascade underpinning the disease.

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Genetic mosaicism associated with early Gorlin syndrome

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A 12 year old patient with multiple papular and nodular skin lesions since birth, all of them located on the right side of her body, is presented. She has unilateral, segmentally arranged basaloid skin tumors, ipsilateral, palmoplantar pits of rather large size being distributed along Blaschko's lines and an ipsilateral odontogenic keratocyst. In addition, she presents a family history of Gorlin Syndrome since her father suffers from this disease.
A molecular study of PTCH1 gene, which is associated with Gorlin syndrome, was performed by PCR and sequencing using patient's DNA extracted from peripheral blood and DNA extracted from biopsies of healthy and affected skin. In all cases was detected the c.3062A> G; p.Y1021C mutation. Furthermore, the presence of a second novel mutation (c.543_549delGGCACTC; p.S181SfsX36) that produces a stop codon and generates a truncated protein was detected only in the DNA obtained from the affected skin. A family study showed up that the father only had the mutation c.3062A> G; p.Y1021C.

Sample	Result
Peripheral blood DNA	c.3062A>G p.Y1021C
Healthy skin biopsy DNA	c.3062A>G p.Y1021C
Affected skin biopsy DNA	c.3062A>G p.Y1021C c.543_549delGGCACTC p.S181SfsX36

We conclude that c.3062A> G; p.Y1021C mutation could be the cause of familiar disease while c.543_549delGGCACTC; p.S181SfsX36 mutation could be a de novo mutation that generates a genetic mosaicism since it does not appear in every patient's cells. The presence of both mutations would make the syndrome more aggressive and be responsible of the early appearance of the disease in cells that carry both mutations.

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P01.137

A replication study for four keloid loci at 1q41, 3q22.3-23 and 15q21.3 in the Japanese population

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Keloid is a dermal fibroproliferative growth that results from dysfunction of the wound healing processes. A previous genome-wide association study (GWAS) reported four novel keloid-susceptibility loci at 1q41, 3q22.3-23 and 15q21.3 (Nakashima M, *et al.* Nature Genet. 2010;42,768-772). Here, we investigated the association of these loci with keloid by using an independent Japanese sample set. We performed case-control association analysis using 204 patients with keloid subjects in Nippon Medical School Hospital. The four SNPs at 1q41, 3q22.3-23 and 15q21.3 were genotyped using Small Amplicon Genotyping method. In this study, the two from four loci identified by previous GWAS, rs873549 on chromosome 1 (odds ratio (OR) = 2.09) and rs8032158 located in *NEDD4* on chromosome 15 (OR= 3.02) were significantly associated in keloid compared with control. Especially, the risk for keloid development (OR= 3.55) or the scar severity scale (OR= 4.73) increased with an increasing number of risk allele in rs8032158. Individuals with two risk alleles of the two loci showed higher risk of developing keloid than those with one risk allele alone. Our findings elucidated the significance of genetic variation at these two loci in keloid in the Japanese population.

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P01.138

Seeking pemphigus vulgaris causative variations in the ST18 gene

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Pemphigus vulgaris (PV) is a severe autoimmune blistering disease caused by anti-epithelial antibodies, leading to disruption of cell–cell adhesion. The disease is exceedingly rare worldwide, but shows a 40-fold higher prevalence in Jewish populations. In a previous genome-wide association study (GWAS) in a cohort of 100 PV patients, we identified a risk allele in the vicinity of the Suppression of Tumorigenicity 18 (ST18) gene that was shown to confer a 6-fold increased risk for PV in Jews. ST18 regulates apoptosis and inflammation, two processes of direct relevance to the pathogenesis of PV. ST18 was also found to be overexpressed in the skin of PV patients as compared with healthy individuals, further supporting a role for this gene in PV (Sarig et al. 2012). In an attempt to identify ST18-associated causative variants, we performed targeted next-generation sequencing of the entire ST18 gene, including nearby regulatory regions (total of 0.47Mb) in 16 affected subjects. A case-control association analysis of 789 variants, found within the sequenced region, revealed a large genomic haplotype block strongly associated with the propensity to develop PV (p-value 0.003). This haplotype has a frequency of 50% in the sequenced cohort and only 8% in 1000 genomes sequences. The block is located within an intron of the ST18 gene that harbors a lincRNA gene. A known SNP in this lincRNA that modifies its secondary structure is enriched in the PV cohort and may be implicated in regulation of ST18 expression.

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P02.001

Duplication of the Rubinstein-Taybi region on 16p13.3 due to suspected germline mosaicism in two adult brothers

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Duplications of chromosomal region 16p13.3 encompassing the CREBBP gene, which is mutated in Rubinstein-Taybi syndrome, have recently been shown to cause a recognizable syndrome characterized by intellectual disability of varying degree and facial dysmorphisms. Here we report two brothers aged 30-31 years carrying a 2.3 Mb duplication of the critical region of 16p13.3 detected by molecular karyotyping.
Both patients show the facial features already described in the 16p13.3 duplication syndrome including a long face, midfacial hypoplasia, pronounced ptosis and microretrognathia with a dimple. However, the dysmorphic features are more pronounced than in the younger patients described so far in the literature. Additional to the characteristic facial features the brothers also show camptodactyly of the fingers and toes and contractures of the large joints. Both display a urogenital malformation and developed scoliosis and abdominal obesity in adolescence. While one of the brothers has mild intellectual disability the other is moderately to severely disabled, with no speech and only limited ambulation. This difference in severity is not explained by additional pathogenic copy number variants.
Analysis of the parents did not reveal any evidence for somatic mosaicism of the 16p13.3. duplication or a chromosomal rearrangement, thus suggesting germline mosaicism as the underlying cause. Germline mosaicism for chromosomal rearrangements has rarely been reported, but the family described here demonstrates that it has to be considered when several siblings appear to be affected with the same condition. In addition, this report extends the phenotype of the 16p13.3 duplication into adulthood.

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P02.002

Refinement of genotype-phenotype correlation in 3 patients with 1q24.3q25.1 microdeletion supports the role of CENPL and DNM3 genes.

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1q24.3q25.1 microdeletion syndrome associates prenatal onset growth deficiency, intellectual disability, facial dysmorphism and small hands and feet. A 1.9 Mb minimal critical region (chr1: 171869242-173832704, Hg 19) containing 13 genes was recently defined for this syndrome. Here we describe three new cases of 1q24.3q25.1 deletion, including the smallest deletion reported to date. Patients presented with the clinical common characteristics including small hands and feet, clinodactyly, absence of ear lobe, broad nasal bridge and moderate intellectual disability. Homogeneous growth retardation of prenatal onset was present in patients 1 and 2 whereas growth retardation appeared later in infancy in patient 3. Array CGH and FISH allowed the characterization of these deletions. Patients 1 and 2 showed a 9.2 Mb and a 3 Mb deletion respectively, both encompassing completely the minimum critical region containing DNM3 and CENPL genes. Patient 3 carried a 6 Mb deletion overlapping only the proximal 1.2 Mb of the critical region and sparing the CENPL gene. These data combined with those of the literature strongly impute CENPL, encoding centromeric protein L, in fetal growth. They also allow to specify the supposed function of other genes within the deletion: DNM3 (Dynamin3) involved in myelination could explain intellectual disability and microRNAs 214 and 199A2 may explain facial dysmorphism based on knocked-out mice experiments. By describing the smallest deletion of the locus and cutting the minimum critical region, we were able to refine the genotype/phenotype correlation for 1q24.3q25.1 deletion and propose CENPL as main candidate gene for growth deficiency in this syndrome.

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P02.003

Short report: Delineation of a new chromosome 20q11.2 duplication syndrome including the *ASLX1* gene

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The current use of microarray-based comparative genomic hybridization allowed the identification of novel cytogenetic abnormalities. Partial trisomies of the long arm of chromosome 20 are rare. Here we report on three males with a *de novo* overlapping 7.5, 9.78 and 10 Mb duplication of chromosome 20q11.2. Together with another patient previously published in the literature with overlapping 20q11 microduplication, we showed that such patients display common clinical features including striking similar dysmorphic features with metopic ridging/trigonocephaly, full eyelids, epicanthal folds, moderate developmental delay, short hands, cryptorchidism, and normal growth to mild growth retardation. The minimal critical region of the duplication to 7.5 Mb encompassing 173 genes including *ASLX1* gene (MIM612990). *ASLX1* is a human homolog of the Drosophila *asx* gene which is an homeotic gene. D*e novo* heterozygous nonsense or truncating mutations have been reported recently in patients with Borhing-Opitz syndrome (MIM605039), a severe developmental and malformation disorder.
Because of craniofacial features in common with Borhing-Opitz syndrome, in particular metopic ridging/trigonocephaly, we suggest that duplication of *ASXL1* may contribute to the phenotype. These observations suggest a novel recognizable microduplication syndrome, and reporting of additional patients with molecular characterization will allow more detailed genotype-phenotype correlations.

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P02.004

22q11.2DS: MLPA and FISH results

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Aim: MLPA-analysis was performed on the group of probands with typical phenotype previously tested for 22q11.2 microdeletion by FISH in order to confirm data, delineate deletion size and make an effort to reveal atypical distal deletions. Materials and methods: A group of 61 probands with phenotype suggestive of 22q11.2 deletion syndrome (22q11.2DS) (CHD, facial anomalies, cleft palate, hypocalcemia) was analyzed by MLPA-analysis with SALSA MLPA P250-B1 DiGeorge (MRC-Holland). All patients previously underwent standard karyotyping in order to exclude other chromosomal aberrations. FISH-analysis with TUPLE1/ARSA (Abbott, Vysis) was performed in cultured lymphocytes. 37 patients were not deleted, 24 patients were found to carry 22q11.2 deletion. DNA for MLPA-analysis was extracted from EDTA-blood samples and from samples of Carnoy's fixated lymphocytes left over from karyotyping and FISH-analysis. Results: MLPA-analysis confirmed all 24 FISH-diagnosed cases of 22q11.2 deletion: in 19 cases A-B-C deletion was revealed, four patients had A-B deletion and one patient had A deletion. In our study MLPA-test didn't reveal nor atypical deletions neither duplications in the remaining 37 patients. DNA from EDTA-blood samples, Carnoy's cell suspension and samples from both materials analyzed simultaneously showed respective results. Conclusion: MLPA-analysis is considered to be a reliable, accurate and cost-effective method with high sensitivity and specificity for 22q11.2 microdeletion detection. MLPA with DNA-samples extracted from Carnoy's cell suspension showed reliable results. Thus, this material can be used in cases when patient or EDTA-blood sample is not available, limited amounts of accessible material and in archive cases.

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P02.005

Further delineation of the natural history of 49, XXXXY syndrome

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49,XXXXY syndrome is a rare sex chromosome aneuploidy occurring in 1:85 000-1:100 000 male births. He has to be distinguished from Klinefelter syndrome. Data on this aneuploidy in adulthood are limited, with most of the literature data based on paediatric patients.
We report a new male patient whose 49,XXXXY diagnosis was formally made at the age of 54 years. A neurologist who examined for the first time the patient for tremor ordered the karyotype analysis. So far, no medical follow-up was performed specifically for his condition. This man presented with eunuchoid body habitus, facial features with epicanthus, hypertelorism, up-slanting palpebral fissures and features of chronic hypoandrogenism with muscular weakness, sparse body hair, dry skin with abnormal healing of skin wounds. Endocrine evaluation confirmed a hypergonadotropic hypogonadism with two testes (one cryptorchid testis). Bone mineral density showed an osteoporosis. There was no cardiac defect. He had moderate intellectual deficiency with more affected verbal skills. There were no behavioural problems and he had social and occupational capabilities with adaptive functioning. A recent deep vein thrombosis was diagnosed in his left leg. Unusually, in addition to moderate deafness, he developed progressively a severe vision impairment leading to blindness.
There have been very few reports of adult individuals with 49,XXXXY syndrome and to our knowledge only one 47-year-old male patient (Akiyama et al, 2011, J Dermatol, 38: 414). This kind of report may contribute to improved management of prospective medical healthcare associated with this condition in older individuals.

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P02.006

4q21 deletion syndrome: involvement of *PRKG2* in short stature and new boundaries for mental retardation and speech delay.

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Patients with interstitial 4q21-q22 deletions show several characteristic features such as neonatal muscular hypotonia, small hands and feet, severe growth restriction, mental delay, severe psychomotor retardation, absent or severely delayed speech and facial dysmorphism with a broad forehead, frontal bossing, hypertelorism, short philtrum, and downturned corners of the mouth. Many cases were described in the literature, and, recently, a study analysed nine patients with overlapping *de novo* 4q21 deletions of different sizes, ranging from 3.2 Mb to 15.2 Mb (Bonnet, 2010). They defined a minimal critical interval of 1.37 Mb, containing 5 genes, *PRKG2, RASGEF1B, HNRNPD, HNRPDL* and *ENOPH1*. Two of these genes, *PRKG2* and *RASGEF1B* were considered to be the most promising candidates for growth restriction, mental retardation, and absent or severely delayed speech. We present a study on a child with a *de novo* 4q21.22 deletion, facial anomalies, developmental delay, and mental retardation. The deletion carried by our patient is the smallest described in this region. It overlaps the minimal critical region previously described thus defining new boundaries for this emergent 4q21 deletion syndrome. Unlike all previously described cases, our patient is of normal height and does not show any growth restriction. Interestingly, in his case, the *PRKG2* gene is not deleted. We thus propose *PRKG2* to be the gene responsible for the short stature of the patients when deleted, and *RASGEF1B* and *HNRNPD* as candidate genes for the mental retardation and speech delay, two features present in all described patients without exception

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5q31.3 microdeletion syndrome: clinical and molecular characterization of two further cases

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The 5q31.3 microdeletion syndrome has recently emerged as a distinct clinical entity, and we report two new patients with de novo deletions of this region, bringing the total to seven. The phenotype is characterized by marked hypotonia, apnoea, developmental delay and feeding difficulties. Both patients had abnormal movements which did not correlate with epileptiform activity on electroencephalogram (EEG). Developmental brain changes on neuroimaging consist of abnormalities predominantly affecting the white matter and frontal lobes. The 5q31.3 deleted regions overlap those of previously reported cases, and allow further refinement of the shortest region of overlap (SRO) to 101kb, including only three genes. Of these the purine-rich element binding protein A (PURA) gene has an established role in brain development, and we propose that haploinsufficiency for this gene is primarily responsible for the neurodevelopmental features observed.

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P02.008

14 new patients with 6q16 deletion: a new minimal critical region and first fetopathological data

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6q16 deletions are associated with a Prader-Willi-Like (PWL) phenotype. A role of several candidate genes has been suggested: SIM1 for obesity and GRIK2 for behavioural problems. However, few patients have been precisely described using high resolution microarray. We report 14 new patients with de novo 6q16 deletions all characterized by oligonucleotide or SNP microarray, among them the first fetus with a complete fetal examination. Nine patients display a PWL phenotype and allow refining of the critical region for this condition. The fetus exhibits dysmorphic facial features and particular cerebellar and cerebral abnormalities with neuronal ectopia. Deletion size in living patients ranges from 2.20 to 7.84 Mb whereas the fetus carries the largest deletion (14 Mb). Genotype-phenotype correlations reveal a novel minimal critical region for PWL phenotype comprising SIM1 and MCHR2 genes. This study confirms, on the largest series reported to date, the strong association between PWL phenotype and 6q16 deletions as previously observed. The genes within the minimal critical region that we described seem to play a critical role in the phenotype. Also, we presented a detailed report of the first fetopathological examination of a fetus deleted for this region.

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P02.009

Aarskog-Scott syndrome: cases with clinical and molecular diagnosis

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Aarskog-Scott syndrome (AAS) is an X-linked disorder characterized by short stature, hypertelorism, shawl scrotum, and brachydactyly. There is wide phenotypic variability. Additional features include joint hyperextensibility, short nose, widow's peak, and inguinal hernia. While most patients do not have mental retardation, they exhibit neurobehavioral features. Carrier females are clinically diagnosed by widow's peak or short stature or by molecular testing whenever there is an affected male in the family. Prevalance of AAS is unknown, but less than 100 cases have been reported after 1970. *FGD1* gene was found responsible from X-linked AAS phenotype in 1994 by a group of researchers (Pasteris et al.). Thirty different mutations including missense, frameshift, nonsense, splice site, in-frame and gross deletions have been reported up till then in more than 40 families (HGMD, 14 December 2012).

We here present the clinical and molecular findings of four additional patients/families with Aarskog-Scott syndrome. Four different mutations in *FGD1* gene, including three novels, were identified in our study group. These novel mutations include two frameshift [(c.2034delA (p.Lys699LysfsX13), c.1815dupG (p.Thr606AspfsX28)] and one missense mutation [(c.1555C>T (p.Arg519Cys)]. The (c.1829G>A/p.R610Q) mutation has been previously described. In three families, familial inheritance was observed, while one case was isolated. In total, 14 additional AAS cases and 13 obligate female carriers were ascertained by pedigree analysis. Further clinical/molecular studies are underway. Clinical findings of affected males and carrier females will be discussed along with molecular data in view of the literature.

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P02.010

An Alström Syndrome case with immunodeficiency

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Alström syndrome (AS) is a very rare autosomal recessive genetic disorder characterized mainly by obesity, hearing loss, retinopathy, cardiomyopathy, recurrent pulmonary infections, progressive renal and hepatic dysfunction and endocrinological features. It is caused by mutations in the ALMS1 gene. Here we present an AS female with immunodeficiency. The proband is a 4.5-year-old female born to nonconsanguineous parents. She was first admitted to the hospital at 6 months old because of developmental delay and pulmonary infection. She had a history of multiple hospitalizations because of recurrent pulmonary infections. Throughout this period immunodeficiency, primary hypothyroidism, growth hormone deficiency were detected. Imaging analysis revealed cortical atrophy, ventricular dilatation and bitemporal lobe hypoplasia in cranial MRI; patent foramen ovale in echocardiography; hepatomegaly with hepatosteatosis in USG; ulnar hypoplasia, short upper limb, coxa valga, metaphyseal widening in distal femur and proximal tibia and Erlenmeyer flask bone deformity in both femur in X-Ray examinations; generalized osteoporosis in DEXA. Her physical examination showed generalized alopecia, blue sclera, jagged teeth, short neck, joint laxity, truncal obesity and generalized hypotonia. Her developmental milestones were delayed. Eye examination revealed pigmentary retinopathy and salt-pepper appearance. She had sensorineural hearing loss. Ankara Developmental Screening Inventory test showed 30 % delay in general, cognitive and linguistic development areas. Her karyotype was 46,XX. Molecular analysis revealed one heterozygous ALMS1 mutation (c.5969C>G p.Ser1990X). This result along with the clinical features, confirmed the clinical diagnosis of AS as it has been stated by Marshall et al. AS cases are suggested to be investigated for concomitant immunodeficiency.

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P02.011

FAM20A mutations behind amelogenesis imperfecta and gingival hyperplasia

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Amelogeneses imperfectae (AI) are a heterogeneous group of rare diseases affecting the formation/mineralization of tooth enamel and transmitted according to various mode of inheritance (X-linked, autosomal dominant or recessive). These diseases exist either in isolation, with clinical manifestations limited to the oral cavity, or associated to other symptoms in syndromes as for example AI and cone-rod dystrophy, AI and epilepsy. Mutations in many genes coding for either enamel matrix proteins, enamel matrix degrading proteins, or proteins involved in hydroxyapatite formation and growth, and mineralization processes have been identified as being responsible for the clinical enamel phenotypes (hypoplastic, hypomineralized, hypomature) encountered. We explored the genotype and orodental and extraoral phenotype in a cohort of patients presenting recessive hypoplastic amelogenesis imperfecta and gingival hyperplasia caused by FAM20A gene mutations. DNA sequencing revealed two compound heterozygous and two homozygous FAM20A mutations in 6 affected individuals from 4 unrelated families (2 of them consanguineous) of various ethnic backgrounds. The orodental phenotype was strikingly similar with absent enamel, gingival hyperplasia but also intrapulpal calcifications, dental follicles hyperplasia, unerupted impacted teeth, root anomalies. Anatomopathology analysis of the gingiva demonstrated signs of discrete leuco-parakeratosis and minimal chronic inflammation.
Mouse teeth transcriptome data indicated that Fam20a as well as Fam20b, Fam20c were expressed as early as E14.5 cap tooth stage.
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P02.012

About 9 patients with ARC syndrome (Arthrogryposis-Renal dysfunction -Cholestasis syndrome): focus on prenatal findings

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ARC syndrome is a severe autosomal recessive multisystem disorder including arthrogryposis, renal tubular acidosis and neonatal cholestatic jaundice. Other features variably reported are icthyosis, mild dysmorphic signs, anomalies of corpus callosum, recurrent infections, failure to thrive and grey platelets due to defect in platelet alpha-granules biogenesis. This syndrome affects newborns who usually die in the first year of life of infections or life-threatening haemorrhage due to platelet dysfunctions. Mutations in VPS33B and VIPAR genes have been reported in this syndrome.
We report on 9 patients from 5 pedigrees of various origins with this syndrome. We will particularly focus on prenatal findings. Transient intestinal hyperechogenicity and hypomobility were frequent findings in our patients. One patient presented with increased nuchal translucency. We will discuss the amniotic enzymatic profil.

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P02.013

Viennese "Fools Tower" Collection: Birth Defects and Malformation Syndromes in Archived Fetal Specimens

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Background
A Pathologic-anatomical collection of Viennese Natural-Historical Museum has accumulated since 1796 a remarkable variety of exhibits, including a rich collection of birth defects. Formerly, the specifications of birth defects were only descriptive; nowadays, many of them are syndromologically classifiable.
Aim
Our aim is to closer determine the nature of developmental anomalies in some specified series of the Viennese Pathologic-anatomic Collection. This diagnostic work-up could help providing extraordinary learn-samples for health professionals dealing with birth defects/syndromes. Considering the rareness and age of this material, the work could be of a great international importance as well.
Material and Methods
Preliminary phase included an overall visual inspection of all (circa 1000) fetal/pediatric exhibits of the museum, in parallel with a key words-search in the museum´s electronic database. Some exhibits already underwent detailed objective evaluation.
Results
The first inspection allowed us to select 5 main "working groups" according to the leading type of external defect: 1. Orofacial clefts, non-midline, 2. Middle-line facial defects, 3. Disorders of extremities and skeletal dysplasias, 4. Congenital tumors, 5. Miscellaneous syndromes. The number of the selected exhibits counted circa 140. Already within this "first-look phase" we have clinically specified some developmental pathologies that we present here as the initial findings; among them e.g. Rosenak-, Piepcorn-, Roberts-, OFD2-, Delleman-, Beckwith-Wiedemann-, Smith-Lemli-Opitz-, Meckel-syndromes and others.
Conclusion/Plans
In further investigations all selected exhibits will undergo a detailed objective reevaluation and documentation, including X-ray, autopsies, histological and eventually DNA analysis to identify or confirm a suspected chromosomal, microdeletion or monogenic syndrome.

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P02.014

The ARHGAP24 gene is disrupted in two sisters with septate uterus

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Uterine malformations, among them septate uterus, are a frequent cause of recurrent miscarriages. We report here two sisters, both presenting with septate uterus, in whom we identified an apparently balanced reciprocal translocation t(2;4)(q35;q21.3). Array CGH analysis excluded a microdeletion/microduplication at breakpoints. Breakpoints were mapped by FISH and sequenced by long-range PCR. The breakpoint in chromosome 2 occurred in a non-coding region while those in chromosome 4 disrupted the *ARHGAP24* gene in the 7th intron. This translocation revealed two potential candidate genes in 4q21 region: the *ARHGAP24* disrupted gene and the *MAPK10* gene located close to the breakpoint, which might be altered through a position effect. *ARHGAP24* is involved in the regulation of uterine contractions and thus appeared to be the best candidate explaining uterine malformations. *MAPK10* is also a good candidate since impairment in MAPK signalling was implicated in gonadal development disorders. Next, we performed qPCR and direct sequencing of *ARHGAP24* and *MAPK10* in 27 patients with isolated septate uterus. No point mutations or intragenic deletions/duplications were identified.

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P02.015

New critical region for recently defined 4q21 microdeletion syndrome

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4q21 deletions have been reported in a number of patients with characteristic features (facial dysmorphism, hypotonia, small hands and feet, short fingers, mild-severe growth delay, ID, severely delayed speech). We report on a new case of 4q21.22 microdeletion and suggest a new critical region for 4q21 microdeletion syndrome. *De novo* interstitial 724Kb deletion of 4q21.22 (83273844 - 84097897 bp, hg18) was detected by arrayCGH (105K) in a patient with considerable hypotonia, severely retarded psychomotor development and delayed speech, short stature (3-10 centile) and dysmorphic features (slant up palpebral fissures, posteriorly rotated ears, short concave nose with anteverted nostrils, short philtrum, prognathia, downturned corners of the mouth, single transverse palmar crease, small hands and feet, short toes).
Recently critical region for 4q21 microdeletion syndrome was defined by analyzing published cases and DECIPHER reports 1571 and 4539 (Bonnet et al., 2010). The overlapping region was from 82332981 to 83182488 bp with candidate genes *RASGEF1B* and *PRKG2*. Our reported deletion does not overlap with defined region. However, clinical features of our patient coincide with 4q21 microdeletion syndrome. We suggest other critical region (chr4:83273844 - 84066553) for this syndrome, shared by previously reported 4q21 deletions, DECIPHER cases 4539 and 4665, and our reported deletion. This region includes up to 10 RefSeq genes. Strong candidate genes are brain expressed *LIN54*, heterogeneous nuclear riboproteins *HNRPDL* and *HNRNPD* - regulators of cell cycle, transcription and mRNR stabilization. Further analysis of genes` candidates` function and expression is necessary for refining minimal deletion region leading to 4q21 microdeletion syndrome.

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P02.016

Blepharophimosis and multiple congenital alterations in a boy with a 6p25 tetrasomy.

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We report a 6-year-old boy referred for clinical assessment because of failure to thrive, psychomotor delay, left renal agenesis and mild pulmonary stenosis. He presented with distinctive facial features: short and narrow palpebral fissures, ptosis, a bulbous nose and a small mouth.
A customized 60.000 oligonucleotide array-CGH (KaryoArray®v3.0, Agilent) revealed a partial tetrasomy of 6p25.1→6p25.3 of about 5.96 Mb. This region contains at least 5 *OMIM* genes including *FOXC1.* Subtelomeric metaphase *FISH* analysis (RH40931, Kreatech) showed hybridization on both 6p subtelomeres, but a brighter signal pattern was observed on one homologue. Interphase *FISH* with the same probe demonstrated four 6p signals. These findings are consistent with a 6p25.1→ 6p25.3 tandem triplication.
Partial trisomy of 6p25 is a chromosome abnormality characterized by low birth weight, psychomotor delay, growth retardation, congenital heart defects, and renal anomalies. Affected individuals present with craniofacial dysmorphism consisting in blepharophimosis, palpebral ptosis, dysplastic and low set ears, small and prominent chin and a bulbous nose. Partial tetrasomy of 6p25 is an extremely rare condition and up to date only one girl with a 6p25 tetrasomy has been described in the literature, interestingly showing strikingly similar facial features.
We would like to highlight the distinctive facial features associated with 6p25 tetrasomy and the need to consider 6p25 chromosome abnormalities in the differential diagnosis of individuals with blepharophimosis and psychomotor delay in addition to other more common entities such as Blepharophimosis-ptosis-epicanthus inversus syndrome (*BPES*) and Ohdo syndrome.

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P02.017

Microduplication of 17q22q23.2 due to a supernumerary marker chromosome in a girl with macrocephaly and global developmental delay

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Small supernumerary marker chromosomes (sSMC), defined as additional structurally rearranged chromosomes that cannot be identified by conventional chromosome-banding alone, are often seen in patients with developmental disorders. The risk and severity of the abnormal phenotype depends on the size of the sSMC and extent of the mosaicism. Application of genome-wide array technology enables full characterization of sSMC.
We report a 15-months old girl with sSMC derived from chromosome 17. The clinical features include global developmental delay, hypotonia, feeding problems, recurrent bronchiolitis, macrocephaly, and distinct facial features. A 47,XX,+mar karyotype was found in all examined metaphases from peripheral blood. Fluorescent in situ hybridization (FISH) studies demonstrated that sSMC derived from chromosome 17 and showed no hybridization signal for subcentromeric probes. Microarray analysis (185K array, Agilent Technologies) showed two gains of about 2Mb at band 17q11, and 10,5 Mb at band 17q22q23.3 (arr[hg18] 17q11(22,367,302-24,320,006)×3,17q22 q23.3(49,265,451-59,786,509)×3). FISH analysis using MPO probe confirmed that sSMC contains17q22 region.
The clinical data of 17q11 and 17q22q23.3 microduplication are scarce. Alvarado et al. reported 17q23.1q23.2 microduplication as a potential CNV locus for isolated clubfoot and Radio et al. described a boy with 17q23.2 microduplication who presented with intellectual disability, brain, heart and skeletal anomalies. Main candidate genes are TBX2 and TBX4. Although the present patient has a duplication of both genes, she shares neither the clubfoot nor the heart abnormalities.
In conclusion, application of genome-wide array technology can reveal unpredicted complexity of the sSMC containing non-contiguous regions and increases the knowledge of the clinical picture of interstitial microduplications.

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P02.018

Array-CGH analysis suggests genetic heterogeneity in Rhombencephalosynapsis

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Rhombencephalosynapsis (RS) is an uncommon, but increasingly recognized, cerebellar malformation defined by vermian agenesis with fusion of the hemispheres. The embryologic and genetic mechanisms involved are still unknown, and to date, no animal models are available.

To detect RS candidate genes, we performed Agilent oligonucleotidic arrays throughout 57 RS patients. Four different unbalanced rearrangements were found out: a 16p11.2 deletion, a 14q12-q21.2 deletion, an unbalanced translocation t(2p; 10q) and particular one microdeletion of 2,7 Mb in the 16p13.11 region containing 2 candidate genes for RS : *c16orf45* and *NDE1.* The sequencing of *c16orf45* performed on the whole cohort did not reveal any mutation. Using *in situ* hybridization to localize gene expression on a chick embryo model, we confirmed the neural expression of *NDE1* and *c16orf45*.

From this first microarray scan series of rhombencephalosynapsis, it could be suggested that its genetic causes are likely heterogeneous.

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P02.019

Chromosomal microarrays in the clinical context: a critical appraisal of algorithms for results interpretation

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Chromosomal microarray analysis has become a first tier examination in intellectual disability, either isolated or combined with congenital malformations. The analysis of its results is still complex and often non-univocal though, making its application in the clinical practice challenging. In the attempt to guide the interpretation, a number of algorithms have been proposed and published over years.. In order to evaluate the clinical utility of the application of array-CGH in our clinical practice, and to appreciate the extent of agreement on the evaluation of the CNVs in literature, we retrospectively analysed a cohort of 74 individuals affected by intellectual disability and malformations, phenotypically and genotypically analysed in the years 2005-2010. We compared our own evaluation against 6 algorithms published in the same time frame. We detected 41 CNV in 31 patients, with a CNV detection rate of 42%; 9.5% of patients had more than one CNV. In 47% of cases the CNV had been deemed pathogenic, in 5% benign and in 48% some degree of uncertainty (VOUS) remained. When comparing our evaluation with the different algorithms, and the algorithms themselves to each other, we found that only in a third of cases the assessment was fully concordant, in another third at least one algorithm was discordant, in the last third 2 or 3 algorithms were discordant. Furthermore, we noticed that over the years the CNVs' categorization is more and more cautious with a progressive widening of the VOUS categories and a parallel decrease of the certainties.

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P02.020

Characterization of a new Complex Chromosome Rearrangement (CCR) between chromosome 3 and chromosome 8

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Background: Complex Chromosome Rearrangements (CCRs) are defined as structural chromosomal rearrangements with at least three breakpoints and exchange of genetic material between two or more chromosomes. To date, more than 255 cases of CCRs involving three or more chromosomes have been reported, although their incidence seems to be increasing because of the impact of the application of new molecular technologies. Nevertheless, CCRs involving two chromosomes are still very rare.

Objective: To identify the origin and chromosomes involved in a CCRs.

Material and Methods: Here we present a malformed newborn female with a partial trisomy of the long arm of a chromosome 3, which at first was considered to be a derivative of a balanced maternal translocation and, after the application of high resolution cytogenetic techniques, array-CGH analysis, Fluorescence in situ Hybridization (FISH) and Multi Color Banding FISH (MCB), was found to be a CCR derived from a maternal insertion.

Conclusion: With the presentation of this case, we would like to reinforce the power of MCB and array-CGH for clarifying any type of CCRs, as previously proposed by Pellestor et al. [2011] and Kang S-HHL [2010], but also to support the value of a high resolution G-Band karyotype and the idea that "all techniques are complementary and provide different information, essential to understand all kinds of CCR".References: Kang S-HL et al., 2010; Lee et al., 2010; Madan K, 2012; Viaggi et al. 2012; Pellestor et al. 2011 and Kang S-HHL et al., 2010.

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P02.021

Defining the minimal critical region for the 3p deletion syndrome.

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The 3p deletion syndrome is a rare disorder characterized by mental and psychomotor retardation, microcephaly, short stature, hypotonia, postaxial polydactyly and dysmorphic craniofacial features, such as ptosis and blepharophimosis. Most of the reported cases have a large terminal 3p deletion, ranging from 6Mb to 12Mb, but the clinical use of microarray-based analysis has recently led to the identification of smaller interstitial deletions on 3p25, from 643Kb to 1.6Mb.

We report the case of a 2 year old male patient with the typical features of the 3p-syndrome: psychomotor retardation, craniofacial dysmorphism, postaxial polydactyly and hypotonia. Array-CGH analysis detected a de novo 371 Kb deletion on 3p25.3, including few genes whose function is still partially unknown. Among the deleted region, MTMR14 (myotubularin related protein 14) appears to be the best candidate gene to explain some of the clinical features, such as hypotonia and some craniofacial dysmorphisms, since it plays a critical role in muscle calcium homeostasis and Mtmr14-null mice showed muscle weakness and increased fatigue compared to wildtype mice. Few cases of 3p25 deletion with features of the 3p-syndrome have been reported so far in the literature. The deletion of 371Kb we observed in our patient overlaps with those previously reported and enables to better define the minimal critical region responsible for 3p-syndrome.

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P02.022

Expanding the clinical phenotype at the 3q13.31 locus with a new case of microdeletion and first characterization of the reciprocal duplication.

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Congenital deletions of 3q13.31 region have been recently described as a novel microdeletion syndrome characterized by developmental delay, postnatal overgrowth, hypoplastic male genitalia and characteristic facial features. A common critical region of overlapping of 580 kb was delineated including two strong candidate genes for developmental delay: *DRD3* and *ZBTB20*.

In this report, we describe a new case of 3q13.31 microdeletion identified by array-CGH in a 16 year-old girl sharing clinical features commonly observed in the 3q13.31 microdeletion syndrome. This girl had a microdeletion of 7.39 Mb spanning the common critical region of overlapping.

More interestingly, we report for the first time the existence of a microduplication reciprocal to the microdeletion syndrome. This familial 2.76 Mb microduplication identified by array-CGH was carried by two brothers and their father. The phenotype shared by the brothers was resembling to the phenotype related to the 3q13.31 microdeletion syndrome including especially developmental delay, behavioral abnormalities and obesity. This mi-

croduplication involves three strong candidate genes for the developmental delay *ZBTB20, LSAMP* and *GAP43*. Further molecular characterization showed that *DRD3*, another strong candidate gene for developmental delay, was not included in the duplicated region. A dosage alteration of this gene cannot be completely excluded as the duplication was inverted at proximity of this gene, as shown by *FISH* analysis. Finally, we hypothesized that the phenotype shared by the two brothers could be related to a gene dosage imbalance even if gene expression could not be measured in target tissues such as brain or adipocytes.

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P02.023

The 3q13.31 Microdeletion syndrome: A new patient molecularly characterised using array-CGH

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Microdeletions in 3q13.31 have been reported in only relatively few patients to date and only a subset of cases have been characterised at molecular resolution. The deletion size for most patients is larger than 5 Mb, some smaller deletions allowed previously to define a smallest region of overlap (SRO).
Apart from intellectual disability / developmental delay (ID/DD) of varying degrees, which is common to all reported patients, the clinical spectrum comprises speech delay, muscular hypotonia, skull malformations, ocular malformations, skeletal malformations and dysmorphic features.
Here, we report a female patient with a 3.4-Mb *de novo* deletion of 3q13.31. She was born at 38 week of gestation, birth weight 3940g (+1 SD), birth height 51cm (normal), occipiofrontal circumference (OFC) 37cm (+1 SD).
Her clinical presentation at the age of 21 month includes: weight 1100g (-1 SD), height 82cm (normal), OFC 51cm (+2,25 SD), mild DD, severe muscular hypotonia, macrocephaly, strabismus, hypermetropia and dysmorphic signs including hypertelorisme, anti-mongoloid slanted eyes, everted upper lip with a "tented" appearance and retrognathia.
The deleted segment of chromosome 3 (chr3:112.144.025-115.514.432) contains 24 RefSeq genes and comprises the previously defined SRO.Our patient with a relatively small deletion of 3.4-Mb confirms the pertinence of the previously delineated SRO and helps to identify the core phenotype of the 3q13.31 microdeletion syndrome.

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P02.024

15q11 microduplication in a boy with psychomotor delay and PWS-like phenotype

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In the literature, the duplications of Prader-Willi/Angelman syndrome critical region 15q11q13 are separate genomic syndrome (OMIM number: 608636). The patients exhibit predominant different neurodevelopmental disorders such as autism, seizures, developmental delay, clumsiness, schizophrenia or schizoaffective patterns. Minor dysmorphic features such as macrocephaly, down-slanting palpebral fissures, broad nasal bridge, pouting lips, expressionless face, frontal tuft of hair were presented in many cases in various combinations.
We report the 9 years old boy for developmental delay, only slight dysmorphic facial features and obesity with a PWS-like phenotype. His height is 140 cm, weight 51 kg (>75thcentile) and OFC 54 cm (>90thcentile). He had facial dysmorphisms reminiscent of PWS with small almond-shaped eyes, small down-turned mouth, small hands and feet, generalized obesity (BMI=26, >97thcentile). He also presented developmental delay (QIP=96, QIG=87), especially of speech (QIV=85) and he followed speech therapies. His mother has similar dysmorphic phenotype: small stature (151 cm), obesity (102 kg) and learning difficulties at school. The family history was significant for obesity (maternal grandfather).Tests for Prader syndrome were FISH using Vysis-Abbott Prader-Willi/Angelman Region LSI SNRPN(SO)/CEP15(SG)/PML(SO), LSI D15S11(SO)/CEP15(SG) and LSI D15S11(SO)/CEP15(SG)/PML(SO). The genetic analyses with normal results included chromosome analyses (550 band level) for PWS.
Array comparative genomic hybridization (aCGH) was carried out on the SurePrint G3 Unrestricted CGH ISCA v2, 4x180K Agilent oligoarray CGH, according to manufacture's instruction and analyzed by CytoGenomics v2.0.6.0 (Agilent Technologies, CA, USA). ArrayCGH detected a 1.5Mb duplication on 15q11 (chr15:22,373,313-23,889,744 GRCh37/hg19). We report and compare our propositus with previously reported patients with 15q11 duplication.

V. Belengeanu: None. **M. Stoian:** None. **C. Popescu:** None. **S. Farcas:** None. **N. Andreescu:** None. **A. Belengeanu:** None. **D. Misceo:** None.

P02.025

Confirmation of atypical features in auriculocondylar syndrome caused by recessive PLCB4 mutations

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Auriculocondylar syndrome (ACS; MIM 602483 and 614669) is a branchial arch syndrome typically inherited in an autosomal dominant fashion. Patients with ACS display the following core symptoms with varying severity: a specific malformation of the external ear, known as a 'question mark ear', micrognathia and mandibular condyle hypoplasia. Recently, phospholipase C, β 4 (PLCB4) mutations were identified as the major cause of autosomal dominant ACS, with mutations of the PLCB4 catalytic domain predicted to have a dominant negative effect. In addition, one ACS patient born to related parents harbored a homozygous partial deletion of PLCB4, and presented core features of ACS plus central apnea and macropenis; these features had not been previously reported in association with ACS. This case suggested autosomal recessive inheritance of ACS, with complete loss of function of PLCB4. We herein describe two patients with ACS caused by PLCB4 compound heterozygous splice site mutations. The patients were born to the same unrelated parents, and the parents did not show any features of ACS. Sequencing of PLCB4 revealed the compound heterozygous mutations c.854-1G>A and c.1238+1G>C in both of the patients, with each parent harboring one of the mutations, indicating autosomal recessive ACS. Both of the patients reported here had central apneas, gastrointestinal transit defects and macroenis, in addition to typical craniofacial features of ACS. This is the first example of patients with ACS caused by compound heterozygous splice site mutations in PLCB4, and the second autosomal recessive cases of ACS confirmed by the molecular analysis.

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P02.026

Map of Autosomal Recessive Genetic Diseases in Saudi Arabia: Concepts and Future Directions

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Saudi Arabia has a population of 27.1 million and an overall consanguinity rate of 56%. Prevalence of many autosomal recessive disorders is higher than other known populations. This is attributable to the high rate of consanguineous marriages, the tribal structure, and the large family size. Founder mutations have been recognized in many autosomal recessive disorders, many of which are overrepresented within certain tribes. On the other hand, allelic heterogeneity is also observed among common and rare autosomal recessive conditions. **With the adoption of more advanced molecular techniques in the country in recent years in conjunction with international collaboration, t**he mapping of various autosomal recessive disorders has dramatically increased. Different genetic concepts pertinent to this highly inbred population are discussed here. Addressing such genetic diseases at the national level will become a cornerstone of strategic health care initiatives in the 21st century. Current efforts are hampered by many socio-cultural and health care related factors. Education about genetic diseases, establishment of a "national registry" and mutational database, and enhanced healthcare access are crucial for success of any preventative campaign.

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P02.027

Baraitser-Winter syndrome: genotype-phenotype correlation and clinical variability of the recurrent *ACTB* mutation

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ACTB and ACTG1 mutations have recently been reported to cause Baraitser-Winter syndrome (BRWS) - a rare condition characterized by ptosis, colobomata, neuronal migration disorder, distinct facial anomalies, and intellectual disability (Riviere et al. PMID:22366783). ACTB and ACTG1 code for the beta- and
gamma isoforms of actin - two nearly identical highly conserved proteins that differ only by four amino acids.
The ACTB mutations c.586C>T and c.587G>A, both affecting the Arg196 codon, are recurrent. One of the patients carrying the ACTB
mutation c.587G>A (p.Arg196His) was previously diagnosed with Fryns-Aftimos syndrome (FAS). Expanding our cohort of BRWS patients we observed the c.586C>T(p.Arg196Cys) mutation in four patients with classical BRWS and in one of the severely affected patients assigned as having FAS, who shows malformations beyond the BRWS spectrum, like double halluces (Der Kaloustian et al. PMID:11311002). Furthermore, we identified a novel ACTB mutation c.359C>T (p.Thr120Ile) in a patient strikingly resembling an individual reported by Der Kaloustian et al. This patient exhibits double halluces.
The ACTB mutation c.359C>T (p.Thr120Ile) is analogous to the ACTG1 mutation NM_001614.1:c.359C>T previously reported in a patient with classical BRWS (significantly milder affected than patient analyzed here).Taking together, BRWS and FAS represent the severity range of the same clinical entity. Despite the structural similarity of beta- and gamma-actins and their overlapping expression pattern, mutations in ACTB cause a distinctly more severe phenotype than ACTG1 mutations. The recurrent ACTB mutation c.586C>T (p.Arg196Cys) may result in classical BRWS phenotype as well as in severe BRWS formerly known as FAS.

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P02.028

Bardet Biedel Syndrome in South Africa: The Clinical Phenotype of a Single Mutation in BBS 10.

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Background: Bardet Biedel syndrome (BBS) is a pleiotropic disorder characterized by obesity, polydactyly, intellectual disability and a progressive retinopathy. It is a highly heterogenous autosomal recessive disease. Homozygosity for single mutation in BBS 10 has been identified in a number of affected individuals tested in South Africa.
Objectives: To delineate the ethnic distribution and clinical phenotype in a cohort of South African BBS patients with the K2431fsX15 mutation in BBS 10 and review the implications for genetic testing and counseling in this disorder in South Africa.
Method: A descriptive cross sectional study collating clinical data retrospectively in a genetically homogenous group of BBS patients from South Africa.
Results: A total of 38 patients from 37 families were tested. 27 of these (71%) were homozygous for the K2431fsX15 BBS 10 mutation. BBS is more common in Black South Africans from different language groups (32 of 37 families). Of those homozygous for this mutation, 26 (96%) shared this ethnicity. The phenotype showed characteristic variability. The onset of visual disability was early in our cohort and renal abnormalities were infrequently encountered.
Conclusions: The high frequency of homozygosity for a single mutation in an ethnic subset of the South African population is suggestive of a founder effect. This has allowed establishment of a cost effective diagnostic test for a single mutation with a high yield in our local population. Appreciation of the phenotype may improve earlier recognition of the disorder to allow for appropriate intervention.

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P02.029

Trisomy 11p15.5 and monosomy 13q34 in a girl with Beckwith-Wiedemann syndrome and Factor VII deficiency.

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Here we report an one-year-old patient recruited to genetic analysis because of developmental delay and the presence of specific for Beckwith-Wiedemann syndrome (BWS) phenotype (five major and four minor clinical criteria of BWS was stated). Moreover, she had reduced level of Factor VII. Initial conventional karyotyping was normal. MS-MLPA (methylation specific multiplex ligation-dependent probe amplification) revealed increased hybridization signals as well as aberrant methylation of specific probes in 11p15.5. The duplication was than confirmed by aCGH indicating 4.68 Mb distal duplication in 11p15.5; besides 2.96 Mb distal deletion within 13q34 was identified. Most of the patient's clinical findings are typical of BWS, whereas developmental delay and distinctive craniofacial phenotype (prominent occiput, flat forehead with frontal bossing, round face with full cheeks, hypertelorism, flat nasal bridge and short palpebral fissures) are the features typical of trisomy 11p15. Reduced level of Factor VII might be explained by deletion of 13q34 in combination with a mutation in the factor gene located in that region on the other chromosome 13, considering that Factor VII deficiency is a recessive disorder. To our knowledge this is a first report of 11p15.5 duplication associated with deletion of 13q34 and Factor VII deficiency. The study underlines the importance of detailed clinical description and indicates that aCGH should be performed in all BWS patients with copy-number abnormalities detected by MS-MLPA and normal conventional karyotype to avoid overlooking of a cryptic unbalanced translocation.
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P02.030

Different methylation patterns in Beckwith-Wiedemann syndrome due to mosaicism for UPD of chromosome11p15

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Molecular abnormalities in the 11p15.5 imprinted gene cluster lead to Beckwith-Wiedemann syndrome (BWS), characterized by overgrowth, macroglossia, abdominal wall defects and embryonal tumors. The most common defect in BWS is loss of methylation at imprinting 11p15 control region (~50% of cases). Approximately 20% of individuals fulfilling diagnostic criteria for BWS have paternal UPD for 11p15 critical region. We report eight cases with BWS due to patUPD of 11p15 with the aim to evaluate the percentage of mosaicism. The diagnosis in our patients was established by methylation sensitive multiplex ligation-dependent probe amplification (MS-MLPA) on leukocyte DNA. Microsatellite analysis of chromosome region 11p15, performed in 6 families, confirmed mosaicism for segmental isodisomy at the short arm of chromosome 11. Different methylation pattern in both, H19DMR and KvDMR, domains was observed for each patient, but there has been no correlation between the percentage of mosaicism and methylation index. Clinical presentation of our patients includes variable manifestation of BWS. Only macroglossia and hemihyperplasia were constant symptoms in almost all cases. Clinical heterogeneity is likely due to the variable levels of mosaicism in different tissues. Testing of other tissues than blood in BWS individuals with complex clinical presentations could be a valuable diagnostic tool to improve the detection rate of mosaic paternal UPD. Identification of individuals with mosaicism for paternal UPD is an important goal as identifies individuals who require surveillance due to increased tumor risk.
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P02.031

Beckwith-Wiedeman syndrome - diagnostic experiences in Slovakia

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Beckwith-Wiedeman syndrome (BWS) is a rare genetic disease associated with owergrowth and predisposition to tumor development in childhood. The incidence of BWS in different ethnic group is estimated to be 1 out of 13 700 (Weksberg, 2010).
We present prevalence data in Slovakia, some of clinical data, diagnostic approaches and testing strategy for patient with BWS phenotype. Basic information about BWS prevalence in Slovakia was obtain from Genetic register and by personal communication with Slovak clinical geneticist.
The diagnosis of BWS was reported in 17 cases in 2006, determined solely on the basis of clinical signs EMG syndrome. The total capture of BWS in 2011 includes 25 cases. Early BWS clinical diagnosis within 1 year of life and varying severity of clinical symptoms, different molecular pathology in 3 female cases is documented. Loss of methylation at KVDMR1 was confirmed in mild BWS phenotype with occurence of neuroblastoma. Mutation CDKN1C - c.517dupG (p.Va1173GlyfsX68) was detected in newborn patient with serious omphalocele and renal dysplasia.Generally, in patient with BWS phenotype, except of chromosomal analysis, determination of altered methylation, microdeletion at imprinting center 1(IC1)and/ or (IC2) or mutation in CDKN1 by DNA tests helped confirm BWS diagnosis definitelly. Recent scientific findings and clinical experiences together with activities Eu in the field of the rare diseases pointed to an expanding range of medically indication for genetic testing, which may have important role for health care management, including BWS patients

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P02.032

Blepharophimosis, ptosis, epicanthosis inversus syndrome (BPES) with FOX L2 mutation and congenital hydronephrosis - a new association.

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Blepharophimosis, ptosis, epicanthosis inversus syndrome (BPES) (OMIM 110100) is an autosomal recessively inherited congenital malformation of eyelids which can potentially lead to loss of vision. Squint, refractory errors and amblyopia can develop as complications. Diagnosis is based on clinical findings of bilateral blepharophimosis, ptosis, epicanthus inversus and telecanthus. Type includes these four major criteria. Type includes additional feature of premature ovarian failure and female infertility. We report for the first time a 1 year old male child with congenital hydronephrosis associated with typical features of BPES. He was the first-born to non-consanguineous parents. His father had similar but less severe features. Growth and development were appropriate for age. Echocardiogram was normal. Congenital bilateral hydronephrosis and reduced kidney function were confirmed by renal dynamic scan. Sequencing was performed on FOX L2, the only gene associated with BPES. The normal gene has 14 Alanine repeats. Sequencing of the only intron of FOX L2 revealed the patient to be heterozygous for mutation p.Ala225_Ala234dup (c.672_701dup) which is a recurrent 30 bp duplication, an expansion of the poly-Ala tract. It represents 30% of mutations found in BPES patients and is usually associated with Type. Some additional atypical findings like growth hormone deficiency, ventricular septal defect, Burkitt's lymphoma and Duane syndrome, associated with this mutation have been reported previously. The atypical finding of hydronephrosis may have resulted from a pleiotropic effect of the mutation. It is not possible to know the type of BPES in our patient as no female family member is affected.

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P02.033

Study of 100 fetuses with syndromic brain malformations by aCGH.

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Congenital malformations affect approximately 2.5% of stillborns. Among these defects, brain abnormalities are the main malformations detected prenatally. Mostly (60%) are sporadic without explanation. Improvements of ultrasound prenatal screening combined to 3rd trimester fetal Magnetic Resonance Imaging (MRI) increase detection cerebral malformations. In most cases termination of pregnancy was elected. Foetopathological examination identified other associated malformations in approximately 50% of the cases. No explanation can be offered for about 50% of cases.
An increasing number of congenital malformations have been identified as a result of a genomic disorder using new techniques analysis of a whole genome. We study a cohort of 100 fetuses with a syndromic cerebral malformation divided into 4 groups (neural tube defects, cerebellum hypoplasia, commissural abnormalities, or microcephaly) by aCGH. The aim of this study was to identify potential new genes involved in brain development.
The cases were included with the participation of fetal pathologists, cytogenetics laboratories and biobanks and thanks to the collaboration of the French Society of fetal pathology (SOFFOET). Fetal DNA was extracted, and aCGH was performed on 180K chips (Agilent). We identified 11 de novo pathogenic CNVs and 3 inherited pathogenic CNVs. Several CNVs included a gene already known to be involved in the brain development such as NDE1 or NFIA. In particular we described the first female fetus with cerebellar hypoplasia and partial de novo OPHN1 deletion. We also identified several CNVs highlighting new interesting loci. Here, we present our results and discuss the new CNVs identified.

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P02.034

Associated malformations among patients with CAKUT (congenital anomalies of kidney and urinary tract)

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Infants with congenital anomalies of kidney and urinary tract (CAKUT) often have other associated malformations. The purpose of this investigation was to assess the prevalence and the types of associated malformations in CAKUT in a defined population. Each affected child was examined by a geneticist, all elective terminations were ascertained, and the surveillance for malformations was continued until 2 years of age. The associated malformations in
CAKUT were collected in all livebirths, stillbirths and terminations of pregnancy during 26 years in 347,810 consecutive births in the area covered by our population based registry of congenital malformations. Of the 1252

infants with CAKUT born during this period (prevalence at birth of 36.0 per 10,000), 410 (32.7 %) had associated malformations. There were 142 (34.6%) patients with chromosomal abnormalities including 15 trisomies 18, and 72 (17.6%) nonchromosomal recognized dysmorphic conditions. There were no predominant recognised dysmorphic conditions, but VA(C)TER(L) association. However, other recognised dysmorphic conditions were registered including Meckel syndrome, osteochondrodysplasia, and caudal regression syndrome. Hundred ninety six (47.8 %) of the patients had multiple congenital anomalies, non syndromic, non chromosomal (MCA). Malformations in the ear, the digestive, the central nervous, and the cardiovascular systems were the most common other malformations. Prenatal diagnosis was performed in 57.8 % of dysmorphic syndromes with CAKUT. The overall prevalence of associated malformations, which was one in three infants, emphasizes the need for a thorough investigation of infants with CAKUT. A routine screening for other malformations may be considered in infants and in fetuses with CAKUT.

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P02.035

Congenital heart defect in a patient with a complex rearrangement and a 13q deletion: delineating a new critical region.

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13q deletion syndrome is a rare genetic disorder caused by deletions of the long arm of chromosome 13. Based on the size and location of the deletion, this syndrome can be divided into three groups, group 3 corresponds to the distal deletion of band 13q33-34. Patients with 13q deletion display a variety of phenotypic features, including growth retardation, microcephaly, facial dysmorphisms, congenital heart, brain and kidney defects. We report the case of a dysmorphic 7-year old male patient displaying polymalformative features and carrying a deletion of approximately 8.57 Mb on 13q33.1q34 (group3), including 20 genes. The phenotype of our patient includes intrauterine growth retardation, developmental retardation, hypospadias, microcephaly, facial anomalies and a congenital heart defect. The deletion size of our patient was further delineated by array-CGH, and precisely located on chromosome 13: 103.941.149-112.512.705 (hg19). Futhermore, we can reduce the critical region to 3 Mb instead of 6 Mb (Huang, 2012). This new chromosomal region contains only 12 genes including *MYO16*, *COL4A1*; *COL4A2*, *ING1* and *ARHGEF7*.
On a cytogenetic point of view, the chromosome 13 of our patient appeared to be a derivative chromosome 13 coming from a complex maternal chromosomal rearrangement involving three chromosomes and four breakpoints. The maternal formula is : 46,XX,t(7;8)(q36;p21),ins(8;13)(p21;q33.1q34). We will discuss the involvement of the deleted genes in the phenotype of our patient and we will also describe the precise mechanisms leading to this rearrangement.

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P02.036

A heterozygous frame-shift mutation in CAV1 is associated with a severe congenital lipodystrophy syndrome

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We investigated a single female patient and unaffected parents presenting with a neonatal progeroid disorder, lipodystrophy, cutis marmorata, feeding disorder and failure to thrive by whole-genome sequencing. This revealed a de novo, heterozygous, frame-shift mutation in the Caveolin1 gene (CAV1), which introduces an immediate stop into the protein (c.385_386delTT; p.Phe129X). Mutations in CAV1, encoding the main component of the caveolae in plasma membranes, cause Berardinelli-Seip congenital lipodystrophy type 3. Although this disorder is recessive, heterozygous carriers either show a reduced phenotype of partial lipodystrophy or no phenotype. We could not identify a second mutation in CAV1 in our patient. Studying mRNA extracted from whole blood from the patient revealed that both MT and WT CAV1 were transcribed, and that RNA from the mutant allele was not degraded by nonsense mediated RNA decay. We therefore hypothesize that the mutant protein is expressed in our patient and acts in a dominant negative fashion, leading to a severe lipodystrophy phenotype. Introduction of this mutant CAV1 could disturb the stable and functional hetero-oligomeric complex formed by Cav1 and Cav2, or disturb cellular localization. We will therefore introduce MT and WT CAV1 in a stable cell line to study the effect of the mutant protein on wild type function and localization.
In conclusion, we suggest that the severe phenotype observed in our patient might be caused by a dominant-negative effect of MT CAV1, and that the presence of a partial lipodystrophy phenotype found in some patients might be dependent on the type of CAV1 mutation.

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P02.037

Searching for the disease gene in a cerebellar ataxia family

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Autosomal recessive ataxia is characterized by progressive lack of coordination of muscle movements. We studied two brothers at the age of 38 and 40 years with initial diagnosis of cerebellar ataxia. The parents were second cousins. The patients walked and spoke single words by the age of 2 years, and progressive ataxic gait and visual impairment began in early childhood. At present the patients have ataxic gait, dysmetria, dysdiadochokinesia, scanning speech and intellectual disability. Additionally, eye examination showed bilateral horizontal nystagmus, posterior subcapsular cataract, optic disk hypoplasia, optic atrophy, thinning of the retinal arterioles, and retinal bone spicule pigmentation.
Genotyping data generated by SNP genome scan for the parents and the affected sibs were used to calculate multipoint LOD scores. Four loci yielded the highest score of 2.66. The largest locus was maximally 52 Mb at chromosome 4q12-24. The others were at 6q25.1-25.2, 12p13.31-p13.1, and 22q12.1 and 0.55 to 5.5 Mb in size. We consider the largest locus (4q12-24) the most likely disease locus but will search for the genetic defect underlying the disease in all four candidate loci. No similar diseases were mapped to any of the loci. The four candidate loci harbor a total of 638 genes. The abundance of the genes discouraged us from applying candidate gene approach, and we launched exome sequencing. Exome sequencing data will be evaluated for those regions, and Sanger sequencing will be performed to validate candidate mutations.

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P02.038

Disruption in *TMCO1* causes autosomal recessive cerebro-facio-thoracic dysplasia

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Cerebro-facio-thoracic dysplasia (CFTD; MIM 213980) is a multiple congenital anomaly and intellectual disability syndrome involving the cranium, face and thorax. The characteristic features are cranial involvement with macrocrania at birth, brachycephaly, various CT/MRI findings frequently involving corpus callosum and septum pellucidum, flat face, hypertelorism, epicanthal folds, synophrys, broad nasal bridge, cleft lip and cleft palate, low-set, posteriorly rotated ears, short neck, and multiple costal and vertebral anomalies. The underlying genetic defect remains unknown. Using a combination of homozygosity mapping and whole-exome sequencing, we identified a homozygous nonsense founder mutation, p.R87X (c.259 C>T), in the human transmembrane and coiled-coil domains protein 1 (*TMCO1*) in four out of five families with Turkish origin. RNA studies on lymphocytes indicated that the mutated TMCO1 transcript is degraded by nonsense-mediated mRNA decay. *TMCO1* gene on chromosome 1q24 was excluded by homozygosity mapping and DNA sequencing analysis in the fifth family with characteristic findings of CFTD. These data provided the first evidence for genetic heterogeneity in this spectrum. Another founder *TMCO1* mutation has recently been reported to cause a unique genetic condition, TMCO1-defect syndrome (MIM 614132), characterized by craniofacial dysmorphism, skeletal anomalies and intellectual disability in 11 individuals in the Old Order Amish of

northeastern Ohio. Phenotype comparison between Turkish and American families clearly demonstrated that TMCO1-defect syndrome meets diagnostic criteria for CFTD which was first described by Pascual-Castroviejo et al. in 1975. Our study demonstrated that TMCO1-defect syndrome, initially believed to represent a distinct disorder, indeed belongs to the genetically heterogeneous cerebro-facio-thoracic dysplasia spectrum.

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P02.039

An intriguing hotspot of recurrent mutations in CHD7 IVS25 revealed by minigene assay is observed in CHARGE syndrome

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CHARGE syndrome (CS) is a rare usually sporadic disorder with multiple congenital anomalies mainly due to de novo truncating mutations of CHD7 gene.

Here we report a series of 20 CS patients carrying a nucleotide variation located in CHD7 IVS25 (c.5405-7G>A, c.5405-13G>A, c.5405-17G>A or c.5405-18C>A). Among these 4 different variants, two of them (-13G>A and -18C>A) were first isolated in our laboratory, the others were previously classified as variants with unknown clinical significance (-7G>A) and pathogenic (-17G>A). In each case, bioinformatics tools clearly indicated an activation of a newly created acceptor splice site. Splicing defect was then evaluated by minigene assays using an exon-trap derived construct: pSpliceExpress (Kishore et al., 2008). Our study demonstrates that alternative acceptor splice sites generated by the four IVS25 variants were exclusively used by splicing machinery giving rise to a longer exon 26. With the exception of the -17G>A which preserves the normal ORF, the three others correspond to frameshift mutations. Although all patients fulfilled diagnostic criteria of CS we observed phenotypic variability, especially within 2 cases where the mutation (c.5405-17G>A) was inherited from a mildly affected parent. Recurrence of these variations could be explained by a particular genomic context: i) the weakness of the exon 26 natural acceptor site, ii) the lack of a real lariat sequence detected within hundred nucleotides upstream exon/intron boundary.

These data prove the pathogenicity of all the studied variants and indicate the presence of a mutation hotspot in CHD7 IVS25 which is involved in 7% of CS french patients.

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P02.040

Transcriptome analysis of wildtype and Chd7$^{Whi/Whi}$ embryos reveals a misregulation of several genes involved in neural crest cell processes.

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CHARGE syndrome (***c***oloboma, ***h***eart defects, ***a***tresia of the choanae, ***r***etarded growth and development, ***g***enital hypoplasia and ***e***ar anomalies) is an autosomal dominant malformation syndrome due to mutations in the chromodomain helicase DNA-binding member *CHD7*. A well established mouse model for this disease is the *Whirligig* mouse (Chd7$^{Whi/+}$), carrying a heterozygous nonsense mutation in exon 11 of the *Chd7* gene. To get molecular insights on the pathogenesis of CHARGE syndrome, we performed a genome-wide microarray expression analysis on wildtype, Chd7$^{Whi/+}$ and Chd7$^{Whi/Whi}$ mouse embryos at day 9.5.We identified 98 genes showing greater than 2 fold differences in expression (log2 fold-change) and a P-value to false discovery rate (FDR) < 0.05 between wildtype and Chd7$^{Whi/Whi}$ embryos. The validation of the microarray data was performed by quantitative real time PCR (qRT-PCR). Interestingly, most genes are involved in neural crest cell induction, formation and migration.

In summary, we could show that misregulation of genes involved in neural crest cell processes is a mechanism behind CHARGE syndrome, demonstrating that CHARGE syndrome belongs to the neurocrestopathies.

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P02.041

Identification of the variations in *CPT1B* and *CHKB* genes in Turkish narcolepsy patients

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The rs55770917 variation between *CPT1B* and *CHKB* genes in Japanese and Koreans are common genetic susceptibility factors to narcolepsy. In this study, variations in *CHKB* and *CPT1B* susceptibility genes were examined in Turkish narcolepsy patients.

The *CPT1B* and *CHKB* genes were sequenced in narcolepsy patients (n= 37), and, when available, their parents. Controls (n= 100) were tested for detected variations. rs5770911, rs2269381 and rs2269382 were detected together in three patients and 11 controls, rs55770917 being susceptibility SNP. In addition to this haplotype, we detected the indel variation (rs144647670) in the 5' upstream region of the human *CHKB* gene in the narcoleptic patients and control subjects carrying four variants together. There was no significant difference between patients and control subjects regarding the presence of variant haplotype and allele frequencies of (p =0.62). There was no significant association between rs5770917 and narcolepsy in Turkish patients. However, in our study we found novel haplotype consisting of the indel variation, which had not been detected in previous studies on Japanese and Korean populations, as well as observing four SNPs in *CHKB/CPT1B* .

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P02.042

ALG3-CDG (CDG-Id) presenting with chondrodysplasia punctata: a case report.

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Chondrodysplasia punctata (CDP) is defined by the radiographic appearance of abnormal cartilaginous stippling. It is generally associated with short stature, maxillofacial hypoplasia and the possible occurrence of multiple

congenital abnormalities (MCA). CDP has been reported in various congenital disorders such as chromosomal abnormalities, disruptions of vitamin K metabolism, and inborn errors of metabolism including peroxisomal disorders, defects of cholesterol biosynthesis and lysosomal storage diseases.
We report two siblings presenting with skeletal dysplasia, CDP and MCA. Patient 1 presented with severe skeletal abnormalities, stippling, vermis hypoplasia, partial corpus callosum agenesis and hepatic fibrosis; the pregnancy was terminated. His sister (Patient 2), showing a similar phenotype (skeletal dysplasia, dysmorphic features, cataract, cerebellar and corpus callosum abnormalities, liver enlargement), died in the neonatal period.
A large metabolic assessment was performed in Patient 2. Screening for congenital disorders of glycosylation (CDG) by Western blot was consistent with "type I" CDG. Phosphomannomutase and phosphomannose isomerase enzymatic assays, performed on patient's fibroblasts, were normal as well as *PMM2* molecular analysis. The analysis of the dolichol linked oligosaccharide precursors was consistent with ALG3-CDG (CDG-Id) and molecular analysis of the *ALG3* gene found a homozygous mutation (p.G96R; c.286G>A), thus confirming the diagnosis.
CDG are a group of metabolic disorders presenting with very heterogeneous multisystem clinical manifestations. To the best of our knowledge, only seven other patients with ALG3-CDG have been described to date. This is the first report of a CDG syndrome presenting with CDP. Screening for CDG should be systematically performed in patients presenting with unexplained CDP.

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P02.043
Phenotypic spectrum and diagnostic considerations in children with somatic chromosomal mosaicism

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Chromosomal mosaicism typically presents with developmental delay, dysmorphic features, asymmetry and characteristic variation in skin pigmentation following Blaschko's lines. Seizures and other congenital anomalies may also be present. Array-CGH in blood detects mosaicism down to a level of around 10%, but the abnormal cell line may not be present in blood cell lineages. In these circumstances the diagnosis can be confirmed by carrying out array-CGH on cultured skin fibroblasts. We present several cases which exhibit the wide phenotypic spectrum of chromosomal mosaicism. This includes presentation with typical features such as pigmentary abnormalities or hemihypertrophy, but also an unusual presentation with complex congenital malformations and developmental delay with none of the canonical features. Array-CGH in blood was normal in the majority of cases regardless of age at testing, and the investigative route to diagnosis was therefore variable and complex. The abnormal cell lines included mosaicism for diploid/triploidy, trisomy and large chromosomal imbalance. There was little or no phenotypic correlation with the precise chromosomal abnormality. We also discuss the differential diagnoses including Hypomelanosis of Ito, Incontinentia pigmenti and Nevus depigmentosus. The low sibling recurrence risk attached to the diagnosis of mosaicism makes confirmation important for affected families. Atypical clinical features may lead to a diagnostic odyssey before confirmation, frequently as a result of skin biopsy. This series of cases suggests that skin biopsy should be considered at an earlier stage in a wider spectrum of clinical presentations than is usual in current practice.

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P02.044
Orofacial clefts associated with other congenital abnormalities seen at a South African multidisciplinary clinic

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Clefts involving the lip and/or palate (CLP) or isolated clefts of the palate (CP) are among the most common congenital anomalies and require complex multi-professional treatment. The incidence varies according to geographical region, ethnicity and socio-economic status with a general accepted incidence in the Caucasian population of 1:800 to 1:1000. The incidence in the black South African population is unsure but estimated to be lower. Approximately 70% of CLP cases are non-syndromic, occurring as an isolated condition while the remaining 30% are present in association with abnormalities occurring outside the region of the cleft. Over 300 syndromes are known to be associated with CLP.
A retrospective analysis was done of the records of all patients who were registered at the Facial Cleft Deformity Clinic (FCDC) of the University of Pretoria since its founding in 1983.
Of the 3971 patients seen up to date 21% had associated abnormalities. These abnormalities were subdivided according to known described syndromes as part of a Mendelian disorder, demonstrated chromosomal defects; facial oblique clefts; skull synostosis and other unspecified congenital abnormalities.
Advances in the understanding of the aetiology of CLP continue and will prompt more accurate methods of genetic screening, molecular diagnosis and identification of at risk individuals, genetic counselling and prenatal diagnosis.

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P02.045
Drawbacks of genotype-phenotype correlation in Cleidocranial Dysplasia in eleven families

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Cleidocranial dysplasia (CCD) is a dominantly inherited autosomal disorder characterized by abnormally large, wide-open fontanels, delayed closure of the cranial sutures, mid-face hypoplasia, abnormal dentition, clavicular hypoplasia/aplasia and other skeletal abnormalities. Diagnosis could easily be determined by clinical and radiological findings. Affected individuals are shorter than their unaffected sibs. It is reported to be a rare disorder with a frequency of one in a million individuals but the incidence could be higher, since relatively mild or absent medical comlications may go underdiagnosed. Presently, *RUNX2*, encoding an essential transcription factor for osteoblast differentiation, is the only gene associated with CCD. Mutation detection rate is reported to be 60%-70% in affected individuals describing total of 177 mutations in the coding and important splice sites regions of *RUNX2*. The phenotypic spectrum of CCD ranges from a single clinical finding of a primary dental anomalies to a full-blown CCD phenotype, no clear phenotype-genotype correlation has yet been established. One recent article , suggested an association between dental alterations and mutations on runt domain of *RUNX2* gene.
We will report clinical/radiological and molecular findings of 15 affected cases from 11 families. Novel mutations identified in *RUNX2* gene will be discussed in view of the already reported mutations.
We anticipate that our findings will help to gain more perspective for the investigations on genotype/phenotype corelation studies on CCD and its drawbacks.

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P02.046
A de novo 6q25.3 deletion comprising *ARID1B* responsible for Coffin-Siris syndrome

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6q25.3 microdeletions identified on array CGH have been reported in association with intellectual disability, acquired microcephaly, dysmorphic features, structural anomalies of the brain and non specific multiple organ anomalies. *ARID1B*, comprised in this deletion, had been identified as the potential candidate gene to explain this distinctive phenotype. Coffin-Siris syndrome (CSS) is a rare, clinically heterogeneous disorder often considered in the setting of cognitive/developmental delay and 5th finger/nail hypoplasia. Due to the clinical variability, this diagnosis can be difficult to confirm clinically and the existence of this disorder as a specific diagnosis has even been at times an issue of debate. Recently, haploinsufficiency of *ARID1B*, now known as one of the SWI/SNF chromatin-remodeling complex member, has been identified as causing CSS. Therefore the 6q25.3 deletion

distinct phenotype should be considered as CSS too.
We report on an 8 months old male, born to healthy unrelated parents. Complex heart malformation had been identified on antenatal scans and parents declined invasive testing. He was born with normal growth parameters. Examination revealed slight dysmorphic features comprising a hirsute forehead, down-slanting palpebral fissures, full lips, deep palmar and plantar creases and small fifth toenails. Tight aortic coarctation was associated with a small mitral valve and poor function of the lateral left ventricle wall. Brain MRI revealed hypoplasia of the corpus callosum. Array-CGH revealed a de novo 69kb deletion at 6q25.3, resulting in partial deletion of *ARID1B*. Since *ARID1B* has also been involved in childhood neuroblastoma, we will discuss about clinical management and genetic counselling implications.

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P02.047

Apoptosis is responsible for neutropenia in Cohen syndrome

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Cohen syndrome (CS) is an autosomal recessive disorder, displaying intellectual disability, microcephaly, truncal obesity, retinal dystrophy and intermittent neutropenia. CS is due to mutations in the *VPS13B* gene, encoding a crucial Golgi-membrane protein. Neutropenia being very rarely described in syndromology, we hypothesized that neutropenia could be a clue to approach the pathophysiology of CS. According to studies in other congenital neutropenias, it could be due to maturation arrest of neutrophils, dysfunctions or apoptosis of mature neutrophils. We confirmed that granulopoïesis was normal in CS patients; neutrophils display normal morphology, chemotaxis, phagocytosis and generation of reactive oxygen species. Transmission electron microscopy revealed a disrupted Golgi apparatus morphology. We then isolated $CD16^+$ neutrophils from CS patients and healthy donors and showed by Annexin V-propidium iodide and Hoescht staining that CS neutrophils displayed an increased apoptosis rate compared to healthy donors. This exaggerated apoptosis was observed only in neutropenic patients, and was specific of neutrophils, as CS $CD3^+$ lymphocytes and $CD14^+$ monocytes were not affected. For most of CS patients, in contrast to healthy donors, GM-CSF had no protector effect despite normal GM-CSF serum level, receptor mRNA expression and signaling. *VPS13B* mRNA expression dramatically decreased during spontaneous apoptosis of normal neutrophils whereas mRNA expressions of 4 other genes (*ELA2, HPN3, MPO* and *LYZ*) were stable, confirming the correlation between VPS13B and apoptosis. As congenital neutropenia can result from increased endoplasmic reticulum stress, experiments are in progress to determine its involvement in CS. These results may give clue for understanding other CS features.

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P02.048

When transmission modifies the complexity of familial complexe rearrangements

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Complex Chromosome Rearrangements (CCR) are rare events, defined by the occurrence of at least 3 chromosome breakpoints. The degree of complexity of CCRs can be very high and the related meiotic disturbances result in important chromosomal imbalance in gametes. During meiosis, crossing-overs between involved chromosomes can modify the complexity of the rearrangement with numerous possibilities of chromosomal imbalance in gametes. We report 2 familial cases in which meiotic events result in a new chromosome rearrangement. Chromosomal analyses were performed using classical cytogenetic procedures (R- and G-banding) and various FISH techniques (chromosome painting and array-CGH). In addition, we developed 2 additional methodological approaches, the „chromosome walking" and Array Painting, for the breakpoint studies. In our first case, karyotyping was done following the discovery of a multiple malformation syndrome on ultrasound examination. The fetal karyotype showed an unbalanced CCR with 3 breakpoints. Subsequent analysis of the mother's chromosomes revealed an apparently balanced CCR with 4 breakpoints. For the second case, a male carrier of a simple reciprocal translocation inherited through his mother fathered a girl with a more complex rearrangement involving a third chromosome. In the 2 cases, a re-building of the initial rearrangement occurred through meiotic recombinations, giving rise to simpler or more complicated karyotypes. For both cases, breakpoint studies led to an understanding of the involved mechanisms underlying the simplification or complexification of the parental rearrangement.

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P02.049

Prenatal diagnosis of paternal uniparental isodisomy of chromosome 14 (patiUPD14) with chromosomal micro-array (CMA) in a fetus with multiple congenital abnormalities.

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Case report
The patient, a 43 years old primigravid woman was referred at 12 weeks of gestation for prenatal diagnosis because an ultrasound scan identified an increased nuchal translucency (3.8 mm, CRL 59 mm). The karyotype on uncultured chorionic villi was normal: 46,XX (15 cells, RHG banding). There were no particularities in the familial history nor consanguinity.
CMA was performed with PrecytoNEM (derived from Agilent 60K, resolution 400 Kb) and didn't show any unbalanced abnormality.
At 23 WG, a subsequent ultrasound showed multiple abnormalities. There was a polyhydramnios, a generalized oedema, dysmorphic features with long filtrum, small rounded ears and prefrontal oedema, a very short femur length (<1° percentile) in contrast with the body weight estimated at the 83° percentile, an abdominal wall eventration and large feet with abnormal hallux implantation.
Amniocentesis was performed to exclude a tetrasomy 12p (Pallister-Killian syndrome) possibly undiagnosed on chorionic villi because of a chromosomal feto-placental discrepancy: the karyotype was again normal and the Fish analysis ruled out tetrasomy 12p. The parents opted for termination of pregnancy after counselling about the poor prognosis of the fetal malformations. Post-mortem examination evoked patUPD14.
The CMA with Affymetrix CytoScan (SNP-array) on the amniotic fluid again did not show any pathogenic CNV but exhibited a complete isodisomy for chromosome 14. Paternal iUPD14 was confirmed by parental microsatellite segregation.
Discussion
We discuss the different underlying causes for patUPD. Although it's an uncommon finding in fetal malformations, SNP's CMA allowing both UPD and CNV detection may be helpful in such conditions.

A. Bazin: None. **D. Trost:** None. **P. Kleinfinger:** None. **L. Lohmann:** None. **B. Bessières:** None. **F. Guis:** None.

P02.050

Impaired embryogenesis (plurimalformative syndromes) as a leading cause of altered anthropometric parameters in patients with congenital anomalies of the kidney and urinary tract

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Introduction: Congenital anomalies of the kidney and urinary tract (CAKUT) comprise all the defects that appear during the embryonic development of the kidney and lead to altered renal structure and/or function. Material and method: We studied 209 children with CAKUT admitted to our Nephrology Department between January 1st 2004 and December 31st 2009. The mean age of patients at admission was 7.12 ± 5.88 years. We assessed the anthropometric status of our lot by calculating the Z score for weight and height, we evaluated CAKUT that were associated with malnourishment, and how renal failure influenced height and/or weight. Results: Distribution of the patients according to sex and renal anomaly showed that the dia-

gnosis of certain CAKUT is more frequent in the female sex: congenital hydronephrosis (17.22%), vesicouretheral reflux (14.35%), abnormal kidney position (10.52%), renal dys/hypoplasia (7.17%), double collecting system (6.69%), renal agenesis (4.78%), only the ureteropelvic junction obstruction (11.96%) was more frequent in the male sex. Analysis of anthropometric indices enabled us to obtain the weight and height Z score histogram, demonstrating the group's uniformity and highlighting the cases (5.26%) with 2nd and 3rd stage malnourishment that were associated with:(a) the following CAKUT: vesicoureteral reflux, renal dysplasia, neurogenic bladder, bladderepispadias extrophy syndrome, Prune-Belly syndrome, double collecting system and polycystic kidney disease; and (b) renal failure stages 4 and 5. Conclusion: Two factors were implicated in altered anthropometric parameters in our patients: impaired embryogenesis (plurimalformative syndromes) and chronic renal failure.

C. Daescu: None. **O. Marginean:** None. **M. Puiu:** None. **A. Craciun:** None. **T. Marcovici:** None. **A. Militaru:** None. **O. Belei:** None. **D. Chiru:** None. **C. Pienar:** None. **A. Chirita-Emandi:** None.

P02.051

Is Arg5 in HOX_DNA binding domain of *HOXB1* hot spot for congenital facial paralysis mimicking Moebius syndrome?

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Congenital facial paralysis (CFP) and Moebius syndrome (MBS; MIM 157900) are highly sporadic, formulating **classical** gene identification **algorithms** difficult, assigning candidate gene approach compatible. Mutant mice for *Hoxb1* were long before reported to present features of facial nerve defects, resembling MBS, only very recently associated with human phenotype. Currently, homozygous c.619C>T in *HOXB1* revealed in the two CFP affected individuals from two different families, both from the same geographic origin, altering arginine to cysteine at 207 (p.arg207cys) corresponds *arg*5 residue of the HOXB1 homeodomain.
We have screened our cohort of 33 sporadic MBS and CFP patients for mutation on *HOXB1* and identified homozygous c.620G>A in one consanguineous family. Alteration changes the same neutral-polar arginine, via second nucleotide, resulting to basic-polar histidine (p.arg207his). Clinical investigation of our case presented left esotropia, right flattened/broad nasal bridge, external ear defects, high arched palate, bilateral cranial nerve VII dysfunction, diagnosed as CFP. We further screened *HOXB1* in 56 DNA samples of MBS cases, referred to us from Radbound University Nijmegen-Holland, none found to carry any pathological alteration. It is surprising that, such a rare disorder with three only identified mutation striking the same conserved amino acid, delegating *arg*5 the achilles of HOXB1 protein. Up to date, out of 267 families with MBS or with the facial weakness, component of MBS (177 from Webb et al.2012, 56 from Nijmegan group and 34 from our center), only three reported to carry mutations in *HOXB1* gene, figuring the prevalence of *HOXB1* mutation frequency to be 1.12%.

O.Z. Uyguner: None. **H. Ozgur:** None. **U. Altunoglu:** None. **G. Toksoy:** None. **S. Basaran:** None. **H. van Bokhoven:** None. **H. Kayserili:** None.

P02.052

Congenital heart defects in recurrent reciprocal 1q21.1 deletion and duplication syndromes: Rare association with pulmonary valve stenosis

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Microdeletion 1q21.1 (del 1q21.1) and the reciprocal microduplication 1q21.1 (dup 1q21.1) are newly recognized genomic disorders, characterized by developmental delay, dysmorphic features and congenital malformations. Congenital heart defect (CHD) is a major feature of del 1q21.1, and has been occasionally reported in dup 1q21.1. We report here a family segregating del 1q21.1 in 3 members. Two of the affected family members had CHD, including the proband with syndromic atrial septal defect, pulmonary valve stenosis (PVS), and muscular ventricular septal defects, and the maternal uncle with non-syndromic PVS. This finding prompted investigation of the role of recurrent rearrangements of chromosome 1q21.1 in the pathogenesis of PVS. We gathered 38 patients with PVS (11 syndromic and 27 non-syndromic), and searched for genomic rearrangements of 1q21.1. A dup 1q21.1 was detected in a single sporadic non-syndromic patient. Review of the CHDs in published del 1q21.1 and dup 1q21.1 subjects showed a great heterogeneity in anatomic types. In conclusion, the present family illustrates recurrent CHD in del 1q21.1, expressing either as syndromic in one family member or as non-syndromic in the another one. The spectrum of CHDs associated with del 1q21.1 and dup 1q21.1 can occasionally include PVS.

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P02.053

A second case of Cornelia de Lange Syndrome with mutation in the SMC3 gene confirms this gene as a cause of the syndrome.

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Cornelia de Lange syndrome (CdLS) is an inherited disorder characterized by facial dysmorphism, growth and cognitive impairment, limb malformations and multiple organ involvement. Mutations in five genes that encode components of cohesin complex account for 70% of patients with CdLS-like phenotype. However, in one of the genes, *SMC3*, only one mutation in a mildly affected adult has been reported.
Here, we report on the second novel mutation in the SMC3 gene in a boy with CdLS. He showed classical CdLS phenotype with typical facial dysmorphism, multiple musculoskeletal anomalies, hirsutism and severe developmental delay. He also had myopia, moderate hearing loss, mild pulmonary and supravalvular aortic stenosis, pyloric stenosis, vertebral anomalies, and gastroesophageal reflux disease. Molecular analysis identified a *de novo* mutation in exon 22 (c.2494_2499del) of the *SMC3* gene, which predicted in-frame deletions of two residues highly conserved and mapped to the C-terminal coiled-coil domain, p.(Leu832_Asn833del). Structural analysis of the mutant SMC3 protein showed a serious shift in the relative position of the accompanying residues, leading to the displacement of the corresponding residues of the two antiparallel helices that form the coiled coil.
Our case demonstrates that mutations in the *SMC3* gene may cause a mild to moderate phenotype, as it has been previously reported in some patients with mutations in the *SMC1A* gene. Finally, we recommend to perform mutation screening of *SMC3* in patients with CdLS phenotype in whom no mutation is identified in the other known CdLS genes.

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P02.054

SNP arrays in the diagnostic strategy of corpus callosum agenesis associated with intellectual disability.

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Corpus callosum agenesis is the most common cerebral malformation in patients with intellectual disability (CCA-ID) with a prevalence of 2-3%. Known genetic causes are heterogeneous and in most cases, the etiologies remain unknown.
In order to achieve a genetic diagnosis, we performed chromosome analyses on microarrays (CMA) on 51 patients with CCA-ID and no known causes. We used Illumina CytoSNP-12 SNP arrays which contain 300k probes.
We found 21 different CNVs (41%) not reported in control subjects of the Database of Genomic Variants (DGV). Among these CNVs, 8 deletions (16%) were de novo and considered to be likely pathogenic, with sizes varying

from 1,3Mb to 24Mb. No de novo duplications were found.
Moreover, 10 CNVs were also carried by healthy parents, and therefore, could not be considered as the main causes of the phenotype. We were not able to recover blood samples of the parents to verify the 3 remaining CNVs.
Thus, CMA seems to be a powerful tool in the diagnostic strategy of patients with CCA-ID and no etiological diagnosis. However, the causes of CCA-ID remain unknown in most patients. In a near future, new techniques such as exome sequencing, or massively parallel sequencing on selected genes panels, will certainly improve the detection rate of the genetic causes of CCA-ID.

B. Keren: None. **M. Moutard:** None. **C. Mignot:** None. **A. Jacquette:** None. **S. Chantot-Bastaraud:** None. **P. Charles:** None. **S. Whalen:** None. **E. Fonteneau:** None. **C. Mach:** None. **M. Gérard:** None. **A. Faudet:** None. **A. Brice:** None. **C. Depienne:** None. **T. Billette de Villemeur:** None. **D. Héron:** None.

P02.055

Molecular Test Results of Syndromic Craniosynostosis Patients: genotype-phenotype correlations

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Synostosis is the premature fusion of cranial sutures in the brain vault producing continued growth at the position of the open cranium suture in parallel to brain growth resulting in morphological deformation called Craniosynostosis. It is observed in 1/2100-1/2500 live births, occurring in both syndromic and non-syndromic forms and addressed in approximately 180 different syndromes. Recent studies have shown that notably in 20% of cases are caused by single gene mutations or chromosome abnormalities. *FGFR2*, *FGFR3*, *TWIST1* and *EFNB1* are listed to be the most common causative genes in craniosynostosis, though rarely involved many others like *FGFR1*, *MSX2* are already known and growing number of novel genes are intensely being identified. Mutations in *FGFR2*, *FGFR3*, *TWIST1* are involved in syndromic and lesser extent in non syndromic forms while *EFNB1* are solely recognized to be associated with Craniofrontonasal Syndrome (CFNS).
Thirty craniosynostosis patients, except CFNS, where chromosomal abnormalities were previously excluded, are recruited to our research study with their families. Our workflow will be targeted mutation screening for common genes, *FGFR2* and *FGFR3*, mutation negative patients will be subject to deletion/duplication analysis by craniofrontonasal MLPA kit, which will follow by *MSX2* sequencing and targeted mutation screening for *FGFR1*.
Our investigation is ongoing presently. We anticipate that our results will foster the acknowledged molecular diagnostic flow charts in craniosynostosis and further delineate genotype-phenotype relationship. Undefined cases will be esteemed subjects for novel gene identification by next generation sequencing.

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P02.056

A new syndrome of intellectual disability, postnatal microcephaly, progressive ataxia and spasticity and hair anomalies caused by CTNNB1 haploinsufficiency.

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We report on a 5 ½ year-old girl patient with a new syndrome characterized by developmental delay, postnatal microcephaly, progressive ataxia and spasticity, light skin and sparse, fair hair, caused by a small de novo 3p22 microdeletion encompassing the entire genomic sequence of the gene CTNNB1. CTNNB1 encodes β-catenin which has been shown to be essential for dendritic development of postnatal neurons in vivo and is also required in the skin for the differentiation of stem cells into hair follicles. Conditional β-catenin knock out mice have brain malformations, neuronal loss, impaired craniofacial development and hair follicle morphogenesis defects, a phenotype reminiscent of that of the patient presented here. In addition, point mutations of CTNNB1 have been recently reported in 3 patients with intellectual disability, using an exome sequencing strategy. Altogether, the data presented here provide compelling evidence that haploinsufficiency of CTNNB1 in human causes a new syndrome combining intellectual disability, postnatal microcephaly, progressive ataxia and spasticity, and skin and hair anomalies.

E. Dubruc: None. **A. Putoux:** None. **A. Labalme:** None. **C. Rougeot:** None. **D. Sanlaville:** None. **P. Edery:** None.

P02.057

The First Reported Surviving Patient with Skeletal Abnormalities and Craniosynostosis due to Homozygous Mutations in the *CYP26B1* Gene

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In 2011, Laue et al reported two families with a lethal syndrome of craniosynostosis and multiple skeletal abnormalities due to mutations in the *CYP26B1* gene which encodes the retinoic acid-degrading enzyme CYP26B1.
We present a 22 year old female who is the first child of consanguineous parents. She was born with craniosynostosis involving the coronal and lambdoid sutures, together with limited elbow extension and finger contractures. She was thought to have hypoplastic external genitalia. CT scan of the head showed complex craniosynsotosis with a large unossified area of skull and multiple craniolacunae along the fused lambdoid sutures and in the occiput. Urinary steroid profile was normal. An initial diagnosis of Antley Bixler syndrome was made. Early milestones were normal, but she later had moderate developmental delay. She attended a school for moderate learning disabilities, and has since studied life skills at college. She is currently seeking employment and hopes to live independently. She menstruates normally. She has had one fracture, following a fall, but no other fractures. She has bilateral conductive hearing loss and has bone anchored hearing aids.
CYP26B1 analysis showed novel homozygous c.1303G>A (p.Gly435Ser) mutations. Both parents were confirmed as heterozygotes.
This patient is only the third reported case worldwide and the only case to survive beyond infancy. The diagnosis is important because it allows accurate genetic counselling and may have implications for on-going management since continued elevated retinoic acid may lead to decreased bone mineral density.
Reference: Laue et al AJHG (2011) **89** 595-606

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P02.058

A novel ALMS1 splice mutation in a non-obese juvenile-onset insulin-dependent syndromic diabetic patient

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Insulin-dependent juvenile-onset diabetes may occur in the context of rare syndromic presentations suggesting monogenic inheritance rather than common multifactorial autoimmune type 1 diabetes. We report the case of a Lebanese patient diagnosed with juvenile-onset insulin-dependent diabetes presenting ketoacidosis, early onset retinopathy with optic atrophy, hearing loss, diabetes insipidus, epilepsy, and normal weight and stature, who later developed insulin-resistance. Despite similarities with Wolfram syndrome, we excluded the *WFS1* gene as responsible for this disease. Using combined linkage and candidate gene study, we selected *ALMS1*, responsible for Alström syndrome, as a candidate gene. We identified a novel splice mutation in intron 18 located 3 bp before the intron/exon junction (IVS18-3T>G), resulting in exon 19 skipping and consequent frameshift generating a truncated protein (V3958*fs*3964X). Alström syndrome is a rare genetic disorder characterized by retinal degeneration, deafness, truncal obesity, short stature, type 2 diabetes and insulin resistance. The patient's clinical presentation significantly differed from typical Alström syndrome by the absence of truncal obesity and short stature, and by the presence of ketoacidotic insulin-dependent diabetes from onset, optic atrophy and diabetes insipidus. Our observation broadens the clinical spectrum of Alström syndrome and suggests that *ALMS1* mutations may be considered in patients who initially present with an acute onset of insulin-dependent diabetes. Our study outlines the value of alternative strategies targeting patients who do not present the typical disease manifestations, in order to identify new mutations responsible for variant presentations of the disease.

M. Sanyoura: None. **C. Woudstra:** None. **G. Halabi:** None. **P. Baz:** None. **V. Senée:** None. **P. Guillausseau:** None. **P. Zalloua:** None. **C. Julier:** None.

P02.059

Does epigenetics contribute to the phenotypic variability in the DiGeorge syndrome

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The 22q11 deletion syndrome (22q11DS), also known as velocardiofacial (VCFS) or DiGeorge (DGS) syndrome, is a common microdeletion syndrome in human, occurring with a prevalence of one in 4000 live births. Phenotype is highly variable among patients, independentely of the size of the deletion. Symptoms include congenital heart defects, atypical face appearance, cleft palate, hypocalcemia, immunodeficiency due to thymus aplasia or hypoplasia and cognitive and behavioral abnormalities.
The deletion results from non-allelic homologous recombination during meiosis and involves highly homologous sequences called Low Copy Repeats (LCRs). Approximately, 87% of patients carry a 3-Mb deletion, known as the Typically Deleted Region (TDR). In 8% a smaller deletion of 1.5-Mb is observed, with the same clinical signs, delimiting the 22q11DS minimal DiGeorge Critical Region (DGCR).
The involvement of epigenetic mechanisms has never been described in the DiGeorge syndrome. We hypothesize that epigenetic changes at the site of recombination, inside and outside of the TDR may play an important role in this pathology and could contribute to phenotype variability. We wish to determine the impact of haploinsuffciency of different genes deleted by the microdeletion such as HIRA and DGCR8 at early differentiation stage using induced pluripotent stem cells derived from patients. More specifically, we will investigate their role during the neuronal differentiation in order to understand their contribution to the mental manifestation of DGS.
Preliminary results will be presented and discussed.

C. Badja: None. **M. Artufel:** None. **M. Deveze:** None. **E. Barruet:** None. **C. El-yazidi:** None. **A. Tasmadjian:** None. **B. Binetruy:** None. **N. Philip:** None. **F. Magdinier:** None.

P02.060

Down Syndrome associated with neonate infectious pathology

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Introduction: The Down syndrome represents a complex and interdisciplinary pathology during the infant period, and even more so for the neonate.
Aim: The authors are set to analyze the pathology associated with Down syndrome, that lead to increase in hospitalization, complications, as well as the immediate and long term prognosis of the patients.
Material and method: The study took place in the our Department over a four year period. 36 patients were included, with clinical characteristics associated to specific caryotype alterations: trisomy 21 in 34 children (94,44%) and only 2 cases (4,46%)with mosaicism.
Results: The study group contained 22 neonates with gestational age below 37 weeks and 12 full term neonates, 77.77% presenting intrauterine growth retardation.
The pathology associated was: ventricular septal defect - 44.44%, atrial septal defect - 19.44%, atrio-ventricular canal defect - 16.66%, musculoskeletal malformations (congenital foot deformity - 30.55% cases, polydactyly - 8.66%, syndactyly - 5.55%).
All patients presented feeding deficit, absence of sucking reflex - 72.22%, suck-swallow incoordination.
Neonatal sepsis occurred in 36.11%, infectious pneumonia - 16.66%, ulcero-necrotic enterocolitis - 11.11%. In 16 cases (44.44%) with gestational age below 32 weeks, and birth weight below 1500g the evolution was slow, with hospitalization between 45-60 days, and 3 cases (8.33%) deceased with associated pathology: heart malformations, neonatal sepsis and ulcero-necrotic enterocolitis.
Conclusions: Extreme prematurity associated with cardiac or digestive malformations, and/or neonatal sepsis represented the major cause of death.
In the absence of associated infectious pathology the evolution was favorable, requiring fewer days of hospital care.

M. Boia: None. **D. Iacob:** None. **A. Manea:** None. **I. Andrei:** None.

P02.061

A corepressor gene mapping to chromosome 21 affects mitochondrial function in Down syndrome fetuses

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The upregulation of chromosome 21 (Hsa21) genes causes the downregulation of several nuclear-encoded mitochondrial genes (NEMG) in heart tissue of human fetuses with Down syndrome (DS). We investigated the consequences of NEMG downregulation on the mitochondrial function in trisomic human fetal fibroblasts (DS-HFF).
Together with the upregulation of Hsa21 genes and the dysregulation of mitochondrial genes in DS-HFF we found an impairment of the mitochondrial function characterized by decreased respiratory activity, enhanced ROS production, increased levels of intra-mitochondrial calcium and abnormal cristae morphology. The mitochondrial dysfunction was more pronounced in fibroblasts derived from cardiopathic trisomic fetuses suggesting that an altered bioenergetics background might induce a more severe DS phenotype.
To unravel the molecular mechanisms underlying the mitochondrial dysfunction we focused on the transcriptional coactivator PGC-1α, which controls the expression of several NEMG, and on its repressor RIP140, which maps to Hsa21 and is known to affect oxidative metabolism and mitochondrial biogenesis. We found that PGC-1α was downregulated while RIP140 was upregulated in all independent DS-HFF cultures we tested. RIP140 silencing in DS-HFF caused a time- and siRNA concentration-dependent increase of PGC-1α expression and the decrease of ROS production.
These findings suggest that the upregulation of the Hsa21 gene RIP140 contributes to the mitochondrial dysfunction observed in DS and have potential therapeutic implications since drugs affecting the RIP140-PGC1α pathway might rescue the impaired mitochondrial phenotype.

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P02.062

Exome sequencing identifies a novel *EP300* frame shift mutation in a patient with features reminiscent of Cornelia de Lange syndrome

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Rubinstein-Taybi syndrome (RTS) and Cornelia de Lange syndrome (CdLS) are genetically heterogeneous multiple anomalies syndromes, each has a distinctive facial gestalt. Two genes (*CREBBP* and *EP300*) are known to cause RTS and five genes (*NIPBL, SMC1A, SMC3, RAD21* and *HDAC8*) have been associated with CdLS. A diagnosis of RTS or CdLS is molecularly confirmed in only 65% of clinically identified cases. This suggests that other causative genes must exist for both conditions. In addition, although *EP300* and *CREBBP* encode homologous proteins and perform similar functions, only eight patients with *EP300* mutations have been reported. This suggests that RTS due to *EP300* could be escaping clinical recognition.
We report on a child with multiple congenital abnormalities and intellectual disability whose complex phenotype is highly reminiscent of CdLS. However, no mutations in CdLS genes were identified. Rather, a novel *EP300* mutation was found on whole exome sequencing.
Possible links between *EP300* and genes causing CdLS are evident in the literature. *EP300* and *HDAC8* are involved in the regulation of p53 transcriptional activity. In addition, p300 and other chromatin associated proteins including NIPBL, SMCA1, SMC3, have been found at enhancer regions in different cell types. This suggests that *EP300* and CdLS-related genes might be involved in a shared pathway, and their phenotypic effects might overlap.
As whole exome sequencing becomes more widely utilized, we will likely have a better understanding of the diverse phenotypes associated with *EP300* mutations. In the meantime, our findings suggest that EP300 is likely another candidate gene for CdLS.

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P02.063

Unusual combination of epilepsy phenotypes and a SCNM1 mutation in a three - generational family: photosensitivity, persistent at high age, and talking induced myoclonic jaw- jerks

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We analysed the clinical phenotypes, evolution, and genetics of a large family presented through a single proband who suffered increasingly from jerks in her jaw especially when speaking in front of the classroom and who appeared to be photosensitive, both clinically and in EEG recordings. Speaking provoked this phenomenon, and it was not present on awakening. We ascertained eighteen family members of whom eight had either similar jaw or limb jerks. Nine family members (including the eight affected by epilepsy) showed photosensitivity in the EEG in all but two younger cases (14 and 24 yrs respectively), and this biomarker was (still) present at older age (average 55; range 50 - 58 years). We performed a linkage scan using PPR as affection status and detected complete segregation for four chromosomal regions. The proband was then investigated by Whole Exome Sequencing, which identified 4 novel, predicted damaging mutations in different genes, all located in one of the four linkage peaks. An Arg267Cys mutation in the SCNM1 gene was carried in heterozygous state in all photosensitive family members. The gene regulates splicing of voltage gated ion channels and the mutation indeed affected the level of splicing of the SCN1A splice variant that was also carried by 7 of the affected family members. No other SCNM1 mutations were found in unrelated PPR European patients, suggesting that SCNM1 is a private mutation of this family and may be causing this unique combination of phenotypes.

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P02.064

Copy number variation profiling of patients with Esophageal Atresia and VACTERL.

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Esophageal Atresia (EA) with or without Tracheo-Esophageal Fistula (TEF) are common congenital anomalies whose cause is unknown in over 90% of affected patients. EA/TEF can be present either as an isolated defect or in association with other developmental defects: e.g. as one of the core features of the VACTERL (Vertebral, Anal, Cardiac, TEF, Renal and Limb anomalies) association. The hypothesis that genetic defects contribute to both EA/TEF and VACTERL etiology is supported by the fact that EA/TEF is a variable feature of several known monogenetic syndromes. Among these possible defects are Copy Number Variations (CNVs). As de novo CNVs can help to identify causal genes or affected biological pathways, the recurrence of unique and rare inherited CNVs may, in combination with other factors, predispose for the development of EA/TEF and other features of the VACTERL association. We therefore profiled 255 affected individuals with SNP-arrays. All had one or more large CNV, most of them were known polymorphisms. We observed six unique loci with a de novo CNV: 4p15, 4q35, 5q11, 6q23, 7p22 and 8q13. We also identified over 300 inherited CNVs which were either absent or uncommon in published control cohorts and our in-house database. Interestingly, 45 of these inherited variants were recurrent in our patient cohort. Ingenuity Pathway Analysis revealed enrichment of several biological functions including embryonic and digestive tract development. Using our genome-wide approach we identified several loci that may impact biological pathways disturbed in EA/TEF or VACTERL association patients.

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P02.065

Molecular genetic testing of Fanconi anemia: experience of the Italian Research Group on Fanconi Anemia

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Fanconi Anemia (FA) is a rare genetic disorder of hematopoiesis where 15 complementation groups have already been described. Previous studies of FA genes mutations revealed high heterogeneity and interpopulation differences but an extensive analysis has not be documented in Italian population. In this work we characterized 104 consecutive Italian patients with a suspected clinical diagnosis of FA. Using a strategy based on complementation and Western blot analyses in lymphoblast cell lines, we defined candidate genes for the mutation screening. This allows us to detect 178 mutant alleles and to identify 94 families with mutations in the FANCA (81), FANCG (9), FANCD2 (2), FANCB (1) and FANCC (1) genes. Consistent with the wide spectrum of mutations already reported for FA, we identified 39 novel mutations that were almost all "private". Regarding FANCA, we detect 15 (11,1%) intragenic deletions. Western blot analyses show that in all cases with al least one missense mutation the FANCA protein is stably expressed. Moreover, in cell lines with a defective FANCG, the expression of the FANCA protein was always reduced consistent with the stabilizing effect of FANCG on FANCA. Eleven patients with negative DEB test but a strong FA suspicion resulted to be hematopoietic mosaics. In conclusion this approach led to the identification of FA mutations in the majority of cases, allowing us to correctly diagnose patients and to expand the spectrum of Italian mutations. It would be interesting to understand whether expression of the FANCA protein, even if mutated, correlates with a mild phenotype.

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Sex reversal associated with Fanconi Anemia. A case report.

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Fanconi anemia (FA) is one of the best defined inherited bone marrow failure syndromes. It is usually inherited as an autosomal recessive trait, but in a small subset of FA cases it can be X-linked. FA patients show marked clinical heterogeneity. Characteristic features include a progressive bone marrow failure (BMF) and an increased predisposition to malignancy. Affected individuals may also exhibit one or more congenital/developmental abnormalities including abnormal skin pigmentation, skeletal, genitourinary and gastrointestinal abnormalities.

We report a 4 year-old girl presented for thrombopenia associated with skeletal malformations referred to our Cytogenetic Laboratory of Pasteur Institute of Tunis for suspicion of FA. The main clinical feature was growth failure and radial agenesis.

Induced chromosomal breakage study was performed using mitomycin (MMC). It was carried out on peripheral blood samples obtained from the patient and a counterpart culture of control.

Cytogenetic test showed a significantly elevated level of chromosome breakage compared to controls with a percentage of unstable mitoses of 100%. According to this sensitivity to MMC, this patient was diagnosed as FA, but the RHG-banded metaphases showed a 46,XY karyotype suggesting a state of an unusual sex reversion associated with FA disorder.

As our knowledge, this is the first reported case with a 46,XY girl associated with FA.

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Clinical and genetic study of Brazilian patients with fetal alcohol spectrum disorders

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Introduction: The teratogenicity of alcohol comprises a spectrum of anomalies known as Fetal Alcohol Spectrum Disorders - FASD; the most severe phenotype is the Fetal Alcohol Syndrome. Objectives: 1) Study patients with FASD according to the Washington Criteria Diagnosis; 2) Establish the frequency of the polymorphisms of the genes of the enzymes ADH (ADH1B*3, ADH1B*3, ADH1C*2) and ALDH (ALDH2*2) in FASD patients; 3) Compare the FASD patients genotype to the control group. Results: 28 Brazilian patients aged 11 months to 17 years were evaluated according to the Washington Criteria protocol: 35,7% (10 patients) were classified in the A category; 32,1% (9 patients) category C ; the remaining patients were classified in the categories E, F, H and J. The distribution of the polymorphisms were: ADH1B*2: genotype ADH1B*1/1 85,19% and ADH1B*1/2 14,81%. ADH1B*3: genotype ADH1B*1/1 52,17%, ADH1B*1/3 43,48% and ADH1B*3/3 4,34%. ADH1C*2: genotype ADH1C*1/1 65,38%; ADH1C*1/2 19,24% and ADH1C*2/2 10,89%.ALDH2*2: genotype ALDH2*1/1 98,02% and ALDH2*1/2 15,38. There were no significant differences in the distribution of genotypes ADH1B*2 and ALDH2*2 between patients and the Brazilian population. The ADH1B * 3 allele was significantly more frequent among patients (p<0,001) and the ADH1C * 2 allele was significantly less frequent among patients (p=0,0058) compared to the healthy Brazilian population. Conclusion: Although it is a small sample, we may suggest that the ADH1B * 3 allele may be a risk factor and the ADH1C * 2 allele a protective factor to the Fetal Alcohol Spectrum disorders in the Brazilian population. Sponsor: FAPESP 2011/08960-8

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P02.068

New Italian patient with Filippi syndrome: a rare condition of unknown genetic origin

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Filippi syndrome is a very rare condition described so far in no more than 30 patients. The most likely way of inheritance is autosomal recessive but the molecular defect is still unknown.
We describe a 12-year-old girl, first child of non-consanguineous healthy parents, born at term after uncomplicated pregnancy. She has typical facial dysmorphism including deep-set eyes, synophrys, broad nasal bridge, thin alae nasi, thin upper lip and prognathism. Her ears are small with prominent helix. Bilateral syndactyly of fingers 3-4 (including osseous) and toes 2-3 was evident. Growth is constantly delayed with short stature (-3SD) and postnatal microcephaly, while cognitive and speech delay is in the moderate range. Ectodermal features included abnormal teeth, hypodontia and thin hair. Additional features included congenital heart defects (Ebstein's anomaly and inter-atrial septal defect) and generalized epilepsy responsive to drugs treatment.
We matched her clinical characteristics with those of 26 patients reported in the literature with Filippi syndrome and noticed a broad overlap with a restricted group of them (n=20), including the first reported patients with this condition. Tentative diagnostic criteria for Filippi syndrome are proposed: IUGR/postnatal growth deficiency (including microcephaly), typical facial dysmorphism, syndactyly of fingers and toes (mainly involving fingers 3-4 and toes 2-3) and cognitive impairment with speech delay. Ectodermal defects and abnormal genitalia support the diagnosis.
The identification of a homogeneous group of patients sharing features of Filippi syndrome will ease genetic investigations aimed at the identification of the disease-causative gene(s).

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P02.069

Mutations in FLNA cause different disorders. Report of two novel mutations and associated phenotypes

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Filamin A is an actin binding protein, a product of X-linked FLNA gene. It interacts with multiple cellular components including intracellular signalling molecules, channels, receptors and transcription factors. The protein plays an important role in stabilisation of cytoskeleton, cell signalling, adhesion, migration, transcription and organ development. Mutations in FLNA resulting in a loss-of-function cause defective neuronal migration in the form of periventricular nodular heterotopia. Mutations resulting in a gain of function underlie a diversity of otopalatodigital spectrum of disorders featuring a skeletal dysplasia and various cardiac, genitourinary and intestinal malformations. To a large extent the pathogenic mechanisms leading to these phenotypes are still unresolved and new patients with different mutations allow better understanding of these processes.
We present two families with different disorders caused by mutations in FLNA. In one family mother and daughter both developed epilepsy during childhood associated with bilateral prominent periventricular nodular heterotopia. In the second family a 15-years-old boy was diagnosed with frontometaphyseal dysplasia without inner organ abnormalities. Sequencing of FLNA confirmed presence of missense mutations. In the former we found both affected individuals were heterozygous for c.82A->G. In the second case the proband was hemizygous for a c.745G->T mutation that predicts the substitution p.D249Y. Both are novel mutations with the first being predicted to substitute an initiator methionine residue for one transcript of FLNA. The second mutation predicts the substitution of a residue in the actin binding domain in a manner very similar to other FMD causing variants. Functional investigations in both families are ongoing.

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P02.070

Isolated urogenital malformations caused by novel biallelic FRAS1 mutations

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Fraser syndrome (FS) is a rare autosomal recessive disorder characterized by cryptophthalmos, syndactyly, and urogenital malformations. So far mutations in the genes FRAS1, FREM2 and GRIP1 have been identified in humans with FS, while FREM1 biallelic mutations were reported in "bifid nose, renal agenesis, and anorectal malformations" (BNAR) syndrome and Manitoba-oculo-tricho-anal (MOTA) syndrome.
We report on two female foetuses of healthy, unrelated parents diagnosed with bilateral renal and ureteral agenesis. Additional findings were vaginal atresia and tracheobronchial forgut duplication in the first fetus and hypoplastic bladder, uterus unicornis (left side) and aplasia of the right tube and dislocation of the right ovar into the upper segment of the abdomen. This spectrum of urogenital anomalies fit well into the Fraser syndrome spectrum, but characteristic features such as cryptophthalmos or syndactyly were absent. We therefore performed exome sequencing in one fetus and revealed novel compound heterozygous FRAS1 mutations: a 2bp deletion resulting in a premature stop codon (c.1146_1147delTG) and a missense mutation affecting a highly conserved amino acid (c.2017T>G).
Since the majority of FS mutations represent premature stop mutations, we assume that compound heterozygosity with this specific missense mutation may explain the atypical phenotype in our family. Notably, single heterozygous FRAS1 and FREM2 missense mutations were recently described in unilateral non-syndromic CAKUT (congenital abnormalities of the kidney and urinary tract).
Our finding therefore expands the phenotype of biallelic FRAS1 mutations to non-syndromic congenital malformations of the genitourinary tract, which is important for prenatal diagnosis and genetic counseling.

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Molecular and expression analysis extends the phenotypic spectrum of GLI3 mutations

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The phenotypic spectrum of GLI3 mutations includes Greig cephalopolysyndactyly (GCPS) and Pallister-Hall (PHS) syndromes. PHS first described as a neonatally lethal condition associates hypothalamic hamartoma, bifid epiglottis, postaxial or central polydactyly, intrauterine growth retardation and anal atresia. GCPS is less severe and combines polysyndactyly of hands and feet and craniofacial features. Phenotype-genotype correlations have been found both for the location and the nature of GLI3 mutations, suggesting different roles of GLI3 during development.
Here we report on the molecular and clinical study of 71 cases from 51 families with either a GLI3 mutation (66 GCPS or PHS), or a large deletion encompassing the GLI3 gene (5 GCPS cases). Most of mutations are novel and consistent with the genotype-phenotype correlation. We describe PHS patients ranging from a mild/incomplete phenotype to severe malformations extending the phenotype to agnathia with absent oral cavity, skeletal anomalies with oligosyndactyly. GLI3 expression studied by in situ hybridisation during human development confirmed its early expression in target tissues including in pharyngeal arches, and later in mandible. Our results emphasise on the possible lethality of GLI3 mutations, extend the phenotypic spectrum of malformations to severe craniofacial and reductional limb defects and further show the overlap between PHS, SLO and OFD syndromes.

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P02.072

Two cases of Heterotaxy with Neural Tube Defect: Further evidence of an uncommon association

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Heterotaxy is a multiple congenital anomaly syndrome characterized by complex cardiovascular malformations and visceral situs anomalies. It results from failure to establish normal left-right asymmetry during embryonic development. Multiple anomalies have been reported in associated with heterotaxy; however, there have been few studies considering the relationship between heterotaxy and midline anomalies in general, and heterotaxy and neural tube defect specifically. We describe two cases, one prenatal and one pediatric, with the combination of heterotaxy and neural tube defect. At present, the genetic basis of the phenotypes in our cases is unknown. Mutations in ZIC3, which have been reported in mice with heterotaxy and NTDs, have been ruled out. These cases add support to the hypothesis that the mid-line development plays a significant role in establishing laterality and that there are common mechanisms involved in both heterotaxy and neural tube defects.

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P02.073

New candidate genes in holoprosencephaly: results from homozygosity mapping in six inbred families

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Holoprosencephaly (HPE) is a congenital malformation of the human brain due to an imperfect division of the forebrain during early development. Multiple genetic defects have been identified as involved in this process. It is now currently admitted that HPE is a multihit pathology caused by at least two or more dysfunctional events involving at least 4 major genes (*SHH, ZIC2*, *SIX3* and *TGIF*) and 10 minor genes belonging to different signaling pathways. However, the mutations and deletions in these genes represent only 30% of HPE cases. Recessive inheritance of HPE can also be suspected in consanguineous families with intrafamilial recurrence. Homozygosity mapping was undertaken in six families with history of consanguineous marriage to search for regions harboring mutations that are identical by descent. Parents and affected children were genotyped on HumanCytoSNP-12 arrays (Illumina). We first determined the population ancestry of each family with OriginMineR [de Tayrac, ASHG 2012] to estimate their specific SNP allele frequencies. Inbreeding coefficients of affected children were estimated from their genomic data by the FEstim method [Leutenegger *et al.*, 2003]. Using the genomic inbreeding coefficient we performed homozygosity mapping without relying on the genealogical information. In parallel we detected the runs of homozygosity across the genome for each individual (PLINK). Three regions of interest were detected by both methods on chromosomes 1, 6 and 10. We applied Endeavor software to prioritize genes in these regions. One of them was previously identified by CGH array on chromosome 6 (*DLL1*) and other good candidate genes will be presented.

M. de Tayrac: None. **M. Babron:** None. **C. Dubourg:** None. **C. Mouden:** None. **R. Bouvet:** None. **S. Gazal:** None. **M. Sahbatou:** None. **L. Ratié:** None. **V. Dupé:** None. **J. Mosser:** None. **S. Odent:** None. **V. David:** None.

P02.074

Molecular investigation in a group of patients with Holoprosencephaly

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Holoprosencephaly (HPE) is the most common forebrain developmental anomaly in humans. Classic HPE occur with a prevalence of 1 in 250 during early embryogenesis, decreasing throughout gestation to a frequency of 1 in 16000 live births. The etiology of HPE is complex, with both environmental and genetic factors being implicated. Three levels of increasing severity are described in HPE: lobar, semilobar and alobar. Another milder subtype of HPE called the middle interhemispheric variant (MIHF) or syntelencephaly, has now been recognized. In the present work we analyzed a group of 70 individuals within the spectrum of holoprosencephaly through molecular techniques. We first performed Sanger sequencing for *SHH, SIX3, TGIF* and *ZIC2* genes. Then, we performed MLPA and array-CGH. The sequencing showed mutations on *SHH, ZIC2*, and *SIX3* genes. MLPA analysis showed a deletion on *SHH* and *TGIF*, and a duplication on *ZIC2*. Array-CGH showed deletions on *SIX3* as well as in other regions of the genome. From the total of 70 individuals, 29% presented a variation. Whereas the etiology of HPE is not completely defined our data are consistent with the literature. A new study is being designed and will use Next Generation Sequencing, and bioinformatics tools to analyze genes that have not been studied that may vary in HPE.

L.A. Ribeiro-Bicudo: None. **B.F. Gamba:** None. **A.B. Rocha:** None. **A.C. Gaspar:** None. **V.T. do Ó:** None. **A. Richieri-Costa:** None.

P02.075

Investigation into the genetic basis of holoprosencephaly-diencephalic hypothalamic hamartoma

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Holoprosencephaly-diencephalic hamartoma (HDH) is a rare disorder involving a hypothalamic hamartoma (benign embryonic tumour of the hypothalamus), holoprosencephaly and various cephalic and extra-cephalic anomalies. Only a few cases of HDH have been reported, most of which are sporadic. The etiology of this syndrome remains unknown and specific causative genes have yet to be identified. We report on two siblings from a non-consanguineous Irish family with a syndrome consistent with familial HDH causing early neonatal death. Externally, both children had bilateral severe microphthalmia with a central cleft lip and cleft palate. Internally, both babies had an abnormal skull base, holoprosencephaly and a hypothalamic hamartomatous mass. One child also had cardiac anomalies. Recurrence of HDH is extremely rare, and suggests a recessive disease gene in the family described here. Diagnostic mutation screening was performed and excluded *GLI3*, *SOX2*, *STRA6*, *ROR2*, *HOXD13*, *SHH*, *ZIC2*, *SIX3* and *TGIF* as possible causative genes in this family. Whole exome sequencing of DNA from the two affected siblings and their parents has been performed. Analysis of the exome data is in progress to identify recessive variants that segregate with the multiple malformation syndrome in this family.

J.P. Casey: None. **P. McGettigan:** None. **C. Keohane:** None. **E. Dempsey:** None. **D. McKenna:** None. **T.J. Browne:** None. **N. Bermingham:** None. **S.A. Lynch:** None. **A. Green:** None. **S. Ennis:** None.

P02.076

A case of hypomyelination - oligodontia - extrapyramidal signs - ataxia syndrome (Pol3 - associated hypomyelinating disorder)

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A 4-year-old girl was born to non-consanguineous Caucasian parents. Her physical and psychomotor development was normal until the age of 8 months, but moderate muscle hypotonia and slight convergent strabismus were noted by a neurologist. At 8 months of age, short periods of postural tremor of the head and hands appeared. The girl began sitting independently at about 9 months old, walking with holding at 1 year 1 month, and speaking at the age of 1 year. When she began walking, constant postural tremor of head and legs and titubation were observed. The dentition was significantly delayed and abnormal. The central incisors were unerupted for almost 4 years, and the first canine erupted when she was two. Since the age of 2 years, the patient was walking independently, but ataxia was present. She was speaking single sentences with dysarthria. At 3 y.o. strabismus increased and myopia was diagnosed. Neurological examination revealed convergent strabismus, horizontal nystagmus when she focused to the left and right, early signs of supranuclear gaze palsy - vertical nystagmus and need to lift up the head to focus on a floor. The head, hands and body postural tremor, moderate muscle hypotonia and bilateral Babinsky signs were observed. MRI demonstrated diffuse hypomyelinization with prevalence of low T2 signal from frontal lobe white matter and optic radiation. Hypomyelination and/or hypodontia syndrome is caused by mutations of POLR3A and POLR3B genes. In this case we performed a sequencing of exon 15 of POLR3B. The heterozygous mutation c.1568T>A (p.Val523Glu) was found.

M.A. Bulatnikova: None. **A.A. Vasilishina:** None. **A.B. Smolyaninov:** None. **V.I. Larionova:** None.

P02.077

Comprehensive methylation testing broadens the epigenetic and clinical description of Imprinting Disorders.

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Imprinting disorders (IDs) are associated with mutations and epimutations affecting imprinted genes, whose expression is restricted by parent of origin. ID diagnosis is challenging because their clinical features are heterogeneous and partially overlapping, and their underlying molecular defects include mutation, epimutation, copy number and chromosomal errors, and can be further complicated by somatic mosaicism and multi-locus methylation defects. It is currently unclear to what extent the phenotypic heterogeneity of IDs reflects their underlying molecular pathophysiology, particularly for multilocus methylation defects (MLMD). To address these issues we performed comprehensive methylation analysis of imprinted genes in 285 patients with clinical features of IDs, with or without a positive molecular diagnosis.
20 of 91 (22%) ID patients had MLMD - ie, additional imprinting anomalies independent from their primary clinical presentation - including 6/35 SRS patients (17%), 8/29 BWS patients (28%), 4/8 (50%) TND patients and 1/11 (9%) PHP1b patients.
The frequency of developmental delay was higher (67%) in MLMD than 'isolated' SRS (26%), and 5/6 SRS-HIL cases had atypical clinical features, compared with 23% of isolated SRS. Compared to 3/11 of 'isolated' BWS (hypomethylation of ICR2), 8/8 of BWS-MLMD had developmental delay ($P = 0.004$); and 6/8 BWS- MLMD had atypical congenital anomalies compared with 4/13 'isolated' BWS. While glycaemia was similar in all TND patients, congenital anomalies were more frequent in those with MLMD, as was assisted reproduction. These findings highlight the heterogeneous molecular anomalies and variable clinical phenotypes of IDs and indicate the value of comprehensive molecular investigations.

D.J.G. Mackay: None. **R.L. Poole:** None. **L.E. Docherty:** None. **A. Al Sayegh:** None. **A. Caliebe:** None. **C.L.S. Turner:** None. **E.L. Baple:** None. **E.L. Wakeling:** None. **S.E. Flanagan:** None. **A.T. Hattersley:** None. **S. Ennis:** None. **I.K. Temple:** None.

P02.078

Molecular study of Incontinencia Pigmenti in two males

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Incontinencia Pigmenti (IP) is a genodermatosis disease associated with mutations in NEMO gene which is situated on X chromosome. A pathogenic mutation on this gene is usually lethal in males. We have analyzed NEMO gene in two males with a suspect of IP in order to look for some alterations which could explain the altered phenotypes.
We have studied two males that present an IP phenotype. The first one (A) is less than 1 year old, the second case (B) is 20 months old. We have analyzed the 10 exons of the NEMO gene by PCR and direct sequencing.
In case A, we have observed that one of the two copies of the NEMO gene is deleted from 4 to 10 exons. In case B, we have only observed a heterozygous codon 326 deletion (exon 8 p.K325_327delK). This mutation has
not been found in his parents. The karyotypes of both patients showed alterations. In case A, a Klinefelter syndrome (XXY) was detected. In case B we observed a trisomy XXY in the 2%, a XYY in the 1% and a XXYYY in the 1% of the total cells.
We present two unusual cases of males carrying NEMO mutations. One of them carry Klinefelter syndrome with two copies of chromosome X. In patient B, the trisomy can not invalidate the possible lethal effect of the mutation found, due to the few percentage of cells with trisomy.
Therefore we can speculate that a mosaicism might explain this case.

D. Martín-Sánchez: None. **E. Bueno-Martínez:** None. **A. Torrelo:** None. **E. Fernández-López:** None. **J. Cañueto:** None. **Á. Hernández-Martín:** None. **R. González-Sarmiento:** None.

P02.079

Mosaic 8p interstitial deletion with absent nails: genotype/phenotype correlation

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The aim of this study was to correlate genotype/phenotype in interstitial deletion of chromosome 8p and to compare the sensitivity of FISH and array CGH in detection of low mosaic cell line. We reported on a male patient presented at the age of 40 days with CHD and recurrent chest infection. He was lethargic with infrequent myoclonic spasm. Patient had facial dysmorphism ,microcephaly.He suffered of CHD with VSD, ASD, pulmonary stenosis and dilated right ventricle. He had intractable convulsions, hypogenesis of corpus callosum, defects in myelination and mild reduce cerebellar size for his age. Both hands and feet showed absent nails (total anonychia) and abnormal increasing palmar and planter creases. He had a karyotype 46,XY,del(8)(p21.2p23). Both parents had normal karyotype. FISH analysis using subtelomere 8 and locus specific identifier for LPL at 8p22 revealed interstitial deletion involving (8)(p22) and presence of subtelomere 8p and 15% normal cell line. Nimblegen microarray analysis indicated 18.5Mb interstitial deletion of chromosome 8p spanning cytoband (8)(p23.2-p21.3) and involving GATA4 gene.
We concluded that a post zygotic deletion occurred in this infant, FISH analysis is a powerful tool in detection of low mosaic cell lines. The array CGH can identify precisely the break points and the exact deleted region. We suggest that the gene responsible for nail formation lies within chromosome 8p cytoband p23.2-p21.3. DNA sequencing for the other allele is recommended to delineate the cause of absent nails whether it is due to haploinsufficiency or autosomal recessive.

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P02.080

Growth pattern in Kabuki Syndrome

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Kabuki syndrome is a multiple congenital anomaly/mental retardation syndrome including characteristic facial dysmorphology, developmental delay and postnatal growth retardation. Kabuki syndrome is caused by mutations in the MLL2 gene in about 75% of cases. One of the key features in individuals with Kabuki syndrome is postnatal growth restriction. However, information on the specific growth pattern of children with Kabuki syndrome is scarce. Only one Japanese report summarizes data in a growth chart (Niikawa et al., 1988). We present a limited report on growth data in a group of individuals with a genetically confirmed diagnosis of Kabuki syndrome. The data showed that postnatal growth retardation is a clinical feature in all cases. Postnatal height SDS ranged from -2,5 to 3,3 (mean -2,3) and the current height SDS ranged from -6,24 to -0,09 (mean -2,8) in all individuals. 26 (=70,3%) patients had a height SDS below -2 SD and thus fulfilled the definition of short stature. Since all Kabuki syndrome patients have a growth deflection during childhood and a growth spurt deficiency, a defect in the growth hormone/IGF-I axis is very likely. Further research is warranted to clarify the cause of this postnatal growth restriction. We hypothesize an altered body composition similar to that in children with Prader-Willi syndrome. Therefore, we will start a study including growth hormone therapy in children with Kabuki syndrome.

C. Schrander-Stumpel: None. **N. Schott:** None. **N. Cramers:** None. **W. Gerver:** None. **C. Fauth:** None. **K. Devriendt:** None.

P02.081

Distal interphalangeal joint contracture and disappearance of flexion crease in paediatric patients with Kabuki syndrome

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As orthopaedic complications of the Kabuki syndrome, scoliosis, abnormality or dislocation of the hip joint, patellar dislocation, and a shortened fifth finger have been reported. However, there has been no report on Kabuki patients with abnormalities of the finger joints. We examined the contracture, flexion angle, and flexion crease on the third and fourth fingers of 6 MLL2 mutation-positive children with Kabuki syndrome (ages, 4-13 years). The flexion angle of the DIP joint ranged from 120 to 170 degrees, with a visible limitation of flexion in 2 patients and a moderate limitation of flexion in 2 other patients. The flexion crease of the DIP joint on the third and fourth fingers was absent in 5 of the 6 patients. Radiographs of 4 patients revealed no morphological abnormality in the third and fourth fingers.
In this study, disappearance of the flexion crease of the DIP joint was observed in 5 of the 6 patients, and limitation of flexion was found in 4 patients. There was no distinctive finding in any of the other small joints. Laboratory findings during the clinical course revealed no morphological abnormality of the osteoarticular joints. Since there has been no report of a syndrome characterized by the contracture and disappearance of the flexion crease on the third and fourth fingers, we propose that the distal interphalangeal joint contracture of the 3rd and 4th finger and disappearance of flection crease are diagnostic features of Kabuki syndrome.

S. Mizuno: None. **Y. Muramatsu:** None. **N. Miyake:** None. **N. Matsumoto:** None.

P02.082

Kabuki Syndrome: genotypic spectrum and genotype-phenotype correlations in a cohort of 200 KS patients.

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Kabuki syndrome (KS, MIM: 147920) is a clinically recognizable syndrome of multiple congenital anomalies and mental retardation affecting approximately 1:30,000 live births. Key features are a characteristic face, growth retardation, developmental delay and additional features such as hypodontia and persistent foetal fingertip pads. Earlier, a major gene causing KS was identified through exome sequencing, reporting mutations in the histone methyl transferase (HMT) gene *MLL2* in 40-70% of KS patients. Recently deletions and mutations in a second gene *KDM6A* were identified as a rare cause of KS. In the current large cohort of >200 suspected KS patients from mostly European descent we evaluated the significance of mutations and/or deletions in *MLL2* and tested a subset of the MLL2 negative patients for the *KDM6A* gene. Several potential *MLL2* splice-site variants were assessed at the RNA level and allele-specific PCR was used to determine parental origin of some mutations. Phenotypic characteristics were compared between carriers and non-carriers as well as between the different types of mutations.

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P02.083

A comprehensive study on Kabuki syndrome: diagnostics, modeling, and therapy

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Kabuki syndrome (KS) is characterized by peculiar facial gestalt, mental retardation, and congenital multiorgan anomalies. *MLL2* is mutated in 60% of KS patients. The majority of them are *de novo* mutations, and in few cases *MLL2* exons microdeletion/duplication. A second gene for KS is *KDM6A*. For 30% of patients the underlying genetic cause remains unidentified.
We have enrolled nearly 400 KS patients; all were subjected to *MLL2* and *KDM6A* screening. In agreement with previous reports we found that about 65% have *MLL2* mutations, mainly nonsense and frameshift mutations, leading to a premature termination codon. *KDM6A* alterations were found in a very few patients. RT-PCR and direct sequencing from KS patients with *MLL2* splice site mutations showed that these variants cause aberrant splicing resulting in a truncating and not functional protein. Using a large collection of KS cell lines (skin fibroblast and EBV-immortalized lymphocytes) we showed that *MLL2* mRNAs bearing premature stop codon are degraded by the NMD contributing to MLL2 protein haploinsufficiency. The high number of protein truncation mutation suggests that a number of KS patients may benefit from a readthrough therapy that could restore the endogenous levels of MLL2 and KDM6A proteins. We tested 14 *MLL2* and 2 *KDM6A* nonsense mutations for their response to drug readthrough treatment by a reporter luciferase vector system. We found that 11/14 (79%) of analyzed *MLL2* and 1/2 *KDM6A* mutations displayed a significant level of readthrough in response to gentamicin suggesting that this strategy is effective and has important implications for therapy.

B. Mandriani: None. **L. Micale:** None. **C. Fusco:** None. **B. Augello:** None. **M. Pellico:** None. **F. Zucchetti:** None. **S. Maitz:** None. **E. Biamino:** None. **M. Silengo:** None. **A. Selicorni:** None. **L. Zelante:** None. **G. Merla:** None.

P02.084

A de novo 3.5 Mb microdeletion of 8p11 explains hypogonadotropic hypogonadism in a 19 years old male

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GnRH deficiency with anosmia is characterized by absent or incomplete puberty, low or normal LH/FSH in the context of low testosterone and normal pituitary function and is known as Kallmann syndrom (KS). Mutations in the *FGFR1* gene are associated with Kallmann syndrome type 2, with and without anosmia. However, deletions of the *FGFR1* as a cause of KS have been described in only a few patients.
Here we report a case of KS in a 19 years old male due to a 3.5 Mb contigious deletion of chromosome 8p11. At 17 years he was referred for investigation due to absence of pubertal spurt. Clinical investigation revealed pubertal development in accordance to Tanner I, stature -3SD, low FSH, LH and testosterone, and normal finding on MRT. There was no effect on the testicles size after treatment with testosterone and investigation was completed with microarray analysis due to suspicion of Kleinfelter syndrome. The microarray analysis with 6.0 SNP chip (Affimetrix) revealed a 3.5 Mb deletion of chromosome 8p11.21p11.23 encompassing 35 genes. *FGFR1* was one of the deleted genes. The deletion was later shown to have arisen *de novo* in the patient. The clinical picture with anosmia, delayed skeletal age, history of cleft lip and palate and absent puberty is in agreement with the diagnosis of Kallmann syndrome.
In conclusion, deletions of the *FGFR1* gene are a rare cause of Kallmann syndrome but should be excluded in patients with Kallmann syndrome and no mutation in one of the Kallmann syndrome connected genes.

E. Kuchinskaya: None.

P02.085

Case report of a patient with KID syndrome associated with Dandy-Walker malformation and generalized contractures

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Keratitis-Ichthyosis-Deafness (KID) syndrome is a rare congenital disorder characterized by the triad: vascularizing keratitis, skin lesions and hearing loss. Other rare manifestations have been also described such as Dandy-Walker malformation (DWM). About 100 patients have been described and most of them have *de novo* or, less frequently, inherited heterozygous mutations in the *GJB2* gene encoding connexin 26.
DWM suspected at the end of the pregnancy leading to the birth of our patient who presented with congenital ichthyosis. No ocular or hearing anomalies were noted. He developed progressively severe contractures of the upper and lower limbs. Progressively others symptoms were detected: vascularizing keratitis, deafness and enamel defect. We examined the boy aged 9 in our Center for Rare Genetic Ophthalmologic Diseases (CARGO) because of severe keratitis. Direct sequencing of the *GJB2* revealed a de novo p.D50A mutation, confirming the diagnosis of KID.
KID syndrome is defined by Keratitis, Ichtyosis and Deafness, but other rare features are described. To date, DWM was reported in 10 KID patients and initially considered as a coincidental finding rather than a real association. Some cases of lower limb contractures have been reported but seem to be much less severe compared to our patient.
The patient reported herein revealed atypical features and widened the manifestations of KID syndrome clinical spectrum. Moreover, mutation identification optimized the genetic counseling of the family and helped in the clinical care although the keratitis and contractures control remain difficult.

S. Scheidecker: None. **V. Pelletier:** None. **Y. Alembik:** None. **D. Lipsker:** None. **M. Louha:** None. **L. Jonard:** None. **H. Dollfus:** None.

P02.086

Kleefstra syndrome: the rediscovery of an old syndrome through array Comparative Genome Hybridization technology (array-CGH)

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High resolution allowing the identification of Copy Number Variations (CNVs), greatly assisted the recognition of Kleefstra syndrome. Previously named 9q subtelomeric deletion syndrome (9Qstds), Kleefstra syndrome is either caused by a submicroscopic deletion in chromosomal region 9q34.4 or an intragenic mutation in the euchromatin histone methyltransferase 1 (EHMT1) gene, resulting in EHMT1 haploinsufficiency. Since the early 1990s, 85 patients have been described, of which the majority (85%) had a 9q34.3 microdeletion).
We present eight newly diagnosed patients who were referred for genetic evaluation due to developmental delay (DD), mental retardation (MR), various congenital anomalies and/or dysmorphic features. In all cases previous standard karyotype was negative. Agilent arrays 4x180k and 1x244k (>170.000 and > 236.000 probes respectively, average resolution 8.9kb) were used. The analysis revealed seven patients with an interstitial 9q34.4 microdeletion (two de novo and one maternal) ranging in size from 0.6Mb to 5.41Mb and in 5 of 7 patients comprising the EHMT1 gene. One patient had a duplication (1 Mb). Other important genes in the same area are the following: AGPAT2 (congenital generalized lypodystrophy), NOTCH1 (aortic valve disease), LHX3 (pituitary hormone deficiency with rigid cervical spine). Additional pathogenetic aberrations which were also observed in our patients (del3p14.1, del6p13.3, del8q24.3, del10q26.3, del15q11.1, del18p11.32-p11.21) might contribute to the patients' severe phenotype, acting as additional modifiers of their clinical manifestations. Alternatively the 9q34 defect acts as a modifier of the main clinical phenotype as is probably the case with the patient carrying the 9q34 duplication.

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P02.088

Elevated Congenital Malformations Rates and Chornobyl Ionizing Radiation in Rivne-Polissia, Ukraine

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In 2000, population-based epidemiological surveillance of congenital malformations (CM) implementing methods and reporting data to the European network of companion registries (EUROCAT) was initiated in several provinces in Ukraine. Analysis of Rivne (R)-Volyn provinces combined 2000-2002 data and later of 2000-2006 solely R data, demonstrated elevated rates of neural tube defects (NTD) and microcephaly. An analysis of 2000-2009 R data of 145,437 pregnancy outcomes confirms the prior observations and indicates that these CM are statistically significantly more prevalent in the Rivne-Polissia (RP) region, an ecologically distinct zone (wetlands) inhabited by a population isolate (Polischuks). The RP region is among the most severely impacted zones by Chornobyl ionizing radiation (IR) in Ukraine. Analysis of stillbirths shows higher rates in RP than non-RP and higher rates than in the adjoining province to the south of R.
The rates in R of NTD, microcephaly, conjoined twins and teratomas are among the highest in Europe. All four categories of CM are prevalent among females. Surveys indicate that alcohol use by pregnant women is not a likely cause of microcephaly. Analyzes of 6026 recordings of whole body counts of incorporated IR, including from 1157 pregnant women indicate that incorporation levels are substantially higher among those residing in RP.
We conclude that the data is sufficiently robust to continue the CM surveillance and expand current international partnerships to include prospective investigations of specific teratogenic risks posed by persisting IR in RP and other teratogenic risks factors.

W. Wertelecki: None. **L.S. Yevtushok:** None. **S.F. Lapchenko:** None. **N.O. Zymak-Zakutnia:** None.

P02.089

A novel *EFTUD2* splice mutation causing Mandibulofacial Dysostosis - Microcephaly

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Mandibulofacial Dysostosis - Microcephaly (OMIM #610536) is a rare craniofacial dysostosis accompanied by microcephaly, intellectual disability and other malformations. *EFTUD2* , a spliceosomal gene, was recently identified by exome sequencing as causal of this multiple malformation syndrome in several publications. 28 cases have been published so far. All mutations are private, and all with available parental samples are *de novo*, except one. We describe a 3,5years old boy with progressive microcephaly but no structural abnormalities on cerebral MRI. He has psychomotor delay, especially expressive language delay, and walked at 36 months of age. His ears have a characteristic appearance with microtia, square ear lobes, ear fistulas and a preauricular tag present at birth. He has generalised epilepsy and unilateral hearing loss. There are no other malformations. His phenotype will be thoroughly described. *EFTUD2* sequencing revealed a heterozygous mutation in intron 25. This sequence variant has not been reported earlier, but interference with splicing is expected. mRNA analyses are in progress. Parental samples are normal, thus proven *de novo.*
New publications also link this syndrome to esophagal atresia, and a wide range of other malformations. Patients without microcephaly are also described. Importantly, *EFTUD2* mutations will be a differential diagnosis to well known syndromes in the group of craniofacial dysostoses and the oculoauriculovertebral spectre.

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P02.090

Marshall syndrome: A recessive form caused by *COL11A1* gene mutation

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Marshall syndrome is a form of skeletal dysplasia due to several gene mutations. Recently, compound heterozygous mutations in the *COL11A1* gene was described in two unrelated patients as a cause of fibrochondrogenesis. Subsequently, dominant and recessive forms of fibrochondrogenesis resulting from mutations at a second locus, *COL11A2*, were also described. Mutations in these two genes along with *COL2A1*were previously reported to cause autosomal dominant forms of both Stickler and Marshall syndromes.
Here we report the first evidence that adds *COL11A1* defect as a cause of Marshall syndrome with a recessive mode of inheritance. The clinical findings in the two brothers of deafness, flat midface, myopia, and ectodermal abnormalities (patient 1) are highly suggestive of Marshall syndrome. Among their remaining five family members, the parents and 2 adult siblings were heterozygote carriers. All the four carriers had mild short stature and thick calvaria. In addition, the parents had mild mixed hearing loss. Direct *COL11A1* gene sequencing of our patients revealed a homozygous missense mutation c.2702G>A (p.Gly901Glu) in exon 35.
In conclusions, these findings expand the list of autosomal recessive genes of the clinically overlapping Stickler and Marshall syndromes to include *COL11A1*. Furthermore, it broadens the clinical spectrum of the recessive form of type XI collagenopathy to include Marshall syndrome.

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P02.092

Maternal consumption of clay during pregnancy: an unexpected cause of recurrent congenital microcephaly with intracranial calcifications in babies from French Guyana (pseudo-Aicardi-Goutière syndrome)

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Eating clay („pemba") during pregnancy is a traditional behavior in the Bushinengue population living on the border of the Maroni river, in the French department of Guiana. Clay consist in aluminium silicate. It is a powerful chelator of iron, and this practice (linked to traditional medicine), is responsible for a high incidence of severe anemia of pregnancy in this area. We report on two sibs born to a pemba-eater mother. The first child was born at term with IUGR severe microcephaly. Intracranial „calcifications" were observed by ultrasound screening during the second trimester. CT scan confirmed massive radio-opaque deposits in the brain basis. The clinical diagnosis of TORCH or Aicardi-Goutières syndromes were suggested initially. The child survived with major developmental delay. At age 7y, she has an OFC of 39 cm (-10 SD) and a height of 10 cm (-4SD). CSF interferon and TORCH screening were negative. Recurrence of microcephaly during the second pregnancy lead to TOP, after diagnosis of a similar microcephaly. Neuropathological examination confirmed severe microcephaly, with extensive microcalcifications dispersed throughout the brain. Electron microscopy made it possible to visualize intraneuronal aluminium silicate deposits, resembling aluminium deposition observed in post-vaccinal myofasciitis. The most likely mechanism to explain this recurrence is an association of IUGR secondary to severe maternal anemia combined with accumulation of exogenous silicates in the neural cells. This appears to be the first description of fetal brain disruption secondary to ingested clay. The syndrome superficially mimics Aicardi-Goutières syndrome, and convey a high risk of recurrence.

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P02.093

Congenital primary microcephaly and type B-like brachydactyly , a new syndrome?

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We report a 10 year-old girl with primary microcephaly and brachydactyly. Microcephaly was suspected during the pregnancy by echography. At birth, at term, head circumference was 29cm (-5 SD), weight 2770g, length 50cm . Pregnancy was normal (no drug nor alcohol) and there were no perinatal problems and no feeding difficulties. She is the first child of unrelated parents none of the members of this family have brachydactyly or microcephaly.
She started walking at 8 month-old, first words were around 1 year.
She followed mainstream schooling. In 2nd grade, problems with motricity and writing fatigability were observed. Clinical examination at 8 year-old: showed a child of medium stature with generalised amyotrophy and microcephaly 41,5cm (-8 SD). Neurological examination was normal. She had facial dysmorphism with upslanted palpebral fissures and microdontia. She has bilateral brachydactyly ressembling type B of hands and feet, respecting thumbs but involving halluces, discovered at birth.
On X rays, the terminal phalanges were missing on most digits, intermediate phalanges were hypoplastic or aplastic, with anonychia and cone-shaped epiphyses. MRI revealed a brain of reduced volume but normal structure and gyration. CGH array (180 k), caryotype and mitomicyn-induced chromosome breakage test were normal.
To our knowledge it is the first described case of severe congenital microcephaly with normal IQ associated with brachydactyly and anonychia. This disorder is clinically distinct from Jawad syndrome (due to mutations in RBBP8/CTIP - currently under sequencing), which shows mental retardation and ungual hypoplasia, and Teebi anonychia-microcephaly syndrome, which has much milder anomalies.

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P02.094

Evaluation of recurrent microdeletions and microduplications of 16p

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Chromosome 16 is rich in segmental duplications that mediate recurrent genomic rearrangements and the proximal short arm of chromosome 16 is particularly susceptible to such rearrangements. In the last few years, several patients with recurrent deletions and duplications from 16p11.2 to 16p13.11 have been described, with the 16p11.2 locus alone encompassing several distinct copy number changes.
Interestingly, the reported phenotypes within the distinct deletion and duplication regions are not consistent and the copy number changes have been observed in ‚phenotypically normal' individuals. The inconsistencies in clinical presentation and the presence of the copy number change in unaffected family members could be due to factors such as incomplete penetrance, variable expressivity or a failure to recognise subtle manifestations of the

phenotype. There is recent evidence to suggest that recurrent rearrangements such as these may be influenced by a two hit model for a neurodevelopmental phenotype, where a sequence variant is unmasked by the copy number change. Whatever the mechanism may be, this region poses a challenge for diagnostic interpretation.
Due to the diversity of published clinical features for these recurrent rearrangements and reports of asymptomatic parental inheritance, we present a retrospective evaluation of published cases and our own clinical patient data to identify the common clinical features, assist with genetic counselling and to standardise the reporting of 16p copy number changes within the diagnostic laboratory.

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P02.095

Molecular and clinical characteristics of three patients with partially overlapping interstitial deletion at 13q12.11-13q12.12.

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There are only a few descriptions of a deletion at 13q12.11. Here we report the molecular and phenotypic description of three unrelated patients with partially overlapping microdeletions at 13q12.11-q12.12.
The first patient, with the smallest, 1.55 Mb, deletion at 13q12.12, presented with moderate developmental delay, dysmorphic features, muscular hypotonia and bilateral coloboma of the iris and retina. The second patient, with larger, 3.12Mb deletion, at 13q12.11, presented with narrowed palpebral fissures, developmental delay, café-au-lait spots, small teeth, cryptorchidism and multicystic right kidney. Recently, in the same region, 2.1 Mb deletion have been described by der Kaloustian VM [2001]. His patient resembled our case but no renal defects were revealed.
The last patient presented with the most striking dysmorphic features including blepharophimosis, pronounced developmental delay, microcephaly (-4.3SD), muscular hypotonia, cardiac malformation (PDA that required cardiac surgery in the first year of life). He was suspected of Down syndrome in infancy then Coffin-Lowry, ATRX and Cohen syndromes were considered in his differential diagnosis. Application of array CGH reveled the 5.7Mb deletion at 13q12.11-q12.12 overlapping with both deletions presented in our previous two patients. He had no renal or retinal defects.
We hope that our detailed description of 13q12 deletion phenotype will contribute to the further genotype-phenotype delineation of that microdeletion.
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P02.096

Deletion 6q24.3-q25.1: a recognizable phenotype with facial dysmorphism, cardiac defect, loose skin and joints without cognitive impairment.

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Interstitial deletions of the long arm of chromosome 6 are rare and associated with variable phenotype depending on breakpoints, localization and size of the deletion. From approximately sixty cases reported in the literature, only a minority have been characterized with high resolution techniques, making genotype-phenotype correlations difficult. We report a familial 6q24.3-q25.1 deletion, identified by CGH array, spanning 1.7 Mb (chr6 (hg18):148 727 249-150 400 356), transmitted from an affected father to his affected daughter. Congenital polyvalvular disease, dysplastic ears, long philtrum, thin upper lip, redundant skin, hypermobile joints in childhood, early delayed motor milestones with normal cognitive development were the most remarkable clinical features shared by both patients. Sixteen OMIM genes are included in the deleted region. One of them, the *TAB2* gene, has been recently reported to cause cardiac defect. Our patients share some clinical features with other patients previously described with partially overlapping deletions. Among them, all patients (6) analysed by CGH array present a facial dysmorphism with similar features to ours and 5 patients whose deletion encompass *TAB2* have a cardiac defect. All patients, except one familial case, have mild or moderate developmental delay. Hypermobile joints and redundant skin have only been reported twice in patients with 6q24-q25 deletion in studies where the mapping of breakpoints was not performed preventing from targeting a causative gene. This observation allows us to describe a new recognizable (6q24.3-6q25.1) microdeletion syndrome without developmental delay, characterized by dysmorphic features, congenital heart defect and loose skin and joints in childhood.

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P02.097

Xp11.23p11.22 microduplication: genotype - phenotype correlations in 17 new patients

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Array CGH has improved the detection of submicroscopic chromosome X imbalances such as duplications of the short arm of the X chromosome. They can result either from an intrachomosomal rearrangement of the X chromosome or from an unbalanced translocation between an X chromosome and an autosome or with a Y chromosome. In 2009, Giorda et al. identified an inherited or de novo recurrent Xp11.23p11.22 microduplication in eight patients (two males and six females) from a large cohort of patients presenting with syndromic intellectual disability. This 4.5 Mb microduplication is mediated by nonallelic homologous recombination between D-REP and P-REP segmental duplications. This duplicated region contains many genes such as PQBP1, FTSJ1, SYP and SHROOM4 responsible for intellectual disability and BMP15 involved in ovarian dysgenesis.
So far, fourteen females and five males with this microduplication have been reported in the literature. Associated clinical manifestations include language impairment, mostly severe, moderate to severe intellectual disability and abnormal electroencephalogram. Other features such as epilepsy, overweight and early puberty have also been reported. Here, we report on seventeen new patients (nine females and eight males) presenting with an Xp11.23p11.22 microduplication detected by array CGH. The 4.5 Mb recurrent duplication was identified in five patients whereas twelve patients harbor an atypical duplication with various breakpoints. These new patients offer the opportunity to better define the phenotype associated with this microduplication and to identify minimal critical regions highlighting some possible candidate gene(s) for specific clinical feature(s).

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P02.098

Mosaic Variegated Aneuploidy syndrome: a new case of *CEP57* mutation in a 4-years-old girl and review of the literature.

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Mosaic Variegated Aneuploidy (MVA) syndrome is a rare autosomal recessive disorder characterized by constitutional aneuploidy and predisposition to cancer. Typically, individuals with MVA display growth retardation, facial dysmorphism, variable congenital defects and developmental delay. MVA is a heterogenetic condition caused by mutations in *BUB1B* and *CEP57* genes, both involved in mitotic spindle and microtubule stabilization.
We report a 4-year-old girl, second child of first cousin parents, presenting with pre and post natal growth retardation, dysmorphic features including mild rhizomelic shortening of the upper limbs and mild mental delay. During follow-up, the proband does not show tumor development.
Diagnosis of MVA syndrome was suspected based on cytogenetic data. Molecular investigations identified a homozygous 11 base pair insertion in *CEP57* gene. This insertion, reported in three of the four previously *CEP57* mutation-positive individuals, is the most common causal *CEP57* mutation in MVA syndrome.
When compared to the literature, our report confirms certain clinical features in *CEP57* mutated MVA cases, such as growth retardation with relative sparing of the head and normal or mildly delayed development. Rhizomelic shortening of uppers limbs has already been observed in two other *CEP57* mutation carriers and may be a specific feature.
In contrast with the *BUB1B* mutated probands, so far no cancer has been reported in *CEP57* mutation-positive individuals.
Since few cases of patients with MVA syndrome caused by *CEP57* mutations have been reported, the careful description of further cases is important to better delineate the phenotypic spectrum and confirm phenotype genotype-correlation.

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P02.099

Lines of Blaschko as a manifestation of (functional) mosaicism in 4 female cases with different disorders

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Patterned pigmentary disturbances are seen in a large variety of human genetic disorders. Cytogenetic studies have provided evidence that such skin lesions often reflect chromosomal mosaicism.
In women X-inactivation results in functional mosaicism, and in X-linked skin disorders this can manifest itself by the appearance of pigmented skin striations following Blaschko`s lines. This has been reported for several X-linked diseases, like Incontinentia Pigmenti, caused by mutations in the NEMO gene, Focal Dermal Hypoplasia, caused by PORCN mutations, Christ-Siemens-Touraine syndrome etc. In these conditions heterozygous females often show a Blaschko-linear pattern of Lyonization.
With techniques like SNP array copy number variations can be detected containing genes on the X-chromosome, that lead to severe diseases in males, but in females can lead to the typical skin changes.
Also mosaics of other (cryptic) chromosome abnormalities can lead to the same skin lesions.
We show four female patients with streaky hyperpigmentation following the lines of Blaschko, with or without accompanying symptoms; one with a de novo deletion on Xq26.2-26.3, containing the PHF6 gene, responsible for the Borjeson-Forssman-Lehmann syndrome in males, and the HPRT1 gene, responsible for the Lesch-Nyhan syndrome in males. The second patient shows a hypomorphic mutation in the NEMO gene, giving rise to Hypohydrotic Ectodermal Dysplasia with Immune-deficiency in males. The third patient is a girl with a mosaic trisomy 14, and in the fourth patient a mosaic maternal isodisomy 11q13.4-qter was found by SNP array analysis.

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P02.100

ZEB2 zinc-finger missense mutations lead to hypomorphic alleles and a mild Mowat-Wilson syndrome

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Mowat-Wilson syndrome (MWS) is an intellectual disability (ID) - distinctive facial gestalt -multiple congenital anomaly syndrome, with features of microcephaly, epilepsy, corpus callosum agenesis, conotruncal heart defects, urogenital malformations and Hirschsprung disease (HSRC). MWS is caused by de novo heterozygous mutations in the ZEB2 gene. The majority of mutations lead to haplo-insufficiency through premature stop codons or large gene deletions. Only three missense mutations have been reported so far, none of which resides in a known functional domain of ZEB2 protein.
In this study, we report and analyze the functional consequences of the first missense mutations, p.Tyr1055Cys, p.Ser1071Pro, and p.His1045Arg, identified in the highly conserved C-zinc finger (C-ZF) domain of ZEB2, in three unrelated patients. Their phenotypes included a facial gestalt reminiscent of MWS and moderate ID, but no microcephaly, heart defects, or HSRC. In-vitro studies showed that all three mutations prevented binding and repression of the E-cadherin promoter, the best-characterized ZEB2 target gene. Taking advantage of zebrafish morphant technology, we performed rescue experiments using wild-type and mutant human ZEB2 mRNAs. Variable, mutation-dependent, embryo rescue, correlating with the severity of patients' phenotype, was observed.
Our data provide evidence that these missense mutations cause a partial loss of function, suggesting that ZEB2 role is not restricted to repression of E-cadherin. Functional domains other than C-ZF may play a role in early embryonic development. Finally, these findings broaden the clinical spectrum of ZEB2-mutations, indicating that MWS ought to be considered in patients with moderate ID, suggestive facial gestalt, and no congenital malformation.

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P02.101

New case supporting the evidence that LAPS and Myhre syndromes are clinical variants of the same disorder caused by mutations in *SMAD4*

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We report the case of a boy of Scandinavian descent diagnosed with Myhre syndrome (MS) at the age of ten years. The patient was initially diagnosed with CHARGE syndrome based on the association of unilateral choanal atresia, patent ductus arteriosus, soft cleft palate with swallowing difficulties necessitating PEG, growth retardation, cryptorchidism, bilateral hearing loss and immunoglobulin deficiency explaining his recurrent bacterial respiratory tract infections. The absence of iris- and/or retinal coloboma, normal semicircular canals and no typical dysmorphic features, however, made CHARGE a less likely diagnosis. Furthermore, the patient has progressive restrictive pulmonary disease, not explained by his immunodeficiency, as well as delayed wound healing and chronic pericarditis requiring permanent immune suppression. MS was suspected based on the presence of typical dysmorphic features, short stature, hearing impairment and limited joint mobility. A heterozygous mutation in *SMAD4* affecting the codon for Ile500 (c.1498A>G, p.Ile500Val) confirmed the diagnosis. He also has signs of precocious puberty with so far no evidence of hormonal imbalance. This is in line with a previous report suggesting hypothalamic-hypophyseal malfunction in MS [Asakura et al., 2012]. The presence of respiratory problems and chronic pericarditis supports a previously reported observation that LAPS syndrome (**L**aryngotracheal stenosis, **A**rthropathy, **P**rognathism, and **S**hort stature) and MS are in fact phenotypic variants of the same disorder [Lindor et al., 2012]. This case is particular by the finding of immunoglobulin deficiency, chronic immune pericarditis and delayed wound healing indicating a role for the SMAD4 and TGF Beta Signaling in immune and inflammatory response.

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P02.102

A new autosomal recessive disorder with hydrops fetalis, arthrogryposis multiplex congenita, neuronal migration disorder, adrenal and pulmonary hypoplasia and renal abnormalities

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Objectives: We report male and a female siblings, born to a consanguineous Turkish couple, with hydrops fetalis (HF), neuronal migration disorder, joint contractures, pulmonary and adrenal hypoplasia and renal abnormalities. To our best knowledge this is a hitherto new, autosomal recessive condition.

The parents were healthy and first cousins of Turkish descent. Patient A, a male, was born at 31.8 weeks gestation following a pregnancy complicated with HF, mild ventriculomegaly, misalignment of the skull bones and lumbar lordosis. He died shortly after delivery and autopsy showed HF, cleft palate, ambiguous genitalia, bilateral talipes equinovarus, camptodactyly of the fingers and clenched fists. There was a butterfly T5 vertebra and hypoplastic lungs, kidneys and adrenal glands. The brain showed megalencephaly, abnormal gyration and an unusual pattern of cytoarchitextural disturbance in the cerebral cortex. The karyotype was 46, XY.

Patient B, a female, the product of the couple's second pregnancy, was found to have an increased nuchal translucency and body edema on ultrasound at 12 weeks gestation. Ultrasound at 20 weeks gestation showed bilateral talipes equinovarus, flexed wrists, echogenic kidneys, pericardial effusion and an abnormal cerebral configuration. The pregnancy was terminated at 21 weeks gestation and the autopsy showed the same findings as in her late brother apart from her female internal and external genitalia. Microarray analysis was normal and female. Extensive investigations done on both siblings failed to identify an eatiology. The couple subsequently had three unaffected pregnancies. Investigations are currently ongoing to identify the causative gene.

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P02.103

Nicolaides-Baraitser syndrome due to a large intragenic SMARCA2 deletion

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Nicolaides-Baraitser syndrome (NBS) is usually caused by SMARCA2 mutations and is characterized by short stature, hypotrichosis, mental retardation and typical facial appearance. We report a case of NBS due to an intragenic SMARCA2 deletion detected by oligonucleotide SNP array CGH. A 24-month-old girl is the only child of healthy parents born from the second pregnancy at 37 weeks with low birth weight. She had poor appetite and low weight gain. Her parents have noted hair loss, short stature and development delay. Physical examination showed low height (3d percentile) and weight (<3rd percentile) and small head circumference (<3rd percentile). The child had pectus excavatum, umbilical and bilateral inguinal hernia. She presented with sparse and thin (vellus) hair over the scalp with normal eyebrows/eyelashes, partial alopecia (occipital region), triangular face, downslanting palpebral fissures, broad nasal tip with anteverted alae nasi, thin upper and thick prominent lower lip, broad and long philtrum, wide mouth, large protruding tongue. The patient had diffuse muscle hypotonia. She was sociable, good-natured and smiling. Seizures, speech delay, enlarged kidneys with a thickened renal parenchyma and incomplete myelination were observed. High-resolution (~1kb) oligonucleotide SNP array (CGH) analysis has shown the presence of a deletion within 9p24.3. The size of the deletion was estimated as 88 kb spanning 21 exons of SMARCA2. To date, this is the first NBS case associated with such a large intragenic SMARCA2 deletion. The index case shows that high-resolution oligonucleotide SNP array analysis is able to detect intragenic deletions causing extremely rare monogenic conditions.

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P02.104

Nijmegen Breakage Syndrome: a possibilities of postnatal and prenatal diagnostics in Belarus

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"Nijmegen Breakage Syndrome" (NBS, MIM 251260) is autosomal recessive disorder with distinct phenotype. Patients selected for moleqular testing based for well-known criteria: microcephaly, mental, growth delay, typical facial features, immunodeficiency, chromosome instability (CIN). We present the scheme of NBS diagnostics used in Belarus. Results. Patients (4 sporadic cases, 2 sibs) were born at term. Variations of birth weight were 2300-3330g; length 48-52cm; OFC 28-31cm. Age of diagnosis detection was: at infancy (1), first year (3), during 3-6 years (2). All showed severe microcephaly, characteristic facial appearance, growth, mental retardation, anemia, sinopulmonary infections, humoral/cellular immunodeficiency, normal karyotype. One had polydactyly. Frequency of chromosomal rearrangements was 13-23%; typical aberrations presented were inv(7); t(7;14); t(14;14); del(14); dic(7;7). DNA testing revealed mutations in exon 6 NBS gene: c.657_661del5 (2 sporadic cases); c.764-768delAAAAC (familial case). All patients were homozygous. Prenatal DNA study was performed in one family: fetus was heterozygous for mutant allele c.657_661del5. Familial case. Proband - fetus (G2) with ventricular erlangement, microcephaly detected by sonography during 1-2st trimesters (karyotype 46,XX). Parents refused from pregnancy termination. Newborn presented low weight, microcephaly. Elder child (6 years old), firstly counseled at same time, showed microcephaly, dysmorphisms, growth and mental delay, recurrent infections, CIN. We have established NBS based on characteristic pattern of clinical, cytogenetical, immunological data, confirmed by mutation analysis. Patients care: follow-up to monitor mentality, growth, immunological status, malignancy. Diagnostics. Genetic counseling of affected children with normal karyotype>NBS suspected>CIN investigation>Test positive>DNA investigation>NBS confirmation>Genetic prognoses>Prenatal NBS diagnostics in families with known mutations.

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P02.105

Identification of two novel mutations in the *NOG* gene in patients with Symphalangism syndrome

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Background

NOG maps to human chromosome 17q22, which encodes a protein named noggin that is essential to bone and joint development. The NOG gene mutations, which lead to aberrant functions of the noggin protein, give rise to different digit anomaly syndromes. One of these syndromes is a syndrome with autosomal dominant proximal symphalangism and congenital conductive hearing impairment. We investigated the NOG gene in 3 patients from 3 unrelated families, who were clinically diagnosed with proximal symphalangism and conductive hearing loss.

Result

All patients had bilateral conductive hearing loss and symphalangism. Exploratory tympanotomy was done in two patients. Stapes ankylosis was identified in both patients. Hyperopia was identified in two out of three patients. Syndactyly was identified in one patient.

Mutation analysis of the NOG gene was performed in all patients. Missense mutations of the NOG gene were identified in two out of three patients. The mutations were causing p.P187A and p.C228S, respectively. We could not find any pathological mutations of NOG gene in one patient.

Conclusion

We identified two novel mutations causing p.P187A and p.C228S in patients with symphalangism spectrum disorder. The NOG gene mutations give rise to a various spectrum of clinical findings. These two novel mutations and clinical manifestations in patients will contribute to understanding genotype/phenotype correlation of the NOG mutations.

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P02.106

Multiple hemangiomatosis in a child with Noonan syndrome due to a SHOC2 mutation

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Noonan syndrome (NS) and RASopathies are recognizable on clinical history and characteristic dysmorphologic features, with usual skin expression. Classical skin features are deep plantar and palmar creases, icthyosis, eczema, keratosis pilaris, cafe-au-lait spots, and lentigines. Skin vascular abnormalities are not commonly described. We report an atypical case of NS with multifocal hemangiomatosis and a SHOC2 mutation. The girl is the first born of non-consanguineous parents without family history of vascular anomalies. The pregnancy was marked by enlarged nuchal translucency. At birth, macrosomia and disseminated small infantile hemangiomas was noted, with more than 50 millimetric angiomas. No hepatic or brain angiomas were found by US and MRI. Clinical examination of the girl showed widely spaced nipples, short neck and posteriorly rotated ears. Sequential gene exploration of RASopathies found a mutation in SHOC2 gene. SHOC2 is associated with the phenotype of Noonan syndrome with loose anlagen hair. Multifocal hemangiomatosis is not associated at our knowledge in Noonan syndrome or other rasopathies, but PTPN11 (SHP-2) has been shown to regulate vascular signalling and angiogenic events in MAP Kinase pathway (Mannell and Krötz, 2012). Vascular malformations have already been described in Noonan's syndrome : multiple pulmonary arteriovenous fistulas, moya-moya, aneurysms and aortic dilatation, additionally to the known cardiac malformations. Vascular tumors, such as hemangiomatosis, especially diffuse, may be another part of RASopathies spectrum.

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P02.107

Prevalence and frequency monogenetic syndromes of multiple congenital malformations in population of Rostov region (Russia)

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A medical genetically study of the population of the Rostov region was carried out (12 districts - 497,460 total surveyed persons, 97,345 surveyed children (aged 0 to 18 years)). Information on births in the studied 12 districts of the Rostov region allocated for the period 2000-2011, of the accounting monitoring of congenital malformations (CM) (born alive and stillborn 64,215 people). Monogenic syndromes of multiple congenital malformations (MSMCM) was examined. The overall prevalence of MSMCM was 1:2211 persons. The proportion of children was 58%, the share of adult age was 42%. As the severity of the CM is variable, children with this condition may not survive to adulthood. Therefore, a more reliable assessment of the prevalence of MSMCM for the child population. The overall prevalence of MSMCM was 1:716 children. Among the syndromes MSMCM with autosomal dominant (AD) inheritance type was identified 29 diseases in 85 children, with autosomal recessive (AR) inheritance type - 17 diseases in 44 children, with X-linked type of inheritance - 4 of diseases in 7 children. Among patients with MSMCM with AR inheritance type children are predominat (72%). Population frequency of MSMCM was 160:100,000. Population frequency were revealed for some MSMCM: Noonan syndrome (frequency of 9:100,000), hemifacial microsomia (8:100,000), constricting bands, congenital (8:100,000), DiGeorge syndrome (8:100,000), Tel Hashomer camptodactyly syndrome (6:100,000), Coffin-Siris syndrome (6:100,000), Waardenburg syndrome, type I (5:100,000), Holt-Oram syndrome (5:100,000), Dubowitz syndrome (5:100,000), Cousin syndrome (5:100,000), Seckel syndrome (5:100,000), CHARGE syndrome (5:100,000), VATER-association (5:100,000). These results agree with data previously surveyed populations of Russia.

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P02.108

Further expanding both the genotype and phenotype associated with germline NRAS mutation

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We present a case of a female infant who presented small for gestational age with multiple abnormalities including; polyhydramnios, intracerebral arachnoid cyst, bilateral cystic hygroma and hydronephrosis on prenatal ultrasound. She had a normal karyotype on amniocentesis. Mother declined prenatal genetic testing for Noonan syndrome. On follow up after birth she was notably dysmorphic with wide sutures, reddish blonde and sparse hair which was abnormal in texture. She struggled with hypoglycemia and developed apneic spells and episodes of seizure. She was hypotonic, did not feed well and required NG tube placement. Further investigations revealed duplication of the collecting system with moderate hydronephrosis, ureteroceles and small cortical cysts bilaterally. Brain imaging was abnormal and echocardiogram identified an ASD and bicommissural aortic valve. She failed her newborn hearing screen and suffers from numerous urinary tract and skin infections. Her milestones are delayed and she has ongoing GI issues. Molecular genetic testing for Noonan syndrome identified a previously uncharacterized germline mutation in the neuroblastoma RAS viral (v-ras) oncogene (NRAS). Previously thought to be restricted to somatic mutations, three novel germline NRAS mutations have recently been reported in nine patients presenting with features suggestive of the Noonan syndrome spectrum. Although our patient shares many of the previously reported characteristic features, our patient is the tenth world wide to be reported, harbors yet another novel de novo NRAS mutation and demonstrates a more severe phenotype which was evident prenatally.

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P02.109

Molecular spectrum in a group of Spanish and German patients with RASopathies

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INTRODUCTION: RASopathies, including Noonan (NS), Cardiofaciocutaneous (CFCS), Costello (CS), LEOPARD (LS), Legius syndromes and Neurofibromatosis type I (NF1), are due to mutations in twelve different genes of the RAS/MAPK pathway. Our aim is to know the genotype in a RASopathy group of patients.
MATERIAL AND METHODS: We reviewed the charts of 67 patients with RASopathy (36 from Spain and 21 from Germany) and analyzed its molecular basis.
RESULTS: 35 males and 32 females. Median age at referral: 1.5 years. Positive family history: 13 (19.4%). Phenotype: NS 52 (77.6%), CFCS 9 (13.4%), LS 2 (3%), CS 2 (3%), and Legius syndrome 2 (3%). In 57 patients (85 %) a pathogenic mutation was identified. In 10 PTPN11 mutation negative patients (9 NS / 1 CFC) molecular analysis is ongoing (15%). Mutations were indentified in 43/52 NS (82.7%): 36 PTPN11 (83.7%), 4 NF1 (9.3%), 2 SOS1 (4.6%) and 1 SHOC2 (2.3%); 8/9 CFCS (89%): 4 BRAF (50%), 2 KRAS (25%), 1 MEK1 (12.5%) and 1 RAF1 (12.5%). Y279C PTPN11 mutation was in both LS, HRAS in 2 CS and SPRED1 in 2 Legius syndrome. Higher proportion of PTPN11 mutations was in exon 3, 8 and 13 (34.3%, 28.6% and 22.9% respectively).
CONCLUSION: Compare to previous reports, we find higher PTPN11 mutation detection rate (83.7% vs 50%), maybe due to stringent criteria, and similar molecular CFCS data and PTPN11 hotspots. The lower rate of SOS1 mutations might be associated to the previous lack of access to the study of other RAS/MAPK genes.
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P02.110

Novel substitutions in patients with a RASopathy phenotype: pathogenic mutations or benign polymorphisms?

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The RASopathies are a group of congenital syndromes with multiple anomalies, including dysmorphic craniofacial features, cardiac, skin and musculoskeletal defects and developmental delay. The RASopathy disorders involve such syndromes as: Noonan (NS), Cardiofaciocutaneous (CFCS), LEOPARD (LS) and other. They result from germline mutations in genes encoding components of the Ras/MAPK signaling cascade. We present a group of eight unrelated patients with novel substitutions in different Ras/MAPK genes, causative of NS, LS or CFCS phenotype. The identified variants were heterozygous missense substitutions: c.166A>G in *PTPN11*, c.784A>T, c.1913C>T in *RAF1*, c.2165G>A, c.2371C>A in *SOS1* and c.785A>C, c.1502A>C, c.1783T>C in *BRAF*. All were absent in the control alleles. To predict whether the novel variant is tolerated or deleterious, we used the combined results of four computer algorithms: SIFT, PMut, PolyPhen-2 and Mutation Taster, which confirmed pathogenicity of all *BRAF* mutations and one *RAF1* substitution (c.784A>T), in accordance to observed CFCS and LS symptoms, respectively. The two *SOS1* substitutions, found in two patients and their parents, were recognized recently as SNPs of unknown clinical consequence. The results for the remaining two variants (c.166A>G and c.1913C>T) were ambiguous, mostly suggesting them as possibly benign. Nonetheless, the detailed clinical manifestations of all patients suggest a Ras/MAPK pathway defect. We presume that introduction of novel mutations in the Ras/MAPK genes might contribute to defining possible differences in the features presented by the patients and enable more precise phenotype-genotype correlations in the RASopathies. The research was supported by NCN Project UMO-2011/03/N/NZ2/00516 and EU Structural Funds Project POIG.02.01.00-14-059/09.

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P02.111

Refinement of diagnostic criteria and molecular study of the FLNA gene in fetal OPD spectrum disorders

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Otopalatodigital syndrome type 1 (OPD1), and 2 (OPD2), Melnick-Needles syndrome (MNS), and frontometaphyseal dysplasia (FMD), belong to OPD syndrome spectrum disorders, caused by mutations in the *FLNA* gene. Overlapping phenotypes are described. Males are more severely affected than females, up to perinatal death of affected males with MNS. We report the first series of 9 fetuses (1 female and 8 males) sent to our laboratory for *FLNA* analysis with a phenotype belonging to the OPD spectrum.
We studied the whole coding sequence of *FLNA* by using either dHPLC or HRM and PCR-sequencing on both strands. A missense mutation was identified in 3 male fetuses: the MNS recurrent mutation p.Ser1199Leu and 2 novel variants in exons 4 and 34. A synonymous variant was identified in 2 brothers but RNA studies did not show any splicing effect.
In our series, the mutation rate is lower than that of postnatal series. The diagnosis is probably more difficult to refine clinically given the absence of certain diagnostic criteria. Hypertelorism, cleft palate, abnormal hands and feet, heart defects and brain anomalies are relevant criteria for fetal OPD2 diagnosis. Radiological signs are more difficult to identify. For the MNS, abnormality of the anterior segment of the eye seems to be a good criterion for the diagnostic orientation. Cleft palate may also be seen, although not originally described in this syndrome. The diagnosis of OPD spectrum disorders is important in fœtopathology because it will provide affected families to appropriate genetic counseling for future pregnancies.

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P02.112

Further Delineation of the Johnston-Biesecker Multiple congenital anomalies-hypotonia-seizures syndrome caused by a single PIGA mutation

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Somatic mutations in the X-linked PIGA gene [MIM 311770] encoding phosphatidylinositol glycan class A are known to cause paroxysmal nocturnal hemoglobinuria, while germline mutations were assumed embryonic lethal. Johnston and colleagues only recently described a PIGA nonsense mutation p.Arg412* with residual function in a family segregating with an X-linked lethal disorder involving cleft palate, neonatal seizures, contractures, central nervous system structural malformations and other anomalies (Johnston et al. Am J Hum Genet 2012). While
three boys of this family were affected, two carrier females with 100% skewing of X-inactivation were healthy. We now identified the same p.Arg412* mutation in an unrelated boy by whole exome sequencing.
Like the previous cases this boy was born with macrosomia, short neck, facial dysmorphism and cerebral anomalies such as hypoplastic cerebellum and immature white-matter. The neonatal course was complicated by absence of spontaneous movements, respiratory insufficiency and intractable myoclonic-tonic seizures. The boy developed sepsis from necrotizing enterocolitis and deceased at the age of 15 days.
In addition to the reported features this boy showed hepatosplenomegaly and remarkable short tubular bones verified by X-ray measurements to approximately correspond to the 3rd centile. The healthy carrier mother showed only mild skewing of X-inactivation in blood (83:17). The family history was remarkable for one male stillbirth of the sister of the maternal grandmother. We therefore confirm that boys with the PIGA p.Arg412* mutation show a distinct pattern of morphological anomalies and are prenatally viable, but die soon after birth due to respiratory failure and intractable seizures.

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P02.113

A clinical and molecular study of Pitt-Hopkins syndrome in Japan

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Pitt-Hopkins syndrome (PTHS) is characterized by severe motor and mental retardation, a characteristic facial features and episodic hyperventilation. Language development is almost absent. Characteristic facial features include a coarse face, a broad nasal bridge and a wide mouth with prominent Cupid's bow. Researchers revealed that microdeletion of chromosome 18q21.1 including *TCF4* in patients with PTHS. De novo missense mutations in *TCF4* gene were identified. *TCF4* is a class I basic helix-loop-helix transcription-factor and involved in neuronal development. Pitt-Hopkins-like syndrome-1 is caused by mutation in the *CNTNAP2* gene on chromosome 7q35, and Pitt-Hopkins-like syndrome-2 is caused by mutation in the *NRXN1* gene on chromosome 2p16.3.
The number of the patients with PTHS increased. So far, reports of Japanese patients with PTHS were few. We studied Japanese patients with PTHS and found 10 novel mutations. Most of them have been diagnosed as atypical Rett syndrome or Angelman syndrome. We studied patients by direct sequencing of coding regions and MLPA analysis for deletions. Most of the mutations generated premature stop codons. Some missense mutations were localized in the highly conserved amino acid in the bHLH domain. All patients showed typical features of PTHS and had severe intellectual disability. Incidence of breathing abnormality and epileptic seizures were low among our patients. However, some typical patients did not have *TCF4* mutations. Further genetic analysis including next generation sequencing is proceeding.

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P02.114

Towards the identification of genetic mechanisms underlying Poland Anomaly

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Poland Anomaly (PA) is a congenital disorder presenting with agenesis/hypoplasia of the pectoralis major muscle variably associated with thoracic and/or upper limb anomalies, incidence 1/30000 births, higher prevalence in males. Most cases are sporadic, but familial recurrence, with different inheritance patterns, has been observed. The PA genetic etiology remains unknown. Since PA involved structures may originate from the same embryonic tissues, genes controlling cell proliferation, migration, and differentiation of these tissues might be involved. Alternatively, one common assumption is that PA may origin from an embryonic vascular insult indicating genes controlling vessels development as indirectly involved.
We recruited a large cohort of PA patients (more than 200, about 10% familial) classified into homogeneous subgroups based on their phenotype features. Karyotype analysis performed in 128 patients has not shown any relevant alterations. The arrayCGH revealed the presence of chromosome anomalies in 19 out of 119 analyzed patients: 10 genomic duplications, some of which overlap common copy number variant (CNV) regions, and 9 heterozygous deletions in different regions outside evident CNVs.
Bioinformatic analysis of arrayCGH data indicates gene enrichment in different pathways including those involved in cell-cell adhesion and muscle structure development.
The screening of these genes in our cohort of patients will contribute to confirm their roles in PA. Although clinical and genetic heterogeneity make the task difficult, the use of next generation sequencing (NGS), the availability of a large cohort of patients and familial cases with dominant inheritance and full penetrance, will be helpful to identify PA causative genes.

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P02.115

Ciliopathies polydactyly variability illustrated by *LZTFL1* related phenotype

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Polydactyly is a common skeletal feature in ciliopathies and recognized as a hallmark diagnostic feature such as for example in Bardet-Bield syndrome (BBS). Polydactyly is mainly postaxial in ciliopathies, except in syndromes related to the GLI3 pathway (such as Greig, acrocallosal and hydrolethalus syndromes) for which lower limb polydactyly is preaxial whereas postaxial for the upper limb. This presentation has also been reported for Oro-Facial-Digital (OFD) syndromes in particular type IV, with *TCTN3* mutations known to impact the transduction of SHH signaling. Moreover, mesoaxial polydactyly has been reported mainly in other ciliopathies: Pallister-Hall syndrome with mutations in *GLI3* and several types of OFD syndromes in particular type VI (unknown genetic basis to date).
In our cohort of patients presenting with BBS and carrying mutations in *BBS1* to *BBS16* genes, 89% had at least one extra digit that was always postaxial.
Recently, we reported on a BBS family mutated in *LZTFL1* (*BBS17*) presenting with mesoaxial polydactyly as an outstanding manifestation (Marion et al., 2012). Herein, we report on twin sisters presenting with classical BBS and mesoaxial polydactyly. They are compound heterozygotes for novel *LZTFL1* mutations confirming this clinical finding as highly suggestive for *BBS17*.
LZTFL1 was identified as an important negative regulator of the BBSome ciliary trafficking and SHH pathway signaling. The association of mesoaxial polydactyly and mutations in *LZTFL1* confirms the impact of SHH pathway in the development of the limbs and patterning of the digits.

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P02.116

Clinical features in patients with *PTEN* germline mutation diagnosed in childhood, except familial Cowden syndrome and Autism Spectrum Disorder.

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PTEN gene (MIM 601628) is implicated in *PTEN* hamartoma tumor syndromes (PHTS) including Cowden syndrome (CS) and Bannayan-Riley-Ruvalcaba syndrome (BBRS). BBRS, considered as the childhood form, is characterized by macrocephaly, intestinal hamartomatous polyposis, lipomas and pigmentation macules of the penis. Half of the patients have also mild to severe mental retardation. Recently, children with macrocephaly and autism spectrum disorders (ASD) without other symptoms of PHTS have been reported.
We report 6 children carrying a *PTEN* mutation, in the absence of ASD or family history of CS, who were diagnosed from 1 January 2009 to 31 December 2012. The raison of referral was lipoma (1/6), isolated macrocephaly (1/6), developmental delay (3/6) and severe facial arteriovenous malformation (1/6). Only three patients partially met the clinical diagnosis of BBRS. All patients had macrocephaly at the time of diagnosis (average at +6.5DS). Three patients had also overgrowth without advanced bone age. Four patients had cerebral MRI that showed enlarged cerebral ventricles or prominent subarachnoid spaces (4/4), abnormal periventricular white matter (2/4), abnormal cortical pattern (2/4) and thick corpus callosum (1/4). Concerning the mutations, 2 patients carried a nonsens mutation, 4 patients a missense mutation. The mutation occurred de novo in 3 patients. The two others related patients had a familial history of macrocephaly and mild developmental delay without cancer history.
This report emphasizes the variability of phenotype associated with *PTEN* mutation in children. Only half of the patients presented several criteria of BBRS. Macrocephaly was the most constant manifestation. Cerebral MRI showed nonspecific anomalies.

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P02.117

Intra- and inter-familiar variability in Saethre-Chotzen syndrome

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Background: Saethre-Chotzen syndrome (SCS) is a rare syndromic craniosynostosis, commonly involving the coronal suture and usually associated with facial craniofacial dysmorphism and digit abnormalities. Hearing loss, ophthalmologic complications and increased intracranial pressure may occur. SCS is caused by loss-of-function mutations in *TWIST1* and is inherited in an autosomal dominant manner. Many individuals diagnosed with SCS have an affected parent.
Methods: A retrospective analysis was performed on all patients with a confirmed *TWIST1* gene abnormality who attended our department over a 15-year period. Each patient's mutation and clinical features, as well as surgical interventions and sequelae, were recorded.
Results: Eleven patients were identified. Eight cases were familiar (corresponding to two families) and three cases were sporadic. *TWIST1* point mutations were found in ten cases and one had a 7p21.2-p15.3 deletion. The

clinical phenotype was highly variable. Craniosynostosis was confirmed in most cases (mostly bi- or uni-coronal; but complex craniosynostoses were reported in 3 cases). Half presented the classic craniofacial dysmorphism. In one case, only mild ear abnormalities were detected. One sporadic case was detected prenatally and resulted in an early termination of pregnancy. Five patients had received surgical intervention. Learning difficulties and/or developmental delay were found in five patients and hearing loss in three. Intracranial hypertension and ophthalmologic complications were not common.
Conclusion: Our patients displays an extreme intra- and inter-familiar variability. Many cases may be mild and not identified before the birth of a more severely affected child. Molecular testing is recommended for genetic counseling and detection of at-risk relatives.

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P02.118

LMX1B mutation and focal segmental glomerulosclerosis without extra-renal involvement

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Primary focal segmental glomerulosclerosis (FSGS) is a clinicopathological entity characterized by sclerotic glomerular lesions and isolated proteinuria or steroid-resistant nephrotic syndrome. The recent identification of causative podocyte gene mutations in familial forms of FSGS, some of which are associated with various extra-renal features, has helped decipher the glomerular filtration barrier pathophysiology. LMX1B mutations lead to Nail-Patella syndrome (OMIM#161200), characterized by dysplasia of the patellae, nails and elbows, and FSGS with specific glomerular basement membrane lesions by electron microscopy. Using a combination of linkage analysis and exome sequencing, we unexpectedly identified a novel LMX1B mutation segregating with the disease in a pedigree of 5 patients with autosomal dominant FSGS but no glomerular basement membrane anomaly suggestive of Nail-Patella-like renal disease by electron microscopy, and no extra-renal features. Subsequently, we screened 73 additional unrelated families with FSGS and found mutations of the same residue that segregate in 2 families. LMX1B encodes a homeodomain-containing transcription factor that is essential during development. To make functional predictions, we mapped the mutated residue onto a LMX1B in silico homology model. This residue is predicted to belong to a homeodomain. The model suggests this residue plays an important role in strengthening the interaction between the LMX1B homeobox domain and DNA molecule. Both mutations are expected to diminish such interactions. This novel finding demonstrates that isolated FSGS could be due to mutations in genes also involved in syndromic forms and highlights the need to include these genes in all next-generation sequencing diagnosis approaches in FSGS.

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P02.119

Comparison of genetic changes in schistosome-related squamous and transitional bladder cancers using FISH

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Abstract
The development of bladder tumors has been associated with a number of causative agents, including schistosomiasis. Schistosome-related bladder tumors show different epidemiological, histopathological, and clinical characteristics compared with non schistosome-related bladder cancers, occurring in younger patients, and being predominantly of squamous cell type.
The present study was carried out to investigate whether there are significant genetic differences between schistosome transitional and squamous subgroups of bladder carcinoma. We have used dual color FISH to determine the cellular copy number of three recognized tumor-related genes (HER-2/neu, MYC, and p53) in relation to chromosomes 8 and 17 centromeres in 75 Egyptian patients with schistosomal associated bladder carcinoma. Forty-one of these tumors were squamous cell carcinomas; the remaining 34 were of transitional cell carcinoma.
Our results demonstrated that, different histological subgroups of bladder tumors are characterized by distinct patterns of genetic alterations. Transitional cell carcinoma showed 1.54 and 2.02 times of Her-2/neu and c-myc gene amplification respectively as squamous cell tumors. For p53, TCC showed 0.67 times susceptible to p53 deletion as the SCC group.
The genetic changes found in transitional cell group are similar to those reported in the non-schistosome-related transitional cell tumors, but differ from tumors exhibiting squamous differentiation.

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P02.120

A Simpson-Golabi-Behmel patient with biliary cirrhosis and successful hepatic transplantation

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Simpson-Golabi-Behmel syndrome (SGBS) -OMIM 312870- is a rare X-linked inherited overgrowth syndrome caused by mutation in GPC3 or GPC4 gene. Affected patients present a variable phenotype with pre- and post-natal macrosomia, distinctive facial dysmorphism and numerous congenital anomalies such as diaphragmatic hernia, heart defect, renal defect, genito-urinary tract malformations, gastrointestinal abnormalities, skeletal and hands abnormalities Intelectual disability is not constant. About 10% of patients have an increased risk of developing embryonic tumors in early childhood. Only one case of biliary disease (choledocal cyst) has been described.
GPC3 is localised on Xq26 and encodes for Glypican-3 protein, a glycosylphosphatidylinositol-linked cell surface heparin sulphate proteoglycan
We report here a SGBS patient, who harbours a GPC3 mutation, with neonatal liver disease, and who developed an early biliary cirrhosis. Liver transplantation was discussed, together with the risk of cancer and developmental delay, and successfully performed when he was 19 months old.

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P02.121

Sirenomelia in Cali, Colombia.

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Sirenomelia is a very rare limb anomaly in which the normally paired lower limbs are replaced by a single midline limb, with a prevalence of 0.98 per 100,000. One hospital in the city of Cali, Colombia, of the ECLAMC (Latin-American Collaborative Study of Congenital Malformations) network, reported the unusual occurrence of 4 cases of sirenomelia within a 55-day period in 2005. The follow-up to 2013, shows 4 new cases from that state, in 483,524 births, yielding a prevalence of 0.83 per 100,000 births in the following years. It further describes the characteristics of the cases: according to the Foster Classification 4 Were Dipus, 2 unipus and 2 Apus; according to Stocker and Heifetz Were Clasification 2 type I, 1 type III, 1 type IV, 1 type V, 3 type VI; 3 of cases had other anatomical defects; none of the studied cases had chromosomal abnormalities. Conclusion. The findings suggest that the first 4 cases were a cluster of sirenomelia in Cali, Colombia. We report 8 cases of sirenomelia, a rare congenital defect.

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P02.122

Novel phenotypic features associated with increased dosage of SOX3

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SOX3 belongs to the Sry-related-HMG-box-containing group of transcription factors critical in vertebrate development. SOX3 plays a key role in the development of the hypothalamic-pituitary axis and gonadal differentiation. Disruption of SOX3, either by direct involvement or copy number variation of its regulatory region, has been implicated in several human conditions. Duplications of chromosome Xq27.1 involving the SOX3 gene result in X-linked panhypopituitarism in males, with some phenotypic variability depending on the extent of the copy number change. Additionally, duplications of the regulatory region surrounding SOX3 have been observed in patients with XX male sex reversal. We present a boy with partial absence of the corpus callosum, dyslalia, mild developmental delay, divergent strabismus and bilateral retinal colobomas associated with a complex duplication/triplication of chromosome Xq27.1 involving only the SOX3 gene. His mother, a carrier of the SOX3 change, has normal intellect and development, but also had bilateral retinal colobomas and mild dyslalia. This is the first time a CNV involving SOX3 has been reported with an ocular phenotype. This may suggest that ocular development is more tolerant of SOX3 dosage changes than the hypothalamus and pituitary gland. Our data are consistent with Sox3 transgenic animal models and underscores the importance of Sox3 dosage in the development of the brain, eyes and hypothalamic-pituitary axis.

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P02.123

Use of Array-CGH to identify new candidate loci in a cohort of 89 children presenting with syndromic obesity

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Syndromic obesity is defined by the association of obesity (BMI>30 in adults and above IOTF 30 chart in children) with one or more abnormalities including predominantly developmental delay, dysmorphic features and/or congenital malformations. Over 25 syndromic forms of obesity have been identified. However, most of cases remain of unknown etiology. The aim of this study was to identify new candidate loci associated with syndromic obesity in order to trigger new candidate genes and to better understand molecular mechanisms involved in this pathology. To achieve our objectives, we performed high-density oligonucleotide array-CGH in a cohort of 89 children presenting with syndromic obesity of unknown etiology, after exhaustive clinical, biological and molecular studies. Chromosomal aberrations were detected in 36/89 children (40%) occurring throughout the genome. Considering aberrations greater than 500 kb, this rate was reduced to 19/89 (21%). Among the entire cohort, only 4/89 (4.5 %) had *de novo* anomalies. However, we could not exclude every inherited anomaly as some of the parents carriers presented with obesity and/or intellectual disability. Even if no recurrent locus was identified (except for the 16p11.2 locus), our results further support that chromosomal rearrangements are more frequently associated with syndromic obesity with a variety of contributory genes having relevance to either obesity or developmental delay. Pathogenicity is however difficult to assert in these disorders suspected to be multifactorial. In conclusion, these findings show the importance of array-CGH in the etiological diagnosis of syndromic obesity allowing an appropriate genetic counseling in some families.

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P02.124

A rare combination of Syntelencephaly, Hemiparesis and Wormian bone

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Syntelencephaly is a rare anomaly characterized by fusion of the hemispheres in the posterior frontal and parietal regions and is considered a new variant of holoprosencephaly.
Wormian bones are accessory bones that occur within cranial suture and fontanelles, most commonly within the posterior sutures. They occur more frequently in disorders that have reduced cranial ossification, hypotonia or decreased movement, thereby resulting in deformational brachycephaly.
Here, we report a one-week- old male who presented with abnormal head shape and concern for craniosynostosis. He had brachycephaly, alopecia, right hemiparesis and closed anterior fontanelle. Three dimentional CT scan revealed absence of the anterior fontanelle, sagittal wormian bone and split metopic suture. Alopecia was noted in the skin overlying the wormian bone. MRI scanning showed deficient formation of the interhemispheric fissure with fusion of parietal lobes and agenesis of corpus callosum. We designed an array which includes 44K ISCA probes covering whole genome and 14,217 additional probes for intronic and exonic regions of the all known 226 skeletal dysplasias genes. We detected no significant copy number changes in our patient. To our knowledge, there is no published report showing syntelencephaly with wormian bone referring our case as the first report in this rare combination.

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P02.125

Ectrodactyly in Townes-Brocks syndrome: expanding the phenotype to limb abnormalities

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Townes-Brocks syndrome (TBS) is a variable autosomal dominant condition characterized by external ear anomalies with hearing loss, hand anomalies, and anorectal malformations. Other features are renal and urogenital anomalies, cardiac malformations, mild feet anomalies and intellectual disability has been reported in some cases. We report a patient with bilateral split feet. Other abnormalities included long and thin thumbs, anal imperforation, renal hypoplasia with cysts, and bilateral preauricular tags. A ventricular septal defect and a profound bilateral deafness were subsequently identified. Townes-Brocks syndrome was suspected on the association of renal, auricular, anal and hand abnormalities, although ectrodactyly is usually not a feature in TBS. A de novo SALL1 mutation, c.826 C>T, leading to a premature stop codon, was indeed identified, confirming therefore the TBS diagnosis. To our knowledge, ectrodactyly has only been described once in TBS (Van Bever et al, 2009). This description of the second case of ectrodactyly in a TBS patient confirms that the diagnosis of TBS should be considered when such limbs abnormalities are associated with other classical features of the syndrome.

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P02.126

Townes-Brocks syndrome: Clinical and molecular analyses in a series of 12 patients with *SALL1* mutations.

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We report on a series of 12 patients presenting with Townes-Brocks syndrome (TBS) and *SALL1* mutations.

TBS is an autosomal dominant Multiple Congenital Anomalies (MCA) condition. It is classically characterized by a triad associating dysplastic ears and/or hearing loss (90% of cases); thumb anomalies (81%) and anal defects (68%). Other features may include foot anomalies (50%), renal and genitourinary malformations (39%) and congenital heart defects (12%).

SALL1 encodes a zinc finger transcriptional repressor implicated in heterochromatin-dependent silencing process.

Here we report on 12 patients (3F/9M from 10 families) with identified *SALL1* mutations and very variable phenotypes. Most mutations (8/10) are novel. Two index patients carry the recurrent Arg276X mutation. The typical triad was found in only 50% cases (6/12). However 100% had ears anomalies (12/12), which were unilateral in 2 patients. Other malformations were thumb malformations (7/12 = 58%), anal (6/12 = 50%), renal (6/12 = 50%) and cardiac (1/12 = 8%) defects. Feet anomalies were observed in 4 cases (33%).

Furthermore one female patient underwent pregnancy termination because of renal agenesis/dysplasia with oligohydramnios in her fetus.

We confirm in this series that *SALL1* mutations cause a range of very variable phenotypes. However, in our series dysplastic or malformed ears and/or hearing loss were constant, even if very mild in some cases. We conclude that ears have to be carefully examined in patients with either anal or renal malformations, and that TBS have to be considered event if the classical triad is not fully present.

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P02.127

Large segment pure trisomy 3q resulting from a familial 3;14 balanced translocation

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Partial trisomy 3q has been reported in the literature on many occasions but less frequently without additional chromosomal abnormalities. Cases typically involve 3q21 to qter with a proposed critical region of 3q26.3 to 3q29 with the resulting phenotype including facial dysmorphisms, microcephaly, heart defects, genitourinary abnormalities, malformations of the extremities, intellectual disability and growth failure.

Here, we report on a prenatally detected case of pure, partial trisomy 3q involving 3q13.2 to qter [46,XY,der(14)t(3;14(9q13.2;p13) in a pregnancy of a woman known to be a balanced translocation carrier [46,XX,t(3;14)(q13.2;p13)]. A prenatal ultrasound identified numerous abnormalities including an increased nuchal translucency, symmetrically small growth, a complex heart defect, a bilateral cleft lip and palate, hypospadias, a small stomach bubble and a small cerebellum. The male baby was born at 38.5 weeks by a spontaneous vaginal delivery. The prenatal abnormalities were confirmed postnatally and the heart defect was confirmed to be Tetralogy of Fallot with pulmonary stenosis. The genitalia were ambiguous and the testes were not identifiable on ultrasound. Additional findings included hip dysplasia, unusual hands and feet, dysmorphic facies, anemia and thrombocytopenia. The child survived to 2.5 months of age.

To our knowledge, this is one of the few reported cases of partial trisomy 3q involving such an extensive segment of 3q and the only case reported in more recent literature. Additionally, it is one of the few reported cases of pure trisomy 3q. The phenotype reported here is consistent with previous reports, providing further evidence for a predictable partial trisomy 3q phenotype.

L.S. Penney: None. **L.M. Currie:** None.

P02.128

Unexpected exome sequencing result: *De novo* trichorhinophalangeal syndrome type I (TRPS I) mutation in an infant with congenital scoliosis, mild developmental delay, and history of consanguinity. A clinical diagnostic problem solved by this new technique

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We report a 7½ mo WF with congenital scoliosis, a family history of consanguinity and physical findings which raised suspicions of an autosomal recessive disorder. However the exome sequencing showed an unexpected autosomal dominant syndrome. The proband was considered normal until age 5m when congenital scoliosis with some developmental delay and GER was detected. Good growth, wide face, nevus flammeus in forehead, prominent philtrum, epicanthus and tendency to telecanthus, minor syndactyly toes 2-3, and RL in the right 3rd finger were found.

The exome sequencing demonstrated that the proband has a *de novo,* previously unreported, single nucleotide missense mutation in exon 5 of the *TRPS1* gene (c.2627 C>T). The result of this mutation is a phenylalanine replacing the normal serine at position 876 (p.S876F) of the TRPS1 protein. This transition, from a polar serine to a non-polar, aromatic phenylalanine, may affect the function of one of the protein's two nuclear localization signals (NLSs). This signal is located ten residues away from the mutation. Rossi et al. (2007) reported a missense mutation within the other TRPS1 NLS of a patient with mild TRPS I. The mutation prevented the TRPS1 protein (a transcription factor) from being transported into the nucleus, reducing the amount available for transcription initiation. We hypothesize that our patient has a similar situation. However, since the mutation is not within the NLS, it is hindering transport into the nucleus to an even lesser extent, producing an even milder phenotype without the classical features of TRPS.

Y. Lacassie: None. **I. Casci:** None. **W.K. Accousti:** None.

P02.129

A case of non-penetrance in an 8-year-old with a pathogenic TSC2 mutation: re-thinking the spectrum of TSC associated disease

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Tuberous sclerosis complex (TSC) is an autosomal dominant genetic condition with widely variable expressivity, and is generally considered 100% penetrant. Here, we present a three-generation family in whom a novel 2bp deletion in TSC-2 was found following the diagnosis of a proband. Six family members tested positive for the deletion. Of these, five showed either clinical or radiographic features of TSC, although only one met full diagnostic criteria aside from the proband. One family member - an 8-year-old boy - showed non-penetrance of TSC features after thorough evaluation, including MRI brain, echocardiogram, abdominal ultrasound, and full dermatologic and ophthalmologic exams. This example adds to the growing body of literature suggesting a broader spectrum of TSC associated disease than was previously appreciated, and presents the second reported case of non-penetrance of TSC in an individual with a familial pathogenic mutation after a full diagnostic workup. These findings illustrate the importance of molecular testing for all first-degree family members of individuals with known TSC mutations, even in the absence of clinical or radiographic findings. A positive result necessitates further screening for TSC related complications in these individuals, and allows for appropriate genetic counseling.

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P02.130

When one is not enough: why we need a much longer follow-up to rule out tuberous sclerosis after a prenatal diagnosis of rhabdomyoma

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In recent years, improvements in prenatal ultrasound screening techniques have led to increasingly early detection of foetal cardiac tumors. After their diagnosis appropriate multidisciplinary counselling to parents should be

made available to explain prognosis, management options and perinatal treatments. Counselling may be challenging after a diagnosis of foetal cardiac rhabdomyomatosis, for its frequent association with tuberous sclerosis complex (TSC). Thus when foetal cardiac tumors are detected, an additional diagnosis of TSC have to be searched, and TSC1 and TSC2 gene analysis could be prompted up.
From then on, in the absence of any other signs, only careful and periodic evaluations could allow to determine whether the cardiac tumor is isolated or is associated with TSC.
But even in newborns the diagnosis of TSC may be confounded: many findings traditionally regarded as among the most specific for TSC become apparent only in late childhood or adulthood, limiting their usefulness for early diagnosis. In the absence of an overt TSC diagnosis it is therefore essential to begin a regular schedule of follow up examinations, aimed at recognizing the onset of typical signs of TSC and to diagnose possible complications which might otherwise escape notice.
We propose, starting from the description of two illustrative cases, a protocol for a follow up, which includes a complete evaluations at birth and one years of age, followed by neurological yearly examination until age 5, when a comprehensive clinical and instrumental negative revaluation can allow to considered negligible the risk of developing other signs of the condition.

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P02.131
Loss of function of the E3 ubiquitin-protein ligase UBE3B causes Kaufman oculocerebrofacial syndrome

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Kaufman oculocerebrofacial syndrome (KOS) is a developmental disorder characterized by reduced growth, microcephaly, ocular anomalies (microcornea, strabismus, myopia, and pale optic disk), distinctive facial features (narrow palpebral fissures, telecanthus, sparse and laterally broad eyebrows, preauricular tags, and micrognathia), mental retardation, and generalized hypotonia. KOS is a rare, probably under-recognized condition, with less than ten cases reported to date. Here we investigate the molecular cause underlying KOS.
We used an exome sequencing approach on a single affected individual of an Italian consanguineous family coupled to mutation scanning by Sanger sequencing on a second unrelated subject with clinical features fitting the disorder.
Exome sequencing identified homozygosity for a novel truncating mutation (c.556C>T, p.Arg186stop) in *UBE3B*, which encodes for a widely expressed HECT domain E3 ubiquitin-protein ligase. Homozigosity for a different nonsense lesion affecting the gene (c.1166G>A, p.Trp389stop) was documented in the second affected subject, supporting the recessive mode of inheritance of the disorder. Mutation scanning of the entire *UBE3B* coding sequence on a opportunely selected cohort of subjects with features overlapping, in part, those recurring in KOS did not reveal disease-causing mutations, suggesting phenotypic homogeneity of *UBE3B* lesions.
Our data provide evidence that KOS is caused by UBE3B loss of function, and further demonstrate the impact of misregulation of protein ubiquitination on development and growth. The available clinical records, including those referring to four *UBE3B* mutation-positive subjects recently described as belonging to a previously unreported entity, which fit KOS, document the clinical homogeneity of this disorder.

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P02.132
Natural course of Weaver syndrome : about two cases

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Weaver syndrome is a rare, mostly sporadic, overgrowth syndrome characterized by prenatal onset overgrowth, macrocephaly, typical face, frequent mild intellectual impairment and marked advanced bone age. EZH2, the gene primarily involved in this syndrome, was recently identified [Tatton-Brown et al. (2011); Oncotarget 2:1127-1133]. Since the first delineation by Weaver et al. in 1974, approximately 50 patients have been reported. Twenty-three of them have been ascertained by genetic studies. For most of them, clinical features are not detailed and no follow-up data are available.
We report here on phenotypic evolution from birth of 2 sporadic cases of Weaver syndrome in patients originating from Reunion Island harbouring a different EZH2 mutation, and describe previously unreported features.
Case 1 was born with 3 parameters above the 97th centile, in a context of gestational diabetes. She is now 2 years old, has typical overgrowth, hypertelorism, retrognathism and advanced bone age. She has normal neurological development.
Case 2 was born with normal weight and height, macrocephaly and arthrogryposis. She subsequently developed generalized overgrowth with advanced bone age and mild learning disability, which evoked the diagnosis of Sotos syndrome. By her teens, we observe a normalization of her growth parameters and bone age. She is now 14 years old. Atypical findings are bifid uvula, sandal gap, abnormally shaped teeth and multiple fractures despite normal calcium metabolism.
Frequency of the disease on Reunion Island (2 cases out of 839 500 inhabitants), the possibly normal final height, and inconstant intellectual disability together suggest an underestimation of Weaver syndrome.

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P02.133
Whole exome sequencing unravels the molecular bases of OFD syndromes

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Oral-facial-digital syndromes (OFD) are characterized by malformations of the face, oral cavity and extremities. Various forms give rise to clinical differentiation in 13 subtypes. While OFD I presents with an X-linked dominant mode of inheritance and lethality in males, other subtypes occur sporadically or with autosomal recessive transmission. To date, a causative gene has only been found for OFD I with the OFD1 ciliary gene. In order to determine the molecular basis of OFD syndromes, we performed exome sequencing on 16 OFD cases: 2 OFD II, 8 OFD VI, and 6 unclassified types (4 with cerebellar hypoplasia, 1 with severe microcephaly, 1 with chondrodysplasia), combined with homozygosity mapping in 4 consanguineous families. Causative mutations in 4 genes were uncovered in 11 out of 16 cases.
First, we found two OFD1 mutations undetected by Sanger sequencing in two patients with cerebellar hypoplasia. We also identified mutations in 2 genes previously reported in Joubert syndrome, one of them being the major OFDVI disease causing gene (6/8 cases). A novel gene mutated in the patient with chondrodysplasia is involved in intraflagellar transport. Finally, in the patient with microcephaly, we identified a homozygous nonsense mutation in a novel ciliary gene that, similarly to OFD1, binds to the BBSome and localizes to centriolar satellites. Congruently, altering levels of this gene

in a cellular model impacts the pericentrosomal distribution of centriolar satellites.
Altogether these results confirm centriolar satellites dysfunction as a major pathomechanism of OFD syndromes, their large genetic heterogeneity and extensive overlap with other ciliopathies.

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P02.134

Wiedemann-Steiner Syndrome: Expanding the phenotypic spectrum of individuals with MLL mutations, identified through clinician phenotyping versus the DDD project

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In 2000 Steiner & Marques reported a girl with short stature, unusual faces and intellectual disability who developed hypertrichosis cubiti and hypertrichosis of the legs and back. They reported similarities to an individual previously reported by Wiedemann with pre- and post- natal growth deficiency, developmental delay and a distinctive facial appearance. Three further individuals were subsequently reported with a distinctive facial appearance, hypertrichosis and severe developmental delay under the classification of Wiedemann-Steiner syndrome.
In 2012 our group reported that de novo mutations in the histone methyltransferase MLL underlie a distinct phenotype of hypertrichosis cubiti, short stature, intellectual disability and a distinctive facial appearance consistent with a diagnosis of Wiedemann-Steiner syndrome. Other associated features were feeding difficulties, behavioural difficulties, skeletal abnormalities and cardiac defects. However due to the small number of individuals with MLL mutations, information regarding the associated phenotypic and mutational spectrum was limited.
We have screened further individuals for mutations in MLL and recruitment to our study is on going. We have also identified individuals with MLL mutations through the Deciphering Developmental Disorders (DDD) project, which utilises array CGH analysis and exome sequencing in individuals with developmental disorders.
The expansion of the clinical spectrum including detailed information on growth, behaviour and feeding together with the mutational spectrum seen in individuals with MLL mutations will be presented. The phenotype of those individuals identified through non-targeted screening, as part of the DDD project, will be compared with the phenotype of those individuals identified following clinician phenotyping.

W.D. Jones: None. **M. McEntagart:** None. **C. Deshpande:** None. **M. Holder-Espinasse:** None. **S. Smithson:** None. **F.V. Elmslie:** None. **K. Tatton Brown:** None. **D.R. FitzPatrick:** None. **D.D.D. Project:** None. **M.A. Simpson:** None.

P02.135

Smaller and larger deletions of the Williams Beuren syndrome region implicate genes involved in mild facial phenotype, epilepsy and autistic traits

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Williams Beuren syndrome is a multisystemic disorder caused by a hemizygous deletion of 1.5Mb on chromosome 7q11.23 spanning 28 genes. A few patients with larger and smaller WBS deletion have been reported. They show clinical features that vary between isolated SVAS to the full spectrum of WBS phenotype, associated with epilepsy or autism spectrum behavior.
We describe four patients with atypical 7q11.23 deletions identified by multiple-ligation probe amplification analysis and fine mapping by quantitative real-time PCR. Two carry an approximately 3.5 Mb larger deletion towards the telomere that includes *HIP1* and *YWHAG* genes. Other two carry a shorter deletion of approximately 1.2 Mb at centromeric side that excludes the distal WBS genes *BAZ1B* and *FZD9*, respectively.
Along with previously reported cases, genotype-phenotype correlation in the patients described here further suggests that haploinsufficiency of *HIP1* and *YWHAG* might cause the severe neurological and neuropsychological deficits including epilepsy and autistic traits and that the preservation of *BAZ1B* and *FZD9* genes may be related to mild facial features and moderate neuropsychological deficits. This report highlights the importance to characterize additional patients with 7q11.23 atypical deletions and to compare neuropsychological and clinical features between individuals with larger and smaller WBS deletion to shed light on the pathogenic role of genes within and flanking the Williams Beuren syndrome region.

C. Fusco: None. **L. Micale:** None. **B. Augello:** None. **M. Pellico:** None. **D. Menghini:** None. **P. Alfieri:** None. **M. Digilio:** None. **B. Mandriani:** None. **S. Vicari:** None. **G. merla:** None.

P02.136

Atypical interstitial deletion of 7q11.23 containing whole ELN and partial LIMK1: Phenotype comparison with typical Williams syndrome

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Williams syndrome (WS) is a contiguous gene syndrome commonly caused by a 1.5-Mb deletion involving ELN located at 7q11.23. Over 99% of individuals with WS can be diagnosed using a commercially available fluorescence in situ hybridisation (FISH) probe for ELN and LIMK1. This report documents the case of a 7-year-old girl with a WS-like phenotype. Commercial FISH failed to detect the deletion, but subsequent analysis revealed an atypical interstitial 1.1-Mb deletion of 7q11.23 containing the whole ELN gene and a part of the LIMK1 gene.
She was born of healthy parents at 40 weeks gestation. She had failure to thrive and severe developmental delay. Her craniofacial dysmorphology was slightly different from that seen in WS, but she had a history of peripheral pulmonary stenosis, rectal prolapse, and skeletal features similar to those seen in WS. She also had hyperacusis, tactile hyperesthesia, and extreme fearfulness, but did not have overfriendliness. Her motor, cognitive, and language delays were universal, and at the chronological age of 5 years and 11 months, her developmental age was 1 year and 8 months.
Microarray analysis demonstrated that more genes were involved in the upstream region and were conserved in the downstream region in this case than in cases of typical WS. FISH and microarray analysis should be performed even when an individual shows subtle features of WS. Such analyses will also help define the phenotype-genotype correlation in WS.

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P02.137

Williams-Beuren syndrome in the Tunisian population: A cohort of 33 patients

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Williams-Beuren syndrome (WBS) is a rare multi-system genomic disorder, caused by 7q11.23 microdeletion with a prevalence of 1/7500-1/20,000 live births.
Clinical phenotype includes typical facial dysmorphism (elfin face), mental retardation associated with a peculiar neuropsychological profile and congenital heart defects. Other signs are occasional like ocular, skeletal, renal and dental anomalies.
Here in, we present 33 WBS Tunisian patients. The mean age at diagnosis was 4,7 years. All patients showed facial dysmorphisms.

67% (21/33) have cardiovascular anomaly, supravalvular aortic stenosis (10/21) and peripheral pulmonary stenosis (11/21) are equally found.
Various degrees of mental retardation were present and a normal intelligence was found in a single patient; evaluation of developmental milestones revealed various grades of developmental delay in all the patients younger than 6 years. The unique cognitive profile was found in 15/25 of our patients.
Ocular anomalies (12/33) were less frequent than described, the skeletal anomalies too (10/33). Dental malformations are frequent (23/28).
Idiopathic hypercalcemia was present in 50% of children less than one year (3/6).
WBS is a rare disorder, cardinal signs (facial dysmorphism, mental retardation and cardiovascular defects) were found in our patient n the same proportions than described. The occasional clinical signs have proportion different of precedent reported like hypercalcemia, ocular and dental anomalies.
The identification of the different clinical signs in WBS patients permits to establish a strategy of follow up.

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P02.138

Translocation of X;autosome and pattern of X-inactivation

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The aim of this study was to correlate genotype/phenotype in Xp terminal deletion and to reveal the pattern of X inactivation in X autosome translocation. We report on a 4 years old girl and presented with developmental delay, seizures and obvious autistic behavior .She had dysmorphic facies and large hairy nevus in her forearm. Her Brain CT showed complete agenesis of the corpus callosum and EEG records showed bilateral epileptogenic focus. She has a karyotype of 45,X,t(X;14)(p22;p11), both parents had normal karyotype. FISH analysis using Xp subtelomere, Shox, XIST gene, and subtelomere 14 revealed Ish t(X;14)(Xpter-,Xp22-). The active centromere belongs to the chromosome 14. Array CGH using Agilent detected a 2Mb loss in Xp22.33 and 4,5Mb gain in Xp22.2p22.12. The deleted region contains 12 genes of which CSFRA and Shox are known as OMIM Morbid Genes. The duplicated region contains 7 OMIM morbid Genes. We used late replicating chromatin technique to detect the pattern of X inactivation. we found that 70% of the translocated X is the inactive one, in the same time the translocated chromosome 14 escape inactivation, while in 30% the normal X is the inactivate one leaving the abnormal X as the active one. The clinical picture may be affected by the haploinsufficiency of the genes that are known to escape X inactivation and lie within the deleted region (Xp22.33), or it is affected by duplicated genes (Xp22.2p22.12) on the abnormal X when it was the active one. We recommend study of gene expression of those genes.

A.M. Mohamed: None. **M.S. Zaki:** None. **A.K. Kamel:** None. **G. Gillesson:** None. **A. Caliebe:** None. **M. Mekkawy:** None. **P. Safwat:** None. **R. Siebert:** None. **I. Mazen:** None.

P02.139

Developmental delay , facial dysmorphism and hypotonia in a 9 month old Tunisian girl: a Zellweger-like syndrome?

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A 9 month old girl born by caesarean section to non consanguineous Tunisian parents is referred for developmental delay, hypotonia, failure to thrive and dysmorphic features with high forehead, broad nasal bridge hypertelorism and micrognathia. These clinical features are typically seen in the Cerebro-hepato-renal syndrome (also known as Zellweger syndrome(ZS)). However, she had no hepatosplenomegaly. She was hospitalised since birth for recurrent respiratory distress. Brain imaging showed agenesis of the corpus callosum and karyotype was normal. Metabolic investigations showed normal very long chain fatty acids and normal plasmalogen synthesis. No mitochondrial defect could be detected. Transmission electron microscopy did not show abnormal ultrastructural morphological changes of the plasma membrane, endoplasmic reticulum, mitochondria and peroxysomes. No particular deposits were noted in the cytosol and intracellular compartments. The girl died at the age of 20 months after a severe infectious respiratory distress.
Interestingly, similar clinical conditions have been reported (two unrelated Polish children (1) and two sibs born to consanguineous Jewish parents (2)). This highlights the existence of a common genetic defect, more likely to be transmitted in an autosomal recessive pattern, underlying this novel syndrome expanding the spectrum of the Zellweger phenotype.

References:

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2. Ahn JK et al. A new autosomal recessive syndrome with Zellweger-like manifestations. Am J Med Genet A. 2003 ;119A(3):352-5.

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P03.01

Gene conversion by non-allelic homologous recombination between CRYBB2 and CRYBB2P responsible for autosomal-dominant congenital cerulean cataract in a Bulgarian child

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Congenital cataracts are among the most common causes of blindness in children world-wide. We report on a 3-year old Bulgarian girl with an autosomal-dominant cerulean cataract. After obtaining informed consent from her parents, genomic DNA was isolated from blood and was scanned on an Illumina iScan system using a HumanCytoSNP-12 BeadChip as a scanning panel. A large ~200-kb deletion was detected in chromosome 22 encompassing the *LRP5L* gene. Although mutations in the gene that it shares similarity with - *LRP5,* lead to another ophthalmological disorder, familial exudative vitreoretinopathy, it is unlikely that the loss of *LRP5L* itself has resulted in the observed phenotype, as this gene encodes only one of the four extracellular beta-propeller domains of LRP5, and does not have the other functional domains, nor the extracellular signal peptide sequence. *LRP5L* is, however, located just between the *CRYBB2*, mutations in which lead to cerulean cataracts, and the pseudogene *CRYBB2P*, which shares 97% sequence homology to *CRYBB2*. Non-allelic homologous recombination introducing the accumulated mutations of the *CRYBB2P* pseudogene into the sequence of *CRYBB2* would lead exactly to the loss of *LRP5L* situated between them. RT-qPCR analysis of the parents showed that both had two copies of the *LRP5L* gene, while the girl had only one. This proves that gene conversion between the paralogous *CRYBB2* and the pseudogene *CRYBB2P* is the mechanism causing cerulean cataracts, as previously theorised.

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P03.02

Identification of Novel Homozygous Deletions in Consanguineous Pedigrees as a Shortcut to Candidate Gene Discovery in Hereditary Blindness

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Purpose: To identify the underlying genetic cause in 25 pre-screened patients of consanguineous origin, diagnosed with autosomal recessive retinitis pigmentosa (ARRP) or Leber Congenital Amaurosis (LCA) using identity-by-descent (IBD) mapping. To demonstrate the power of mapping of homozygous deletions as a shortcut to candidate gene identification in retinal dystrophies (RDs).
Methods: IBD mapping was performed by genome-wide SNP chip analysis, data analysis by PLINK. Deletions were confirmed by conventional PCR, segregation analysis was performed by qPCR.
Results: Homozygous deletions were identified in 3 out of 25 families. The first deletion (133 kb) removes the first non-coding exon of the known gene *EYS*. The second deletion (112 kb) disrupts the last 3 exons of *RERG*. The third one (416 kb) is a partial deletion of *GRID2*, which leads to an in-frame

deletion (p.Gly30_Glu81del).
Non-coding deletions of *EYS* have not yet been described. As to the *RERG* and *GRID2* genes, both are regulated by the transcription factor CRX (Corbo et al., 2010). The *RERG* deletion was found in a homozygous state in unaffected siblings however, whose clinical status needs to be revisited. *GRID2* encodes a neurotransmitter receptor. While a homozygous mutation in mouse was found to be lethal, the in-frame deletion found here might represent a hypomorphic allele.
Conclusions: This study revealed involvement of a homozygous 5'UTR deletion of *EYS* in ARRP, and uncovered potential novel candidate genes for RDs. We demonstrated that homozygous deletion detection in consanguineous families might be a powerful approach for gene discovery in hereditary blindness.

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P03.03
Correlation between phenotype and genotype in diabetic retinopathy

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Diabetic retinopathy (DR), a microvascular complication of diabetes, is one of the most frequent eye diseases in the world. Different patterns of progression are observed in patients with similar metabolic control and duration of the disease, suggesting the involvement of genetic components.
A case-control association study was performed to identify genetic biomarkers that can predict DR progression in type 2 diabetic patients.
A population of 307 type 2 diabetic patients with DR, followed-up during a 2 years prospective study, was classified in 3 different phenotypes of DR progression (phenotypes A, B, C) based on non-invasive methods.
The genes ACE, AGER, AKR1B1, ICAM1, MTHFR, NOS1, NOS3, PPARGC1A, TGFB1, TNF and VEGFA were chosen as candidates based on literature searches. The SNPs described for these genes were filtered through bioinformatics tools to identify the polymorphisms with a high probability of association with pathogenic traits. The 172 SNPs selected were genotyped in the 307 patient samples using the TaqMan OpenArray Genotyping Platform from Life Technologies. The genotype distribution was analyzed for the 3 phenotypes using the Pearson test and/or the Fisher's exact test. Phenotype A, the one with a lower DR progression, was used as the reference.
Statistically significant differences between the reference and the worst phenotypes were found for SNPs in ACE, AGER and NOS1. The identification of these biomarkers, associated with a more rapid DR progression and vision worsening, could open new perspectives for the clinical management of these patients.

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P03.05
USHER Syndrome Clinical Aspects in Spain: Genotype-phenotype correlation of USHA2 gene frequent mutations

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Usher syndrome is an autosomal recessive disorder characterized by retinitis pigmentosa and bilateral sensorineural hearing loss with (type I), without (type II) or variable (type III) impairment of vestibular function.
We aim to describe: 1-the cardinal phenotypic characteristics and differences among Usher I and II of a Spanish Usher patients cohort and 2-to perform a phenotype-genotype correlation for most frequent mutations in USHA2 gene.
Methods: 201 unrelated Usher Spanish families (271 patients) were studied using genotyping microarray (Usher, Asper-Biotech) and/or automatic sequencing of Usher genes.
Diagnosis and type classification was based on pedigree data, ophthalmologic, audiological and vestibular examination.
Phenotype (Usher I -N=56- or Usher II -N=215- clear diagnosis patients) and phenotype-genotype correlation for c.2299delG and p.Cys759Phe were assessed.
Results: Frequent USH2A gene mutations, carrier frequencies: c.2299delG=36.9% (31/84) and p.Cys759Phe=15.5% (13/84).
Ages at Diagnosis: Usher I=14.6yr; Usher II=27.4yr; c.2299delG=27.5yr; p.Cys759Phe=34.2yr (p=0.06)
Night blindness onset: Usher I=9.8yr; Usher II=17.8yr; c.2299delG=19.1yr; p.Cys759Phe=25.2yr (p=0.08)
Visual field loss onset: Usher I=11.7yr; Usher II=22.4yr; c.2299delG=21.4yr; p.Cys759Phe=27.7yr (p=0.07)
Visual acuity loss onset: Usher I=13.7yr; Usher II=25.5yr (p<0.00); c.2299delG=24.2yr; p.Cys759Phe=30.7yr
Hearing loss age and severity: c.2299delG=8.5yr; mild 15.4%, moderate 71.8% and profound 12.8%; p.Cys759Phe=29.2yr; mild 45.5% and moderated 55.5%. Onset p<10-4.
Conclusions
Visual and audiological impairment is more severe on Usher type I than on type II, p<10-4 for every item analysed.
c.2299delG associated phenotype is similar to the general phenotype found on Usher II patients. Although is only significant for audiological impairment (p<10-4), p.Cys759Phe seems to be associated with milder Usher II phenotype.

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P03.06
Disease mechanism in the Usher syndrome

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Aim: To obtain a better understanding of the unknown disease mechanisms, which leads to development of blindness and deafness in Usher syndrome (USH).
Background:.USH is clinically is divided into three types, USH1, USH2 and USH3. To date 9 genes and 3 novel loci have been identified for USH syndrome.
1) At the Kennedy Center we have a database with Usher affected persons and many of these have not been genotyped. We will try to genotype as many as possible by screening the DNA for all known USH mutations.
2) We have a Danish consanguineous family of Dutch descent with 5 affected. We performed homozygosity mapping with SNP array and identified a homozygous region, where all affected were homozygous and the non-affected heterozygous. The region was located at chr 15: 60.51 Mb- 67.84 Mb. Some of this region overlapped both *USH1H* locus, *DFNB48* and *USH2* locus and we screened all the genes in this overlapped region but did not identified any pathogenic variants. We performed then a targeted Next Generation Sequencing of the region and did not identify any mutation either. Exome sequencing has also been performed.
3) For the purpose of making a new treatment with aminoglycoside, which can suppress a premature stopcodon so the translation will continue and a partial functionally protein will be made. We will try this setup with two known USH genes both *in vitro* and *in vivo.*

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P03.07
CYP1B1 mutational screening in a portuguese cohort of primary congenital glaucoma patients

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Primary congenital glaucoma (PCG), a rare but severe form of glaucoma, is characterized by a high intraocular pressure during the neonatal or early infantile period. If not promptly treated, the optic nerves are irreversibly damaged, causing permanent vision loss.
Mutations in CYP1B1 gene are the major cause of PCG. To determine the incidence and spectrum of CYP1B1 mutations in Portuguese PCG patients, translated and untranslated regions from 29 affected individuals were analyzed by direct sequencing.
Nine disease causing mutations were identified in 69% (20/29) of the patients. CYP1B1 mutations were homozygous in 31% (9/29) and compound heterozygous in 38% (11/29). Found mutations included 5 frameshifts (c880delG, c1410del13, c1546dup10, c1691delG, c1749dup27), 3 missenses (Leu378Gln, Glu387Lys, Pro437Leu) and 1 nonsense (Arg444Term). The c880delG mutation was identified as a predominant CYP1B1 allele

among the Portuguese patients. All mutations segregated with the disease phenotype, being consistent with the autosomal recessive form, described for the disease, in complete penetrance.
Despite the high incidence of CYP1B1 mutations in our patients, 31% (9/29) were negative for mutations in this gene. These patients represent an exciting opportunity to explore alternative pathogenetic mechanisms involved in PCG and, therefore, their whole exomes are now being sequenced.
Since mutations in CYP1B1 gene are the major cause of PCG in the Portuguese population, CYP1B1 mutational screening in PCG patients and at-risk relatives, should be performed as a tool to assist the medical community in the management of this disease. Early diagnosis, along with prompt and surgical intervention, result in better prognosis.

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P03.08
Prevalence of RDS peripherin mutations in Tunisian families with autosomal retinal dystrophies

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Purpose: To evaluate the relevance of RDS gene in Tunisian families displaying an inter- and intra- familial clinical heterogeneity in retinal dystrophies.
Methods: Blood samples were obtained from four families including 12 available patients presenting with 6 retinal dystrophies, and 3 healthy relatives. Each patient underwent a detailed clinical examination. Genomic DNA was extracted from the blood samples and screened for mutations in RDS peripherin gene.
Results: Sequence analysis of the human RDS/peripherin gene led to the identification of five non disease-causing changes Vall06Val, Glu304Gln, Lys310Arg, Gly338Asp and IVS3+15 C>T. None of them could explain the significant phenotypic differences between retinitis pigmentosa, fundus flavimaculatus, bull's eye maculopathy, related Stargardt disease retinopathies and retinitis punctata albescens.
Conclusions: This study showed that RDS mutations have a low prevalence in retinal dystrophies. The frequency of peripherin mutations appears to be similar to Italian (0-1.4%), Indonesian and Japenese populations (0-1.9%). To our knowledge, this is the first report to identify frequencies of RDS mutations in retinal dystrophies patients in Tunisia.

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P03.09
Genome-wide association study in animal model of vision disorders

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Purebred dogs suffer from hereditary vision disorders, which resembles corresponding disorders in humans. Our study has focused on the genetics of retinal dysplasia (RD) and vitreous degeneration (VD) in two different dog breeds, American Cocker Spaniel (ACS) and Italian Greyhound (IT), respectively. Both disorders are known to be inherited in the breeds, but the genetic background has remained unknown. Both disorders may cause vision deterioration and secondary complications may include glaucoma, lens luxation and retinal detachment, which in turn may lead to a complete blindness. We have established a large sample cohort for multifocal RD in ACS and for VD in IT. A genome-wide association study (GWAS) was performed using canine specific SNP array to map the associated loci in both disorders.
The GWAS data identified a genome-widely significant association for MRD in ACS on canine chromosome 22 (CFA22) with p_{raw}=1.9*10-5, p_{genome}=0.01 and a tentative association, with a 2 Mb homozygous haplotype, for VD in IT on CFA15 with p_{raw}=5.1x10-5, p_{genome}=0.27. Further replication studies are being performed to confirm and to define the associated regions.
Two novel loci were mapped for two canine vision disorders. Ongoing candidate gene sequencing is likely to open new insights to the molecular background of the studied conditions. The identification of genes behind these disorders will establish a large animal model for the corresponding human disorders and the associated genes can be studied in humans. While the associated genes will become candidates for human studies, a genetic test can be offered for the studied breeds.

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P03.10
Run of homozygosity and candidate gene sequencing identify the first unequivocal stop mutation in CNGB1 responsible for retinitis pigmentosa 45

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Retinitis pigmentosa (RP) is a group of inherited disorders in which abnormalities of the photoreceptors or the retinal pigment epithelium (RPE) of the retina lead to progressive visual loss. RP are genetically heterogeneous disorders caused by more than 40 different genes for isolated RP and more than 50 different genes for syndromic RP.
Mutations in CNGB1 encoding the beta-subunit of the rod cGMP-gated channel are responsible for an autosomal recessive form of RP (RP45). However, mutations in CNGB1 have been reported only three times in the literature.
We studied a 51-year-old patient of French origin affected with an isolated RP and born to consanguineous parents (f = 1/32).
Run of homozygosity (ROH) was done using SNP-array (CytoSNP12, Illumina, with 300,000 markers including 200,000 SNP) in the proband and her 2 healthy brothers. After segregation analysis, the largest LOH that was only present in the affected patient was a 23MB region at chromosome 16q12.1q23 containing CNGB1.
Sequencing of CNGB1 evidenced a homozygous stop mutation, p.W313X.
Until now, only 3 homozygous mutations have been reported in CNGB1: two missense mutations (Bareil et al, 2001;Simpson, 2011) and one frameshift mutation in exon 32 (Kondo et al, 2004; Becirovic, 2010).
The mutation reported here is therefore the fourth pathogenic mutation in CNGB1 and the first unequivocal stop mutation in this gene confirming its implication in RP 45.

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P03.11
Isolated dominant familial ectopia lentis and FBN1 mutation: variability of expression and interest in clinical surveillance

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Most of the time, congenital ectopia lentis (EL) are part of syndromic diseases such as Marfan Syndrome, Homocystinuria, Weill-Marchesani Syndrome, etc. However, in 8% of cases, some can be isolated and are mostly familial. Both inheritance are observed: 64% of them, autosomal dominant, are linked to FBN1 while 18%, autosomal recessive, are linked to ADAMTSL4.
We report the case of a FBN1 mutation associated with a familial isolated EL.
The diagnosis of isolated EL was made in a ten years old propositus, during a routine eye examination. The dominant character of the disease was established through the production of the Pedigree. Therefore, an ophthalmologic examination was performed in the first-degree relatives and FBN1 molecular analysis was performed in the propositus.
A heterozygous missense mutation was identified. This mutation was previously described in another Chinese family by positional cloning. Therefore, this is the first time it is reported in a Caucasian family, using direct FBN1 sequencing. Then, it was researched in relatives at risk of developing an EL, especially in asymptomatic subjects who also received ophthalmologic examination.
Because this affection can go unnoticed to become evident and symptomatic at a variable age, the molecular diagnosis allowed us, in this case, to provide a better surveillance of mutated patients, together with an early detection of EL, then to prevent them from serious complications, and, on the other hand, to reassure non-mutated subjects.

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P03.12

Severe Congenital High Myopia Mapped to Chromosome 1.

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Myopia is the most common human eye disorder and constitutes a significant public health concern. Specifically, high myopia is a leading cause of visual impairment and blindness worldwide because of its associated ocular comorbidities of retinal detachment, macular degeneration, premature cataract, and glaucoma. We have previously demonstrated that monogenic high myopia can be caused from mutations in LEPREL1, encoding a collagen hydroxylase. Two remotely related consanguineous Israeli Bedouin families presented with an apparently autosomal-recessive phenotype of non-syndromic severe myopia. Using polymorphic markers, homozygosity of affected individuals at loci of candidate genes, including LEPREL1, was ruled out. Genome wide linkage analysis identified a ~4 Mb disease-associated locus on chromosome 1 between rs1970168 and rs636877. Whole exome sequencing of affected individuals is underway.

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P03.13

A genome-wide association study provides evidence for association of chromosome 8p23 (MYP10) and 10q21.1(MYP15) with high myopia

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PURPOSE. Myopia is a complex trait affected by both genetic and environmental factors. High myopia is associated with increased risk of sight-threatening eye disorders such as retinal detachment. The purpose of this genome-wide association study was to identify susceptibility genes contributing to high myopia in the French population.
METHODS. High myopic cases were genotyped using Affymetrix SNP 6.0 chips and population controls were selected from GABRIEL French dataset in which samples were genotyped by Illumina Human610 quad array. The association study was conducted using 152,234 single nucleotide polymorphisms that were present on both manufacturers' chips in 192 high myopic cases and 1064 controls to identify associated regions.
RESULTS. Statistical risk associations were found in the 8p23 (MYP10) and 10q21.1 (MYP15) regions. Rs189798 (8p23) and rs11005665 (10q21.1) showed the strongest associations in the associated regions (P=6.64x10-7 and P=2.77x10-5, respectively). The imputed results at 8p23 showed 2 peaks of interest. One region spanned 130kb including rs189798 between MIR4660 and PPP1R3B with the most significant association at rs17155227 (P=1.12x10-10). The second novel peak located in the middle of MIR124-1 and MSRA gene was only 4kb in length with the highest association at rs55864141 (P=1.36x10-7). The peak of the imputed 10q21.1 region was located in the same region as non-imputed dataset between ZWINT and MIR3924, and was 160kb in length with rs3107503 having the lowest P value (P=1.62x10-7).
CONCLUSION. we found that 8p23 (MYP10) and 10p21.1 (MYP15) are associated with high myopia in French population and significantly refined 2 associated loci.

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P03.14

Vitamin D receptor signaling and the axial myopia in children

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The main reason for myopia in children is the eyeball overgrowth in the anteroposterior direction. The aim of this study was to investigate an association of gene polymorphisms *COL1A1* G-1997T, G+1245T, *VDR* A-3731G, T+61968C, and *APOA1* G-75A, C+83T with the development and severity of axial myopia in children. We examined 53 girls and 24 boys aged 4 to 17 yo: 19 (38 eyes) - with high myopia, 44 (88 eyes) – with medium myopia, and 14 (28 eyes) - with emmetropia. Data of medical history, family history of myopia, ocular tonometry, and A-scan ultrasound biometry were collected for multivariate statistics. RESULTS: The medium myopia was associated with the carrying of *VDR*-3731A-allele and +61968TT-genotype. Also, it was associated with the carrying of *APOA1*-75A-allele, and the female sex. The high myopia was associated with the carrying of *VDR*-3731A-allele, the carrying of *APOA1*-75A-allele, and the carrying of *COL1A1*-1997T-allele. Collagen typeI alpha1 is the main component of the eye sclera matrix, and is known as an eye growth GO-signal. Apolipoprotein A1 is the transporter of cholesterol, a precursor of the $25(OH)_2$vitaminD_3, and is supposed to be a STOP-signal of eye growth. The function of the vitamin D receptor in eye tissues is not clear. But it is known, that receptor activated with $25(OH)_2$vitaminD_3 inhibits indirectly expression of collagen typeI. So, we hypothesize that vitamin D receptor might be a STOP-signal of the eye growth, and *VDR* polymorphism together with vitamin D or ultraviolet deficiency might be a cause of axial myopia in children.

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P03.15

Molecular Analysis of TYR, P, TYRP1 , SLC45A2 and GPR143 Genes in the Italian Reference Centre for Patients with Oculocutaneous and Ocular Albinism.

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Since April 2006 a cohort of 216 OCA or OA subjects were recruited from the Medical Genetic Unit and the Department of Pediatric Ophtalmology of the Niguarda Ca' Granda Hospital of Milan (Italy).
TYR, P-protein, TYRP1, SLC45A2 and GPR143 genes, associated to the different phenotypes OCA1, OCA2, OCA3, OCA4 and OA1, have been characterized by sequencing analysis.
Furthermore in order to detect the presence of TYR and P genomic rearrangements (deletion/duplication), MLPA (Multiplex Ligation-dependent Probe Amplification) assay was performed.
After clinical examination and instrumental evaluation by our Ophthalmologic Centre, all patients received a genetic counselling and signed an informed consent.
Up to date the complete analysis of the four genes associated with OCA phenotype was performed on 117 patients. We identified TYR mutations/deletions in 84 patients, P mutations/deletions in 19 patients, TYRP1 pathological alterations in 2 children, SLC45A2 mutations in 5 patients.
Only 7 OCA patients (6%) remained molecularly undiagnosed
We also analyzed for GPR143 gene 8 male patients with a probable OA1 phenotype, possibly X-linked segregating, we found pathological DNA variation in all of them.
The mutations identified in this study are missense and nonsense, small deletions/duplications, splicing defects and gross deletions.
TYR gene sequencing has already been performed on the remaining 91 OCA patients and no mutations have been identified. The analysis of the other genes is currently in progress. Our preliminary results indicate that the OCA1 frequency reaches at least the 40% in our country.

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P03.16

Peripapillary Chorioretinal Lacunae in an Autosomal Microdeletion Syndrome

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Chorioretinal lacunae (PCRL) consist of well-circumscribed, full-thickness defects of retinal pigment epithelium and choroid, with intact overlying retina. Although pathognomonic for Aicardi syndrome (AS), PCRL are seen in other conditions including microcephaly with chorioretinal degeneration, oral-facial-digital, oculo-auricular syndromes.
AS is characterized by agenesis of corpus callosum, seizures and PCRL. Inheritance is presumed X-linked dominant but causative gene is unknown. We present girl without the classic AS triad who had PCRL due to chromosome 3 microdeletion.
18 month old girl was evaluated for speech, motor delays, staring spells. She was born at 32 weeks gestation to pregnancy complicated by preeclampsia. At birth, was noted to have left hand preaxial polydactyly, atrial-septal defect, pulmonary valvular stenosis. Examination disclosed flat nasal bridge,

epicanthal folds, wide mouth, retromicrognathia, truncal hypotonia. Retinal examination showed bilateral PCRL, which consisted of depigmented areas with variably pigmentated borders, surrounding optic discs. MRI revealed mild thinning of corpus callosum and diffuse hypomyelination.
Genomic hybridization microarray demonstrated 6-megabase interstitial deletion of chromosome 3, spanning 3q21.3 to 3q22.1.
Conclusions: PCRL are not unique to Aicardi syndrome.
AS is thought to arise from gene mutation on X-chromosome. Our patient's large autosomal deletion comprised 66 genes, none known to be associated with chorioretinal lacunae. It is possible that PCRL in our and patients with AS may result from complex gene interactions between the X chromosome and autosomes.
Because our patient had autosomal microdeletion, we recommend chromosomal microarray be performed in patients with PCRL when neurological features do not correspond to those in AS.

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P03.17
Early-onset neurological presentation of *OPA1*- associated autosomal dominant optic atrophy with variable expressivity

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OPA1 encodes a protein involved in regulation of mitochondrial inner membrane fusion and cristae remodeling. Mutations in the *OPA1* gene are the most common identifiable cause of autosomal dominant optic atrophy (DOA), which is characterized by childhood-adulthood onset of selective retinal ganglion cell loss, temporal pallor of the optic nerve and a typical color vision deficit with reduced acuity. Dominant optic atrophy plus applies to a subgroup of patients manifesting extraocular neurologic signs.
We describe a girl presenting with infantile onset optic atrophy, developmental delay, ataxia, tremor and hypotonia.
She was born at term to unrelated healthy parents. Her mother noticed abnormal eye movements since early infancy. Strabismus, hypotonia and motor delay were diagnosed at the age of 1y. At 18m optic atrophy, reduced visual acuity, eye lid retraction and developmental delay were diagnosed. At 2y ataxia and tremor were noted . Brain MRI at 4 years showed atrophy of the optic nerve and chiasm.
A known disease causing heterozygous missense mutation A1146G (I382M) was found in the *OPA1* gene by exome sequencing. Her healthy mother and aunt carry the same mutation.
This is a description of an unusually early infantile onset, *OPA1* associated optic atrophy plus, in a girl whose asymptomatic mother carries the same mutation. There is one report regarding early onset severe clinical manifestation of OPA1+ associated with compound heterozygosity for OPA1 mutations. We postulate that a further explanation could be involvement of another gene and epigenetic factors in the mechanism of the disease.

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P03.18
Age related macular degeneration and association of CFH Y402H, rs1410966, LOC387715 A69S, rs11200638 and complement component 3 (R102G) polymorphisms in Turkish Population

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Age related macular degeneration (AMD) is a complex multifactorial disease. In this study, it is aimed to establish a relation between single nucleotide polymorphisms of genes (in Complement Factor H (CFH) rs1061170 and rs1410966, in HTRA1 rs11200638 and rs10490924, in C3 gene rs2230199) which are among the suspected genes in AMD disease. 100 healthy control individuals and 199 patients who have long AMD history were involved in this study. Statistically meaningful difference has been observed (p=0.002) between patient and control groups when 183 AMD patients were evaluated for Y402H polymorphism. The difference between groups has been found statistically meaningful for LOC387715 (rs10490924). In our study on RS11200638 , 56.4% GG, 34.3 %AG, 9.3% AA genotypes and GG 26.4 %, AG 47.2 %, AA 26.4% genotypes were determined for patient and control groups, respectively. Evaluations of results have revealed that there is meaningful difference in distruption of genotype for rs11200638 polymorphism between patient and control groups (p=0.0001). But no meaningful difference has been observed for distruption of genotypes between patient and control groups when rs2230199 polymorphism is considered (p=0.981). A meaningful difference has been obtained for distruption of genotypes between patient and control groups for rs1410996 polymorphism (p=0.013). Rs2230199 has given statistically meaningless results in our study on the contrary to the findings obtained for Caucasians. The results that we obtained during this study will be useful for scientific studies which are concentrated on geriatric diseases because of ageing world population in the sense of transferring the data of Turkish population.

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P03.19
Investigating the complex genetic architecture of Age-Related Hearing Impairment by a Genome-Wide Association Study in the European population

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Age-Related Hearing Impairment (ARHI) or presbyacusis is the most common sensory impairment in elderly and the leading cause of adult-onset hearing loss. It is a severe social and health problem. ARHI is a complex disease with both genetic and environmental factors contributing to the degeneration of the cochlear cells, but remains poorly studied from the genetic side. To further elucidate the genetic factors for ARHI, we performed a Genome-Wide Association Study (GWAS) using the Illumina CNV370 quad chip and the Illumina HumanOmniExpress BeadChip on 1560 samples, representing the 20% extremes of a population of 2161 Antwerp subjects aged 55-65 years for 3 audiometric phenotypes, as scored by principal component (PC) analysis. We tested association with ARHI by linear regression between each genotype and each PC-score. In addition, we adjusted for environmental factors (PLINK software) and for population stratification (EMMAX software). Previously reported susceptibility genes for ARHI could not be replicated. To more deeply investigate the genetic architecture of ARHI, we executed pathway-analysis (MAGENTA software), examined the role of rare variants per gene (SKAT software) and analysed gene-gene interaction (SIXPAC software). GCTA (Genome-wide Complex Trait Analysis software) estimated that 16 % of the phenotypic variance could be explained by the SNPs analysed in our GWAS. This analysis proves for the first time that ARHI is a highly complex disease, involving many genetic factors with small effect sizes, while there is no evidence for major genetic risk factors with high effect sizes.

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P03.20
Heterozygous mutation IVS1+1G>A (GJB2 gene) is associated with age-related hearing impairment (ARHI) in the Yakut population isolate (Eastern Siberia)

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Age-Related Hearing Impairment (ARHI 1 and ARHI 2: OMIM 612448, 612976) is one of the frequent sensory disorders registered in 50% individuals to the age of 80 years. ARHI is multifactorial disorder due to environmental and poor-known genetic components. In this study, we present the data on age-related hearing impairment of 48 carriers of mutation IVS1+1G>A (GJB2 gene) and 166 subjects with GJB2-genotype wt/wt in the Yakuts, Siberian population isolate (Eastern Siberia). The GJB2 gene resequencing and detailed audiometric analysis in the frequency range 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 kHz were performed in all examined subjects. Comprehensive molecular and audiological analysis of all individuals allows examination for the presence of genotype-phenotype correlations of mutation IVS1+1G>A and hearing of subjects from examined groups. We revealed the linear dependence between increase of average hearing thresholds at speech frequencies (0.5,1.0,2.0,4.0 kHz) and age of individuals with genotype IVS1+1G>A/wt (r=0.571, p=0.001 for males and r=0.523, p=0.000007 for females). Moreover, the average hearing thresholds on high frequencies (4.0 and 8.0 kHz) in individuals with genotype IVS1+1G>A/wt (both sexes) were significantly worse than in individuals with genotype wt/wt (p<0.05). Age of hearing loss manifestation in individuals with genotype IVS1+1G>A/wt was estimated to be ~ 40 years (r=0.572, p=0.0001). These findings demonstrate that the IVS1+1G>A mutation (GJB2) is associated with age-related hearing impairment (ARHI) of the IVS1+1G>A carriers in the Yakuts.

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P03.21

Mutations in PXDN cause complex microphthalmia, anterior segment dysgenesis, hypotonia and developmental delays

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Anophthalmia and microphthalmia (A/M) are common birth defects that are significant because of visual loss. Mutations in SOX2 and other genes can cause A/M, but there is high genetic heterogeneity and more than 50% of affected individuals do not receive a molecular genetic diagnosis. We used exome sequencing to study a Caucasian family with two children who had complex A/M with anterior segment dysgenesis, sclerocornea, microphthalmia, developmental delays and hypotonia. The older male sibling had glaucoma. Analysis with ANNOVAR revealed both sibs shared 262 sequence variants. 11 genes had two novel variants and 4 of these genes were expressed in the eye. Sanger sequencing showed two Peroxidasin (PXDN) mutations in both sibs - a maternally inherited nonsense mutation, c.C1021T predicting p.R341* and a paternally inherited, frameshift mutation, c.2375_2397del, predicting premature protein truncation. Mutations in the PXDN gene were previously described in three families with congenital cataracts, microcornea, sclerocornea and developmental glaucoma that had no extraocular anomalies (Khan et al., 2011). The gene is expressed in corneal epithelium and is secreted to the extracellular matrix, thus the eye defects may result from impaired adhesion in the anterior segment or an inability to metabolize reactive oxygen intermediates. Our finding of a broader phenotype for PXDN than previously appreciated is characteristic for exome sequencing, which has proven to be highly effective for mutation detection in “atypical” presentations. We conclude that PXDN mutations should be considered in complex microphthalmia with anterior segment dysgenesis. Khan et al., Am J Hum Genet 2011;89:464.

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P03.22

Diagnosis of Bardet-Bield Syndrome by a custom multiplexing mutation panel

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Introduction: Bardet-Biedl Syndrome (BBS) is a pleiotropic ciliopathy characterized by inducing alterations in diverse body systems leading to a wide range of clinical features. Its prevalence is estimated at 1/150,000. Its main features are obesity, progressive pigmentary retinopathy, post-axial polydactyly, polycystic kidneys, hypogenitalism, and learning disabilities. Pigmentary retinopathy is always present in adults. Progressive vision loss, some degree of intellectual deficit and obesity can severely impair the life quality of the affected persons. BBS is a genetically heterogeneous disease transmitted in most families as autosomal recessive disease. To date, fourteen genes have been associated with BBS, representing up to 80% of the cases.

Method: We developed a custom multiplex mutation panel (CGC Mutation Panel - Pat. Pending) that contains a total of 129 point mutations identified on the 13 principle genes involved in Bardet-Biedl syndrome: BBS1, BBS2, BBS3/ARL6, BBS4, BBS4, BBS6/MKKS, BBS7, BBS8/TTC8, BBS9/B1, BBS10, BBS11/TRIM32, BBS12 and BBS13/MKS1.

Results: From the 30 samples tested we detected the following mutations on 4 cases:

c.1169T>G (p.Met390Arg) mutation in heterozygosity in the BBS1 gene

c.1015A>G (p.Ile339Val) in heterozygosity was detected in the MKKS gene

c.1169T>G (p.Met390Arg) in homozygosity was detected in the BBS1 gene

c.951+1G>A mutation in homozygosity was detected in the BBS1 gene

Conclusion: This approach is a valuable diagnostics tool, since it detects the most common mutations associated with Bardet-Biedl syndrome. This panel reduces time and costs to achieve a diagnosis and improves its capability, independently of the sample type, allowing an earlier decision-making process in patient management.

J. Sá: F. Consultant/Advisory Board; Modest; CGC Genetics. **P. Rendeiro:** A. Employment (full or part-time); Modest; CGC Genetics. **A. Lopes:** A. Employment (full or part-time); Modest; CGC Genetics. **L. Lameiras:** A. Employment (full or part-time); Modest; CGC Genetics. **L. Dias:** A. Employment (full or part-time); Modest; CGC Genetics. **A. Palmeiro:** F. Consultant/Advisory Board; Modest; CGC Genetics. **P. Tavares:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; CGC Genetics.

P03.23

Nation-wide genetic screening for patients with bilateral nonsyndromic sensorineural hearing loss (SNHL) in Slovakia

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Introduction: Etiology of hearing impairment is very heterogeneous. Approximately 50% of all deaf born cases are attributed to genetic causes. Exact data about genetic etiology of bilateral sensorineural hearing loss in our country are not fully available. Therefore, **the aim of our study** was to carry out the genetic screening for selected genes in patients with bilateral nonsyndromic SNHL throughout Slovakia.

Patients and Methods: Since 2010 we have recruited more than 560 subjects from all boarding schools for children with hearing disabilities in Slovakia and University Hospital in Bratislava. For genetic testing, 418 unrelated individuals with clinically manifested bilateral SNHL were selected and sequenced for *GJB2* gene. In 206 patients, we have also carried out analyses of *GJB6, GJB3* and *POU3F4* genes using the MLPA methodology.

Results: We identified 14 already known mutations, six polymorphisms and one variant in the *GJB2* gene. Homozygous and compound heterozygous mutations were present in 21% and 11% of the subjects, respectively. Proportion of the subjects without having any pathogenic allele, reached 61%. In one individual, a simultaneous occurrence of the 309 kb large deletion in *GJB6* with the *GJB2* mutation was identified. No mutations were found in other genes investigated.

Conclusions: 1. We have established the mutation profile of *GJB2/GJB6* genes in subjects identified across whole Slovakia, and 2. we have confirmed the genetic etiology of hearing impairment in 32% of individuals studied. 3. Our findings serve as key information for genetic counseling and clinical prognosis of SNHL.

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P03.24

Cytokine Genes Polymorphisms and Expression Pediatric Cochlear Implant Patients: No Association with TNF-α (-308), and IL-6 (174), but the IFN-γ (+874) in Associated with the Disease in Turkish Patients

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The aim of this study was to explore the association between polymorphisms and expressions of three cytokine genes and clinical parameters in patients in children who underwent cochlear implantation because of congenital profound sensorineural hearing loss.
We analyzed three cytokine genes (IL-6, IFN-γ and TNF-α) in 64 cases with congenital sensorineural hearing loss and in 70 healthy controls. Cytokine genotyping was performed by the PCR-SSP method. All data were analyzed using de Finetti program and SPSS version 14.0 for Windows.
No significant differences were detected between the patient group and the healthy controls with respect to the distributions and numbers of genotypes and alleles in TNF-α and IL-6. However, the TT genotype associated with high expression in IFN-γ and T allel frequency were found to be significantly more frequent in the patients group as compared to the controls (p=0.016, 0.023, respectively).
The relationship between cytokine genotypes/expressions and clinical parameters in patients with congenital hearing loss has not been investigated before. Our results suggest that high expression of IFN-γ gene may be associated with susceptibility to disease. Consequently, IFN-γ may be a useful marker for ethiopathogenesis in patients with congenital sensorineural hearing loss.

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P03.25

Clinical and genetic characteristics of patients with POU3F4 X-linked deafness

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Hearing impairment is the most common sensory disorder in humans, affecting approximately one to three in 1000 newborns, with 50 % due to genetic causes. The majority of these cases (70 %) are non-syndromic, about 2 % of these are X-linked. So far five different X-linked loci have been mapped, the causative gene POU3F4 (MIM 300039) has been identified for the gene locus DFNX2. This hearing loss is often progressive, with temporal bone abnormalities and stapes fixation. Temporal bone anomalies include dilation of the fundus of the internal acoustic canal (IAC), that can increase the risk of CSF gusher during stapes or cochlear implant surgery. POU3F4 belongs to a subfamily of transcription factors, which are characterized by two conserved DNA binding domains, a POU and a HOX domain. Several molecular genetic studies have identified mutations in the POU3F4 gene, including partial or complete deletions of the gene, as well as deletions, inversions, and duplications of the DFNX2 genomic region not encompassing the POU3F4 coding sequence. Here we present the clinical characteristics of four patients from independent families. Mutations analysis in the POU3F4 gene was brought by direct sequencing. Sequence analyses revealed a novel deletion of 2600 bp [TAG (Stop) +21nt - 2620nt] and two novel missense mutations c.902C>T, (p.P301L) and c.973 T>A, (p.W325R) and in a fourth patient an already known missense mutation c.907C>T, (p.P303S). Until now three novel strongly conserved mutations in the HOX-domain of the POU3F4 transcription factor were identified leading to an effect in the protein function.

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P03.26

A novel mutation (p.Asp171Asn) in wolframin gene, in a patient presenting Low-Frequency Hearing Loss

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Nonsyndromic sensorineural hearing loss predominantly affecting the high frequencies is a genetically heterogeneous common disorder. On the contrary, low-frequency sensorineural hearing loss (LFSNHL) is an unusual type in which frequencies at 2000 Hz and below are predominantly affected. Most of the families with LFSNHL carry mutations in WFS1 gene that maps to chromosome 4p16 and codes for wolframin, a membrane glycoprotein localized in the endoplasmic reticulum.
A Portuguese NSHL patient was shown to display LFSNHL after evaluation by pure tone audiometry. Blood sample was collected after signing written informed consent. Sequencing of exons 5 and 8 of WFS1 gene was performed after previous testing for mutations in the GJB2 coding exon and for the common GJB6 deletions using sequencing and multiplex-PCR, respectively. One hundred hearing Portuguese controls were sequenced for exon 5.
No mutations were found in GJB2 and GJB6 genes. A novel mutation, c.511G>A, was found in heterozygosity in the exon 5 of the WFS1 gene. At protein level, it causes an alteration of residue 511 of wolframin, occurring in the extracellular N-terminus domain of the protein, by changing an aspartic acid to an asparagine (p.Asp171Asn). This mutation was not present in 100 Portuguese hearing controls.
Since LFSNHL presents a dominant pattern, the auditory phenotype observed in this heterozygous patient might probably be due to the novel mutation p.Asp171Asn. Functional characterization will be performed in order to assess its effect on the mutated protein and to clarify in which way this change leads to LFSNHL.

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P03.27

Loss-of-function mutations in SOX10 cause Kallmann syndrome with deafness

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The SOX10 transcription factor plays a role in the maintenance of progenitor cell multipotency, lineage specification, cell differentiation, and is a major actor in the development of the neural crest. It has been implicated in Waardenburg syndrome (WS), a rare disorder characterized by the association of pigmentation abnormalities and deafness, but SOX10 mutations cause a variable phenotype that spreads over the initial limits of the syndrome definition. Based on recent findings of olfactory bulb agenesis in WS patients, we suspected SOX10 was also involved in Kallmann syndrome (KS). KS is defined by the association of anosmia and hypogonadotropic hypogonadism due to incomplete migration of neuroendocrine GnRH (gonadotropin-releasing hormone)-cells along the olfactory, vomeronasal, and terminal nerves. Mutations in any of the nine causal genes identified to date account for only 30% of the KS cases. KS can be either isolated or associated with a variety of other symptoms including deafness. We report SOX10 loss-of-function mutations in approximately one-third of KS patients with deafness, which defines this gene as the first one with a substantial involvement in this clinical condition. Study of SOX10-null mutant mice revealed a new developmental role of SOX10 in a subpopulation of glial cells called olfactory ensheathing cells. These mice indeed showed an almost complete absence of these cells along the olfactory nerve pathway, as well as defasciculation and misrouting of the nerve fibers, impaired migration of GnRH-cells, and disorganization of

the olfactory nerve layer of the olfactory bulbs.

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P03.28

Exome sequencing and linkage analysis identifies novel N714H mutation in WFS1 as a cause of autosomal dominant hearing loss

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Hearing loss (HI) is a significant medical problem in Poland and worldwide, occurring in 1 of every 500 newborns. The cause of hearing loss can be genetic or environmental. Currently the background of genetic hearing impairment is an area of intensive research conducted by many groups. Because of more than 40 genes involved in pathogenesis of nonsyndromic hearing loss, it is also an extremely heterogeneous trait. The most common variants responsible for an isolated recessive HI are mutations in the GJB2 gene, but there is no single gene accounts for the majority of cases of autosomal dominant (AD) HI. To date, more than 60 loci of ADHI were mapped, but particular genes were identified for only 25. However mutations in 4 of these genes (WFS1, KCNQ4, COCH, and GJB) are more frequent as a causes of ADHI in comparison to the other.
For searching the reason of HI among family members with AD pattern of inheritance, we performed linkage analysis using Affy 10K chip and exome sequencing on Illumina platform. Data analysis revealed novel N714H mutation in WFS1 gene as a plausible reason for HI. Direct sequencing of fragment of 8th exone of WFS1 gene showed perfect co-segregation between N714H mutation and HI among members of this family. N714H mutation is localized in exon 8, which contains the conserved C-terminal domain. Considering fact that the majority of deafness causing mutations have been identified particularly in exon 8, this domain seems to have a crucial function in the cochlea.

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P03.29

Mutations in GJB2 gene - a main cause of non-syndromal hearing loss among Belarus patients

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About 50% of congenital non-syndromal hearing loss (SNHL) are caused by mutations in GJB2 gene. The spectrum and frequencies of the GJB2 mutations depend on ethnic origin; in Europe 35delG recessive mutation is the most frequent. According to our previous data, the carrier rate of 35delG in Belarus is twice higher than European average (5.68%). Screening of 391 patients revealed that 35delG is the main cause of SNHL in Belarus: 45.5% were homozygous, 13.1% heterozygous and for 41.4% the major GJB2 mutation was not found.
Among 35delG heterozygotes with SNHL six other mutations in the GJB2 coding exon 2 were found (combining single-strand conformational polymorphism analysis and sequencing methods). However, for 40 patients - 35delG heterozygotes - still only one mutant GJB2 allele was detected while for 139 patients we did not find any exon 2 GJB2 mutations. Therefore, the aim of the present study was to determine still undetected genetic lesions causing SNHL in these patients. We screened them for the mutation IVS1+1G>A in splicing site (5' UTR of GJB2 gene). Mutation IVS1+1G>A was detected in 5 out of 179 patients, that indicates its high frequency. As a result, for five 35delG heterozygote patients the second mutation was detected; thus, the genetic nature of SNHL for them was proved. Moreover, we estimated that for deaf patient cohort IVS1+1G>A is a third most common GJB2 mutations in Belarus, after 35delG and 312del14. The results obtained are necessary for further genetic testing of Belarus patients with non-syndromal hearing loss.

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P03.30

Simultaneous Screening of 100 Mutations Associated with Non-Syndromic Hearing Loss in Brazil

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Recent advances in molecular genetics have allowed the determination of the genetic cause of non-syndromic hearing loss, but a large percentage of patients still remain with unidentified cause. It points an imminent need for new methodological strategies for the detection of an increased number of mutations in multiple genes. In this work we screened a set of 100 mutations previously described in 20 different genes, using mass spectrometry system MassArray, Sequenom® followed by Sanger sequencing, to evaluate the contribution of selected changes in the etiology of deafness in Brazil. We analyzed a group of 45 individuals with non-syndromic hearing loss that were previously screened for mutations in main deafness genes, and that showed recessive mutations in *GJB2* and *GJB6* in only one allele. We found additional mutations in two genes: *SLC26A4* and *CDH23*. In the *SLC26A4* were identified two mutations in heterozygous, *p.C282Y* in one individual and *p.V609G* in two other individuals. In *CDH23* two missense mutations were found in different individuals, the *p.R301Q* mutation was found in homozygous, explaining the etiology of deafness, and the *p.R1746Q* was found in heterozygous. These results show that *SLC26A4* and *CDH23* mutations are frequent in individuals with non-syndromic hearing loss in Brazil and emphasize the necessity of considering these two genes for diagnosis. The presence of *CDH23* and *SLC26A4* mutations associated with *GJB2* and *GJB6* mutations might suggest gene interaction, producing deafness in a digenic mode of inheritance, but the point need to be proved in further study.

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P03.31

Audiologic characteristics of carriers of a dominant WFS1 mutation

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The WFS1 with chromosomal 4p16.1 position, encodes an endoplasmic reticulum (ER) membrane-embedded protein, called wolframin, which has physiological functions in membrane trafficking, secretion, processing and/or regulation of ER calcium homeostasis. A proper calcium balance plays crucial role in many different cellular functions, including cell-to-cell communication, the tensing (contraction) of muscles, and protein processing. Disturbances in the balance of calcium in the inner ear might interfere with the hearing process.
Mutations in WFS1 gene cause both autosomal dominant low-frequency sensorineural hearing loss (LFSNHL) at the DFNA6/14/38 locus and Wolfram syndrome, characterized by autosomal recessive hearing loss, diabetes mellitus, diabetes insipidus and optic atrophy. The hearing loss caused by dominant WFS1 mutations has been reported to be very characteristic, preferentially affecting the low frequencies without significant influence on the high frequencies.
Using whole exome sequencing we recently found novel WFS1 mutation (p.N714H) segregating in a large family with autosomal dominant HI. The purpose of this study was to evaluate the audiograms in mutation carriers in order to validate the association between WFS1 mutations and low frequency HI. So far we obtained three audiograms. Whereas one of the audiograms was typical with low frequency HI (0.5KHz 45 dB, 8KHz 5 dB) two other were not (0.5KHz 90 dB, 8KHz 70 dB; 0.5KHz 55 dB, 8KHz 120 dB). These data indicate that a flat down-sloping audiograms do not exclude WFS1 defects.

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P03.32

Association of Mitochondrial Uncoupling Protein Gene 2 with Congenital Sensorineural Hearing Loss in Pediatric Patients

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Recently findings suggest that uncoupling proteins (UCPs) may play a protective role against reactive oxygen species in neuron and a thermal signaling role for neuron modulating in vestibular nevre. Uncoupling protein 2 (UCP2) is expressed in inner ear periphery, but the function of UCP2 in inner ear tissues has not been determined yet. The aim of this study was to examine whether UCP2 gene variations are associated with congenital sensorineural hearing loss.

In this study, 64 congenital sensorineural hearing loss children and 80 age and sex matched healthy controls were detected for promoter -866G>A and exon 8 insertion/deletion (I/D) variations of UCP2 gene by using PCR and/or RFLP methods.

Promoter and exon 8 variations of UCP2 gene were showed statistically significant association with congenital sensorineural hearing loss patients. The distribution of II, ID and DD genotypes for the gene was 54.7 %, 26.7 % and 18.6 % in patients compared with 8 %, 30.7 % and 61.3 % in the controls. Insertion homozygote genotype and I allele were found higher in patients ($p<0.0001$). The distribution of UCP2 gene promoter variation GG, GA and AA genotypes was 10.9 %, 45.3 %, 43.8% in patients compared with 21.1 %, 62.5 %, 16.3 % in the controls. Statistically, AA genotype and A allele were found to be increased in patients ($p=0.0003$, $p=0.0010$, respectively).

Our data show that UCP2 gene may play a role for ethiopathogenesis of congenital sensorineural hearing loss. Further studies are needed to elucidate function of UCP2 in sensorineural hearing loss.

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P03.33

Genetic aetiology of Hearing Loss in Cyprus

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Mutations in the *GJB2* (Connexin 26) gene are responsible for more than half of all cases of pre-lingual recessive inherited non-syndromic deafness in Europe. This study presents a mutation analysis of the *GJB2* and *GJB6* genes in 146 Cypriot patients with non-syndromic sensorineural hearing loss (SNHL) compatible with recessive inheritance. Patients were screened for the c.-23+1G>A splice mutation and the coding exon 2 of the *GJB2* gene including also the deletions del*(GJB6-D13S1830)* and del*(GJB6-D13S1854)*. Thirty patients were verified with bi-allelic *GJB2* mutations and with c.35delG as the most dominating one, accounting for 81.7%, followed by p.Arg184Pro (6.7%), p.Leu90Pro (5.0%), p.Glu47stop (1.7%), delGlu120 (1.7%), 167delThr (1.7%) and p.Val178Ala (1.7%).

Ten patients with severe SNHL were also identified with one mutation in the *GJB2* gene only. Interestingly, five of these patients were identified in the heterozygous state with the *GJB2* missense mutation/variant p.Val153Ile, while the rest were identified as heterozygous for p.Val37Ile, c.35delG, p.Leu90Pro, c.-23+1G>A splice mutation and the novel c.-1G>A 5'UTR.

Finally, no *GJB6* mutations or the known del*(GJB6-D13S1830)* and del*(GJB6-D13S1854)* were identified in any of the investigated Cypriot SNHL patients.

This work confirms that the *GJB2* c.35delG mutation is an important pathogenic mutation for hearing loss in the Cypriot population and that the underlying molecular basis of autosomal recessive non-syndromic deafness in Cyprus is genetically relatively homogeneous. This finding will be used towards the effective diagnosis of SNHL, assisting in genetic counseling and used as a potential therapeutic platform.

V. Anastasiadou: None. **G. Tanteles:** None. **C. Costi:** None. **C. Shammas:** None. **E. Spanou:** None. **V. Neocleous:** None. **L.A. Phylactou:** None.

P03.34

Deep-intronic mutations in OPA1 cause optic atrophy and "optic atrophy plus" phenotypes

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Inherited Optic Neuropathies (ION) account for about 7% of legal blindness in the European working-age population. Despite the progress in DNA sequencing, still around 55% of all ION cases remain unsolved. This might partly be due to the fact that important ION mutations are commonly missed by exonic sequencing techniques. Here we demonstrate for the first time that deep-intronic mutations in the most common nuclear ION gene OPA1 cause optic atrophy and "optic atrophy plus" phenotypes in so far unresolved ION patients.

Using cDNA analysis of puromycin treated patient-derived fibroblasts of an index family with a severe multisystemic "optic atrophy plus" phenotype, we identified a deep-intronic OPA1 mutation in intron 4b. The mutation creates a novel splice acceptor site, leading to inclusion of a cryptic exon. This aberrant splice product induces frameshift and subsequent nonsense-mediated mRNA decay (NMD) of the mutated transcript. RFLP screening of intron 4b in 369 patients with unexplained ION (negative for exonic OPA1 mutations) revealed 3 further families with deep-intronic mutations around the same site. In the affected siblings of the index family, the intronic mutation occurred in conjunction with a known compound heterozygous mutation (I382M), which explains the observed severe phenotype. The I382M variant acts as a modifier that might be used as a hint for compound heterozygous mutations in non-coding regions of "optic atrophy plus" patients. We conclude that deep-intronic OPA1 mutations might explain a substantial share of so far unexplained cases with optic atrophy or "optic atrophy plus" phenotypes.

T. Bonifert: None. **M. Synofzik:** None. **L. Schöls:** None. **B. Wissinger:** None.

P03.35

Sensorineural hearing loss in OPA1-linked disorders

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Mutations in OPA1, encoding a mitochondrial dynamin-related GTPase, are reponsible for dominant optic atrophy (DOA). About 20% of OPA1 mutation carriers have complicated forms of DOA associating extra-ocular features such as deafness or neuromuscular disorders. Reviewing the files of 327 patients with an OPA1 mutation, we found 21 patients with hearing loss (6.4%). In 10 patients deafness was detected under age 20 (~48%), in 3 patients over age 20 (~14%) and in 8 patients the age of onset was unknown (38%). The severity of deafness ranged from mild and moderate in 14 cases (66%) to profound in 5 cases (24%). Audiological tests supported the diagnosis of auditory neuropathy in 8 cases.

Seven different OPA1 mutations were identified in deaf patients. Three mutations, p.Arg445His, p.Gly401Asp and p.Leu243*, have been previously reported in patients affected with both DOA and deafness. Two mutations, p.Val291_Phe328del and p.Ile463_Phe464dup, have been reported in patients with isolated DOA but not in association with deafness. Finally, two novel OPA1 mutations, p.Arg437Glu and p.Ala357Leufs*4, are implicated for the first time in DOA and deafness. In the majority of patients with DOA and deafness, visual impairment occurred during the first decade while deafness appeared in late childhood or early adulthood. However, in 54% of patients deafness started prior to visual abnormalities indicating that a careful examination of the optic nerves is needed in young patients with post-lingual neurosensorial hearing loss.

These observations suggest that audition should be carefully tested, including a specific search for auditory neuropahy in OPA1 mutation carriers.

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P03.36

Antisense oligonucleotide-mediated exon skipping to treat 10% of Leber congenital amaurosis cases.

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Purpose: Leber congenital amaurosis (LCA) is a congenital or neonatal

blindness. The most common disease-causing mutation (>10%) is located deep in intron 26 of the *CEP290* gene where it creates a strong splice donor site that leads to the insertion of a cryptic exon encoding a premature stop codon. Our aim was to assess antisense oligonucleotide (AON)-mediated exon skipping to correct the aberrant splicing.

Material and Methods: Patient fibroblasts harbouring the c.2991+1655 A>G mutation and controls were transfected using antisense and sense 2'O-methyl phosphorothioate oligonucleotides designed to target ESE around the mutation. The efficiency of skipping was assessed using qRT-PCR, Western blot and primary cilia counting.

Results and discussion: *CEP290* Expression levels were unchanged when control cells were transfected using the sense or antisens ONs ($p>0.05$). Likewise, no change was noted when patient cells were treated with the sense ONs ($p>0.05$). Conversely, a highly significant increase in expression of the wildtype *CEP290* allele was obtained when cells were treated with AONs ($0.029<p<0.002$) with expression levels reaching that of controls. Western blot analysis evidenced increased levels of CEP290 in patients' cell lines treated with the AONs but not the sense ONs. Finally, primary cilia expression was significantly reduced in patient's fibroblasts compared to controls lines (48.6%±6.5%*vs*83.6%±3.2%; $p=0.0097$**).** Upon transfection with the antisense ONs but not the sense versions, the proportion of ciliated cells increased significantly in patients, reaching levels similar to controls (75.3%±3.5%*vs*78.3%±3.4%; $p=0.624$). These results show therapeutic potential of exon skipping for the treatment of the most common LCA-causing mutation.

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P03.37

Regulatory Mutations in the 5'UTR of *NMNAT1*, encoding the Nuclear Isoform of Nicotinamide Nucleotide Adenylyltransferase 1, cause Leber Congenital Amaurosis

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Leber Congenital Amaurosis (LCA) is the earliest inherited retinal dystrophy (RD). Recently, coding mutations in *NMNAT1* resolved the last remaining locus, LCA9 (Falk *et al*. 2012). Here, we aimed to identify the genetic defect in an LCA9-linked consanguineous Sub-Saharan African family (F1) and determine the contribution of *NMNAT1* mutations in LCA.

Massively parallel sequencing of all exons and promotor regions in 4 identity-by-descent regions of F1 identified a novel homozygous 5'UTR variant in *NMNAT1* (c.-70A>T), which segregated with disease. Subsequent *NMNAT1* Sanger sequencing in 76 probands with LCA/early-onset RD revealed coding mutations in 5 additional probands, and identified a second homozygous 5'UTR variant (c.-69C>T) in a Moroccan consanguineous LCA family (F2). In both F1 and F2, cDNA sequencing detected no other potential pathogenic variants but revealed loss of heterozygosity in heterozygous carriers, suggesting *NMNAT1* mRNA degradation. Subsequently, significantly lower mRNA expression in leukocytes was shown for the homozygous mutants in comparison with healthy controls. Luciferase assays for both adjacent 5'UTR variants in COS-7, KGN and COV434 cell lines revealed significantly lower activity for c.-69C>T in comparison with the wild-type 5'UTR. Of note, both families show LCA with evolutive macular involvement, typical for *NMNAT1*-related disease.

In conclusion, this study sustained the role of coding *NMNAT1* mutations in LCA. Moreover, the identification of two neighboring 5'UTR variants in *NMNAT1* makes this the first study to link 5'UTR regulatory mutations to congenital blindness, and may impact upon the role of 5'UTR mutations in hereditary blindness in general.

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P03.38

Broadening the phenotype of LRP2 mutations: association of a new LRP2 mutation with predominantly ocular phenotype suggestive of Stickler syndrome.

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We studied two siblings from a consanguineous Iraqi family presenting with a syndrome with high myopia, esotropia, vitreous changes, cataract, , and distinct facial features (epicantic folds, a high nasal bridge and a degree of maxillary hypoplasia).

No mutation was identified in COL9A1/2. To identify the causative mutation, we performed exome sequencing of one of the probands, which revealed a new non-synonymous variant in LRP2 that segregated in the family. The variant (c.11483A>G; p.Asp3828Gly), is predicted damaging according to SIFT and Polyphen prediction programs and is conserved among species. Mutations in LRP2 have been shown to cause the Donnai-Barrow syndrome or Facio-oculo-acoustico-renal (FOAR) syndrome, a syndrome presented with facial abnormalities, ocular anomalies, sensorineural hearing loss, proteinuria, and diaphragmatic hernia and absent corpus callosum, although there is variability in the expression of some features (eg absent corpus callosum and proteinuria). This family shows a milder phenotype with some features and similarities to the Donnai-Barrow syndrome, including demonstrated microglobulinuria. The type of mutation (non-synonymous) might explain the milder phenotype. In conclusion, we have identified a new mutation in LRP2 that broadens the phenotypical spectrum associated with LRP2 mutations.

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P03.39

Mainzer-Saldino syndrome is a ciliopathy caused by mutations in the *IFT140* gene.

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Introduction: Ciliopathies is an emerging class of genetic disorders due to altered cilia assembly, maintenance or function. Syndromic ciliopathies affecting bone development have been classified as skeletal ciliopathies. Mutations in genes encoding components of the intraflagellar transport (IFT) complex A, that drives retrograde ciliary transport, are a major cause of skeletal ciliopathies. Mainzer-Saldino syndrome (MSS) is a rare disorder characterized by phalangeal cone-shaped epiphyses, chronic renal failure and early-onset severe retinal dystrophy.

Methods and results: We collected 16 families presenting three diagnostic criteria of MSS. Through ciliome re-sequencing combined to Sanger sequencing, we identified *IFT140* mutations in seven MSS families. The effect of the mutations on the IFT140 localization was assessed using flagged-IFT140 mutant proteins which showed a partial to nearly complete loss of basal body localization associated with an increase of cytoplasm staining while the wild-type Flagged-IFT140 protein predominantly localized to the basal bodies in RPE1 cells. To assess the impact of *IFT140* mutations on ciliogenesis, abundance and morphology of primary cilia were studied in cultured fibroblasts of patients and detected absent cilia in a high proportion of patient cells compared to controls. Ciliary localization of anterograde IFTs were altered in MSS patient fibroblasts supporting the pivotal role of IFT140 in proper development and function of ciliated cells.

Conclusion: Here we report on compound heterozygosity or homozygosity for *IFT140* mutations in seven MSS families. After Sensenbrenner and Jeune syndromes, MSS is the ultimate skeletal ciliopathy ascribed to IFT disorganization.

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P03.40

A missense mutation in the ALDH1A3 gene cause autosomal recessive microphthalmia in a large consaguisnuos family.

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Here we report on large Muslim inbred kindred from Northern Israel with isolated microphthamia/anophthalmia in nine affected individuals. Assuming autosomal recessive mode of inheritance we
performed whole genome linkage analysis, on four DNA samples of affected individuals. Homozygosity mapping techniques were employed and a 1.5MB region, homozygous in all affected individuals, was delineated, further linkage analysis resulted a two point LOD score of 7.9. The region contained 9 genes, one of which; ALDH1A3, was a clear candidate gene. This gene seems to be the key enzyme in the formation of a retinoic acid gradient along the dorso-ventral axis during the early eye development and also in the development of the olfactory system. Sanger sequence analysis revealed a missense mutation, causing a substitution of Valine to Methionine at position 71. Analyzing the Val71Met mutation using Mutation Taster online predicts this variant to be disease casing (probability score:0.92). The effect of the missense mutation on the enzymatic activity was studied in vitro but results showed no changes between mutated and wiled type ALDH1A3. Recently, Lucas et al (2013), have reported on three families with anophthalmia/microphthalmia caused by mutations in ALDH1A3 gene. In summary, we report on nine affected individuals from consanguineous kindred, bearing a missense mutation in
ALDH1A3, a key enzyme in the formation of a retinoic acid gradient during the early eye development.

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P03.41

Genetic analysis of insensitivity to pain in hunting dogs: a model for human sensory neuropathies

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Among different human Hereditary Sensory Autonomic Neuropathies (HSAN) and some forms of Charcot-Marie Tooth diseases (CMT), several are characterized by insensitivity to pain sometimes combined with self-mutilation. Strikingly, several purebred dogs also present similar clinical characteristics reported in the literature for fifty years. In the dog, clinical signs appear in 4 months old puppies and consist of acral analgesia, with or without sudden intense licking, biting and severe self-mutilation of the feet, whereas proprioception, motor abilities and spinal reflexes remain intact. Despite its rare occurrence in the whole dog species, several hunting dog breeds are clearly predisposed with a higher risk, i.e. estimated to 1 % in the French spaniels. Since only a few genes responsible for human homologous neuropathies have been identified, we took advantage of the dog model to search for the genetic causes of this disease in hunting dogs. To this aim, we collected blood samples from hunting breeds of which 30 are affected. Pedigree analyses allowed us to confirm a recessive inherited disease. A Genome Wide Association Study (GWAS) using the Illumina Canine HD SNP array with 24 affected and 40 healthy dogs, allowed us to identify a single 1.5Mb locus not yet described for human HSAN and CMT, with a Bonferroni corrected p-value of 2.5x10-6. We are thoroughly investigating this locus through a capture sequencing strategy since, interestingly, it contains candidate genes not yet associated to human neuropathies and thus constitutes an opportunity to identify new neuropathy genes in humans.

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P03.42

Genes and mutations for olfactory phenotypes

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Genetic variations in the Olfactory Receptor (OR) genes are likely responsible for odorant-specific sensitivity differences (anosmias),while variations in auxiliary genes, mediating neuronal transduction, development and maintenance, are candidates underlying general olfactory sensitivity (GOS) phenotypes, including congenital general anosmia (CGA). We recently generated a comprehensive account of genomic missenese variations in human ORs. We find that each individual harbors, 500-600 allelic variants with different coding sequence, a considerably higher number than locus count (413). With the reported allele-specific sensory neuronal expression, the brain receives distinct neuronal readouts of all such alleles. Further, we identified 239 segregating pseudogenes (SPGs), ORs with both intact and pseudo forms in the population. A "barcode" of missense and nonsense variants thus underlies a highly personalized smell receptor repertoire. One borderline signal, requiring validation, shows lower sensitivity to a specific odorant associated with an OR pseudogene allele. In parallel, we performed a systematic exploration for auxiliary olfactory genes and their variations. This was done using 11 data sources, including RNA-Seq of olfactory epithelium, and literature survey of olfactory-related diseases and mouse phenotypes.
One auxiliary gene variation appears to affect sensitivity to a specific odorant, suggesting a novel odorant processing mechanism. These efforts now also assist in an on-going whole exome sequencing study of 66 Jewish families with CGA, showing various modes of inheritance. The identification of specific pathogenic functional CGA variants will help elucidate the molecular basis of general olfactory sensitivity.

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P03.43

Association of TGFB1 with otosclerosis in a population of clinically and histologically confirmed Hungarian patients

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Otosclerosis is a frequent cause of hearing impairment in the Caucasian population characterized by abnormal resorption and deposition of the bone of the otic capsule. It is a complex disease caused by both environmental and genetic factors. Case-control association studies have implicated several genes. In order to confirm these susceptibility genes for otosclerosis, we studied a unique Hungarian otosclerosis population in which the presence of otosclerotic foci was confirmed by histological investigation. This inclusion criterium has never been used in previous association studies. We examined thirteen single nucleotide polymorphisms (SNPs) in six genes (*COL1A1*, *TGFB1*, *BMP2*, *BMP4*, *AGT* and *RELN)* in 153 otosclerosis patients and 300 unrelated controls. An association between *TGFB1* (rs1800472) and clinically and histologically confirmed otosclerosis was detected, with a similar effect size across histologically confirmed and non-confirmed otosclerosis populations. The fact that other genes did not replicate could be due to different reasons such as lack of power (*BMP2* and *BMP4*) and false positive initial association (*COL1A1* and *AGT)*. However, for *RELN,* with very reasonable power and for which association was robustly replicated in previous studies with several independent populations, this seems very unlikely. The results for *RELN* may reflect the possibility that otosclerosis is clinically heterogeneous with several endophenotypes, each with a different molecular basis.

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P03.44

Study of allelic variants related to Quantitaive Sensory Testing parameters in patients with CRPS

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Background: Complex regional pain syndrome (CRPS) is a chronic pain condition, result of dysfunction in the central or peripheral nervous systems.

Due to the level of difficulty in the evaluation and diagnosis of neuropathic pain, a new technique, called QST (Quantitative Sensory Testing) is being used to measure changes in somatosensory function.
The aim of our study was to investigate the genetic variation of 16 genes involved in nervous system pathways, in patients from Salamanca (Spain), diagnosed CRPS, comparing these results with different parameters of the QST.
Methods: Genomic DNA was extracted from peripheral blood by standard techniques. We selected 16 non-synonymous SNPs. Studies were performed using TaqMan probes for the analysis of the polymorphisms in the following genes:. OPRM1, OPRD1, OPRK, CNR1, DRD2, GABRA, EDN1, TRPV1, NOS3 and BDNF.
Pain evaluation using VAS and QST. Statistical analysis performed with SPSS.
Results and conclusion: Preliminary analysis showed differences (p<0,05) when we compare patients and controls in OPRD1, CNR1, GABRA6 and BDNF genes. Analysis of VAS values showed significant differences in GABRA1 and EDN1 genes.
We also compared SNPs genotypic distribution with different parameters of the QST, and we found significant differences in OPRK, OPRM1 and CNR1 genes.
That support the hypothesis that polymorphism in genes involved in different pathways could be associated with increased susceptibility to develop CRPS and suffer pain. SNPs in these genes are also associated with QST parameters, reinforcing that QST could be an useful tool for the diagnose of neuropathic pain.

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P03.45
A dual approach for comprehensive genetic testing of *ABCA4* in Stargardt disease

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Purpose: Genetic testing of Stargardt disease, a frequent retinal dystrophy, is mostly limited to Asper chip analysis or Sanger sequencing of the coding region. To overcome its cost and limitations, we designed a comprehensive molecular test based on massively parallel sequencing (MPS) and quantitative PCR (qPCR) to identify single nucleotide and copy number variations (CNV) respectively.
Methods:
Following enrichment of the *ABCA4* coding region, pools of 12 patients were sequenced (Miseq, Illumina).
CNV screening comprised 50 qPCR assays, performed in 48 patients with one heterozygous or no *ABCA4* mutation following chip and/or sequencing, and 27 controls (LC480, Roche).
Results: Using MPS, a 20x minimal coverage was reached for 98% of the amplicons. A variant allele frequency threshold of 25% allowed detection of 99% of previously identified variants. In addition, a previously undetected indel mutation was identified in a patient with one known heterozygous mutation.
CNV analysis revealed a novel heterozygous deletion in a patient carrier of a known heterozygous splice site mutation. The deletion covers 3-4 kb, spanning exons 20-22. Breakpoint delineation is ongoing.
Conclusions: MPS for *ABCA4* proves to be a cost-efficient alternative to Sanger sequencing and chip analysis. In addition, qPCR screening identified a novel *ABCA4* deletion, being the second one reported. CNV analysis might be useful in this fraction of patients (30%) in whom only one or no coding mutation can be detected by standard approaches. Finally, the low prevalence of CNVs in unsolved Stargardt cases might point to other mechanisms such as variations in non-coding regions.

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P03.46
DNA Diagnostics of Retinal Stargardt Dystrophy Type 1 in Russia

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Stargardt disease is the most common hereditary early-onset macular degeneration. Autosomal-recessive Stargardt desease type 1 (STGD1) is caused by mutations in the ATP-binding cassette transporter gene (ABCA4). ABCA4 gene mapped to 1p22, encodes 2273 amino-acid, includes 50 exons. There are about 500 mutations in the ABCA4 gene have been reported.
The two groups of unrelated control individuals and the two groups of unrelated STGD1 patients from Russia were analysed for the prevalent European ABCA4 gene mutations: G863A, G1961E, A1038V, L541P, P1380L, T1526M, P2027F, R2107H by MLPA.
In the first control group (882 individuals) the allele frequency of G863A, A1038V and G1961E were 0.17%, 0.51% and 0.62%, respectively. In the second control group (201 individuals) was found L541P only, the allele frequency - 0.25.
In the first patients group (124 individuals) the allele frequency of G863A were 1.2% and G1961E - 7.7%; in the second patients group (110 individuals) allele frequency of A1038V, L541P, P1380L were 17.7%, 18.2% and 1.8%, respectively (p<0.05). Other mutations were not found.
Heterozygous carrier frequency of G863A is high in Europe (1:18 in Northern Europe), but it is significantly lower in Russia (1:294).
L541P is detected in linking with A1038V in the compound heterozygous in 31 patients, and without A1038V in two patients only. One patient had a A1038V alone.
For the first stage STGD1 diagnosis seems comfortable to use MLPA-system to detect the five mutations, that we have found, as a full ABCA4 gene sequencing is expensive.

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P03.47
Whole genome analysis of single nucleotide polymorphisms and copy number variants in fibromyalgia suggest a role for the central nervous system

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Fibromyalgia (FM) is a highly disabling syndrome defined by a low pain threshold and a permanent state of pain. The mechanisms explaining this complex disorder remain unclear and its genetic factors have not been identified. The aim of this study was to elucidate genetic susceptibility factors for FM.
We used the Illumina 1 million duo array to perform a genomewide association study (GWAS), and Agilent's 2X400K platform for array comparative genomic hybridization (aCGH) to identify regions varying in copy number that could be involved in FM susceptibility.
GWAS was performed in 300 FM cases and 203 controls. No SNP reached GWAS significance, but 21 of the most associated SNPs were chosen for replication in over 900 cases and 900 pain free-controls. Four of the strongest associated SNPs selected for replication showed a nominal association in the joint analysis, and one, rs11127292 (MYT1L) was found to be associated to FM with low comorbidities (p=4.28X10-5, OR (95%CI)=0.58 (0.44-0.75). By aCGH, an intronic deletion in NRXN3 showed to be associated to female cases of FM with low levels of comorbidities (p=0.021, OR (95%CI)= 1.46 (1.05-2.04)).
Both GWAS and aCGH results point at a role for the central nervous system in FM genetic susceptibility. If the proposed FM candidate genes are further validated in replication studies, this would highlight a neurocognitive involvement in this disorder, currently considered musculoskeletal and affective.
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P03.48

Analysis of aniridia patients by CGH-array

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Aniridia is a rare genetic disorder due to mutation of the *PAX6* gene.
Among these mutations there are deletions, which are often not detectable through direct sequencing. Identification of deletions of the *PAX6* genomic can be performed by MLPA. However, the MLPA system does not allow a precision identification of the deletion borders. In order to set up a system for a precise identification of deletions and their borders in the *PAX6* genomic region, we used the CGH-array approach. A chip was designed in which oligonucleotides spanning a region of 20 Mb, 10 Mb in position 5' and 10 Mb in position 3', respectively to *PAX6* gene, with a probe average frequency about 500 bp. This chip was used to analyse aniridia patients with deletion of a *PAX6* genomic region previously identified by MLPA. Five individuals show deletions in a position 3' to the last exon of the *PAX6* gene. The common region of these deletions has a size of 154 Kb, and includes elements of transcriptional control of the *PAX6* gene. Our data confirm that aniridia can be due to deletion of transcriptional control elements of *PAX6* gene and demonstrate that the CGH-array approach can be successfully used to identify deletions in the *PAX6* genomic region.

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P04.01

Microdeletion of 6q25.1 and congenital heart defect: a case report

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We describe the case of a girl that was born to nonconsanguineous parents at 39 weeks of gestation by Cesarean section performed for perinatal suffering and intrauterine growth arrest. Birth weight was 2.240 kg. At birth was diagnosed subaortic Perimembranous Ventricular Septal Defect, so therapy with Furosemide e ACE-Inh was immediately started. She had also left renal pielectasy.
Phenotypic examination was remarkable for: flat nasal bridge, epicanthic folds, folded right helix, short neck, small hands and feet, eyelid ptosis, joint laxity, cutis laxa, generalized hypotonia, auxological parameters <3rd centile. Karyotype was normal.
At the age of 18 months she was hospitalized for failure to thrive, recurrent vomiting, food refusal and episodes of arrhythmia. Holter-ECG showed "paroxysmal supraventricular tachycardia" and then beta-blocker therapy was started.
At the age of 32 months brain MRI showed „Expansion of the cisterna magna with a wide communication with the fourth ventricle, hypoplasia of cerebellar hemispheres and vermis (Dandy Walker variant)."
Array-CGH showed a de novo microdeletion in the 6q25.1 region, extended approximately 630 kb.
In the critical region 6q25.1 was identified TAB2 gene (TAK1-binding protein 2), responsible for numerous non-syndromic congenital heart anomalies such as ASD, VSD, aortic stenosis and congenital hypoplasia of the aortic arch. In a family abnormalities in heart rhythm (paroxysmal supraventricular tachycardia) was also described in literature.
Extensive studies carried out in patients with congenital cardiac defects demonstrated the involvement of this gene in the development of cardiac structures.

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P04.02

New mechanisms underlying the variable phenotypes caused by N- and C-terminal SCN5A mutations

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Numerous SCN5A mutations affect the cardiac sodium channel Nav1.5 α-subunit and cause several types of arrhythmias. By expressing N- and C-terminal SCN5A mutations (R104W and R1860GfsX12) with wild-type (WT) channels in HEK cells to mimic the heterozygous state of the patients, we showed that interactions between α-subunits could modulate the channel function, location and potentially the disease phenotypes.
When expressed alone, the N- and C-terminal mutants were mostly degraded and showed either reduced or no INa density. When co-expressed with WT channels, the N-terminal mutant exerted a dominant-negative effect on WT channels by retaining them in the endoplasmic reticulum. In contrast, co-transfection of WT channels with C-terminal mutants almost restored the INa density to the level of WT and increased total and cell surface protein expression of mutant channels, suggesting that WT allow mutant proteins to escape proteasomal degradation. Co-immunoprecipitation studies showed that the WT α-subunits interacted with each other, as well as with the N- and C-terminal mutants. In addition, altered biophysical properties of INa current were found at the heterozygous state for both mutants.
In conclusion, interaction between Nav1.5 α-subunits could explain the variable phenotypes observed in SCN5A-mutation carriers. The N-terminal variant associated with a loss-of-function due to its dominant-negative effect on WT channels lead to a Brugada type-I ECG pattern. In contrast, the partial rescue of the INa current of the C-terminal variant and its altered biophysical properties could explain the complex clinical picture of sick sinus syndrome and atrial fibrillation without Brugada ECG pattern.

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P04.03

Regulation of Expression of CYP19A1, MIF, ABCA1 by the Retinoic Acid Receptor-Related Orphan Receptor α (RORα)

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The RORα is a member of the nuclear receptor superfamily of transcription factors that plays an important role in the regulation of many physiological processes. Melatonin and cholesterol are two known ligands of RORα and both were shown to be important for cardiovascular diseases like atherosclerosis. Therefore, it was suggested that RORα might play a role in the pathogenesis of atherosclerosis. In this study, we identified RORα response elements (ROREs) within the promoter regions of the CYP19A1, MIF and ABCA1 genes. ChIP analysis demonstrated RORα occupancy of the CYP19A1, MIF and ABCA1 promoters in THP-1 and HUVEC cells. Briefly, THP-1 and HUVEC cells were treated with RORα specific ligands (CPG52608 and SR1001) and after the activation of RORα, the expression of the target genes were analyzed. In the result of the analyses, we found that expression of some of target genes were differentiated in THP-1 and HUVEC cells. Furthermore, we investigated whether RORα activity in THP-1 macrophages, which play an important role in the development of atherosclerosis and express target genes of RORα, is affected by the presence of simvastatin. We observed that simvastatin repressed the expression of the studied target genes, and that this repression was partially prevented by RORα ligands (especially by SR1001). These data suggest that CYP19A1, MIF and ABCA1 are direct target genes of RORα. With the demonstration of the genes that involve in the pathogenesis of atherosclerosis might be controlled by an inducible transcription factor, this study offers a potential therapeutic treatment for the disease.

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P04.04

Genetic risk factors for atrial fibrillation in the Japanese population

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The etiology of atrial fibrillation (AF) associates on complex interactions of multiple environmental and genetic factors. We recently reported *in silico* replication study for AF susceptible chromosomal loci identified Caucasian descent by a genome wide association study (GWAS) results in a Japanese population[1)]. Here, to further validate the results of the Japanese GWAS and to identify novel AF susceptible loci, we genotyped the high-ranking single nucleotide polymorphisms in the GWAS with additional samples of approximately 1,600 cases and 17,100 controls in the Japanese. Through combination of the GWAS results, the known AF locus on chromosome 4q25, close to

PITX2, showed extremely strong association ($P < 10^{-150}$). Other three known loci, *CAV1* locus on 7q31, *ZFHX3* locus on 16q22 and *PRRX1* locus on 1q24, were also validated with statistical significance ($P < 1.0 \times 10^{-8}$). Furthermore, we identified two novel association signal on chromosome 10 and 12 ($P < 5 \times 10^{-8}$). Interestingly, the four genes (*PITX2*, *PRRX1*, *ZFHX3* and the gene on chromosome 12) encode transcription related factors with homeobox domain found in proteins related to cardiopulmonary development and/or differentiation. Stratification by age at diagnosis revealed that the greater odds ratios observed in the younger age than the older one on the three loci, *PITX2*, *CAV1*, *2* and chromosome 12. By combination of the six genetic factors, the odds ratio has risen to 4.5. These results indicate that the genetic factors significantly contribute to the etiology of AF in the Japanese.

[1)]Ellinor PT et al., ***Nature Genetics*** 44, 670-675 (2012).

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P04.05

A homozygous mutation in Smoothened, a member of the Sonic Hedgehog (SHH)-GLI pathway is involved in human syndromic atrioventricular septal defect

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Introduction

Atrioventricular septal defect (AVSD) is a common congenital heart disease with a high impact on morbidity. It is often accompanied by other congenital anomalies and previous studies have shown that in these syndromic AVSDs defects in the SHH-GLI signaling pathway are major etiological factors. One crucial member of the SHH-GLI signaling pathway is the receptor protein smoothened. Here we present the first reported homozygous smoothened mutation in two syndromic AVSD patients.

Methods

Two probands, a twin boy and girl, presented with an AVSD, postaxial polydactyly and skin syndactyly of the second and third toes of both feet. The boy also had hypospadias. The parents were consanguineous and they had one healthy older child. Karyotyping was normal and Smith-Lemli-Opitz syndrome was excluded. Exome sequencing was performed and candidate variants were validated.

Results

A novel homozygous missense mutation p.R575W (c.1725C>T) in exon 10 of the gene *SMO* (7q32.3) was detected. Functional studies in fibroblasts of the patients showed normal expression of the SMO protein but an abnormal localization of SMO protein, outside the cilia. Moreover we show severely reduced downstream GLI1 mRNA expression after stimulation with the SHH agonist purmorphamine, These results, together with the previously described association of SHH signalling defects with AVSD and SLOS, suggest that this SMO mutation is involved in syndromic AVSD in humans.

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P04.06

Bicuspid aortic valve morphology in fetal Turner syndrome

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Background

Bicuspid aortic valve (BAV) is a common congenital heart defect. Animal studies demonstrated that BAVs with different leaflet orientations have distinct etiologies. BAV is particularly common in Turner syndrome (TS). As TS patients share a common genetic abnormality (monosomy X), one would expect a common morphogenetic background for BAV and thus the same BAV leaflet orientation. Indeed, 95% of BAVs in TS adults were shown to have anterior-posterior (AP) leaflet orientation. Data on BAV morphology and associated cardiovascular abnormalities in fetal TS are scarce.

Methods and results

We studied post-mortem heart specimens of 36 TS fetuses and one TS newborn. Abnormal aortic valve morphology was observed in 32 (86%): BAV in 28 (76%), unicommissural valve in 2 (5%) and atresia in 2 (5%). In BAV, the leaflets showed AP-orientation in 61% and left-right (LR) orientation in 39%. There were no significant differences in occurrence of additional cardiovascular abnormalities between hearts with AP-BAV and LR-BAV. However, *all* hearts with LR-BAV showed ascending aorta hypoplasia and tubular hypoplasia of the B-segment, as opposed to only 55% and 64% of hearts with AP-BAV, respectively.

Conclusion

The large majority of TS fetuses shows abnormal aortic valve morphology. BAVs with different leaflet orientations are present, implying that haplo-insufficiency of sex-chromosomal genes can lead to different BAV morphology types, and that additional factors have a role in determination of BAV morphology. As the proportion of LR-BAV is higher in TS fetuses than reported in adults, LR-BAV may have a worse prognosis than AP-BAV in TS.

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P04.07

Phenotypic variability in generalized arterial calcification of infancy.

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INTRODUCTION: The generalized arterial calcification of infancy (GACI) is a rare disease, with autosomal recessive inheritance. Eventhough 85% die during the first six months due to heart failure, there are wide phenotypical variability with patients with long term survival. An early treatment with diphosphonates has been used with good results. We present here two unrelated patients with different outcomes.

CASE 1: 5 weeks old newborn, premature of 33 weeks, transferred to our hospital due to heart failure and cardiorrespiratory arrest. The suspected diagnoses were viral myocharditis versus metabolopathy. The boy died four days later. The diagnosis of GACI was done by the anatomophatological study.

CASE 2: Patient born at 32 weeks of gestation with hidrops faetalis. 30 weeks of gestation had detected a pericardial effusion and hyperechogenic arterial in ultrasound. Echocardiography revealed calcifications in aorta and coronary arteries. Computed tomography and ultrasound showed extensive calcifications until the iliac arteries with low flow in renal arteries. The diagnosis of GACI was made and therapy with etidronate was started when the child was 22 days old. All calcifications disappeared when the child was 15 months and the etidronate was stopped in the second year old. Actually, patient is 6 and asymptomatic.

DISCUSSION: A high index of suspicion of these disease is needed in order to use an early treatment with biphosphonates. Also, the genetic counseling is very important for those families. There are some questions not solved like the duration of the treatment and the factors conditioning the evolution.

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P04.08

Whole-exome sequencing in familial calcific aortic valve stenosis

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Calcific aortic valve stenosis (CAVS) is a complex disease under the influence of risk factors (e.g. dyslipidemia) as well as genetic polymorphisms (e.g. in APOA). The identification of rare genetic variants with a strong effect would give major insights into the mechanisms leading the bone-like phenotype. Mutations in the NOTCH1 gene have been linked to aortic valve calcification but they only explain a handful of cases and also lead to congenital anomalies. Our aim is to uncover the first genes involved in 'degenerative' CAVS, using families that appear to be monogenic autosomal dominant forms of CAVS, including a very large pedigree of >50 cases.

First, we sequenced the exome of 20 patients affected by severe CAVS, from 9 families with ≥3 cases (mean depth: ~82X per patient). The hundreds of rare variants were prioritised according to their functional consequence and their segregation with the CAVS phenotype in the families. As a vali-

dation study, we sequenced a target of 500 kb (Agilent SureSelect capture, Illumina HiSeq sequencing) in 475 CAVS cases. The 500-kb target contained exonic regions of candidate genes from our exome sequencing project (69 genes) or the literature (31 genes), and regulatory regions. After applying standard frequency and quality filters, we performed burden tests to identify CAVS disease genes.
The identification of rare genetic variants within this project could lead to the possibility of alternative patient management for a disease that represents a major public health problem in the ageing population.

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P04.09

Homozygous mutation in the Myosin-Binding Protein C gene leading to an early onset of HCM with mild hypertrophy and severe arrhythmias.

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Introduction ~ Heterozygous mutations in the myosin-binding protein (MYBPC3) genes are the most frequent genetic causes of hypertrophic cardiomyopathy (HCM). Patients with MYBPC3 mutations generally have late onset disease and a relative good prognosis. Homozygous or compound heterozygous MYBPC3 mutations have been associated with early onset disease with severe hypertrophy and heart failure.
We will describe here the clinical features of a family with a homozygous mutation (p.Cys566Arg) in MYBPC3.
Case ~ The index patient (an 8 year old girl) presented with a cardiac arrest due to ventricular fibrillation at the age of 8 years. After resuscitation she underwent cardiac evaluation. The electrocardiogram (ECG) showed significant left ventricular hypertrophy (LVH) but echocardiogram and Magnetic Resonance Imaging (MRI) revealed only mild LVH. She fully recovered and underwent ICD implantation. During one year follow-up she had two appropriate ICD shocks for ventricular arrhythmias. The asymptomatic brother of the index patient (age 11 years) showed a similar typical ECG with only mild LVH on echo and MRI. Genetic testing of both the index patient and her brother showed a homozygous mutation (p.Cys566Arg) in the MYBPC3-gene. Both asymptomatic parents were heterozygous carriers of this mutation and their cardiac assessments revealed no abnormalities.
Conclusion ~ Homozygous mutations in the Myosin-Binding Protein C gene could lead to an early onset of HCM with mild hypertrophy and severe ventricular arrhythmias.

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P04.10

Steroid 21-hydroxylase genetic analysis of 50 Tunisian patients

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Congenital adrenal hyperplasia (CAH) is an autosomal recessive disease of steroid biosynthesis in humans. More than 90% of all CAH cases are caused by mutations of the 21-hydroxylase gene (CYP21A2), and approximately 75% of the defective CYP21A2 genes are generated through an intergenic recombination with the neighboring CYP21A1P pseudogene.
In this study, the CYP21A2 gene was genotyped in 50 patients in Tunisia with the clinical diagnosis of 21-hydroxylase deficiency. CYP21A2 mutations were identified in 87% of the alleles. The most common point mutation in our population was the pseudogene specific variant p.Q318X (26%). Three novel single nucleotide polymorphism (SNP) loci were identified in the CYP21A2 gene which seems to be specific for the Tunisian population. The overall concordance between genotype and phenotype was 98%.
With this study the molecular basis of CAH has been characterized, providing useful results for clinicians in terms of prediction of disease severity, genetic and prenatal counseling.

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P04.11

Association of single nucleotide polymorphisms with microalbuminuria in essential hypertension

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Objectives: Microalbuminuria (Malb) is an early marker for cardiovascular diseases and renal risk in hypertension. Several studies have identified different loci and genetic markers for the risk of developing hypertension in Malb, but the genetic basis of Malb is little known. Therefore, the objectives were the identification of genetic variations involved in the development of Malb in essential hypertension.
Methodology: we have designed an experiment which studies 1536 SNPs, selected in based results of prior GWAS, in 960 samples of hypertension Spanish population with a Custom Golden Gate Genotyping Assay of Illumina. Linear regression analysis of quantitative variables and logistic regression analysis of qualitative ones were performed using PLINK v1.07. We adjusted by age, sex, body mass index and systolic blood pressure for all analysis.
Results: we obtained an association between rs12322500(ERC1 gene) and log_UAE, 0.3253(0.1413-0.5093) and OR for Malb presence of 2.657(1.373-5.144); the AA genotype had higher value for UAE than AG/GG genotypes. Other SNP with a significant association was rs1746048(CXCL12 gene) that had for log_UAE 0.7362(0.2441-1.228), an OR for Malb presence of 28.53(3.237-251.4); the AA genotype has higher value for UAE than AG/GG genotypes. Finally, SNP rs12260555(WDFY4 gene) had log_UAE of 0.1367(0.0354-0.2381) and OR for Malb presence of 1.798(1.2-2.694); with the highest levels of UAE in the AC genotype.
Conclusions: this study shows three SNPs strongly associated with UAE and presence of Malb in hypertensive patients. These SNPs are located in genes involved in renal damaged in hypertension. Further functional studies will be necessary to confirm these results.

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P04.12

Introducing a multiplex panel of markers for genetic testing of familial hypertrophic cardiomyopathy based on linkage analysis

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Aims: Familial hypertrophic cardiomyopathy (HCM) is caused by mutations in genes encoding cardiac sarcomere proteins. Nowadays genetic testing of HCM plays an important role in clinical practice by contributing to the diagnosis, prognosis, and screening of high risk individuals. The aim of this study was developing a reliable testing strategy for HCM based on linkage analysis and appropriate for Iranian population.
Methods and Results: six panels of four microsatellite markers surrounding MYH7, MYBPC3, TNNT2, TNNI3, TPM1, and MYL2 genes (24 markers in total) were selected for multiplex PCR and fragment length analysis. Characteristics of markers and informativeness of the panels were evaluated in 50 unrelated Iranians. The efficacy of the strategy was verified in a family with HCM. all markers were highly polymorphic. The panels were informati-

ve in 96-100% of samples. Multipoint linkage analysis excluded the linkage between the disease and all six genes by obtaining maximum LOD score ≤ -2.

Conclusion: This study suggests a reliable genetic testing method based on linkage analysis between 6 sarcomere genes and familial HCM. It could be applied for diagnostic, predictive, or screening testing in clinical setting.

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P04.13

Running for Genotyping in a Heartbeat? Hypertrophic Cardiomyopathy, Molecular Findings

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Hypertrophic Cardiomyopathy (HCM) is the most common cause of sudden death among youngsters and athletes, affecting 1:500 individuals. More than 1000 mutations are associated with this condition, especially in genes encoding sarcomere proteins.

Sudden cardiac death (SDC) is a devastating complication of several cardiovascular diseases, commonly including genetic disorders. The number of SCD in which no specific cause can be confirmed, even thorough a rigorous post-mortem examination, is still highly significant.

Recent guidelines recommend the genetic screening for family members of patients with HCM or victims of SCD.

The identification of alterations in the genes can be extremely important in the management of families, especially because it allows clarification of risk situation of asymptomatic family members and, in cases of SCD, it can provide a diagnostic.

New approaches with panels of genes, such as Sequenom MassArray System and Exome Sequencing Technology, are being developed, allowing an increase of the number of genes to be studied simultaneously and detection of a greater variety of mutations.

During the last 4 years 89 index cases were analysed in the laboratory by direct sequencing and Sequenom MassArray System.

The results of this study demonstrated that the most frequent mutated genes are the *MYBPC3* and *MYH7*, being detected in more than 50% of the analysed cases, as found in the literature.

New generation technologies promise to revolutionize the current knowledge about genetic bases of HCM and SCD and can contribute to clarify genotype-phenotype co-relation or to establish the cause of death in negative autopsies.

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P04.14

Mutations and Deletions mitochondrial DNA in Iranian Hypertrophic Cardiomyopathy (HCM) patients

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Because of the linkage between energy metabolism in the mitochondria and cardiac muscle contraction, it is reasonable to assume that mitochondrial abnormalities may be responsible for some forms of HCM. We analysed the whole mitochondrial genome in a series of 52 patients with HCM for alterations and compared the findings with those of 60 control subjects. An increased number of novel missense mutations could be detected nearly in all genes encoding for protein subunits in patients. Four mutations were found that are unpublished. The c.4384T>C in **tRNA glutamin** , c.9063A>G in **ATPase6** , c.2071 T>C, c.3170C>A, in noncoding MTRNA2 16S Also 33 polymorphisms were identified which had not been published in the MitoMap database. The c.16189T>C mutation in the D-loop region that is associated with susceptibility to DCM could be detected in 19% of patients as well as in 0% of controls. Five different deletions were found in 29 patients. Eighteen patients had 8.5 kb , Twelve 9 kb ,Seven 7.3 kb, Eight 4977 bp common deletion between nt8161-nt13640, and 11 patients had 7.4 kb deletion. Multiple deletions have been found in 21 patients.

Also 43 polymorphisms were found ,10 unpublished polymorphisms were found. A homologous amino acid A5480G, non-homologous substitutions, such as G5466A , an apparently homoplasmic substitution within tRNA genes T4384C, which affects the T_C loop of tRNA glutamine, 3 remarkable mutations (A8860G, A2706G and N3107C) most of these polymorphisms were observed .

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P04.15

Identification of 22q11.2 deletion in patients from adult congenital heart disease clinic - a missed burden in the transition care

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22q11.2 deletion syndrome (22q11DS) is a common genetic diagnosis in patients with congenital heart disease (CHD). It is multi-systemic with both congenital and later-onset features with lifelong consequences. Variable clinical expression and limited awareness contribute to its under-diagnosis. With low childhood mortality, there is an increasing number of diagnosed/undiagnosed adults, posing a hidden challenge in the transition care for patients with CHD. Our objective is to determine the prevalence of 22q11DS in adult patients with conotruncal defects and to delineate their extra-cardiac manifestations. We enrolled patients through an adult CHD clinic by active screening, using fluorescence-PCR and FISH. We have recruited 102 with conotruncal defects in 2012. Twelve patients are diagnosed with 22q11DS. Importantly 11 are not known to have 22q11Ds previously, which translates into a missed diagnosis of 1 in every 10 adults with conotruncal defects. Nine had the cardiac diagnosis of tetralogy, 2 had pulmonary atresia and ventricular septal defect, 1 had interrupted aortic arch; for extra-cardiac manifestations, 2 patients had learning difficulties, 2 had schizophrenia, 2 had history of hypocalcemia, 1 had thyroid nodule, and 1 had brain abscess. Among these 12 patients, only 1 was assessed by the cardiologists to have the typical facial dysmorphism. By cascade family testing, the deletion in 1 patient was found to be inherited from her mother with schizophrenia. The recruitment is ongoing. This study will provide important information on the disease burden of 22q11DS and may highlight an important and actionable gap in the transitional care of patients with CHD.

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P04.16

Identifying genetic determinants of congenital heart defect in Down syndrome

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Congenital heart defect (CHD) occurs in 40% of Down syndrome cases. While carrying three copies of genes or other functional elements on chr21

increases the risk for CHD, trisomy 21 is not sufficient to cause CHD. Thus additional genetic variation and/or environmental factors could contribute to CHD risk. Here we use association studies to identify genomic variations that with trisomy 21, determine the risk for CHD in DS. This GWAS includes 187 DS with CHD (AVSD=69, ASD=53, VSD=65) as cases, and 151 DS without CHD as controls. Chr21 specific association study revealed rs2832616 and rs1943950 (both cis-eQTLs for KRTAP7-1) as CHD risk alleles (adjusted p-values < 0.05). These signals were confirmed in a replication cohort of 92 DS CHD cases and 75 DS controls (nominal p-value = 0.0005). Furthermore rs2183593 and rs7282991 were identified as risk factors for ASD. Since DS is likely to be a disorder of gene expression, 2-locus interaction was applied for whole genome eQTLs. A pair of interacting eQTL on chr2 and chr11 was identified. Furthermore, a search for chr21 risk CNVs for CHD was performed using a customized chr21 array of 135K probes across 55 DS-AVSD and 53 DS controls. It revealed two CNV regions (FDR=0.04) in previously defined CHD minimal region, and another CNV (FDR=0.03) upstream of POFUT2. We propose that the CHD risk of DS is complex and determined by specific SNPs and CNVs variations on chr21 and interaction of non-chr21 genomic variants.

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P04.17

Congenital heart defects are rarely caused by mutations in ACTC1/ACTA2 genes

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Background: It has been shown that some congenital heart defects (CHD) can have genetic background due to missens mutation in cardiomyocyte-specific genes. Thus, cardiac actin was shown to be involved in pathogenesis of CHD and smooth muscle actin in pathogenesis of aortic aneurysm in combination with patent ductus arteriosus (PDA). In present study we investigated whether mutations in human α-cardiac actin (ACTC1) and smooth muscle α-actin (ACTA2) genes can cause ASD or PDA.
Matherial and Methods: Total genomic DNA was extracted from peripheral blood of 86 patients with ASDs and 100 patients with PDA. Coding exons and flanking intron regions of ACTC1 (NM_005159.4) and ACTA2 (NM_001613) were amplified by PCR with specific primers designed according to the corresponding gene reference sequences. PCR fragments were directly sequenced using a BigDye Terminator v3.1 sequencing kit and a 3130 Genetic Analyzer (Applied Biosystems). Sequencing results were analyzed with BioEdit 7.1 software and Sequencing Analysis 5.3.1 software (Applied Biosystems).
Results: Sequence analysis of ACTA2 and ACTC1 did not identify any nucleotide changes that altered the coding sense of the genes. In the case of ACTC1 we have found the earlier described nucleotide polymorphism rs2307493 (NCBI SNP database) resulting in a synonymous substitution.
Conclusion: our results confirmed that the mutations in ACTC1 gene are rare (at least <1%) cause of ASD. In patients with PDA the mutations of ACTC 2 gene are not detected, thus, being excluded from the list of frequent PDA-associated genetic defects.

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P04.18

The genetic basis of complex congenital heart disease: identification of potentially causal variants through whole-genome sequencing

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Background. Genetic risk factors contributing to the multi-factorial etiology of congenital heart disease (CHD) remain poorly understood. The goal of this study is to explore genetic variants underlying the development of complex CHD through whole-genome sequencing (WGS) of a family affected by hypoplastic left heart syndrome (HLHS) and conotruncal defects. We hypothesize that the clustering of these anatomically distinct lesions is attributable to a more proximal defect in cardiogenesis.
Methods. Paired-end, WGS data was generated on the Illumina Genome Analyzer II and Hi-Seq platforms for germline DNA from a proband with HLHS (~36X average depth of genome coverage); her affected half-sisters with tetralogy of Fallot (~38X) and truncus arteriosus (~10X), respectively; her unaffected full brother (~30X); and their shared unaffected father (~33X). Reads were aligned to the human genome reference sequence, followed by realignment and recalibration. Single nucleotide variant and short indel calls were made using the Genome Analysis Toolkit's UnifiedGenotyper. Variants with <1% minor allele frequency and all variants located in previously-published CHD-associated gene regions were identified. These variants were analyzed for co-segregation according to one of three genetic models: 1) autosomal dominant (AD) with incomplete penetrance, 2) AD with gonadal mosaicism, and 3) autosomal recessive. Variants co-segregating according to one of these models and defined as high impact and/or potentially damaging were considered causal candidates.
Results. Variant calling and co-segregation analysis identified a list of likely-damaging variants. Candidate causal variants for complex congenital heart disease will be presented.

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P04.19

Human valve disease is caused by copy number changes in ADAMTS19

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Valve defects are one of the predominant causes of cardiovascular morbidity and mortality. A third of all patients with congenital heart defects exhibit valve defects. Several structural variations have been identified in human disease including valvular heart defects. In a search for causal Copy Number Variation (CNV) we identified a family with a homozygous deletions in ADAMTS19.
We have studied a large patient discovery cohort sampled at the Sainte Justine Hospital, Montreal for disease associated CNVs and searched for small genomic imbalances resulting in homozygous deletions. After independent validation we have screened additional public cohorts for recurrent CNVs. Downstream in vivo modeling was done using antisense gene knockdown in Xenopus tropicalis oocytes.
We identified a homozygous deletion overlapping ADAMTS19 in an inbred family with two affected. The observed phenotype is a pulmonary stenosis, pulmonary artery dilatation and an aortic valve anomaly. This 475kb CNV spans the first seven exons of ADAMTS19 including part of the peptidase domain. Two additional duplications in patients with hypoplastic left heart syndrome have been observed in another cohort. Gene knockdown in Xenopus tropicalis supports a role during heart development.
This CNV is the first homozygous microdeletion observed in a family with valve disease. The importance of extracellular matrix genes in valve pathogenesis has been demonstrated in human and mouse. These findings of loss and gain of function spanning the same gene will enable a better genotype-phenotype understanding and will improve our understanding of normal and abnormal valve development.

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P04.20

Involvement of BAG3 and HSPB7 loci in various etiologies of systolic heart failure: results of a European collaboration assembling more than 2,000 patients

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Background and purpose. Genetic background of multifactorial systolic heart failure (systHF) is poorly understood. However, through a recent GWAS we identified two loci associated with sporadic Dilated Cardiomyopathy (DCM): BAG3 and HSPB7 loci. We hypothesized that the two loci (1) could also be involved in systHF due to coronary artery disease (ischemic-HF) and (2) could be involved in the severity of systHF and not only in the susceptibility to develop HF.

Methods. We genotyped SNPs previously associated with DCM (rs2234962: BAG3 locus, rs10927875 and rs945417: HSPB7 locus) in 1160 European patients with ischemic-HF and 1322 controls. The severity of systHF (including left ventricle ejection fraction and LV end-diastolic diameter or LVEDD) was also analysed in ischemic-HF patients as well as in a European cohort of 1141 DCM patients.
Results. SNPs related to HSPB7 locus were significantly associated with ischemic-HF (MAF of rs10927875 and rs945417 were less frequent in patients than controls, p value 0.0017 and 0.0016 respectively) whereas SNP related to BAG3 locus was not. In patients, the two loci were not associated with severity of HF, except LVEDD that was associated with rs2234962 both in DCM patients and ischemic-HF patients (p=0.0086 and 0.012 respectively).
Conclusions. Out of the two loci previously associated with DCM we observed that HSPB7 locus was also associated with ischemic-HF whereas BAG3 locus was not, suggesting differential involvement according to the underlying cause of HF. Severity of HF was not related to the two loci, except BAG3 locus associated with LV diameter in both populations.

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P04.21

Lack of relationship between CYP3A5 expression and blood pressure in healthy adults in Ashanti, Ghana

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Introduction: CYP3A5 protein substantially increases 6β-hydroxylase activity which stimulates the sodium-retaining actions of the mineralocorticoid receptor, potentially causing high blood pressure. Most Africans are functional CYP3A5 expressers so one would expect to find a relationship between CYP3A5 and blood pressure. Our study investigates this possible explanation for the high incidence of hypertension in Africans, in a Ghanaian population.
Materials and Methods: DNA samples were obtained from 957 of a cohort of 1,013 apparently healthy individuals, who in 2001/02 had been recruited to an epidemiological study of hypertension and salt intake. They were residents of 12 villages in the Ashanti region of Ghana. The extracted DNA was, in 898 individuals, of sufficient quality to allow genotyping at the *CYP3A5*3* and *CYP3A5*6* SNPs by real-time polymerase chain reaction. Blood pressure was measured using an OMRON HEM705CP sphygmomanometer. The participants had sat for at least 5 min before three pressures were obtained one minute apart; the first reading was discarded and the mean of the second and third calculated for analysis. Hypertension was defined as systolic BP ≥140 mmHg.
Results: 244 (27.2%) of the 898 individuals were hypertensive. Although there was a possible relationship between blood pressure and degree of CYP3A5 expression, there was no statistically significant relationship between either systolic or diastolic pressure and *CYP3A5*3* or *CYP3A5*6* genotypes and their haplotypes.
Conclusion: We conclude that there is either no association between CYP3A5 expression and blood pressure or, if there is a relationship, an association that is very weak.

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P04.22

Exome sequencing of multiple affected individuals from an Irish family with Brugada Syndrome uncovers a novel locus for the disorder

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Brugada Syndrome (BrS) is a disorder associated with an increased risk of sudden cardiac death and specific ECG features. Loss-of-function mutations in SCN5A underlie the disorder in ~25% of patients, while other genes have been implicated but account for <1%. We here set out to identify the genetic defect underlying BrS in a 3 generation family with a case who died suddenly at 24 years old.
Individuals from the pedigree were genotyped using the Illumina-HumanOmniExpress SNP-array. Exome-sequencing was carried out in 3 individuals from the pedigree using Agilent SureSelect-Target-Enrichment 50Mb capture followed by sequencing on an Illumina-HiSeq2000. These 3 individuals were selected based on their genetic distance and the presentation of a BrS-ECG at baseline.
Linkage analysis identified a region on chromosome 8 (8p11.21-8q11.23) shared among affected family members. In parallel, comparison of 3 exomes from affected individuals and after filtering out of variants occurring at a MAF of >1% in public and in-house exome/genome databases results in 10 rare variants shared, 2 locate to chromosome 8 locus and reside in 2 different genes, namely HOOK3 and PXDNL. These 2 variants have been found at a MAF of 0.02% and 0.008%, respectively, in the NHLBI-EVP (n=6500 individuals). The function of PXDNL, which appears to be specifically expressed in heart, is yet unknown.
Combining linkage analysis and exome sequencing, we identified a chromosomal interval and a possible novel gene for BrS. Ongoing studies are aimed at investigating further the involvement of this gene in a large set of patients with BrS.

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P04.23

Spectrum of KLHL3 and CUL3 mutations in Familial Hyperkalemic Hypertension

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Familial Hyperkalemic Hypertension (FHHt) is a rare inherited form of arterial hypertension. In 2001, two genes - WNK1 and WNK4 - were identified and subsequently found to regulate renal ion transport. In 2012, we and others identified mutations in two other genes, KLHL3 and CUL3, as responsible for the majority of cases. Kelch-like 3, is an actin-binding protein that recruit substrates for the Cullin3-based ubiquitin-ligase complex. The objective of this study was to compare inheritance and phenotype in one or the other gene.
We identified 20 different missense mutations in the KLHL3 gene in 24 FHHt index cases. The majority of them were located within conserved Kelch motifs at the surface of the molecule. There were 16 dominant, 4 de novo and 4 recessive cases and a large variability of the phenotype. Homozygous subjects had an earlier age at diagnosis (8.4 vs 35.9 years) but comparable blood pressure and biological phenotype. Analysis of the CUL3 gene revealed six de novo and 2 dominant heterozygous splice-site mutations clustered around exon 9 that lead to a loss of 57 residues corresponding to a segment linking BTB and RING-binding domains of the protein. Patients had a young age of diagnosis (10 years) and a severe clinical and biological phenotype. KLHL3 is highly expressed in the distal nephron and its inhibition by RNA interference leads to an increase of Na+-Cl- cotransporter expression at the cell membrane. All mutations should decrease the ubiquitination of this transporter and thus augment its activity in the distal nephron.

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P04.24

Genotype-Phenotype correlations in Hereditary Haemorrhagic Telangiectasia. Data of the French Rendu-Osler-Weber Syndrome cohort.

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INTRODUCTION: Hereditary haemorrhagic telangiectasia (HHT) is a dominantly inherited genetic vascular disorder characterized by recurrent epistaxis, cutaneous telangiectasia and visceral arteriovenous malformations (AVM) that affect many organs, including the lungs, gastrointestinal tract, liver and brain.

AIMS: To study the relationship between the phenotype and genotype in patients with a proven mutation in either ENG, ACVRL1, or less frequently MADH4.

METHOD: We used the French HHT database (CIROCO) developed for the network, which contains detailed clinical features registered from 2005 to 2013.

RESULTS: A total of 1534 HHT patients with a proved mutation were analysed. Among them, the mutated gene was ACVRL1 in 58.4%, ENG in 39.9% and MADH4 in 1.7% of patients.

The nose-bleeding event appears to be a predominant characteristic for each of the HHT populations studied: this symptom was present in 92.4, 93.4 and 88.5% of the cases of patients carrying the ENG, ACVRL1 and MADH4 mutations, respectively. Telangiectasia were present in all groups: ACVRL1 (92.7%), ENG (90.6%) or MADH4 (76%).

Patients with ENG mutations showed a higher prevalence of pulmonary arteriovenous malformations (PAVM) (72.4%) as well as a marked prevalence of cerebral arteriovenous malformations (CAVM) (24.5%). In contrast, the hepatic malformations (HAVM) manifested to be more often related to the presence of the ACVRL1 mutation (66.2%). Structurally, ENG mutations showed to be widely distributed through the gene. In contrast, ACVRL1 mutations occur preferentially in exons 3 and 7.

CONCLUSIONS: This study on a large cohort showed major differences linking the syndrome and the mutated gene.

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P04.25

Genetic variants affecting the expression of DRAM2 at 1p13.3 are associated with acute myocardial infarction with different effects for STEMI and NSTEMI

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Myocardial infarction (MI) due to coronary artery disease (CAD) is the most common cause of death in developed countries. Genetic and non-genetic factors affect risk for MI with roughly equal relative contributions. The heritability of MI has been estimated to be 0.35 and 0.38 for males and females, respectively. Known genetic risk variants explain 10 % of the additive genetic variance in susceptibility to CAD.

Despite advances in understanding the pathophysiology of MI, relatively little attention has been paid to the distinction between ST-segment elevation MI (STEMI) and non-ST-segment elevation MI (NSTEMI). This division is made based on characteristic ECG changes and the two are known to present with different baseline characteristics. It is commonly thought that STEMI results from complete occlusion of a coronary artery whereas NSTEMI is caused by partial or transient blockage. The possible differences in genetic risk factors for STEMI and NSTEMI are virtually unknown.

We performed a genome-wide association study of MI (1579 cases, 1576 controls) with stratification into NSTEMI/STEMI and replicated the results in two independent study samples. We identify a novel risk locus for MI (OR=1.28, $p=7*10^{-8}$) at 1p13.3 containing *DRAM2*, *CEPT1* and *DENND2D*. We show that the association is markedly stronger for NSTEMI (OR=1.56, $p=4*10^{-10}$) than for STEMI (OR=1.11, p=0.20). We further show that the SNPs conferring risk to NSTEMI are also associated with the expression of *DRAM2* and provide evidence suggesting that this locus contains two distinct but partially overlapping association signals.

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P04.26

Compound heterozygous or homozygous truncating *MYBPC3* mutations causes severe cardiomyopathy with left ventricular noncompaction and septal defects resulting in neonatal death

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Background Familial hypertrophic cardiomyopathy (HCM) is a heart muscle disease generally caused by autosomal dominant sarcomeric gene mutations leading to a phenotype in adult age. The most commonly mutated gene (20-40%) in adult-onset HCM is *myosin binding protein C* (*MYBPC3*). Idiopathic HCM in young children is rare. Our goal was to describe four neonates with severe HCM caused by compound heterozygous or homozygous truncating *MYBPC3* mutations and compare these to those described in literature.

Methods Four unrelated neonates with severe unexplained cardiomyopathy were studied. A literature review was performed to identify other patients with biallelic *MYBPC3* mutations.

Results All children presented with feeding difficulties, failure to thrive, dyspnea and died from cardiac failure before age 13 weeks. Left ventricular non-compaction was diagnosed in three patients. In the fourth, noncompaction was not a clear feature, but could not be excluded. All patients had septal defects. Two patients were compound heterozygotes for the c.2373dupG (p.Trp792fsX17) and c.2827C>T (p.Arg943X) mutation. Two were homozygous for the c.2373dupG and the c.2827C>T mutation respectively.

All reported patients with double truncating mutations in *MYBPC3* reported so far (n=21), are diagnosed with severe cardiomyopathy and/or die within the first months of life. In 52% (11/21) septal defects or a patent ductus arteriosus accompanied cardiomyopathy.

Conclusions In contrast to heterozygous mutations, homozygous or compound heterozygous truncating *MYBPC3* mutations cause severe neonatal cardiomyopathy with features of left ventricular noncompaction including septal defects in 60% of patients. Neonates with cardiomyopathy should be investigated for *MYPBC3* and other sarcomeric gene mutations.

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P04.27

Analysis of single and combined genotypes of MTHFR, MTR and MTRR polymorphisms regard to content of sulfur amino acids in plasma of patients with preeclampsia and coronary heart disease

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Hyperhomocysteinemia is generally established etiological factor for thrombosis. We have analyzed common polymorphic variants MTHFR C677T/A1298C, MTR A2756G, MTRR A66G regard to the plasma homocysteine and cysteine level among 115 Ukrainian patients with preeclampsia and coronary artery disease (55 and 71 respectively) and 186 controls (33 pregnant and 83 non-pregnant women, and 70 men). Significant increase of homocysteine level was proved in patients with preeclampsia, whereas moderate hyperhomocysteinemia (within 15-30 mmol/L) was noted just only in 10.91% of patients with ischemic heart disease. Unexpectedly, 41.8% of such patients and less than 4% of controls showed decrease in cysteine concentrations with significant difference in mean values (239.35+47.38 and 270.93+38.28 mmol/L, P<0.05). Hypercysteinemia (>350 mmol/L) was noted in 7.27% cases in the absence of control. No significant differences in prevalence either of C677T or A1298C genotypes of MTHFR gene was established. At the same time, significantly increased proportion of MTR AA and MTRR GG genotypes were recognized as a prominent trait of patients with preeclampsia and coronary artery disease. We found 6 among 81 possible combined genotypes of MTHFR C677T and A1298C, MTR A2756G, MTRR A66G, which more than 5 times increases the risk of preeclampsia and coronary heart disease. Our data suggest a link between hyperhomocysteinemia and hypocysteinemia with carrier status of defined genotypes of methionine synthase, methionine synthase reductase and cystathionine synthase. Reducing the concentration of L-cysteine can lead to disruption of the biosynthesis of endogenous hydrogen sulphide with the loss inherent to his significant vasodilator effect.

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P04.28

Genotype-phenotype discrepancies in Brugada syndrome

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Brugada syndrome is one of common inheritable arrhythmogenic disorders in apparently structurally normal hearts, inherited as an autosomal dominant trait with incomplete penetrance and variable expression. Clinical diagnosis is based on documented ventricular arrhythmias and/or related symptoms, family history AND a type 1 spontaneous or sodium-blocker induced ST-segment ECG elevation. Genetic diagnosis is currently still based on the mutation analysis of the pore-forming α-subunit of the sodium channel gene(*SCN5A*), resulting in a genetic diagnostic yield of approximately 20%.
We performed SCN5A mutation analysis in 135 Brugada syndrome probands of our outclinic patient population. We identified 20 variants of which 17 described mutations and 3 novel potential disease causing variants by *in silico* prediction tools, resulting in a diagnostic yield of respectively 12,6% to 14,8% (3 variants included). Subsequent segregation analysis was performed in 8 large families of the identified SCN5A positive BrS probands to determine genotype-phenotype correlation. A sensitivity of 76% and specificity of 79% was assessed and could be increased up to 85% and 92% respectively with exclusion of the undescribed SCN5A variants. Revision of all ECG data revealed that some ajmaline or baseline ECG negative BrS patients with SCN5A mutations did not meet the stringent diagnostic criteria but demonstrated conduction disease.
These results urge for the need to revise the clinical diagnostic criteria for Brugada syndrome, especially since the ajmaline ECG data are of important added value for this low penetrant disorder in the discovery of novel major molecular genetic pathways in Brugada syndrome.

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P04.29

Whole exome sequencing as a tool to identify novel causal variants and genes for Brugada syndrome

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Brugada syndrome (BrS) is a cardiac channelopathy inherited as an autosomal dominant trait with incomplete penetrance and variable expression. All published BrS-associated genes have been identified by a classical candidate gene approach, leading to a total genetic diagnostic yield of 30% (with 20% of mutations in the SCN5A gene). Currently available genomics and bioinformatics technologies allow us to identify novel and potentially major genes involved in BrS. Hence we can better address the urgent question to what extent BrS can be regarded as a pure Mendelian, monogenic disorder or as an oligo- to polygenic syndrome modulating disease expression.
We report the results of a first panel of exome sequence data of 8 stringently selected, large BrS families. Multiple candidate causal variants in genes already linked with cardiac arrhythmias and in novel cardiac genes were identified and validated with Sanger sequencing. In three families we observed that novel, in silico disease-causing variants in known cardiac arrhythmia genes did not show complete correlation with the clinical diagnosis by segregation analysis in all available family members. This phenomenon is also seen in our diagnostics lab for SCN5A mutation analysis. On one hand these results question the major causality of the analyzed variants but on the other hand they might point in the direction of a polygenic inheritance of this disorder. To further investigate this hypothesis, we currently compare exomic SNP profiles, including the type, relative burden and pattern of variants, within a cardiac arrhythmias candidate gene set between control and BrS-patient samples.

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P04.30

Genetic diversity of plakophilin-2 (PKP2) and desmoglein-2 (DSG2) genes in Russian patients with arrhythmogenic right ventricular dysplasia

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Background: Arrhythmogenic right ventricular dysplasia (ARVD) is an inherited cardiomyopathy characterized by progressive fibro-fatty replacement of the myocardium and high risk of life-threatening ventricular arrhythmias. Mutations in the genes encoding desmosomal proteins cause ARVD. Mutation rate in those genes in Russian cohort had not been studied yet.
Methods: Fifteen unrelated Russian ARVD patients were examined with collecting of personal and family history, physical examination, ECG, Echo-CG, myocardial biopsy and cardiac MRI. Analysis of PKP2 and DSG2 genes was performed by direct sequencing.
Results: Screening of mutations in PKP2 and DSG2 genes in 15 DNA samples was performed. In PKP2 gene we found three rare genetic variants: two truncating mutations (p.W538X and c.1523_1538del) and one missense VUCS p.S140F. All patients carrying 3 genetic variants had an oblivious manifestation at the 46 y.o. with high grade ventricular arrhythmia; ICD was implanted.
In DSG2 gene we did find three missense VUCSes p.S194L, p.V533I and p.N245H. Variants p.S194L and p.N245H were analysed with PolyPhen2 and considered as «probably damaging» and «possibly damaging» respectively. All patients carrying 3 genetic variants had an oblivious manifestation at the 26 y.o. with ventricular tachycardia, RV hiperthrophy; ICD implanted or strongly recommended.
Conclusion: We identified six mutations in 2 most common ARVD genes in Russian unrelated ARVD patients. Always truncated mutations and probably damaging variants account for 40% mutations. This prevalence matches with the prevalence of ARVD9 and ARVD10 (HRS/EHRA Expert Consensus Statement). Observation in healthy ethnically-matched volunteers is in progress now. Functional effect has to be elucidated.

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P04.31

Ebstein anomaly: report of two siblinigs born to unaffected parents.

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Ebstein anomaly is a rare cardiac malformation associated with displacement of the septal leaflet of the tricuspid valve towards the apex at the right ventricle of the heart with variable severity extending from a severely affected fetus to an asymptomatic adult. Ebstein anomaly can be familial or sporadic as well as syndromic and non-syndromic. The majority of cases are sporadic and non-syndromic and the suggested mode of inheritance in familial cases is autosomal dominant (AD) (Sinokovec et al., 2005, Digilio et al., 2011) although cases suggestive of autosomal recessive mode of inheritance have been reported (Chitayat et al.,1992). A few genes including *MYH7* (Postma et al., 2010), *GATA4 and NKX2-5* (Digilio et al., 2011) have been suggested to be candidate genes in isolated, non-syndromic cases. We report two siblings (a boy and a girl) diagnosed prenatally with Ebstein anomaly and other right sided heart lesions. The parents were non-consanguineous of Swiss descents. The couple had 4 miscarriages, the 2 affected children and a healthy child. The father carries an apparently balanced chromosomal rearrangement between chromosomes 17 and 19 and array CGH done on the affected girl was negative. The Ebstein anomaly in this pregnancy seems to have autosomal recessive mode of inheritance although autosomal dominant mode of inheritance with incomplete penetrance in one of the parents cannot be ruled out. Further investigation is being done to identify the causative gene in this family.

S. Jougheh Doust: None. **A. Redington:** None. **D. Chitayat:** None.

P04.32

A Dutch founder mutation in the cardiac regulatory light chain (MYL2).

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Hypertrophic cardiomyopathy (HCM) is a common disease with an estimated prevalence of 0.2% in the general population. In 30%-60% of patients an autosomal dominant gene mutation is identified. Most of the reported mutations are distinct and unique for each family. In addition, clinical heterogeneity and incomplete penetrance are observed in families with a mutation. Occasionally, sudden cardiac death is the first manifestation of the disease.
In this report, we describe the mutation p.Glu22Lys (p.E22K, c.64G>A) in MYL2, a gene that is rarely mutated in HCM as it accounts for less than 1% of identified HCM mutations. We identified the p.E22K mutation in 11 HCM families and show that it is a Dutch founder mutation that probably originated about 425 years ago in the region around Eindhoven in the south of The Netherlands. The availability of multiple families carrying the same founder mutation provides the opportunity to investigate phenotypic differences influenced by external or genetic factors other than the primary mutation, which can improve mechanistic insights and aid in the discovery of prognostic factors. We demonstrate that mutation carriers generally have a benign disease manifestation with variable phenotype expression, reduced penetrance and late onset. However, when a comorbidity factor is present like hypertension, hypothyroidism, coronary artery disease, atrial fibrillation or a second gene mutation, disease penetrance appears to be higher with an earlier onset and sometimes cardiac arrest requiring an implantable cardioverter defibrillator in survivors.

A. Van den Wijngaard: None. **G.R.F. Claes:** None. **I.P.C. Krapels:** None. **M.B. Hoos:** None. **A.T.J.M. Helderman-van den Enden:** None. **Y.E.G. Barrois:** None. **R.J. Jongbloed:** None. **F.H.J. van Tienen:** None. **J.W. Sels:** None. **S.H.H. Kuijpers:** None. **P. Garcia-Pavia:** None. **A. Perrot:** None. **I. Christiaans:** None. **P.G.A. Volders:** None. **H.J.M. Smeets:** None.

P04.33

Idiopathic pleuroparenchymal fibroelastosis: identification of two candidate loci by genome-wide homozygosity mapping in a familial case.

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Idiopathic Pleuroparenchymal Fibroelastosis (IPF) is a rare clinicopathologic entity, characterized by a fibrotic thickening of the pleura and subpleural parenchyma with upper lobe predominance. The pathologic findings include the following: 1) intense fibrosis of the visceral pleura, 2) prominent subpleural fibroelastosis, 3) sparing of the parenchyma distant from the pleura, 4) patchy lymphoplasmatic infiltrates, 5) fibroblastic foci present at the leading edge of the fibrosis. Etiology and physiopathology of this entity are still unknown but genetic origin is suggested by reported affected sib pairs.
Here we described three sibs, a brother and two sisters, affected by a dramatic restrictive syndrome, secondary to bilateral pleural fibrosis and leading to premature death during the fourth decade. For these three sibs, clinical presentation, radiographic and pathologic findings are consistent with IPF. Their unaffected parents were first cousins and the siblings had identical symptoms with similar severe progression which is strongly indicative of a simple autosomal recessive inheritance. We identified by Genome-Wide Homozygosity Mapping two candidate loci, spanning 9,60 Mb on chromosome 5 and 8,97 Mb on chromosome 9. Unfortunately, Whole Exome sequencing strategy failed to identify the causal mutations of this rare fatal disease in our pedigree.

J. Plaisancié: None. **E. Justrabo:** None. **H. Hamel:** None. **P. Calvas:** None. **N. Chassaing:** None. **E. Bieth:** None.

P04.34

Mutation analysis of the *AGXT* gene in combined liver-kidney and isolated liver transplanted six children for primary hyperoxaluria type 1: a single center experience

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Primary hyperoxaluria type I (PH1) is an autosomal recessive rare disorder, characterized by progressive kidney failure, caused by mutations in the alanine: glyoxylate aminotransferase (*AGXT*) gene which leads to the failure of the alanine: glyoxylate aminotransferase activity in the liver. Although targeted sequence analysis of exons 1, 4 and 7 of *AGXT* has been proposed for first line genetic testing, the sequence of the entire coding region is recommended. We aimed to detect the *AGXT* gene mutations causing PH1 in combined liver-kidney, isolated liver, sequential kidney and liver-kidney transplanted six Turkish children (Five male) with phenotypic characteristics of PH1. Median age at diagnosis was 119 months (Range 42-178 months). The entire coding region including exon intron boundaries of the *AGXT* gene were sequenced in patients.
We detected six mutations PH1causing and two minor allele polymorphism in six patients. The entire patients had at least one PH1 related mutation. Patient 1 had homozygous minor allele polymorphisms Pro11Leu in exon 1 and Ile340Met in exon 10, and mutation Met195Arg in exon 5.
Patient 2 had homozygous mutation c. 33_34insC in exon 1. Patient 3 was compound heterozygous for mutations Gly170Arg in exon 4 and c.846+1G>A in intron 8 and heterozygous minor allele polymorphisms Ile340Met in exon 10. Patient 4 had homozygous mutation c.823-824dupAG in exon 8. Patient 5 and 6 had homozygous mutation c.976delG in exon 10. Mutational analysis of the *AGXT* gene in PH1 patients can be a useful tool for establishing the diagnosis and choosing an appropriate therapeutic strategy.

M. Turkmen: None. **S. Agılkaya:** None. **D. Alaygut:** None. **M. Torun Bayram:** None. **B. Kasap:** None. **A. Soylu:** None. **S. Kavukcu:** None. **S. Cingoz:** None.

P04.35

Both genetic and allelic heterogeneity greatly influence the outcome of renal function in polycystic kidney disease patients.

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited disorder affecting 1/400 to 1/1000 individuals worldwide. The disease is genetically heterogeneous with two causative genes, *PKD1* (85% of patients) and *PKD2* (15% of patients). To improve our understan-

ding of the genotype/phenotype correlation in ADPKD, we have collected a large population of more than 1000 patients (including The Genkyst cohort) from the western part of France. We have scanned the whole coding sequences of these two genes and searched for large rearrangements. We have identified a mutated allele in 90% of our patients and reported 351 new mutations (Audrezet et al., Human Mutation, 2012). These molecular data as well as the correlation with the patients' phenotype allowed us to show that *PKD2* mutations are associated with renal survival approximately 20 years longer than that associated with *PKD1* mutations (79.7 years versus 58.4 years). In terms of allelic effect on phenotype, in sharp contrast to previous studies, we demonstrated that it is the type but not the position of the *PKD1* mutation correlates strongly with renal survival. The median age of end stage renal disease (ESRD) onset is 55.6 years in patients with truncating mutation versus 67.9 years in the non-truncating mutation carriers (E Cornec-Le Gall, JASN, in Press).The identification of such genetic factors is crucial as targeted therapies are currently under development.

E. Cornec-le gall: None. **M.P. Audrezet:** None. **J.M. Chen:** None. **Y. Le meur:** None. **C. Ferec:** None. **T. Genkyst working group:** None.

P04.36

Array CGH analysis of Bulgarian patients with multiple congenital pathologies including anomalies of kidney and urinary tract

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Congenital anomalies of the kidney and urinary tract (CAKUT) represent approximately 25% of all pathologies identified in the prenatal period. These conditions constitute a broad range of disorders resulting from the abnormal renal development in embryogenesis. They are one of the most common causes of chronic renal failure in both children and adults. In the present study an array Comparative Genome Hybridization (aCGH) for 10 Bulgarian patients was performed. The children were diagnosed with different manifestations of CAKUT - unilateral agenesis, multiple cysts, VUR, hydronephrosis, etc. Many had accompanying malformations in other organs like heart, skeleton, neuronal tube, etc. All patients were initially screened for mutations in the two most commonly affected genes, TCF2 and PAX2, but no defects were identified.

Using aCGH we identified a variety of genomic rearrangements. Common copy number variations (CNV) were present in all samples. In addition, we found microdeletions and microduplications affecting multiple genes known to be expressed in kidney, which potentially have important role for urinary tract development and function - SUMF1, CROCC, TNS1, NALCN, ANXA6, ELF3, BCAR3 and SKAP2. Their protein products are implicated in cell signaling, cytoskeleton formation, transcription regulation and extracellular matrix rearrangements. To the best of our knowledge, this is the first study to identify a possible link between mutations affecting these genes and CAKUT. Further studies need to be carried out in order to confirm their involvement in the pathogenesis of these disorders.

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P04.37

Mutations in TCF2 and PAX2 genes are a rare cause for Congenital Anomalies of Kidney and Urinary Tract (CAKUT) in Bulgarian patients

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Congenital Abnormalities of Kidney and Urianry Tract (CAKUT) are common pathological conditions diagnosed in 1 out of 500 newborns worldwide. Knowledge of the fundamental causes of CAKUT is essential for understanding the mechanisms of their pathology as well as for reliable consultation, prognosis and treatment.

HNF1B (TCF2) and PAX2 are two of the important transcription factors responsible for correct kidney development. Mutations in the genes for these proteins are the most commonly identified causes for CAKUT. They are found in up to 15 % of the cases.

In the present study 40 patients from 32 families were included - 24 families of Bulgarian, 5 of Turkish and 3 of Roma origin. Each affected individual was tested for the presence of *TCF2* and *PAX2* mutations using direct sequencing of all coding regions and Multiplex Ligation-dependent Probe Amplification (MLPA). In case genetic defect was identified in the index patient we chekked the carrier status of the parents in order to determine its origin and inheritance within the family.

Genetic defects such as missense mutations and deletions of multiple exons were found in only small proportion of the cases (less than 10%). This could be explained either with the presence of as yet unknown defects in other genes involved in urinary tract development or with the different ethnic background of the patients. Including more genes in the panel for CAKUT genetic testing as well as expanding the groups of Roma and Turkish patients would allow us to clarify that.

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P04.38

Genome-wide association analyses identified a locus influencing the ratio urinary calcium/ urinary sodium.

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Concentrations of calcium and sodium in the urine (UCa, UNa) are important biomarkers for various cardiovascular and renal disorders. These traits vary across individuals and part of this variance is genetically determined. Hence, we performed a genome-wide association study (GWAS) for UCa, UNa and UCa/UNa ratio in over 8,000 subjects of European descent.The discovery phase was conducted on 5,150 samples from a Swiss population based cohort (Colaus), with replication in four additional genetic isolates (CROATIA-Korcula, CROATIA-Split, INGI-Val Borbera and INGI-Carlantino) for a total of 3,122 samples. Calcium and sodium were measured in a spot urine sample and both traits, unadjusted and adjusted for urinary creatinine excretion as well as the UCa/UNa ratio were analyzed as outcome variables.

A GWAS was conducted on 2.5M single nucleotide polymorphisms (SNPs) applying a linear regression with additive model on normalized traits adjusted for age, sex and population structure. 143 SNPs with p-value below 1E-5 in 34 loci were selected for replication, and subsequently meta-analyzed. Among loci subjected to replication attempt, one locus reached the recognized genome-wide significant threshold of 1E-8 (five phenotypes), for UCa/UNa ratio. The significant signal (top SNP p-value = 1.5E-10, effect size in original scale in discovery cohort = 3.8E-3) lies within the long arm of chromosome 21 (21q22.3), in a gene-rich region. Studies are ongoing in order to determine which gene of the locus is causative for the trait variation, and the biological relevance of the association.

T. Corre: None. **C. Hayward:** None. **G. Pistis:** None. **N. Pirastu:** None. **S. Ulivi:** None. **Z. Kutalik:** None. **M. Bochud:** None. **O. Polasek:** None. **I. Rudan:** None. **N.D. Hastie:** None. **M. Traglia:** None. **M. Cocca:** None. **P. Gasparini:** None. **D. Toniolo:** None. **S. Bergmann:** None. **P. Vollenweider:** None. **O. Devuyst:** None.

P04.39

Characterization of NEK8/NPHP9 mutations in patients with severe renal cystic hypodysplasia and associated ciliopathy defects

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NEK8/NPHP9 encodes a protein that belongs to the NIMA (Never In Mitosis gene A) kinase family, which is essential for control of cell cycle progres-

sion. NEK8 is composed of a N-terminal serine/threonine kinase domain and a C-terminal RCC1 domain involved in localization of the protein at the centrosome and the base of the cilium. To date only one homozygous NEK8 mutation, located in the RCC1 domain, has been reported in a patient with early onset nephronophthisis, a frequent renal ciliopathy.
Using ciliary gene-enriched exome sequencing, we identified novel recessive NEK8 mutations in 3 cases with very severe overlapping phenotypes including renal cystic dysplasia or hypodysplasia, situs inversus, cardiopathy with hypertrophic septum and paucity of the bile ducts.
Two patients, who died early after birth, carried either compound or homozygous missense mutations. A homozygous splice mutation in intron 1, likely resulting in protein loss, was identified in a fetus who presented agenesis of the vermis, pancreatic cysts/fibrosis and abnormal genitalia in addition to multicystic kidney dysplasia and situs inversus.
We demonstrated that the NEK8 mutations affect localization of the protein at the base of the cilium. Moreover, fibroblasts of the patient carrying the two compound mutations showed defect in ciliogenesis compared to fibroblasts from control individuals.
This study extends the spectrum of ciliopathy defects associated with NEK8 mutations, from nephronophthisis to lethal developmental multisystemic disorders. Further characterization of the functional effect of the mutations and downstream affected pathways will allow to understand the pathophysiological basis if this phenotypic variability.

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P04.40
Molecular investigation of distal renal tubular acidosis in Tunisia, evidence for founders mutations

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Background: Primary distal renal tubular acidosis (dRTA) is a rare genetic disease caused by mutations in different genes involved in the secretion of H+ ions in the intercalated cells of the collecting duct required for final excretion of fixed acids. Both autosomal dominant and recessive forms have been described; the latter is also associated with sensorineural hearing loss.
Methods: Twenty five Tunisian families were analyzed for mutations in the ATP6V1B1 and ATP6V0A4 genes by direct sequencing. Mutation dating for the founder mutations was performed by 2 methods (Kosambi formula and DMLE+2.2 program).
Results: Two founder mutations in ATP6V1B1 gene were found in 16/25 dRTA patients, one of them was estimated to be older than 2400 years. For the remaining patients, two mutations in the ATP6V0A4 gene, one of them being novel, were found in three Tunisian cases. The presence of a heterozygous missense mutation p.T30I, of the ATP6V1B1 gene, was identified in six patients; while no mutations of the second gene were detected. Any deleterious mutations of either ATP6V1B1 or ATP6V0A were found for two probands.
Conclusion: Our study gives evidence of phenotypic and genotypic heterogeneity of dRTA in Tunisian population. Five different mutations were found, two of them were due to a founder effect. Direct screening of these mutations could be a rapid and valuable tool for diagnosis of dRTA in Tunisian and North African populations.

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P04.41
Founder effect of Claudin-19 p.(Gly20Asp) mutation in Spanish families with familial hypomagnesemia with hypercalciuria and nephrocalcinosis

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Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) is an autosomal recessive tubular disorder characterized by excessive renal magnesium and calcium excretion and chronic kidney failure. This disease is caused by mutations in CLDN16 or CLDN19, encoding tight junction proteins claudin-16 and claudin-19, respectively. The identification of several FHHNC patients from Spain carrying missense mutation p.(Gly20Asp) in CLDN19 has raised the question of the origin of this mutation from a common ancestor. The aim of this study was to identify disease causing mutations among a Spanish cohort of 30 unrelated FHHNC families and to determine whether the p.(Gly20Asp) mutation is a founder mutation. The mutational analysis was carried out by sequencing the CLDN16 and CLDN19 exons and their exon-intron boundaries. Determination of the haplotypes was performed by genotyping three short tandem repeats flanking the CLDN19 locus and four single nucleotide polymorphisms located in introns 3 and 4. Our results revealed CLDN19 mutations in all the patients; no CLDN16 mutations were detected. Six different mutations were identified, four of them novel, p.(Ile41Thr), p.(Gly75Cys), p.(Gly75Ser) and p.(Gly122Arg). The p.(Gly20Asp) mutation was found in 28 unrelated patients (93.3%), and was present in homozygous state in 83.3% of the probands. Results of the haplotype analysis indicated that all alleles carrying the p.(Gly20Asp) mutation shared a common haplotype cosegregating with this mutation. These findings are consistent with the existence of a founder effect responsible for FHHNC in our cohort. Testing for the presence of this mutation should be the first genetic screening in Spanish patients.

F. Claverie-Martin: None. **E. Cordoba-Lanus:** None. **E. Martin-Nuñez:** None. **H. Gonzalez-Acosta:** None.

P04.42
A rare penetrant mutation in *NEPH3* gene confers high risk of renal failure in primary hematuric glomerulopathies and of microalbuminuria in the general population

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The wide spectrum of phenotypic heterogeneity, ranging from benign isolated hematuria to severe proteinuria and renal failure, is a critical issue in primary hematuric diseases, such as Thin Basement Membrane Nephropathy (TBMN) and IgA nephropathy. Phenotypic heterogeneity could be explained by the existence of modifier genes. We genotyped a cohort of well-studied adult TBMN patients (sample group 1) from 19 families, with a homogeneous genetic background, for potential functional modifier SNPs expressed in slit diaphragm (SD: pivotal to the structure of the glomerular filtration barrier). We identified six likely variants. When we categorized the patients in "Severe" and "Mild", based on the existence or not of proteinuria and renal failure, variant V353M in *NEPH3* (filtrin) gave suggestive association (genotypic association: p=0.036), where 353V is highly conserved. A pooled hematuric cohort of 524 patients (sample group 2) confirmed the association, under the dominant model (genotypic association: $p=5.0x10^{-3}$, OR=5.95 adjusting for gender/age; allelic association: $p=5.1x10^{-3}$ adjusting for patients' kinships). Genotyping 6531 subjects of the Framingham Heart Study (sample group 3) revealed an association of the homozygous 353M/M genotype with microalbuminuria in this population ($p=1.0x10^{-3}$, OR=12.8 adjusting for gender/age). Co-immunoprecipitation assays showed that 353M causes reduced Neph3 homodimerization and increased Neph3-Nephrin binding, possibly this affecting the long-term SD integrity. In conclusion, genetic association results from three independent sample groups and functional studies support a "rare variant-large effect" phenomenon for *NEPH3*-V353M in renal disease, giving also clues for common pathophysiology in hematuric diseases of glomerular origin.

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P04.43

Molecular diagnosis of hereditary kidney diseases: nephronophthisis, medullary cystic kidney disease, hyperuricemic nephropathies and renal cysts-diabetes syndrome

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Hereditary kidney diseases (HKD) represent a heterogeneous spectrum of disorders and a leading cause of chronic renal failure. Once ultrasound and urinalysis have excluded polycystic kidney and glomerulonephritis, the family history and clinical features can help to distinguish recessive nephronophthisis from dominant nephropathies, but the differential diagnosis is still difficult due to phenotypic overlap.
Aim of this study is to develop and validate a clinical and molecular diagnostic algorithm, useful to drive the genetic screening. DNAs from 30 affected patients were submitted to direct sequencing of *NPHP5*, *UMOD*, *REN* and *HNF1B* genes. Furthermore, deletion analysis by multiplex PCR for *NPHP1* and MLPA for *HNF1B* were performed.
Six causative mutations were identified. A novel heterozygous p.E48K mutation in *REN* was found in a familial case of cystic nephropathy, hyperuricemia, hyperkalemia and anemia. The homozygous p.R489X mutation in *NPHP5* gene was detected in a patient with retinitis pigmentosa and recurrent cholangitis (Senior-Locken Syndrome). The heterozygous p.R295C variant in *HNF1B* was identified in a patient with renal failure and diabetes; this mutation is known to alter the DNA binding domain. The homozygous deletion of multiple exons in *NPHP1* gene was identified in a patient with renal medullary cysts. Heterozygous whole gene deletions of *HNF1B* were detected in a patient with medullary cysts and renal hypoplasia and in a patient with diabetes and cholestasis. Our data confirm the high genetic heterogeneity of HKD and indicate the need to broaden the genes to be screened, a goal that could be achieved thanks to NGS technology.

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P04.44

Nephrogenic diabetes insipidus due to a novel AVPR2 mutation. A case report.

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Nephrogenic diabetes insipidus (NDI) is characterized by poliuria, polydipsia, recurrent bouts of fever and acute hypernatremic dehydration after birth that may cause neurological sequelae. Prevalence is estimated at 1-2/1000000. The disease results from the failure of the renal tubules to respond to antidiuretic hormone. In most cases, the disease is X-linked recessive (90%) and caused by mutations in the gene located on Xq28, coding for the V2 receptor of antidiuretic hormone. AVPR2 is the only gene in which mutations are known to cause X-linked NDI. NDI can also be inherited in an autosomal recessive (9%) or autosomal dominant manner (1%).
We describe the case of a 12 months-old boy came to our observation for persistent fever, hypernatriemia (sodium 159 mmol/L), hyperchloremia, hyperazotemia and a family history of polyuria and polydipsia (mother, maternal aunt and maternal grandmother). At the physical examination the child showed discrete clinical conditions and febrile temperature, marked hypotonia and poor responsiveness to external stimuli, delayed motor development and failure to thrive.
After liberalization of water intake, we have seen the disappearance of fever and hypotonia, restarting of normal caloric intakes and spontaneous normalization of sodium and other electrolytes. At this point, after having achieved a normal hydration we observed polydipsia (1330 cc/die), polyuria (138 ml/Kg/die) and dilute urine (specific gravity <1.010).
Sequencing analysis of AVPR2 gene showed: c.738dupG, p.Arg247Alafs*12. The condition of heterozygous carriers of this duplication was found also in mother, aunt and grandmother: c.[738dupG];[=], p.[Arg247Alafs*12];[(=)].
The mutation that we found has never been described before.

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P04.45

NPHS2 mutations in steroid-resistant nephrotic syndrome: a mutation update and the associated phenotypic spectrum

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Mutations in the human NPHS2 gene encoding podocin are implicated in an autosomal recessive form of non syndromic steroid-resistant nephrotic syndrome (SRNS) in both pediatric and adult patients. Patients with homozygous or compound heterozygous mutations commonly present with SRNS before the age of 6 years and rapidly progress to end-stage kidney disease with a low prevalence of recurrence after renal transplantation. Here we reviewed all the NPHS2 mutations published between 2000 and January 2013, and we also reviewed all mutations described in our personal cohort in Necker hospital. In summary we identified 5 new pathogenic mutations in addition to the 106 already described. The mutations are distributed along the entire coding region and lead to all kinds of alterations including 44 missense, 13 nonsense, 10 small insertions, 22 small deletions, 9 splicing, 2 indel mutations and 1 mutation in the stop-codon. In addition to the 101 likely pathogenic mutations, 36 variants were classified as variants of unknown significance, as these missense changes were not predicted to be deleterious by the Polyphen 2 software and/or variants that were exclusively described in the heterozygous state. Genotype-phenotype analyses established correlations between some variants and age at onset, ethnicity and clinical evolution. Based on this review, we are creating a web database using the Leiden Open Variation Database (LOVD) software that lists all identified variants and will allow the inclusion of future reports.

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P04.46

Bladder exstrophy and other urological defects associated with deletions in 2p14p15 involving OTX1

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Development of the genito-urinary (GU) tract is intricate and defects include cryptorchidism (1:150), hypospadias (1:200), micropenis (1:300), vesicoureteral-reflux (VUR) (1%) and bladder-exstrophy-epispadias (BEE) (1:45000). Using array-comparative-genomic-hybridization (aCGH) we identified five individuals with deletions in the 2p14p15 region (66.0kb-5.6Mb) that include the homeodomain transcription factor orthodenticle-homolog-1 (*OTX1*). Case #1 (BEE and VUR) has the smallest deletion (66kb) encompassing only *OTX1* and was identified from 30 BEE patients using Nimblegen-3x720-aCGH and validated by Agilent-aCGH and CNV-Taqman-assays. Cases #2-5 were identified among 30,183 subjects submitted to Signature Genomics for clinical aCGH testing and had deletions validated by FISH. Cases #2-5 had *de novo* large CNVs (2.39-5.70Mb) and exhibited variable features similar to the 2p15p16.1 microdeletion syndrome including developmental delay, short stature, facial abnormalities, and GU defects. Different GU abnormalities in patients with the same gene defect are not rare. GU defects in our patients include testicular (3/5), penile (3/5), kidney (2/5), bladder (1/5), and VUR (1/5). *Otx1* null mice have prepubescent transient growth retardation and gonadal defects attributed to low levels of pituitary hormones. Two patients have short stature and three have gonadal defects. The presence of GU defects in four of our cohort and in seven of 2p15–p16.1 syndrome cases (one of them has OTX1 deleted) suggest this region encodes genes important for GU development. As conclusion, impairment of OTX1 function may lead to GU tract abnormalities as common as cryptorchirdism to as rare as BBE.

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P04.47

On the origins of renal cell carcinoma, vesicoureteric reflux and C (Opitz trigonocephaly) syndrome: A complex puzzle revealed by the sequencing of an inherited t(2;3) translocation.

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The co-incidence of an inherited balanced translocation through 2q37.3 in patients who developed renal cell carcinoma (RCC) with the finding of genetic linkage in the same chromosomal band in a study of vesicoureteric reflux (VUR), an inherited developmental disorder, suggested the possibility that there might be a gene involved in urinary tract development in that band that could account for both findings. We have identified the breakpoints. On 3q, the gene *CD96* is bisected in an intron, while on 2q the break-point is in an intergenic region 103 kb from *CXCR7*. *CXCR7* expression has been reported to be elevated in > 50% of renal cell carcinomas and the gene is also involved in kidney development. This raises the possibility that the t(2;3) translocation brought an enhancer of CD96 into proximity to *CXCR7*, causing it to be misregulated, leading to a risk of RCC. Mutation of *CD96* was thought to be the cause of some cases of C Syndrome because a t(3;18) translocation was found in a case. The translocation bisected *CD96* and no gene was found at the breakpoint on chromosome 18. However, this is now shown not to be true, because none of the patients with t(2;3) have any features of C Syndrome. The other breakpoint in the C Syndrome case was in 18q12.1, which is now known to contain the desmocollin and desmoglein gene clusters, responsible for tight junction formation in epithelia, and it seems likely the mis-regulation of these genes is the true cause of the syndrome.

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P04.48

Identification of Homozygous Mutations in two Alpha Integrin Encoding Genes in Fetuses with Severe Kidney Development Defects

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Renal hypodysplasia (RHD) is a heterogeneous condition encompassing a spectrum of kidney development defects responsible for pediatric end-stage renal disease and mortality. The occurrence of familial and syndromic cases indicates the existence of a genetic component.
In order to identify genes involved in recessive RHD, we analysed fetuses belonging to consanguineous families by whole exome sequencing. In two families, we identified homozygous mutations in integrin encoding genes that play a crucial role during kidney development: a missense variation in ITGA3, predicted as damaging by Polyphen2 and Sift, in a family with two fetuses with multicystic renal dysplasia and a splicing mutation in ITGA8 in two fetuses from a family with bilateral renal agenesis. These mutations were absent from SNP and in-house databases. Screening of fetuses with similar phenotypes did not allow us to identify any other ITGA3 or ITGA8 mutation.
By reexpressing the mutated ITGA3 protein in MDCK cells, we demonstrated that the L518P mutation alters epithelial morphogenesis (3D culture) and cell adhesion on laminin 5. We also showed that the ITGA8 mutation led to skipping of exon 28, resulting in a protein with in frame deletion of 33 aminoacids.
This is the first report of human mutations in ITGA3 and ITGA8 associated with RHD. However, ITGA3 mutations have been reported in association with another renal pathology, congenital nephrotic syndrome. Experiments are in progress to formally demonstrate the causative effect of these mutations in RHD and to understand the pathophysiological basis of the phenotypic variability associated with ITGA3 mutations.

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P04.49

The MEFV mutation profiles and SAA1 gene BcII polymorphism in chronic renal failure patients that requiring long-term haemodialysis in Turkish population

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Background and Aim: There is an increased mortality risk in chronic renal failure(CRF) patients taht requiring long-term haemodialysis due to the chronic inflammation. The relationship between chronic kidney failure and role of familial genetic markers remains incompletely understood. In the current study it was aimed to find out the prevalence of MEFV mutation profiles and serum amiloid A1 (SAA1) genes BcII polymorphism in CRF patients that requiring long-term haemodialysis. Method: Current cohort includes 242 CRF patients and 245 healthy individuals from the same population. Total genomic DNA was isolated from peripheral blood- EDTA and MEFV mutation analysis was carried out by reverse hybridization StripAssay and real-time techniques. The SAA1 gene was genotyped by BclI/RFLP method. Results: Various common MEFV mutations were detected in high portion of (38%) CRF patients. The most frequent mutations were E148Q and M694V respectivelly. The increased T allele frequency was detected in CRF patients when compared to the health individuals from the same population. Conclusions: The current results indicate that germ-line mutations in both genetic biomarkers (MEFV and SAA1 genes) play crucial role in CRF pathogenesis due to the long-term chronic inflammation in the current CRF cohort.

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P04.50

Autosomal Recessive Steroid Resistant Nephrotic Syndrome (SRNS) in a three generation family: Expanding the genotype:phenotype correlation

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SNRS type 2 (OMIM: 600995), is an autosomal recessive condition characterized by proteinuria, hypoalbuminemia, hyperlipidemia, and oedema. It is resistant to steroid treatment and usually progresses to end-stage renal disease before the second decade. Approximately 20-30% of cases are caused by recessive mutations in the NPHS2 gene1.
We describe a family with three generations of individuals with compound heterozygous mutations in NPHS2. Two individuals developed SNRS in childhood, while one is clinically well.
The proband was diagnosed with SRNS aged 1yr. She was referred to Genetics as her father had the same presentation, requiring kidney transplant at 10yrs. Genetic testing confirmed that both were compound heterozygotes for mutations R138Q and Q215X in the NPHS2 gene. The mother was found to be heterozygous for R138Q mutation. Cascade screening revealed that the paternal grandmother carried the Q215X, and the unaffected paternal grandfather was a compound heterozygote for R138Q and R229Q.
The R138Q mutation has been found in approximately 1/3 of familial cases, and is thought to be a founder mutation in Europe 1,2. It is associated with early onset of disease3. Q215X results in premature termination of the protein4. The R229Q is a common polymorphism with allele frequency of 3.6%, although it can lead to a late onset form in patients who are compound heterozygotes for R229Q and another mutation4.
This case is a striking example of how genetic testing is invaluable to accurately counsel a family with an autosomal recessive condition, which initially appears to be an autosomal dominant pedigree.

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P04.51

Urofacial syndrome is a genetically and phenotypically heterogeneous condition caused by recessive mutations in HPSE2 and LRIG2.

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Urofacial syndrome (UFS) or Ochoa syndrome is a rare autosomal recessive condition characterised by voiding dysfunction of the urinary bladder with a significant risk of childhood renal failure. Most affected individuals have an abnormality of facial movement on expression which distinguishes the condition from other forms of urinary voiding dysfunction.
Previously, we reported that the majority of cases of UFS are caused by biallelic, loss-of-function mutations in *HPSE2*, which encodes heparanase 2. We present an expanded *HPSE2* mutation spectrum for previously unreported cases of UFS. Further, through exome sequencing and autozygosity mapping, we report that UFS is also caused by biallelic mutations in *LRIG2* in three unrelated families. *LRIG2* encodes the leucine-rich repeats and immunoglobulin-like domain 2 protein (LRIG2). The lack of *HPSE2* or *LRIG2* mutations in some cases of classical UFS provides evidence of further genetic heterogeneity.
The urinary tract features of UFS are indistinguishable from non-syndromic forms of voiding dysfunction. We demonstrate that novel missense variants in *LRIG2* may be associated with non-syndromic bladder dysfunction.
Little is known about the functional roles of heparanase 2 and LRIG2. However, both LRIG2 and heparanase-2 were immunodetected in nerve fascicles growing between muscle bundles within the human fetal bladder, directly implicating both molecules in neural development in the lower urinary tract.

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P04.52

The mutational analysis of the ACTN4 gene in patients with focal segmental glomerulosclerosis using HRM method

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Nephrotic syndrome (NS) is characterized by proteinuria, hypalbuminemia and edemas. There are four most important genes that condition the formation of hereditary nephrotic syndrome in adult patients (ACTN4, CD2AP, NPHS2 and TRPC6). The gene ACTN4, which encodes protein α-actinin 4, is responsible for the autosomal dominant form of focal segmental glomerulosclerosis (FSGS).
The mutational analysis of the gene ACTN4 was performed on the set of 48 patients with FSGS/MCD. To investigate the prevalence and possible effect of some substitutions found in FSGS/MCD patients we were also looking for these changes in 154 patients with IgA nephropathy (IgAN) and 55 patients with membranous glomerulonephritis (MGN). 200 unrelated healthy males and females without history of renal disease or abnormal urinary findings were studied as controls. High resolution melting (HRM) analysis and sequencing selected samples were used during this mutation detection.
It was found 20 exonic and intronic substitutions in the set of patients with FSGS/MCD. We found unpublished substitution 2242A>G (p.Asn748Asp) that could have causal associations with FSGS. This change was identified in 59 years old woman with FSGS and positive family history. This substitution was found neither in healthy controls nor in patients with IgAN and patients with MGN. Exon 19 seems to be a variable region due to amount of found polymorphisms. In this exon we also found unpublished substitutions c.2351C>T (p.Ala784Val), c.2378G>A (p.Cys793Tyr) and c.2393G>A (p.Gly798Asp). These changes were neither in healthy controls nor in patients with FSGS/MCD and patients with MGN.
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P04.53

Autosomal recessive hypophosphatemic rickets type 2 (ARHR2) and hypertension due to novel compound heterozygous mutations within the *ENPP1* gene

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Hypophosphatemic rickets is a heterogeneous group of renal phosphate wasting disorders caused by elevated circulating fibroblast growth factor 23 (FGF23) which inhibits phosphate reabsorption and 1,25-dihydroxyvitamin D synthesis in the proximal renal tubules. Mutations in any of the five known genes - *PHEX* (XLH [MIM 307800]), *FGF23* (ADHR [MIM 193100]), *SLC34A3* (HHRH [MIM 241530], *DMP1* (ARHR1[MIM 241520]), and *ENPP1* (ARHR2 [MIM 613312]) causes hypophosphatemia. We report a 13 years old boy with a late onset of rickets, who presented with progressive bone deformity, genu valgus and bone pain since the age of ten years. In addition the patient had bilateral conductive hearing deficit and hypertension. Hypertension was related to hyperreninemia following stenosis of the right renal artery due to myointimal proliferation. Biochemical investigations showed an elevated alkaline phosphatase activity, decreased serum phosphate levels and an elevated FGF23 level. Autosomal dominant hypophosphatemia was excluded by sequence analysis of the *FGF23* gene. Due to the more complex phenotype of the patient we suggested sequencing of the *ENPP1* (ectonucleotide pyrophosphatase/phosphodiesterase 1) gene. Sequence analysis of *ENPP1* revealed one heterozygous non-synonymous single nucleotide variation (SNV) in exon 2 (NM_006208.2:c.275G>A, NP_006199.2:p.Gly92Asp) and one heterozygous splice site mutation in intron 21(NM_006208.2:c.2230+1G>A) of the *ENPP1* gene. Both SNVs were not found in databases of sequence variations (dbSNP137) and of mutations (HGMD), and within 1846 in house exomes. We identified two novel compound heterozygous mutations within the *ENPP1* gene in a boy with autosomal recessive hypophospatemia and a concomitant mild phenotype of generalized arterial calcification of infancy.

K. Kapelari: None. **B. Lorenz-Depiereux:** None. **T. Strom:** None. **E. Steichen-Gersdorf:** None.

P04.54

Mutations in a large VNTR of MUC1 are frequent in autosomal dominant medullary cystic kidney disease (MCKD)

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MCKD is a rare disorder characterized by autosomal dominant tubulo-interstitial kidney disease (TIKD), sometimes associated with renal cysts and gout/hyperuricemia. UMOD mutations have been described in patients with hyperuricemia (MCKD2). Mutations in the MUC1 gene encoding mucin 1 were very recently identified in MCKD1 patients (Kirby et al, Nature Genet, 2013) using a sophisticated approach associating cloning of the genomic region, capillary sequencing and de novo assembly since next generation sequencing failed to identify them. All patients harbor a single cytosine insertion in one copy of the 60-base repeat unit of the long CG-rich coding VNTR of MUC1. The frameshift is predicted to result in a mutant protein which is expressed in the distal tubule and collecting duct of affected individuals.
Using an approach coupling PCR amplification of MwoI digestion (which selectively cleaves the reference sequence) products and primer-extension assay (SNaPshot Multiplex Kit), we tested 21 new families with clinical information for 67 patients but linkage data in only 2 families.
50 pts reached ESRD at a mean age of 46 +/-4 yrs (20-82 yrs) with high

intrafamilial variability. Early gout occurred in only 2 patients and hyperuricemia was generally absent or related to CRF. Renal cysts were present in 10/28 pts.
We detected the presence of the cytosine insertion in 11/21 families. Futhermore, we detected an additional mutation made of a 5C deletion, which is predicted to result in the same mutant protein as the cytosine deletion. These results show that MUC1 mutations represent a frequent cause of MCKD.

V. Moriniere: None. **A. Mégarbané:** None. **S. Lebbah:** None. **A. Gnirke:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; inventors on the C-insertion genotyping assay under patent review, filed by the Broad Institute. **B. Blumenstiel:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; inventors on the C-insertion genotyping assay under patent review, filed by the Broad Institute. **A. Bleyer:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; inventors on the C-insertion genotyping assay under patent review, filed by the Broad Institute. **A. Kirby:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; inventors on the C-insertion genotyping assay under patent review, filed by the Broad Institute. **B. Knebelmann:** None. **C. Antignac:** None.

P04.55

Thin basement membrane nephropathy due to heterozygous *COL4A3/COL4A4* mutations is a more frequent cause of end-stage kidney disease compared to Alport syndrome

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Alport syndrome (AS) is a severe hereditary hematuric nephritis, associated with deafness, eye defects and early progression to end-stage kidney disease (ESKD). About 85% of all AS cases are X-linked due to *COL4A5* mutations (XLAS) and the rest are autosomal recessive due to *COL4A3/COL4A4* mutations (ARAS). Thin basement membrane nephropathy (TBMN) is the leading cause of familial microscopic hematuria (FMH) worldwide and is mostly explained by heterozygous *COL4A3/COL4A4* mutations. We recently showed that TBMN should not be considered as a benign condition, since about half of these patients progress to chronic renal failure after the age of 50 years.
We have studied, genetically and clinically, nine Greek-Cypriot AS families referred to public hospitals: four XLAS families with nine living patients (71%) and five ARAS families with four living patients (29%). *COL4A5*-P628L was found in two of the XLAS families. Only six of these living patients reached ESKD. We have also studied a large number of FMH families. We have found a heterozygous *COL4A3* or *COL4A4* mutation in 213 living patients in 27 families. Mutation *COL4A3*-G1334E was found in 16 families and it accounts for 155 patients. Of these 213 patients, 21 have reached ESKD. Interestingly, we observe that Greek-Cypriots patients with ESKD due to TBMN (21 patients) outnumber by 3.5 times those who reach ESKD due to AS (6 patients). This observation demonstrated again that TBMN is not a benign condition, as usually mentioned in the previous literature. Further investigations are needed in other populations to confirm this epidemiological finding.

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P04.56

Heapatitis C infected patients and liver expression of miR-122, miR-126, miR-136 and miR-181a

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For hepatitis C virus (HCV) it was shown that host cellular microRNA (miRNA), *miR-122*, facilitates replication of viral RNA genome by binding to its 5'-UTR. With respect to the findings *in vitro*, the clinical impact of hepatic *miR-122* on HCV in humans is still largely unclear and little is known about the role of other host/cellular miRNAs in viral infection. We therefore analysed expression of *miR-122*, *miR-126*, *miR-181a* and *miR-136* in HCV infected patients.
Study included liver biopsies of 65 patients infected with HCV of different genotypes (gt: gt1, gt1a, gt1b, gt3 and gt4) and 9 non-infected individuals. Expression analysis of *miR-122*, *miR-126*, *miR-136* and *miR-181a* was performed by qPCR and statistically analysed for differences between gender and age of patients, genotypes, stage of fibrosis, grade of inflammation, serum level of liver enzymes, serum viral load, and presence of steatosis and mode of transmission.
Statistical analyses revealed differential expression of *miR-122*, *miR-126*, *miR-136* and *miR-181a* in HCV infected patients relative to non-infected individuals, between genotypes, and gender. Their expression was associated with the degree of fibrosis and with the grade of inflammatory activity, and correlated to the presence of steatosis of the liver, to the mode of transmission, and to the serum HCV load.
Recent advances in the relationship between HCV infection and miRNAs showed that other miRNAs may be as important as *miR-122* in the fight against HCV. Here we show, that *miR-126*, *miR-181a*, and *miR-136*, might also play a role in HCV infection.

E. Bostjancic: None. **E. Bandelj:** None. **B. Luzar:** None. **M. Poljak:** None. **D. Glavač:** None.

P04.57

Relationship between response to colchicine treatment and MDR1 polymorphism in FMF patients

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Familial Mediterranean Fever (FMF) is a disease, which is encountered with recurrent acute inflammatory episodes. Colchicine, which is used in FMF treatment, prevents leukocyte chemotaxis and collagen migration into the extracellular space. Multi Drug Resistance 1 (MDR1) gene codes for a significant membrane protein, and this controls xenobiotic passage-transport. We investigated the relationship between MDR1 C3435T polymorphism and colchicine response in FMF patients.
Patients were divided into three groups according their responses. Patients, who recovered from episodes with standard colchicine treatment, and had no attack in the last 1 year, were accepted as Complete Responders. Patients, whose episode number and intensity were decreased, but ongoing with the standard treatment, were accepted as "Partial Responders". Patients, whose episodes were not decreased despite the standard treatment, were accepted as "Non-responders". MDR1 C3435T genotype was defined by RT-PCR method.
MDR1 C and T allele frequencies of our FMF patients with colchicine responses of complete, partial and unresponders were C= 0.75 and T= 0.25 in complete responders; C= 0.56 and T=0.44 in partial responders; and C= 0.50 and T= 0.50 in non-responders. While T allele frequency was the lowest in complete responders to colchicine, it was the highest in the non-responders.
According to our results, Multi Drug Resistance 1 gene C3435T polymorphism acts an important role on colchicine response in FMF patients good response to colchicine treatment was related to C allele, whereas poor response was related to T allele in FMF patients.

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P04.58

Mechanism of MDR3 dysfunction in PFIC3 patients

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BACKGROUND: Progressive familial intrahepatic cholestasis type 3 (PFIC3) is a rare liver disease which caused by mutation of the ATP-binding cassette, subfamily B, member 4 gene (*ABCB4*) also known as multidrug resistance 3 (*MDR3*). MDR3 is hepatocellular canalicular transporter associated with not only biliary secretion of phosphatidylcholine but also efflux of drugs and xenobiotics. This study was conducted to investigate the functional characterization of several variants of *MDR3* found in PFIC3 patients.
METHODs: Thirteen nonsynonymous *MDR3* variants found in PFIC patients were selected. Transport activity of each variant was measured through ATP-dependent uptake of paclitaxel using plasma inside-out membrane vesicles. To determine the mechanism through which *MDR3* variants change transport activity, cell surface biotinylation, immunoblotting and immunofluorescence were performed.
RESULTs: Six variants (M1, M5, M7, M8, M11 M12, M13) of *MDR3* showed a significant reduction in the transport activity, compared to the reference (p<0.05). Through cell surface biotinylation, we found that M1 and M11 showed altered protein size and the others showed decreased cell surface expression levels (p<0.05). In particular, three of them (M5, M7, M8) resulted

in decreased total protein levels in immunoblotting (p<0.05) and this result was confirmed by immunofluorescence.
CONCLUSION: Our studies revealed several *MDR3* variants result in changes in the transport activity of MDR3. Decreased transport activity of each variant can be explained by protein truncation (M1, M11), decreased total protein levels (M5, M7, M8), or impairment of membrane trafficking (M12, M13).

T. Kim: None. **J. Kim:** None. **J. Choi:** None.

P04.59
Impact of ABCB4 mutations on 90 Italian adult patients with cholestatic syndromes

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ABCB4 gene encodes for MDR3 protein; its mutations may impair biliary phosphatidylcholine secretion and are well-known as causes of Progressive-familial intrahepatic-cholestasis-type-3 (PFIC3) in children, but their clinical impact in adults with cholangiopathies remains ill-defined.
We consecutively evaluated 2602 subjects with hepatobiliary-disease and 90 underwent sequencing analysis of each of the 27 ABCB4 coding exons including the adjacent intronic regions; 37 had clinical diagnosis of Primary-Sclerosing-Cholangitis (PSC) of which 14 with a concomitant hepatobiliary disease (e.g, Juvenile Cholelithiasis -JC-, Intrahepatic Cholestasis of Pregnancy -ICP-), 17 had Primary-Biliary-Cirrhosis (PBC), 24 had Idiopathic-Cholestasis (IC), 1 had Autoimmune-Hepatitis and 1 had Overlap-Syndrome. Moreover, 4 subjects had experienced ICP and 6 had medical history of JC. We identified 15 different nucleotide changes that have an effect on the primary protein structure, 7 not described previously (p.E616K, p.G634E, p.G811R, p.S849YfsX24, p.L859W, p.M948I and p.Q1181X) and 8 already identified in children and adults with different cholangiopathies; the new nucleotide changes were not detected in 112 ethnically matched healthy subjects or reported as polymorphisms in dbSNP database. A single heterozygous mutation was identified in 15 and two mutations were identified in 3 subjects; among them, 6 had PSC of which 4 (4/14, ≈29%) with concomitant hepatobiliary disease, 3 (≈18%) had PBC, 4 had IC (≈17%), 2 (50%) had ICP and 3 (50%) JC. In summary, single heterozygous mutations in ABCB4 are associated to different cholangiopathies in a relevant percentage of adults, including the phenotype PSC when, in particular, JC and/or ICP are described within the clinical history.

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P04.60
Segmental paternal isodisomy as a cause of juvenile hemochromatosis type 2a

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Juvenile hemochromatosis (JH) represents a group of disorders characterised by iron overload causing liver injury, cardiomyopathy and endocrine abnormalities. Here we describe the genetic basis of JH caused by a homozygous nonsense mutation in *HJV* in a non-consanguineous family.
Hemochromatosis is generally caused by mutations in *HFE*. Recessive forms of JH are associated with mutations in *HAMP, HJV* and *TFR2*. Testing for the prevalent mutations C282Y, H63D, S65C in *HFE* revealed only the heterozygous state for H63D. No other predictably pathogenic variation was detected by *HFE* sequencing. With regard to the patient's age, *HAMP*, *HJV* and *TFR2* were analyzed. A known homozygous mutation c.196G>T, predicted to introduce premature stop codon (p.G66*), was found in *HJV*. The mutation was present only in the patient´s father who turned out to be a heterozygote. Neither MLPA nor real-time PCR revealed copy number variations in the *HJV* gene region. Fragment analysis of six STS markers indicated loss of heterozygocity (LOH) in the middle third of chromosome 1. LOH spanning the region chr1:86,020,000-162,505,000 was confirmed by genomic hybridisation on the Affymetrix Genome-wide human SNP 6.0 array. The patient´s genotypes at the overwhelming majority of the examined SNP loci outside of the LOH region were identical with those on both paternal chromosome 1 alleles.
Conclusion: JH in our patient is caused by duplication of the mutated *HJV* allele due to paternal disomy combined with 1/3 segmental isodisomy. This highlights the importance of testing clinically unrelated parents of homozygotes suffering from autosomal recessive diseases such as JH.

M. Neroldova: None. **S. Frankova:** None. **V. Stranecky:** None. **E. Honsova:** None. **O. Luksan:** None. **M. Benes:** None. **K. Michalova:** None. **S. Kmoch:** None. **M. Jirsa:** None.

P04.61
Viral hepatitis C infection influencing host genetic factors

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Objectives: susceptibility to viral hepatitis C and it clinical outcome could be influenced by patients mutations in detoxification enzyme coding genes and most common monogenic liver diseases.
Material and methods: 74 males and 40 females with chronic viral hepatitis C infection, age 41±13 years, 81 of them underwent antiviral treatment, 16 of them had SVR. From biochemical analysis available were level of iron, ferritin, ALT before and in time of treatment, viral load, and treatment outcome. 300 healthy individuals represented control population (195 males and 105 females) at age 21±3 years. For included individuals were tested mutations in the genes: *GSTP1* (A333G), *GSTA1* (C69T), *MTHFR* (C677T), *HFE* (C282Y, H63D), *UGT1A1* ((TA)7), *ATP7B* (H1069Q) by standard molecular methods.
Results: mutation frequencies in patient group (control group) were as follows: A333G= 0.238 (0.327, p>0.05), C69T=0.318 (0.349, p>0.05), C667T=0.297 (0.359, p>0.05), C282Y=0.053 (0.035, p>0.05), H63D=0.118 (0.121, p>0.05), (TA)7=0.384 (0.35, p>0.05), H1069Q=0.048 (0.013, OR=3.77, p=0.05). With biochemical markers association was found for ferritin with alleles (TA)7 (DOMDEV model, r^2=-289.1, p=0.005) and C282Y (ADD model, r^2=499.3, p=0.02), for ALT before treatment with allele A333G (ADD model, r^2=-36.33, p=0.027), for ALT in treatment with alleles C677T (ADD model, r^2=-23.62, p=0.05) and C282Y (ADD model, r^2=67.21, p=0.03), viral load before treatment with - C677T (ADD model, r^2=0.9, p=0.036).
Conclusions: (TA)7, C282Y, A333G and C677T alleles has impact on inflammation severity in liver during hepatitis C viral infection. C282Y and H1069Q alleles could be influencing susceptibility to hepatitis C virus.

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P04.62
Identification of rare variants in patients with isolated biliary atresia by exome sequencing.

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Biliary atresia (BA) is a severe pediatric liver disease resulting in necro-inflammatory obliteration of the extrahepatic biliary tree and presenting within the first few months of life. The etiology of BA is unknown, with evidence for infectious, environmental, and genetic factors described. Possible genetic risk factors include a BA associated locus flanked by *XPNPEP1* and *ADD3* revealed by GWAS, a report of *JAG1* missense mutations in a small percentage of patients, and a BA-associated deletion on 2q37.3 revealed by CNV analysis. Animal models have suggested candidate genes, but mutations have not been found in humans until now. We performed exome-sequencing on 30 Caucasian, non-syndromic BA patients to further investigate the genetic basis of BA. Exomes were captured using Agilent All Exon V5 71MB kit and sequenced with the Illumina HiSeq platform. The 100bp paired-end reads were aligned to GrCH37 using BWA and processed using Picard and SAMtools. Variants were called using GenomeAnalysis ToolKit. There were

a total of 4,938 loss-of-function or non-synonymous variants in these samples. We filtered across the 1000 Genomes Phase I data and NHBLI Exome Sequencing Project datasets to investigate variants that occur in less than 1% of these populations. We found one likely damaging variant in both *ZEB2* and *ZIC3*, genes previously reported in patients with BA findings. We also found five likely damaging variants across several candidate genes such as *ADD3* and those implicated by animal models of BA. Our results suggest that genetic susceptibility to BA is likely to be highly heterogeneous.

E.A. Tsai: None. **C.M. Growchowski:** None. **L.D. Leonard:** None. **K.M. Loomes:** None. **B.A. Haber:** None. **N.B. Spinner:** None. **M. Devoto:** None.

P04.63

Potential role of ISV-8 poly-T variants of CFTR gene in recurrent idiopathic pancreatitis: A case series of four children

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Background: The 5T variant is a stretch of five contiguous thymidines at the 3' of the intron 8 of CFTR gene that exacerbates skipping of exon 9, resulting in reduced levels of functional CFTR protein. This process seems to be influenced by the number of TG repeats immediately adjacent to 5T. Individuals carring 5T adjacent to either 12 or 13TG repeats are more likely to exhibit an abnormal phenotype (non-classic cystic fibrosis, recurrent pancreatitis) than those with 5T adjacent to <12TG.
Materials and methods: We describe the cases of 4 children (aged 12, 10, 6 and 3 years) affected by recurrent idiopathic pancreatitis. The diagnosis of pancreatitis was made by the presence of typical abdominal pain, serum amylase and/or lipase 3 times greater than the upper limit of normal and characteristic imaging findings. Sequence analysis of PRSS1, SPINK1 and CFTR genes was performed.
Results: Sequence analysis showed the absence of mutations in PRSS1 and SPINK1 genes.
CFTR gene sequencing in three boys showed the presence of following polymorphisms: 5T/5T-12TG/12TG; 5T/5T-11TG/12TG; 5T/9T-13TG/11TG.
The 3-years old girl with the most severe form of idiopathic pancreatitis showed 5T/7T-13TG/9TG. The 5T allele, in *cis* with 13TG, was associated in *trans* with F508del, a severe CFTR mutation.
Conclusions: The high prevalence of 5T carriers makes the assessment of the TG repeat number of great interest as a reliable predictor of the 5T penetrance. Further studies comparing the prevalence of the 5T-poly(TG) haplotypes to the general population are needed to define their role in pancreatic disease.

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P04.64

Novel IL-10RA splice-site mutation causes autosomal recessive inflammatory bowel disease

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Ulcerative colitis and Crohn's disease are chronic inflammatory bowel diseases (IBDs), rarely expressed in an early onset form (EO-IBDs). These early onset forms are characterized by a particularly severe and predominantly pancolitic inflammation, and have been shown to be caused by mutations in IL10RB (IBD25 , MIM123889) and IL-10RA (IBD28 , MIM146933). Eight individuals of a large Bedouin kindred in southern Israel presented with an autosomal recessive phenotype of EO-IBD. Homozygosity of affected individuals and heterozygosity of obligatory carriers was assayed at the IBD25 and IBD28 loci: while there was no association with IBD25, polymorphic markers at the IBD28 locus demonstrated full segregation with the disease phenotype. Sequencing of IL10RA revealed a novel homozygous point mutation. IL-10 is an anti-inflammatory cytokine secreted by a variety of cell types and is critical for maintaining immune homeostasis in the gastrointestinal tract. The mutation is synonymous, but as it is located at the first nucleotide of a conserved splice donor site, it is essential for splice-site recognition. Sequencing of an affected individual's cDNA revealed that destruction of the natural splice site led to the absence of 18 nucleotides of the coding region of IL-10RA, eliminating 6 amino acids. The mutation was not found in the 1000 Genomes Project, NHLBI Exome Sequencing Project or in any of 200 control chromosomes of Bedouin controls.

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P04.65

Two deletions on chromosome 16p13.3 cause autosomal recessive intractable diarrhea of infancy syndrome

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Intractable diarrhea of infancy syndrome (IDIS) is a non-infectious diarrhea lasting more than 2 weeks. Onset is observed within the first few weeks of life leading to malabsorption and failure to thrive, and is associated with great suffering and high mortality. We have encountered 6 IDIS patients from different families of Iraqi jewish origin. SNP analysis together with Exome sequencing detected two different deletion alleles, ΔS and ΔL. Performing long range PCR we established the exact boundaries for the two deletions, which share 1476bp, on the short arm of chromosome 16 (16p13.3).
Using multiplex PCR assays, we determined the form in which these deletions appear in all 6 patients . Full segregation was found among the Iraqi patients with autosomal recessive patterns of inheritance, while the deletions were not found in 200 controls. The shared 1476bp deletion does not encompass any known genes; however, it does include a predicted enhancer. It is possible that the enhancer's target is a transcription factor controlling a developmental event crucial for normal gastrointestinal function. To test this hypothesis, we assessed for the expression of the 1476bp region in a transgenic mouse model and observed reproducible enhancer activity in the stomach, duodenum and pancreas of mouse embryos at days 11.5, 12.5, and 13 not later and neither in grown mice. To further study these deletions we amplified candidate genes around the deletion, but so far we have not observed any differences between patient and control cDNAs.

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P04.66

Toward the genetic basis of oesophageal atresia: clinical and molecular study by next generation sequencing

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Oesophageal Atresia (OA) consists in the interruption of the continuity of the oesophagus sometimes associated with a persistent communication with the trachea (tracheo-oesophageal fistula). Its incidence is 1 in 3500 births, with associated anomalies - cardiac malformations being the most common - present in around half of the cases (syndromic OA). It's aetiology is still unknown: twin and family studies have suggested that genetic factors don't play a major role; however, at least 3 genes are involved in the etiology of syndromic OA: NMYC in Feingold syndrome, SOX2 in anophthalmia-esophageal-genital syndrome and CHD7 in CHARGE association. Moreover, microdeletions of the FOX gene cluster (FOXF1, MTHFSD, FOXC2, FOXL1) were reported to cause a phenotype resembling VACTERL association.
We studied a cohort of 10 subjects with OA, firstly excluding genomic imbalances by array-CGH analysis and later analysing 9 genes, potentially involved in the disorder, by next generation sequencing. Selected genes were those reported as associated with syndromic forms of OA, and those described in literature as potentially causative. We found 50% of cases with predicted damaging variants, among which two in DST, encoding a protein component of desmosomes that anchors intermediate filaments to desmosomal plaques; and two in DSP, encoding a protein member of the plakin protein family of adhesion junction plaque proteins. These mutations were not present in dbSNPs135 nor in the Exome Sequencing Project. Our initial results suggest that, even in a multifactorial condition, high-throughput sequencing

can identify genes that could allow to better understand the pathogenesis of the disease.

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P04.67

Recurrence of Hirschsprung disease due to maternal mosaicism of a novel *RET* gene mutation

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Hirschsprung disease (HSCR, MIM 142623) or aganglionic megacolon is the most frequent genetic cause of congenital intestinal obstruction. Several genes have been implicated in isolated HSCR. Inactivating mutations of *RET*, transmitted in an autosomal dominant fashion with incomplete and sex-dependent penetrance, are identified in about 50% of familial cases.We report a family with recurrence of long-segment HSCR in two siblings. Mutation analysis of *RET* performed by PCR and direct sequencing identified a heterozygous truncating mutation in both
siblings. The same mutation was identified in the unaffected mother but the electropherographic signal intensity of the mutant allele was low, suggesting a mosaic mutation. The same result was obtained when the analysis was carried out in genomic DNA from buccal cells.We are not aware of any other cases of somatic mosaicism for *RET* mutations reported in isolated HSCR familial cases. As the somatic mosaic mutation in the mother was detected by a widely used standard method (direct sequencing), we wonder whether this is a truly unique family or whether the true frequency of somatic and germ-line mosaicism is underestimated in HSCR. Based on these findings we would recommend that germ-line or somatic mosaicism should be specifically looked for in families with recurrence of HSCR in the offspring of unaffected parents.

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P04.68

Dense genotyping of immune-related disease regions identifies nine new risk loci for primary sclerosing cholangitis

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Primary sclerosing cholangitis (PSC) is a severe liver disease of unknown etiology leading to fibrotic destruction of the bile ducts and ultimately the need for liver transplantation. We compared 3,789 European ancestry PSC cases to 25,079 population controls across 130,422 single-nucleotide polymorphisms (SNPs) genotyped using Immunochip. We identified 12 genome-wide significant associations outside the human leukocyte antigen (HLA) complex, nine of which were novel, thereby increasing the number of known PSC risk loci to 16. Despite comorbidity with inflammatory bowel disease (IBD) in 72% of the patients, six of the 12 loci showed significantly stronger association with PSC than IBD, suggesting an overlapping yet distinct genetic architecture. We incorporated pleiotropy with seven diseases clinically co-occurring with PSC and found suggestive evidence for 33 additional PSC risk loci. These findings further complete the genetic risk map of PSC and considerably expand on the relationship between PSC and other immune-mediated diseases.

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P04.69

Telomerase gene copy number is increased in IBD and PSC

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Background: Telomerase is an enzyme complex that lengthens telomeres. It is composed of the catalytic subunit of human telomerase reverse transcriptase (hTERT) and the telomerase RNA component (TERC) encoded by TERC gene. Gene amplifications involving the TERC gene (3q26) are frequent in human tumors. Primary sclerosing cholangitis (PSC) and Inflammatory bowel disease (IBD) are two pre-malignant conditions. The aim of this study was to evaluate the TERC gene copy number and senescence status in peripheral lymphocytes of patients with PSC and IBD, as a possible surrogate marker for increased tendency for malignancy.
Methods: By applying fluorescence in situ hybridization (FISH) to leukocytes of 14 PSC patients, 13 IBD patients (8 with Crohn's disease, 5 with Ulcerative colitis) and 12 healthy controls, we estimated gene dosage of the TERC gene at 3q26.3. The cells in senescence were evaluated from the nuclei fragmentation on the DAPI staining.
Results: The percentage of cells with more than two copies of TERC gene was significantly higher in PSC patients [mean (3 x red signals) 44.7] than in IBD patients [mean 29.6] and controls [mean 3.86] (p value < 0.0001). The TERC gene copy number was also higher in PSC patients with concomitant colitis (64%) despite significantly lower disease activity indices. Significantly more cells in senescence status were also observed in the PSC group.
Conclusion: TERC gene copy number and senescence status are increased in IBD and even more in PSC. These findings may be related to their different tendencies and predisposition to develop malignancy.

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P04.70

Latrophilins a new family of genes contributing to increased airway smooth muscle mass and contraction in asthmatic airways

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Asthma is associated with hyper-contractile airways and structural changes in the airway wall, including increased airway smooth muscle (ASM) mass. The aim of this study was to identify genes which may contribute to these processes in asthmatic airways. To compare the gene expression profiles of human primary ASM cells from asthmatic (n=3) and healthy donors (n=3), Affymetrix GeneChip HuGene_1.0_ST Arrays were used. Partek® analysis revealed 11 differentially expressed genes, which were not previously associated with asthma, including latrophilin 3 (LPHN3), a gene thought to be brain specific, which was up-regulated in asthmatic ASM cells compared to healthy donors (3.4 fold p<0.001, n=3). Using qPCR LPHN3 and the family member LPHN1 were both found to be up-regulated in asthmatics (n=16) compared with healthy donors (n=6, p<0.05 and p<0.01, respectively). The microarray predicted a single splice variant of LPHN3 increased in asthmatics which was confirmed by RT-PCR and sequencing. The LPHN1 ligand, α-LTX (toxin produced by black widow spider), increased proliferation of immortalised ASM cells (n=3, 1.21 & 1.20 fold, 0.3nM & 1nM, p<0.01 respectively) and induced the phosphorylation of AKT at 1 min (1nM). Addition of α-LTX (10nM) caused contraction of human airways, while FLRT3 (LPHN3 ligand) had no direct effect. The enhanced expression of LPHN1 in asthmatic airways may contribute to increased proliferation and contractility of ASM. The functional role of LPHN3 in the airways requires further investigation.
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P04.71

Association of the genes involved in xenobiotic metabolism and antioxidant pathways polymorphic markers with development of chronic obstructive pulmonary disease

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Chronic obstructive pulmonary disease (COPD) is a multifactorial inflammatory disease primarily affecting distal respiratory pathways and lung parenchyma. An oxidant-antioxidant imbalance in the lung contributes to the

development of COPD that is caused by a complex interaction of genetic and environmental risk factors.
The contribution of the 28 polymorphic markers of xenobiotic metabolism and antioxidant pathways genes (CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2E1, CYP2F1, CYP2J2, CYP2S1, GSTM1, GSTT1, GSTP1, NQO1, EPHX1, UGT2B7, SOD1, SOD3, CAT, GPX1) to COPD has been assessed. For this purpose, PCR-RFLP analysis of the xenobiotic metabolism and antioxidant pathways genes polymorphisms in COPD (N=391) and control (N=514) groups has been performed.
The NQO1 rs1131341 was associated with COPD in additive model (OR=2.55 95%CI 1.74-3.75), P=1.012 x 10-6 adjusted for age, sex, pack-years, ethnos). Analysis showed an association of the C-T haplotype of rs1131341 and rs1800566 NQO1 gene polymorphisms
(OR=2.77 95%CI 1.75 - 4.40, P=0.0001) with COPD. The relationship between the CYP2A6 rs71790353 (OR=3.16 95%CI 1.89-5.33, P=0.00001 with normal genotype) and CYP2S1 rs338583 (OR=0.21 95%CI 0.10-0.44, P=0.00001 in recessive model) and COPD risk was found. Significant association with increased rick of COPD was observed for the CAT rs1001179 in recessive model (OR=0.40 95%CI 0.21-0.76, P=0.0035), the EPHX1 rs1051740 in additive model (OR=1.46 95%CI 1.13-1.89, P=0.0034), and the GSTP1 rs1695 in dominant model (OR=1.51 95%CI 1.12-2.04, P=0.00439). We found a significant interaction of the smoking status and CYP1A1 rs1048943 (P=0.047), GPX1 rs1050450 (P=0.041), CYP2F1 rs11399890 (P=0.049) and UGT2B7 rs7439366 (P=0.013).

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P04.72

Dyrk1A (Dual-specificity thyrosine (Y)-phosphorylation regulated kinase 1A) overexpression is linked to Congenital Hypothyroidism in Down Syndrome

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Background: Down Syndrome patients are predisposed to Congenital Hypothyroidism which may aggravate their mental status.
Objective: To understand the thyroidal dysgenesis mechanisms in a Down Syndrome murine model.
Methods: The transgenic Dyrk1a (TgDyrk1a) mouse has three copies of Dyrk1a. We compared T4 and TSH plasmatic levels of 8-12 weeks old TgDyrk1a and wild type (wt) mice, their thyroid structure and their development from embryonic day (E) 13.5 to E17.5 by immunofluorescence with anti-Nkx2.1 antibody, a marker of early thyroid development and anti-T4 antibody for final differentiation. The transcription factors Nkx2-1 and FoxE1 involved in thyroidogenesis and the thyroglobulin necessary for T4 synthesis were studied by qPCR at these stages.
Results: TgDyrk1a mice have a lower plasmatic T4 (2.4 ng/mL versus wt: 3.7 ng/mL; p = 0.019) and an increased plasmatic TSH (114mUI/L versus wt: 73mUI/L). Their thyroidal follicles surface is larger (6955µm^2 versus wt: 5755µm^2). At E15.5 the primary thyroids are larger (5700000 pix^2 vs wt: 2900000 pix^2; p= 0.01) but their follicular differentiation surface is decreased at E17.5 (1070000 pix^2 vs 2800000 pix^2 in wt, p= 0.01). RNA levels of Nkx2-1(p=0.009), FoxE1 (p=0.025) at E13.5, and Thyroglobulin (p=0.04) at E17.5 are increased.
Conclusions: TgDyrk1a mice have abnormal thyroid development with ultimately mild hypothyroidism. An increase of the transcription factors expression and their target genes involved in thyroid development and function was documented. The young adult thyroids phenotype is probably due to a compensation mechanism. We are studying candidate genes as Dyrk1a targets using thyroidal cell lines.

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P04.73

Familial pulmonary arterial hypertension: BMPR2 gene mutation findings in a Venezuelan family

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Objectives: The purpose of this paper is to determine the mutations in the *BMPR2* gene in members of one family with a diagnosis of Familial Pulmonary Arterial Hypertension.
Methods: Clinical Evaluation: Written informed consent was obtained from all family members, who underwent pedigree analysis and clinical assessment in accordance with the requirements of the Ethics Committee of the Genetic Medical Institute of Zulia University. Familial pulmonary arterial hypertension was diagnosed with the use of current international consensus criteria, and was determined through clinical evaluation, chest radiography, electrocardiography, Doppler echocardiography and right-heart catheterization. Other known causes of elevated pulmonary arterial pressure were excluded. The diagnosis was established without knowledge of the genotype status. The molecular analysis of the
BMPR2 gene was performed with collaboration with the Centre for Human Genetics, University of Leuven, Belgium.
Results: Analysis of *BMPR2* sequence was performed in three family members with pulmonary hypertension. All were heterozygous for the c.1471C>T mutation in exon 11 of the *BMPR2* gene (at protein level p.Arg491Trp).
Conclusion: The FPAH is less rare than initially believed, in part because of patient and physician awareness of the familial occurrence of the disorder. It is an autosomal dominant disorder that affects females disproportionately, may occur at any age, and is characterized by reduced penetrance and variable expressivity as is seen in this first Venezuelan family report. These results suggest that other endogenous or exogenous factors modify its expression, as is well known.

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P04.74

Contribution of *LHX4* mutations to pituitary hormone deficits in 350 patients presenting with ectopic posterior pituitary and/or *sella turcica* anomalies

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To date, only 9 unambiguous *LHX4* mutations have been reported in pituitary hormone deficits. These mutations, always found in the heterozygous state, are responsible for a dominant hypopituitarism with variable expressivity. Several patients displayed an ectopic posterior pituitary (EPP) or a hypoplastic *sella turcica*.
The objective of this study was i) to assess the prevalence of *LHX4* mutations in a large group of patients presenting with various pituitary hormone deficits associated with an EPP and/or *sella turcica* anomalies, ii) to provide a most complete description of the *LHX4* associated-phenotypes, and iii) to assess the functional consequences of the identified variants.
We screened the *LHX4* gene in a group of 350 independent probands and assessed the ability of the identified *LHX4* variants to transactivate the *POU1F1* proximal promoter.
In addition to one previously described mutation (c.538-1G>C), we identified 6 novel heterozygous mutations: 1 nonsense (p.Tyr131X), 1 frameshift (p.Arg48ThrfsX151), 1 splice (c.606+1G>T, *de novo*) and 3 missense variations (p.Ala65Val, p.Arg221Gln and p.Arg235Gln). Posterior hypophysis was eutopic in one patient; EPP was noted in four probands. Three patients showed an abnormal *sella turcica*. Short stature at diagnosis ranged from -2SD to -5SD. Incomplete penetrance was observed in one family (p.Tyr131X). Functional assessment of the identified variants is underway.
This study, performed in the largest cohort of patients so far screened for *LHX4* mutations, shows that this gene is responsible for at most 2% (7/350 independent probands) of hypopituitarisms associated with ectopic posterior pituitary and/or *sella turcica* defects.

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P04.75

TSC1 and TSC2 genotypes and clinical efficacy of everolimus in subependymal giant cell astrocytoma (SEGA) and renal angiomyolipoma (AML) associated with Tuberous Sclerosis Complex (TSC)

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TSC is characterized by growth of nonmalignant but often progressive lesions in several organs throughout the body such as the brain (SEGA) and kidneys (AML). Most TSC patients have inactivating mutations in either *TSC1* or *TSC2*, and TSC lesions show constitutive activation of mTOR. The efficacy and safety of everolimus, an mTOR inhibitor, was assessed in two double-blind, placebo-controlled, phase 3 trials: EXIST-1 (SEGA associated with TSC; NCT00789828) and EXIST-2 (AML associated with TSC or sporadic lymphangioleiomyomatosis; NCT00790400). In EXIST-1, patients were randomized to receive 4.5 mg/m^2/day everolimus (n=78) or placebo (n=39) and in EXIST-2, patients were randomized to receive 10 mg/day everolimus (n=79) or placebo (n=39). In each trial, everolimus was superior to placebo for the primary endpoints of SEGA and AML response rates (SEGA: 35.0% vs 0%; P<0.0001; AML: 42.0% vs 0%; P<0.0001) and was associated with an acceptable safety profile. Blood DNA was collected from all patients in both trials and *TSC1* and *TSC2* gene alterations were identified by sequencing of the coding regions for mutations and by Multiplex Ligation-dependent Probe Amplification analysis for genomic deletions. Preliminary results demonstrated no apparent correlation between response rate and mutation categories in either trial (Table). These results suggest that everolimus has a similar clinical benefit in patients, regardless of TSC genotype; further in-depth analysis of genotype-clinical response correlation will be performed.

TSC1/2 Mutation Frequency and Corresponding SEGA and AML Response Rate

EXIST-1				
Gene	Mutation frequency, % (n/N) Everolimus	SEGA response rate, %	Mutation frequency, % (n/N) Placebo	SEGA response rate, %
TSC1	13 (10/77)	50	8 (3/39)	0
TSC2	71 (55/77)	30	74 (29/39)	0
No mutation identified	13 (10/77)	45	18 (7/39)	0
EXIST-2				
Gene	Mutation frequency, % (n/N) Everolimus	AML response rate, %	Mutation frequency, % (n/N) Placebo	AML response rate, %
TSC1	4 (3/79)	50	5 (2/39)	0
TSC2	76 (60/79)	48	69 (27/39)	0
No mutation identified	18 (14/79)	36	26 (10/39)	0

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P04.76

A search for new *CYP3A5* and *CYP3A4* variants as determinants of tacrolimus dose requirements in transplanted patients

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Tacrolimus (Tac) is an immunosuppressive drug used to avoid the rejection of solid organs. *CYP3A5*3* (rs776746) and *CYP3A4*1* (rs2740574) polymorphisms were related with Tac dose requirements. A new variant *CYP3A4* variant (**22*; rs35599367) was recently associated with a significant lower Tac dose requirement. The main aim of this study was to analyze the impact of *CYP3A4*22* on blood Tac levels and dose requirements among Spanish kidney transplanted, and to identify new *CYP3A5* and *CYP3A4* variants that could be related with Tac bioavailability. A total of 206 kidney patients who received a cadaveric kidney and received Tac as primary immunosuppressor were studied. *CYP3A4*22* was genotyped by real time PCR *Taqman* assay, and the *CYP3A5*3* and *CYP3A4*1* polymorphisms through PCR-RFLP. The coding exons (plus intronic flanking bases) of the two genes were sequenced. *CYP3A5**3, *CYP3A4*1* polymorphisms were associated with Tac dose requirements (p<0.05). The *CYP3A4*22* variant did not influence Tac dose and blood levels, nominally and after correcting by the CYP3A5 genotypes. We identified a new missense *CYP3A4* exon 8 variant, p.P227T, in one patient. This was a conserved residue predicted to affect protein function. In conclusion, the absence of *CYP3A5* coding variants suggested that rs776746 (*CYP3A5*1/*3* alleles) was the main determinant of Tac pharmacogenetics in our population. Although rare *CYP3A4* variants were found among our patients, the promoter rs2740574 (*CYP3A4*1/*1B*) was the main modifier of Tac bioavailability at this gene.

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P04.77

A novel homozygous insertion in the NNT gene in a Dutch patient with familial glucocorticoid deficiency (FGD)

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Familial glucocorticoid deficiency (FGD; MIM 202200) is a rare autosomal recessive disorder characterized by undetectable or low levels of plasma cortisol despite high plasma adreno¬corticotropin (ACTH) levels. Affected individuals are resistant to ACTH, i.e. the adrenal cortex is not able to produce cortisol in response to stimulation by ACTH.
FGD is a heterogeneous disorder with known mutations in MC2R, MRAP, STAR, CYP11A1 and MCM4. It has recently been indicated that mutations in NNT (nicotinamide nucleotide transhydro¬genase) are also responsible for FGD. NNT is a highly conserved protein integrated in the inner mitochondrial membrane; it appears to be essential for free radical detoxification in adrenocortical cells. Our study provides support for NNT being involved.
We studied a consanguineous Dutch family with one affected boy with FGD, presenting with convulsions and hypoglycaemia at the age of 12 months. In retrospect, he had had a brownish tan due to increased ACTH level since the age of 4 months. His adrenal ultrasound was normal and no mutations could be detected in MC2R and MRAP. SNP haplotyping (300k array) identified a 6.67 Mb homozygous region harboring the NNT gene. Exome-sequencing revealed a novel homozygous frameshift mutation (NM_012343.3: c.1259dupG). This mutation was validated by Sanger sequencing and both his parents were shown to be heterozygous carriers. The mutation is located in exon 9 and creates a frameshift starting at codon His421 and leading to a premature stop-codon (p.His421Serfs*4). It also lies in the mitochondrial matrix domain, which is known to result in FGD when truncated.

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P04.78

Contribution of *GH* and *GHRHR* mutations to isolated growth hormone deficiency - Identification and functional characterization of new variants

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Although growth hormone (GH) and the GH releasing hormone receptor (GHRHR) have been recognized as key etiologic factors in non-syndromic forms of isolated growth hormone deficiency (IGHD), few mutations have been identified in this rare condition (accounting for 6-12.5% and 0-6.7% of IGHD cases). So far, very few functional studies have been performed.
To assess the contribution of *GH* and *GHRHR* in the pathogenesis of IGHD, we first analyzed these two genes in a large cohort of patients with non-

syndromic forms of IGHD.
GH was studied in 191 independent patients and *GHRHR* was subsequently analyzed in the remaining 144 independent patients (with no identified *GH* defect and available DNA). The GHRH response of GHRHR variants was assessed *in vitro* through a CRE-dependent luciferase assay.
GH mutations were identified in 28 patients (15%), 10 of them (10/191, 5%) representing familial forms of IGHD. These include 7 novel mutations (2 frameshifts, 5 missense). We identified *GHRHR* mutations in 22 patients (15%), 7 of them (7/144, 5%) representing familial cases. The *GHRHR* mutation spectrum (6 truncating, 1 splice, 10 missense) comprises 13 novel mutations. As shown by functional studies, 5 of the *GHRHR* missense variations represent loss-of-function mutations.
Overall, this study performed in a large patient cohort, which identifies molecular defects in *GH* or *GHRHR* in 50 out of 191 independent patients (27%), reveals the importance of those genes in the pathogenesis of non-syndromic IGHD. Noteworthy, 62% (31/50) of the patients with a *GH* or *GHRHR* defect represent sporadic cases.

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P04.79
Short stature caused by a pseudo-isodicentric Xq21.1 chromosome, despite three SHOX genes

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Pseudo-isodicentric X chromosomes of the short arm of chromosome X (i(Xp) chromosomes) are rare. Most i(Xp)chromosomes occur as a mosaic with a 45,X cell line. Patients with this mosaic genotype have ovarian failure and variable height. We report two patients who presented with proportionate short stature without stigmata of Turner syndrome. Both patients also had hypergonadotropic hypogonadism.
Conventional cytogenetic analysis in lymphocytes revealed the presence of a 46,X,pseudo-isodicentric Xq21.1. FISH analysis showed that the X inactive specific transcript (*XIST*) gene is present twice on the i(Xp)chromosome. In patient 1 the i(Xp)chromosome was demonstrated in all cells next to a normal X-chromosome, while in patient 2 a mosaic was seen in which 10% of the cells harboured a 45,X karyotype, and in 90% of the cells a karyotype with the i(Xp)chromosome was shown.
The presence of two *XIST* genes on the i(Xp) chromosome indicates inactivation of the i(Xp), caused by alteration of the chromatin structure, a process described as inactivation enhancement. The degree of inactivation is correlated with the size of the Xq deletion, with larger deletions being more critical. The inactivation might also concern the PAR regions. This would effectively cause haploinsufficiency of the *SHOX* gene, explaining the short stature in these patients.
Both patients were treated with growth hormone, while patient 1 also received low dose estrogens. This resulted in growth acceleration without increase in bone maturation.

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P04.80
A novel chromosomal locus for primary ciliary dyskinesia

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Cilia are essential for fertilization, respiratory clearance, cerebrospinal fluid circulation and establishing laterality. Cilia motility defects cause primary ciliary dyskinesia (PCD, MIM244400), a disorder affecting 1:15,000-30,000 births. In a consguineous Bedouin family with 2 affected and 6 healthy siblings we have searched for the mutation causing the disease. Assuming disease by homozygosity of a mutation from a common ancestor, linkage to the *DNAH5* and *DNAI1* genes was excluded by the finding of heterozygosity of alleles at known polymorphic markers adjacent to these genes. We further searched for homozygous regions consistent with linkage by genotyping the patients and both parents with the Affymetrix (Santa Clara, CA) GeneChip® Human Mapping 250K Sty arrays. Five homozygous regions larger than 18cM were identified using the KinSNP program. To determine linkage to a single region, all family members were tested with both known polymorphic microsatellite markers and with additional markers developed for this purpose. Linkage was identified to a chromosomal locus on chromosome 18p11.1-q13.3. The multipoint Lod score calculated was 2.99, using the Pedtool server assuming recessive inheritance with 99% penetrance and an incidence of 0.01 or 0.001 for the disease allele in the population. The region contains 10 genes encoding ciliary proteins, a putative mutation causing PCD was not identified in any of these.

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P04.81
Surprising genetic heterogeneity for primary ciliary dyskinesia in the Irish Traveller population

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Primary ciliary dyskinesia (PCD) is a life-limiting multi-organ condition characterised by impairment of muco-ciliary clearance. Identification of the PCD disease mutations in the Irish population would facilitate the development of genetic-based diagnostic tests to allow for earlier definitive diagnosis, earlier treatment and improved long-term outcomes. Currently patients have to travel to the UK for confirmation of diagnosis. We aimed to identify the genetic mutation responsible for PCD in the Irish Traveller population (~26,000), an endogamous nomadic group distinct from the Roma gypsies. Our study involves five affected children from three different families. As each recessive disorder in the Irish Traveller population is caused by a common homozygous mutation, we hypothesised that the three families shared the same PCD disease mutation. Exome sequencing was performed for one affected child from each family. Analysis of the exome data did not identify a recessive deleterious mutation common to the affected children from all three families. Analysis of electron microscopy results supported the genetic findings; each family has a different type of ciliary defect suggesting a different disease gene. The findings have led to a change in study design and the exome data from each family is now being analysed independently. We identified a known PCD disease gene in one family, a novel disease gene in the second family and analysis is on-going in the third family. The results to date suggest that there is more than one PCD disease gene in the Irish Traveller population, which is surprising given the limited population size.

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P04.82
First reported spontaneous pregnancy in autosomal recessive pseudohypoaldosteronism type 1 (PHA1B) complicated by placental insufficiency

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Introduction
Pseudohypoaldosteronism (PHA) is characterized by hyperkalaemia, metabolic acidosis and elevated aldosterone levels (Chang 1996). The autosomal recessive form (PHA1B) is due to mutations in three genes, each encoding a sub-unit of the epithelial sodium channel (ENaC) (Chang 1996). This channel is widely expressed on the surface of motile cilia (Enuka 2011) and therefore PHA1B is associated with respiratory infection and infertility.
Case history
We report the case of a 28-year old female with PHA1B, due to homozygous mutations in the *SCNN1A* gene, who became spontaneously pregnant after one year of trying to conceive. A 20-week antenatal scan showed severe intra-uterine growth restriction (IUGR). Continued placental insufficiency and oligohydramnios led to an emergency delivery at 28 weeks. Post-operatively, the mother required treatment for hyperkalaemia with metabolic acidosis as well as a DVT. The baby developed meconium ileus necessitating a bowel resection and a prolonged neonatal intensive care admission.

Discussion
PHA1B causes widespread ciliary dysfunction and infertility (Enuka 2011). To our knowledge, this is the first case of a successful spontaneous pregnancy in a female with PHA1B. We hypothesize that the placental insufficiency in this pregnancy was linked to the maternal PHA1B resulting from the homozygous mutation in the *SCNN1A* gene encoding the α-subunit of ENaC. Therefore, this case illustrates that spontaneous pregnancy is possible in PHA1B. If achieved there should be careful monitoring for the possible complications of maternal metabolic instability and fetal IUGR/prematurity as well as counselling given to the couple to highlight these issues.

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P04.83
Loss of function of DMXL2 causes a complex neuroendocrine disorder with ataxia and mental disability.

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Here, we studied a new phenotype in three brothers born from first cousins of Senegalese origin. Disease onset began in early childhood with growth retardation and asymptomatic hypoglycemia with incomplete suppression of insulin levels. Between 14 and 20 yrs, the three patients developed a slowly progressing non-autoimmune insulin deficient diabetes mellitus, central hypothyroidism and partial hypogonadotrophic hypogonadism. In addition, they exhibited an ataxia and dystonia due to a progressive peripheral sensitive-motor demyelinating polyneuropathy, along with pyramidal manifestations. Brain MRI exposed moderate sub-cortical temporal white matter disease. All three subjects presented a moderately low intelligence quotient, dysarthria, difficulties swallowing and frontal alopecia to different degrees.
A genome mapping followed by high throughput sequencing of two candidate regions found an un-described homozygous in-frame deletion of 15 nucleotides in exon 24 of DMXL2 (c.5827_5841del) leading to the deletion of 5 residues (p.1942_1946del) in the three brothers. The c.5827_5841del homozygous deletion was associated with a significant decrease of DMXL2 mRNA levels in blood lymphocytes. The screening of DMXL2 in 10 additional cases with a similar phenotype did not reveal any new mutation.
DMXL2 encodes for rabconnectin-3α (rbcn3-α) which is a synaptic protein interacting with regulators of the Rab3a "on-off" activity, a vesicle associated protein involved in the regulatory secretion. The production of a Dmxl2-knock-out mouse line confirmed the role of rabconnectin-3α in the central activation of the gonadotropic axis. In-vitro investigation showed its role in regulatory secretion. These data open a new door to understand the postnatal neuroendocrine plasticity leading to puberty.

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P04.84
Copy number variants in patients with short stature

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Height is a highly heritable and classic polygenic trait. Recent GWAS have revealed that at least 180 genetic variants influence adult height. However, these variants explain only about 10% of the phenotypic variation in height. In an effort to identify novel genetic variants associated with short stature (SS), we studied 162 patients from 149 unrelated families with SS. To detect copy number variants (CNVs), whole genome SNP array analysis was performed. All potentially pathogenic CNVs (containing protein-coding genes and not described in the Database of Genomic Variants or an in-house reference) were assessed with Ensembl and DECIPHER for gene content and similar cases, respectively. Segregation analysis was performed if possible. Genes in CNVs were compared with information from GWAS, gene expression in rodents' growth plates, and published information. Known or potentially pathogenic CNVs were detected in 39 families; in 6 families a known cause of SS was found (*SHOX* or *IGF1R*), in two combined with a second, potentially pathogenic CNV. In 33 families one or more potentially pathogenic CNVs (n=40) were detected, several of the deleted or duplicated genes may be considered as potential candidate genes for growth disorders, including four genes associated with height by GWAS (*ADAMTS17*, *PRKG2*/*BMP3*, *PAPPA*, *TULP4*). In 9 families, the CNVs occurred *de novo* or segregated with SS. In conclusion, besides 6 CNVs in 6 families (4%) known to be causative for short stature, 40 CNVs in 33 families (22.1%) with possible pathogenicity were identified. Segregation studies and bioinformatic analysis suggested various potential candidate genes.

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P04.85
A large novel deletion in the NBD2 domain of the ABCA3 gene causes neonatal respiratory distress, SMDP3.

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The hereditary surfactant metabolism dysfunction disorders (SMDP) are genetically heterogeneous resulting in severe respiratory insufficiency in full-term infants. Surfactant, mixture of lipids (90%) and specific proteins, prevents lung collapse by lowering surface tension in the alveoli, at the end of expiration.
SMDP are associated with mutations in genes encoding the surfactant proteins like SP-C and SP-B and lamellar body biogenesis like ABCA3. SMDP caused by *ABCA3* mutations is inherited as an autosomal recessive disorder. with the majority of affected children dying within 3 months of age. Some infants though, will develop pediatric interstitial lung disease (ILD). Some will improve spontaneously. Genotype-phenotype relationship is not clear.
We present a novel mutation in the *ABCA3* gene associated with neonatal death. She was born at term and developed progressive respiratory distress within a few hours. On day 9th she was mechanically ventilated. On day 59 despite extensive treatment the patient died. Sequencing the entire encoding regions of the ABCA3 revealed a novel homozygous nonframeshift deletion of 24bp in exon 26 in a highly conserved region. The deletion results in a protein shorter by eight amino acids in the NBD2 domain. The parents were found to be heterozygous for this deletion.
Functional data and genotype-phenotype relationship studies are needed in order to allow proper counseling and clinical decisions. Until now, no therapies have been shown to be effective. Lung transplantation has been suggested in patients with milder mutations. Prenatal genetic diagnosis has been offered to the parents in our case.

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P04.86
Mutations in *CYP24A1* as a major cause of Idiopathic Infantile Hypercalcemia (IIH).

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Idiopathic infantile hypercalcemia (IIH), also called Lightwood syndrome, is characterized by transient hypercalcemia and hypercalciuria responsible for nephrocalcinosis, with increased levels of 1,25-dihydroxyvitamin D

(1,25-$(OH)_2$-D), and low levels of parathyroid hormone (PTH). Recently mutations in the *CYP24A1* gene encoding for vitamin D-24-hydroxylase, the enzyme responsible for inactivation of the 1,25-$(OH)_2$-D, have been reported, giving a new insight into IIH.
Objectives: To confirm and extend earlier findings, we screened for *CYP24A1* mutations a cohort of 34 patients (index cases) diagnosed with hypercalcemia and low PTH level (<20 pg/mL).
Methods: Biochemical and endocrine analyses were performed using standard methods and *CYP24A* mutations were identified using the routine procedures involved in the laboratory.
Results: We found 9 (5 children and 4 adults) unrelated patients (26.5%) with homozygous (4/9) or compound heterozygous (5/9) *CYP24A1* mutations. We identified seven new mutations and a large deletion encompassing exons 9 to 11. In children, IHH was diagnosed before 2 years (range: birth - 8 months; mean: 4,5 months) with high calcemia level (>3 mmol/l) and low PTH. All presented with nephrocalcinosis. Out of the 4 adults with CYP24 mutations (1 female and 3 males) 2 presented with nephrocalcinosis, and 1 had kidney stones associated with hypercalciuria. All received vitamin D supplementation.
Conclusion: Mutations in *CYP24A1* currently could give a pathogenic explanation for some IIH, and thus constitute caution regarding vitamin D supplementation in infancy.

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P04.87
Genetic testing for MODY: retrospective study of utilisation in the Netherlands

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Genetic testing for maturity-onset diabetes of the young (MODY) may be relevant for treatment of patients and for monitoring of their healthy family members. This study describes the current state of genetic testing for MODY in the Netherlands in terms of volume and test positive rate, medical setting, purpose of the test, and age of patients tested. Some analyses focus on the most prevalent subtype, HNF1A MODY.
In total, 1473 mutation scans for HNF4A, GCK, HNF1A and HNF1B were performed in 2001-2010. Cascade testing for a known mutation in family members occurred 290 times, and did not show an increasing time trend. In total about 400 individuals were identified with a MODY mutation. Testing for HNF1A MODY was mostly requested by internists and paediatricians, often from regional hospitals. A substantial proportion (20-28%) of HNF1A MODY probands was over 40 years old at the time of testing.
The number of individuals genetically tested for MODY so far in the Netherlands is low compared to previously predicted numbers of patients. Primary care physicians and clinical geneticists rarely request genetic testing for HNF1A MODY. In 2009-2010 25% of clinical geneticists' requests concerned cascade testing. Doctors' valuation of the test on the one hand and patients' and family members' response to (an offer of) genetic testing on the other hand need to be investigated. There could be a lack of perceived utility of genetic testing for symptomatic patients and/or presymptomatic family members. Efforts may be needed to develop and implement translational guidelines.

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P04.88
Molecular and genetic analysis of autosomal dominant early-onset diabetes in Tunisian families

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Background: Maturity-onset diabetes of the young (MODY) is a dominantly inherited non-autoimmune early-onset diabetes, that is characterized by clinical and genetic heterogeneity. At least thirteen genetic subtypes have been described worldwide with important impacts on diagnosis strategy, individual and familial prognosis and pharmacogenomics.
Objective: To identify the genetic aetiologies of early-onset diabetes or MODY in Tunisian families.
Methods: Thirteen diabetic probands (mean age at diabetes diagnosis: 26.6±4.9 years) from families with diabetes segregating in 2-to-3 generations were screened for mutations in *HNF1A, HNF4A, INS, IPF1, NEUROD1* and *GCK* genes by Sanger sequencing and MLPA technique. Two informative negative families were investigated by linkage analysis using SNP microarray genotyping (Illumina Cardio-Metabo BeadChip consisting of ~200,000 SNPs). The MAZEL software was used for SNP selection, and 11,993 SNPs were analyzed by parametric and non-parametric methods in each family.
Results: Direct MODY genes sequencing showed only one probably non-damaging missense mutation in *HNF4A* (p.I453V) in one family. Two independent linkage signals were found, each one in a single family: on chromosome 11 (F1:$Z_{max\text{-}bipoint}$ = 1.986; $Z_{multipoint}$= 2.230) and chromosome 17 (F2: $Z_{max\text{-}bipoint}$ = 1.735). In family F1, a non-parametric analysis confirmed the linkage signal on chromosome 11 (p-val<0.005).
Conclusion: A causal mutation in known MODY genes has been excluded in the studied MODY patients. A thorough exploration of genomic intervals identified by our study in two families, through gene targeted sequencing or whole exome sequencing, will be promising to identify the responsible gene(s) and causal mechanism of the disease.

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P04.89
Monogenic diabetes: molecular screening of MODY genes

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Maturity-onset diabetes of the young (MODY) is characterized by early-onset non insulin-dependent diabetes mellitus and autosomal dominant inheritance. Although 12 MODY genes are described, only the most frequent MODY2 and 3 subtypes are usually investigated.
We report a mutation screening of GCK, HNF1A, HNF4A, HNF1B and PDX1 genes (MODY1-5) in 283 patients fitting MODY criteria. Gene mutations are investigated by dHPLC and sequencing; large gene deletions are detected by MLPA. 108 different mutations, 44 of which novel, were detected in 143 probands (50.5%). Among the mutated patients, 79% were GCK/MODY2, 14% HNF1A/MODY3, 3% HNF4A/MODY1, 2% PDX1/MODY4 and 2% HNF1B/MODY5. Among GCK mutations, 59 were missense and 7 were non-sense mutations, 5 were splicing and 4 were small indel, 16 mutations were detected in more than one family. Among HNF1A mutations, 13 were missense, 3 were indel and 2 non-sense, 2 variants affected the HNF-4α binding site of the gene promoter. Also in HNF4A gene a mutation disrupting the HNF-1α binding site in the P2 promoter was detected. A patient showed double heterozygous mutations involving both HNF1A and HNF4A genes. Compound heterozygosis for 2 variants of PDX1 gene was detected in a proband. Heterozygous deletion of whole HNF1B gene was found in 2 patients. Our data broaden the knowledge of the mutations repertoire in these genes and assess the relative prevalence of MODY subtypes in our population. However, about half of patients remains without characterization of the gene defect, indicating that additional genes should be investigated.

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P04.90
Neonatal Diabetes Mellitus in a prospective cohort of 174 patients: frequent association with developmental defects and neuropsychological dysfunction.

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Neonatal diabetes mellitus (NDM) is a rare form of pancreatic beta-cell dysfunction leading to hyperglycaemia early in life. It belongs to monogenic diabetes with genetic alterations impairing either pancreas development or insulin secretion.

We evaluated phenotype-genotype correlations and clinical outcome in 174 probands with NDM diagnosed before one year of age, referred to the French NDM Study Group from 1995 to 2010. No patient had beta-cell autoimmunity and all had normal pancreas morphology. They were prospectively investigated for alterations in chromosome 6q24 (uniparental isodisomy, duplication, methylation deffect) and mutations in genes encoding K_{ATP} channel *(ABCC8, KCNJ11)* and preproinsulin (*INS).*

The genetic cause of NDM was identified in 128/174 (74%) probands and consisted in 6q24 abnormalities (n=40), mutation in *KCNJ11* (n=43), *ABCC8* (n=31) or *INS* (n=14). Refined neuropsychological and psychomotor investigations evidenced disorders ranging from developmental coordination disorder (particularly visual-spatial dyspraxia) and/or attention deficits to developmental delay and epilepsy (DEND syndrome) in all patients with K_{ATP} channel mutations. On the other hand, intra-uterine growth retardation (92%), early age at diagnosis (median 5 days [1-120]), and developmental defects involving heart, kidneys or urinary tract (22%) were core feature of the 6q24 phenotype. Remission of diabetes occurred in 89 (51%) probands, but recurrence probability was high, without difference between 6q24 and K_{ATP} channel probands (82% vs 86%, p=0·36).

In conclusion, age at onset, birth weight or associated features can guide NDM genetic testing. Neuropsychological and developmental defects are frequent, differ according to the genetic aetiology, and deserve multidisciplinary assessment.

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P04.91

Does TCF7L2 gene predicts type 2 diabetes also in Turkish?

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Type 2 Diabetes Mellitus (T2DM) is one of the most challenging health problems of the 21st century and growing anxiously in time. The Turkish population is no exception to this trend. To date, TCF7L2 gene has been shown to be the strongest association with an increased risk of T2DM with well-replicated results in several different populations. TCF7L2 encodes a transcription factor, involved in Wnt-signalling pathway, a major regulator of cell growth and development. Wnt signaling influences endocrine pancreas development and modulates mature β-cell functions including insulin secretion, survival and proliferation. This study provides valuable information about the Turkish population because it is the only association study between TCF7L2 variants and T2DM in a Turkish population to the best of our knowledge. Genotyping was carried out by PCR-RFLP and PCR-SSCP techniques and six common variants (rs7903146 C>T, rs12255372 G>T, rs7901695 T>C, rs11196205 G>C, rs11196213 C>T and rs3814573 C>T substitutions) were genotyped in 169 non-obese diabetic and 119 healthy individuals. rs7903146 and rs12255372 were significantly associated with T2DM (OR:1.9 [95% CI: 1.15-3.19] *p*=0.005 and OR:2.1 [95% CI: 1.25-3.55] *p*=0.002 respectively). Calculated odds ratios were higher compared with current literature. Remarkably, for all studied SNPs, heterozygote and homozygote rare allel frequencies were observed higher than common allels according to other populations. We consider it is because of our genetic heterogeneity resulting from historical importance of our geographical position which in the center of migration roads. Our results indicate that TCF7L2 might be a strong effector on T2DM development in Turkish, too.

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P04.92

Whole-exome sequencing in Czech MODYX families: first results and implications

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Maturity-Onset Diabetes of the Young (MODY) is a heterogeneous group of autosomal dominant diabetes clinically characterised by positive family history of diabetes, age at diagnosis before 25 years and negative beta-cell autoantibodies. Although 13 MODY genes are known, approximately 30% of all cases remain genetically unclarified (MODYX). We aimed to identify the genetic cause of diabetes in 6 MODYX families (22 persons) by whole-exome sequencing (WES).

On average, 99.2% of all exons from each individual were sequenced on an Illumina HiSeq 2000. The detected variants in each sample (average 65,816) underwent co-segregation analyses within the family and filtering against databases dbSNP, Exome Variant Server, 1000 Genome Project, HapMap, LuCamp.

While 154 variants identified in 4 MODYX families are further evaluated and compared with data from other MODYX families, causal mutations in previously known MODY genes were found in two families: First, p.Arg235Trp in the HNF1B gene was detected. Although HNF1B disruption is mostly associated with diabetes and cystic kidney disease (Renal Cysts and Diabetes syndrome), the proband did not display any renal anomaly. A second family carried p.Trp113Leu in the HNF1A gene causing HNF1A-MODY. This mutation escaped the previous testing by direct sequencing due to the presence of 2 formerly unknown polymorphisms under the reverse primer suggesting that only one allele was amplified.

First results from WES in MODYX families pointed out that there is a need for constant awareness and regular re-evaluation of both clinical criteria and primer sequences in routine testing of MODY.

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P04.93

Metabolic and genetic cardiovascular risk factors in diabetes mellitus type 2 patients

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Diabetes mellitus type 2 (DM2) is closely associated with the development of various cardiovascular complications including coronary artery disease (CAD). The aim of this study was to investigate CAD risk factors specific to DM2 patients. STUDY POPULATION: DM2 patients with CAD, among them 41 male and 134 females (average age 52±10), and DM2 patients without CAD, among them 57 males and 127 females (average age 50±10). Polymorphisms of genes *APOA1* G-75A and C+83T, *APOC3* Sst1, *APOE* epsilon, *APOA5* T-1131C and S19W, *ADRB3* W64R, and *ACE* I/D together with medical history, family history of CAD and DM2, the body mass index, and the serum lipid levels, among them total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, very low density lipoprotein cholesterol, triglycerides, and the atherogenic index were studied with the factor analysis and the logistic regression method. RESULTS: The DM2 patients with CAD were older than the DM2 patients without CAD. Also, their body mass index and the levels of high density lipoprotein cholesterol were higher than the same in the DM2 patients without CAD. It is interesting that the elevated levels of high density lipoprotein cholesterol were typical to the female DM2 patients with CAD and not to the males, when analyzed

separately. No one gene polymorphism was associated with higher risk of CAD in the DM2 patients.

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P04.94

The adiponectin variants contribute to the genetic architecture of type 2 diabetes in Turkish

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Adiponectin is a strong candidate gene for type 2 diabetes, since adiponectin modulates insulin sensitivity and plays an important role in regulating energy homeostasis. We aimed to evaluate the contribution of the adiponectin gene polymorphisms in genetic background of type 2 diabetes in a Turkish population. The study included unrelated, non-obese 169 patients and age-BMI-matched 119 non-diabetic individuals with no family history. PCR-RFLP technique was used for ten SNPs genotyping. We detected significant association with type 2 diabetes and SNPs -11391G>A and -11043C>T in proximal promoter region and SNP +276G>T in intron 2 of adiponectin gene (P<0.05). The silence SNP +45T>G in exon 1 and SNP +349A>G in intron 2 showed weak association with disease (P=0.06 and P=0.07, respectively). We consider that SNPs in proximal promoter might be important in disease development by changing adiponectin expression. The effect of other associated SNPs is unclear, possibly that it may be in interaction with other known or unknown SNPs and/or genes. Also, one of rare SNPs, Y111H was associated with fasting insulin (P<0.05) while other SNP R112C was effective on fasting glucose, fasting insulin and HbA1C (P<0.05). We observed that Y111H and R112C polymorphisms may be severely effective on glucose and insulin levels even in heterozygote form in an individual. Especially R112C, was associated with impaired multimerization and possibly with impaired secretion and/or action of adiponectin since its localization. Consequently, adiponectin gene polymorphisms in Turkish population might contribute to genetic background of type 2 diabetes.

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P04.95

Association between TCF7L2 gene polymorphisms and haplotypes with risk of type 2 diabetes in the Iranian population

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TCF7L2 is a transcription factor influencing the transcription of several genes thereby exerting a large variety of functions within the cell. There is strong evidence from several previous studies that two common variants of *TCF7L2* (rs7903146 and rs12255372) are associated with type 2 diabetes. We examined the impact of rs7903146, rs12255372 and rs11196205 variants of TCF7L2 gene and the haplotypes involving these variants on type 2 diabetes in an Iranian genetic association study. We genotyped the three polymorphisms in 537 type 2 diabetic case and 441 control subjects by TaqMan assay. In logistic regression analysis adjusted for age, sex and BMI, the allele frequency of rs7903146 and rs12255372 were significantly different between type 2 diabetes patients and control subject ($p=6.8 \times 10^{-10}$ and $p=9.3 \times 10^{-11}$ respectively). In genotype frequency comparison, the most significant association was obtained under a dominant genetic model for rs7903146 and rs12255372 variants ($p=9.6 \times 10^{-10}$ and $p=3.8 \times 10^{-10}$ respectively). In haplotype analysis the CGG haplotype of TCF7L2 rs7903146-rs11196205-rs12255372 variants showed significant association with type 2 diabetes in our study subjects ($p=1.1 \times 10^{-5}$). In conclusion, we found a strong association of TCF7L2 variants and a three-marker haplotype with development of type 2 diabetes in the Iranian population

P. Keshavarz: None.

P04.96

Does summation of alleles account for genetic risk and genotype-phenotype association in Type 2 Diabetes Mellitus?

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Introduction: Type 2 diabetes (T2DM) and metabolic syndrome are common complex disorders with a high prevalence in the Maltese population. The aim of this study is to further define the genetic interplay between cognate genes from metabolic and inflammatory pathways on the likelihood of developing T2DM in adulthood and to relate the association of certain genetic profiles with defined biological and clinical endpoints.
Method: Eight hundred carefully characterised T2DM cases were recruited. Anthropometric and biochemical parameters, including serum high-sensitivity C-Reactive protein (hsCRP) levels were determined, and genotyping of 43 cognate genes carried out. Neonatal cord blood samples were used as the control reference population in this study.
Results: Ten polymorphisms in metabolic/inflammatory pathways showed significant association with T2DM. Three loci showed significant association with lipid profile, body weight and hsCRP levels. hsCRP levels demonstrated a strong positive correlation with body mass index. Genetic score analysis showed that combining multiple genetic markers results in higher relative risks. The functional significance of these polymorphisms is being further evaluated using targeted siRNA-mediated silencing in cultured monocytes.
Conclusion: A panel of ten candidate genes has consistently demonstrated significant association with type 2 diabetes and metabolic syndrome in the Maltese population. These gene variants serve functional roles in inflammation and adipose tissue function. A recruited cohort of untreated newly-diagnosed T2DM serves to identify and explore genotype-phenotype association. The strong effect sizes of these alleles could be used to develop personal genetic susceptibility profiles for T2DM leading to personalization of care and prevention of chronic complications.

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P04.97

Analysis of the WFS1 gene promoter polymorphisms as putative risk factors of diabetes mellitus

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Wolframin is a transmembrane protein of the endoplasmic reticulum (ER) playing a role in maintaining ER homeostasis. The protein is coded by the *WFS1* gene, loss-of-function mutations are responsible for the monogenic DIDMOAD syndrome (diabetes insipidus, diabetes mellitus, optic atrophy and deafness), whereas polymorphisms of the gene were suggested to be risk factors of diabetes mellitus.
Our aim was to investigate the association of two promoter polymorphisms, the rs4273545 single nucleotide polymorphism (SNP) and the rs148797429 insertion/deletion variant (ins/del) with diabetes mellitus. 452 patients and 484 healthy controls took part in the study, genotypes were analyzed by PCR-RFLP and allele-specific amplification. Statistical analysis was carried out using khi-square statistics, linkage analysis was done by the Haploview software.
The rs4273545 G/T SNP showed a significant association with type 2 diabetes (T2DM), even after correcting for Bonferroni ($p = 0.00034$, OR = 1.343), but not with type 1 form of the disease (T1DM). No significant association was found however between the ins/del and either T1DM or T2DM. Linkage analysis revealed linkage disequilibrium between the SNP and the ins/del ($D' = 0.97$; $R^2 = 0.78$), moreover a combined analysis of the two loci demonstrated that the rs4273545T-rs148797429ins haplotype showed an increased risk for T2DM ($p = 0.0004$, OR = 2.410).
In silico analysis suggested that these two polymorphisms are in a GC-rich region possibly altering the methylation status of the promoter region and influencing the binding of transcription factors (e.g. Sp1). *In vitro* functional analysis using luciferase reporter constructs is in progress.

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P05.001

A combination of inherited CNVs of unknown significance: Could it be responsible for severe phenotype?

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Microarrays can identify CNVs associated with intellectual disability (ID). The assessment of causality of inherited aberrations is difficult. We report an affected boy in whom a combination of inherited CNVs was likely pathogenic.

The patient showed severe ID, speech delay, ADHD, seizures, microcephaly, craniostenosis, strabismus, hypotonia, lower limb deformities, atypical fat distribution, facial dysmorphism (hypertelorism, high nasal bridge, broad nose, small mouth, thin lips, long philtrum, small chin, low-set ears), asthma and allergies. His karyotype was normal. SNP array analysis revealed 4 large CNVs: 0.8 Mb dup5p13.2, 1.4 Mb del13q12.12, 0.9 Mb dup19p13.3, and 2.9 Mb del14q32.2. The large del14q32.2 was maternal and the remaining 3 aberrations were paternal. The parents were mildly affected, both attended a special school. The mother had a similar phenotype with dysmorphism, speech disorder, craniostenosis, asthma and allergy.

The maternal deletion involved zinc finger protein gene BCL11B highly similar to BCL11A, the candidate for the 2p15-p16.1 microdeletion syndrome. The paternal dup19p33.3 affected 33 genes including ID candidates MAP2K2, EEF2 and HDGFRP2.

The presence of 2 CNVs of unknown significance larger than 500 kb is 8x more likely in ID patients than in controls. CNVs tolerated in mothers can cause ID in sons. This and the milder parental affection, large CNV size and involvement of candidate genes suggest that the combination of CNVs could cause the severe phenotype in our patient. The combination might exceed the tolerable load of genetic hits and/or transfer them to a sensitive genetic background.

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Further delineation of 12p13.33 micro deletions: Two families with small overlapping deletions.

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12p13.33 microdeletion, associated with intellectual disability (ID), specific language impairment and psychiatric manifestations has only been described in a handful of case reports. We report 2 families with overlapping, but not identical small microdeletions on chromosome 12p13.33, who presented with features consistent with the previously published cases. Array CGH was performed using BlueGnome Cytochip oligo ISCA (4080-5) 8x60K array. Family one consists of an affected mother in whom the deletion has occurred de novo and her 2 affected daughters. They have a 896kb deletion at 12p13.33 [chr12:1,972,757-2,868,481 (hg18)]. involving the CACNA1C, DCP1B, FKBP4, ITFG2, NRIP2, FOXM1 and C12orf32 genes. The mother has mild ID with prominent psychiatric issues whilst her 2 daughters have mild ID and significant speech issues. Family two consists of an affected mother (parents unavailable for testing) and her affected son and daughter. They have a 1.35Mb deletion at 12p13.32-p13.33 [chr12: 2,117,194-3-,469,086 (hg18)]. Involving the CACNA1C, DCP1B, FKBP4, ITFG2, NRIP2, FOXM,C12orf32, TULP3, TEAD4 and TSPAN9 genes. All have mild ID with prominent speech issues. To date these are the smallest deletions identified in the 12p13.33 region and may assist in localizing the genes responsible for the neurodevelopmental aspects of the phenotype. Despite partial or total deletion of CACNA1C, none of the affected individuals have symptomatic or ECG findings consistent with Brugada syndrome, suggesting that haploinsufficiency of this gene is not enough to cause this condition.

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P05.003

12q12 deletion syndrome: new perspective on the genetic cause of the phenotype

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We present a 9 year old boy with post-natal growth delay (2,5th centile) and delayed skeletal age, mild-moderate intellectual impairment, problems with limb coordination and hypotonia of the limbs. He manifests dysmorphic features: broad forehead, strabismus, low-set ears, broad mouth, small hands and feet, pectus excavatum, widely spaced nipples.

The karyotype of the patient was concluded to be 46,XY,t(7;8)(p12.1;p12), not disrupting any known genes on chr8. The breakpoint on chr7 may be disrupting *POM121L12*, (mapping in progress) which has as yet unknown function. aCGH (244k) performed on the patient detected a deletion of 12q12 (1,1 Mb chr12:44,830,147-45,964,945, hg19), encompassing the genes *NELL2, DBX2* and *ANO6* as well as the pseudogene *PLEKHA9*. Four previously described patients with overlapping deletions share most of the dysmorphic features and the growth- and mental retardation seen in our patient.

We suggest that the loss of *NELL2* may contribute to the growth delay, because it is upregulated in the epiphyseal plates of broilers with excessive growth and is likely a part of the CNS also controlling skeletal development in addition to neuronal differentiation and mitogenesis. It is also coupled to protection and proliferation of neurons in the hippocampus and cerebral cortex, and Nell2-deficient mice have been shown to have impaired spatial learning memory. Thus, *NELL2* may contribute to the intellectual impairment in the patient. *DBX2* might contribute to the psychomotor delay, as *DBX2* is involved in determining the fate of interneurons in the ventral spinal cord controlling motorneurons, as well as in early neural plate patterning.

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P05.004

Dysregulation of *FOXG1* pathway in a 14q12 microdeletion case.

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„*FOXG1* syndrome" includes postnatal microcephaly, severe mental retardation with absence of language and agenesis of the corpus callosum. When the syndrome is associated with large 14q12q13 deletions, the patients present characteristic facial dysmorphism. Although all reports were based on genomic analysis, recently a *FOXG1* regulatory elements deletion associated with a down expression of mRNA suggested an implication of *FOXG1* pathway. Herein, we report the case of a young boy with a phenotype consistent with a „*FOXG1* syndrome". He had a *de novo* translocation t(6;14)(q22.1;q12) associated with a heterozygous 14q12.2q13 deletion encompassing *FOXG1*. Subsequently, we investigated his transcriptomic profile on lymphoblastoïd cell lines and/or fibroblasts and showed that *FOXG1* was commonly down-regulated. Moreover, several other *FOXG1* pathway genes were also disturbed. Our data and review of previous reports highlight dysregulation of *FOXG1* pathway as the cause of the „*FOXG1* syndrome" developmental disorder.

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P05.005

15q11.2 microdeletion (BP1-BP2) and developmental delay, behaviour issues, epilepsy and congenital heart disease: a series of 49 patients

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Chromosome 15's proximal long arm is a region rich in duplicons, defining

five breakpoints for 15q rearrangements. 15q11.2 microdeletion between BP1 and BP2 has been previously associated with developmental delay and behaviour issues. This region contains four highly-conserved and non-imprinted genes: *NIPA1, NIPA2, CYFIP1, TUBGCP5*. Our goal is to refine the phenotype associated with this microdeletion in a large cohort of patients.
Prevalence of this CNV is 0.8% in patients presenting with developmental delay, behaviour issues and/or multiple congenital malformations, analysed by array-CGH in 4 different French Genetic laboratories. After exclusion of patients presenting an associated genetic alteration (known CNV, aneuploidy or point mutation in a known gene), we collected data from 49 unrelated patients.
67.5% of the patients presented with mild or moderate developmental delay, 82% had speech impairment and 60% had behaviour issues (Attention Deficit and Hyperactivity Disorder, Autistic Spectrum Disorder or Obsessive-Compulsive Disorder). Seizures were described in 19% of the patients and 16% had an associated non specific congenital heart disease.
Parents were analysed in 55 % of the families. In those families, 29 % of the microdeletions were inherited from one of both parents, who did not present any of the features associated with the deletion.
Our results support the hypothesis that 15q11.2 (BP1-BP2) microdeletion is associated with developmental delay, abnormal behaviour, generalised epilepsy and congenital heart disease, although this latter finding had not been previously reported.
However, incomplete penetrance and variability of expression amongst patients are frequent and would require further studies to be assessed.

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P05.006

Copy-number variations at 16p11.2 in Estonian patients

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There are many copy-number variations (CNVs) associated with genomic disorders with extreme phenotypic heterogenity. One of such CNVs is duplication-deletion at 16p11.2. This syndrome seems to be as frequent as microdeletion/duplication 22q11.2.
Both deletions and duplications at 16p11.2 have been associated with intellectual disability and language impairment. In case of duplication, underweight and intellectual disability is observed contrary to deletion, where obesity and autistic features are main symptoms. It is shown previously that clinical symptoms in carriers of the deletion/duplication represent opposite manifestation mediated by gene dosage.
From January 2012 until January 2013 we performed 667 CNV analyses (HumanCytoSNP-12 v2-1 BeadChips; Illumina Inc.) and found microdeletion/duplication at 16p11.2 in 10 (1.4%). At the same time we diagnosed in 5 (0,75%) patient with microdeletion/duplication 22q11.2.
Here we describe 11 Estonian patients with CNV at 16p11.2. Three cases were with duplication and 8 with deletion in that region. The length of changes varies between 0.2Mb-0.8Mb.
All patients show developmental delay and language impairment in different severity. As expected, dysmorphic features are presented in very minor scale. In one family two brothers had inherited the deletion from father. In one case the duplication was inherited from mother. All parents are not clinically investigated yet.
16p11.2 microdeletion/duplication syndrome is surprisingly frequent in Estonian population. It seems to be even more frequent CNVs than 22q11.2 microdeletion/duplication. Such finding can be explaned by the fact that since 2012 chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities and/or congenital anomalies.

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P05.007

Systematic phenotyping of the 16p11.2 600 kb deletion and duplication carriers

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The ~600kb 16p11.2 deletions and duplications are among the most frequent genetic etiologies of neurodevelopmental disorders. These recurrent rearrangements are associated with mirror phenotypes such as obesity and underweight, macro- and microcephaly, autism spectrum disorder (ASD) and schizophrenia (SCZ). In 16p11.2, as well as in other genomic disorders, reports of variable expressivity and "incomplete penetrance" have hampered genetic counseling and patient management.
To better understand this variability, we compared the effects of deletions and duplications through a systematic assessment of large cohorts of 16p11.2 rearrangements' carriers (Simons VIP and Europe consortia: n= 287 del, 301 dup). The deletion consistently impacts neurodevelopment, regardless of ascertainment. It is associated with a decrease in full scale IQ (FSIQ) of two standard deviations and frequently causes specific language difficulties. Psychiatric co morbidities are present in the majority of carriers, and seizures are observed in 24% of deletion carriers. We identified an increased velocity of head circumference growth during infancy, which recapitulates the well-documented pattern seen in ASD. In contrast, duplication carriers have a broader range of IQ when compared with deletion carriers (increased rates of FSIQ below 50 and above 100: OR=3.5, p=0.002): this heterogeneity is associated in part with ascertainment method. The association of the duplication with decreased head circumference is similarly influenced by ascertainment, suggesting that the phenotypic heterogeneity might be caused by an interaction between the duplication and other genetic and/or environmental factors. Additional genetic variants that could underlie these observations are currently under investigation.

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P05.008

Chromosome 16q12.1 deletion: a case report adding evidence for a new microdeletion syndrome

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Interstitial deletions of the proximal long arm of chromosome 16, involving bands 16q11 to 16q13, are rarely reported. With the global implementation of the array-Comparative Genomic Hybridization (array-CGH) technique, the reported cases can now be well characterized at a molecular level, with exact breakpoint establishment. Despite some phenotypic variability, the few reported cases with 16q deletion present some common features, like developmental delay, abnormally shaped ears, hypotonia, limb anomalies and dysmorphisms.
We report a 9 year old female patient with a *de novo* 3.9Mb deletion at chromosome 16q12.1, detected by array-CGH using an Agilent 180K oligonucleotide. She presented with short stature, renal bilateral anomaly, development delay, hypotonia, facial dysmorphisms and precocious puberty.
The reported deletion contains 18 genes, one of which is *SALL1*, whose mutations cause Townes-Brocks syndrome (TBS), a rare dominant developmental disorder characterized by anal, renal, limb and ear malformations. The few reported patients with 16q12.1 deletions have in common the loss of *SALL1* gene, but they do not meet the clinical criteria for being diagnosed as having TBS. In the literature, it has been suggested that TBS is caused by a dominant negative mutation of the gene, whereas haploinsufficiency results in a milder phenotype, like the ones observed in patients with the deletion including our patient. The deletion of adjacent genes might contribute to the phenotypic variability that is observed among these patients.
Report of further patients is necessary in order to define the critical region and the candidate genes responsible for this new 16q12.1 microdeletion syndrome.

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P05.009
Duplication-Deletion-Duplication 17p13.3 with the smallest reported deletion of YWHAE

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Microdeletion of 17p13.3 involving YWHAE (14-3-3-epsilon), but distal to PAFAH1B1, is a newly recognized syndrome associated with variable disorders of cortical development and facial dysmorphism. More recently, both microdeletions and microduplications mapping to this region have been identified. Clinical characterization has been reported according to the length and the genes involved in such rearrangements.
we report on a boy presenting with seizure, learning difficulties and foot abnormalities.Array comparative genomic hybridization (oligonucleotide array-CGH Nimblegen) showed a complex rearrangement with six breakpoints on 17p13.3: Duplication-Deletion-Duplication. Deletion of 30 Kb included only the last exon of YWHAE. First duplication of 380 Kb involved NXN, TIMM2, ABR genes and the second of 164 Kb involved CRK, MYO1C, INPP5K, PITPNA genes. Quantitative PCR was performed to confirm the rearrangement and to show that was absent in both parents and therefore occurred de novo.
We are able to review and refine the molecular and clinical features of recently described novel microdeletion and microduplication on 17p13.3. We report the smallest deletion of YWHAE described. This gene is encoding a highly conserved protein that has been shown to play a crucial role in neuronal development and synaptogenesis. We compare critical regions delineated and candidate genes proposed in cohorts with the aim of refining the phenotype-genotype correlation.
Breakpoints of this non recurrent complex genomic rearrangement were not associated with low-copy repeats and are therefore probably due to replicative mechanism such as Fork Stalling and Template Switching (FoSTeS) or Microhomology Mediated Break Induced Replication (MMBIR).

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P05.010
19q13.11 Microdeletion Syndrome due to an apparently balanced *de novo* chromosome rearrangement: Characterized by high-density SNP array analysis

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Background: 19q13.11 Microdeletion Syndrome [OMIM#613026] is an emerging disorder characterized by pre-postnatal growth retardation, developmental delay, microcephaly, minor facial dysmorphic features, and signs of ectodermal dysplasia, extremity malformations, and genital abnormalities in males (hypospadias). Using SNP array we detected a 19q13.11 microdeletion in a patient with apparently balanced *de novo* cytogenetic rearrangement between chromosomes 2p35 and 19q13.11. **Methods and Results:** We study a male patient of 6 years 7 months old age with all principal features of 19q13.11 microdeletion syndrome. By cytogenetic-FISH analysis identify to *de novo* apparently balanced non-reciprocal t(2;19) (p35;q13.11). Using 250 k Nsp SNP array we detect a *de novo* interstitial 19q13.11 pat deletion of ~2.54 Mb. To the further characterized the extent of deletion was analyzed by high-density 6.0 SNP array, and found a 2,489,839 pb deletion, the proximal and distal breakpoints at (33,565,628-36,055,467). We realized genotype-phenotype correlations of our patient with eight previously published cases, and two annotated in DECIPHER database, refining the critical region to ~517 kb (chr19:34,919,268-35,436,076), containing 9 RefSeq genes (*UBA2, WTPI, SCG2B2, ZNF302, ZNF181, ZNF599, LOC400685, LOC100652909,* and *ZNF30*). This genomic region was further analyzed the probability of gene haploinsufficiency; resulting *UBA2* as the most significant gene for the phenotype; including the male genital abnormalities. **Conclusions:** To our knowledge, this is the first case report of 19q13.11 deletion syndrome due to an apparently balanced de novo chromosome rearrangement. In addition, we propose that *UBA2* may be responsible for the core phenotype of this novel microdeletion syndrome.

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P05.011
Inherited 1q21.1q21.2 duplication and 16p11.2 deletion : a two-hits case with severe clinical manifestations

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In a fetus exhibiting absent nasal bone, a proximal duplication of chromosome 1 long arm was first identified on karyotype, associated with a 16p11.2 deletion secondary characterized by array-CGH. Duplication was shown to be paternally inherited. Whole chromosome 1 painting probe in both excluded a translocation or an insertion elsewhere. Oligoarray-CGH on amniotic fluid characterized the 1q21.1q21.2 duplication of 5,8Mb with father's identical breakpoints. Furthermore a 16p11.2 deletion of 545Kb was revealed that was unexpectedly maternally inherited and identical.
Paternal history was noteworthy for placement since 6 months-old in host families because of his parents inability to take care of him. Language delay was noted at 3 years. Proband's mother was adopted at 1 month-old. Language delay and learning difficulties were observed associated with overweight.
In early development, proband presented epilepsy and axial hypotonia followed by psychomotor retardation. Absence of nasal bone was confirmed. The boy exhibited macrocephaly, dysmorphy and congenital anomalies.
Thus, we report a case with two inherited chromosomal rearrangements resulting in exacerbation of neurodevelopmental phenotype compared to his parents. Described 1q21.1 duplication is associated with developmental anomalies and macrocephaly notably because of the *HYDIN* paralog gene (Brunetti-Pierri *et al*, 2008). The 16p11.2 deletion, a novel microdeletion syndrome is associated with abnormal behaviour, mental retardation and macrocephaly (Walters *et al*, 2010, Nature, and Jacquemont *et al* 2011, Nature). We will discuss the fact that occurrence of additional chromosomal events, as described in the proband, may be involved in variable expressivity and clinical heterogeneity as observed in the family.

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P05.012
1q21.1 microduplication syndrome in a boy and his father

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Chromosomal band 1q21.1 can be divided into two distinct regions, proximal and distal, based on segmental duplications that mediate recurrent rearrangements. Microdeletions and microduplications of the distal region within 1q21.1 are susceptibility factors for a variety of neurodevelopmental phenotypes and distinct dysmorphic features.
We present a 6 years old boy with severe intellectual disability, ASD, significant speech delay, hyperactivity and macrocephaly. He comes from 3rd pregnancy complicated by preeclampsia , diabetes and excessive obesity of the mother (there were 2 sponatenous abortions from another partnership of the mother). His early development was delayed- walking unattended at 18 months of age. He exhibits significant facial dysmorphism: macrocephaly with prominent forehead, long face, arched eyebrows, periorbital fullness, epicanthal folds, broad nasal bridge, upturned nose, deep philtrum . His extremities are relatively short with short fingers, he also has hypoplastic genitalia.
The extent of the duplication of our patient is 2.3 Mb , affecting 32 HGNC genes, chr1:144.510.930 - chr1:146.812.122.(hg18)
His father is a carrier of microduplication confirmed by FISH analysis, the exact extend will be confirmed by array CGH method. He has borderline intelectual capacity, his facial features are almost normal.
Consistent phenotypic differences haven't yet been described between people with different-sized microduplications - and people in the same family with the same size microduplication can have very different features.
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P05.013
1q44 microdeletion in a Czech boy

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The 1q44 microdeletion syndrome is a newly described syndrome first reported by De Vries et al (2001). It is associated with facial dysmorphism, developmental delay (in particular delay of expressive speech), seizures and hypotonia. The most common facial features include microcephaly, hypertelorism and thin upper lip. Abnormal corpus callosum (agenesis, hypogenesis or slightly reduced thickness) is observed in some affected patients. Our patient is a 6-year-old boy with a de novo deletion of 1q44 of about 800 kb (242,951,908-243,763,883, hg18). He was born from the 3rd pregnancy (two previous pregnancies ended by spontaneous abortion). The psychomotor development of the boy is delayed (sitting at 12 months, walking attended by both hands from 18 months, unattended at 3 years, he understands simple sentences but does not speak understandable words). He suffers from seizures developed at the age of 2 years (first two seizures were during fever, since 3 years of age the seizures are short, with wrapping up and turning blue in the face). MRI showed normal corpus callosum, but small changes in white matter. The deletion of our patient is one of the shortest published so far, affecting only 5 genes (FAM36A/COX20, NCRNA00201, HNRNPU, EFCAB2 and a part of KIF26B), and his clinical picture helps to define the phenotypic spectrum of the syndrome. Supported by CHERISH, NT/14200 and 00064203.

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P05.014
Fulminant Hepatic Failure associated with Phelan McDermid syndrome

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Phelan McDermid syndrome is caused by 22q13.3 deletion and its typical features are severe intellectual disability, absent or poor speech development, decreased pain-sensitivity, hypotonia and mild facial dysmorphism. The syndrome could also comprise autistic features, seizures or thermoregulation problems. Rare symtoms are hearing loss, genitourinary anomalies and congenital heart defect. Extremely rare is association of Phelan McDermid syndrome and hepatic failure in early childhood. There have been described only 2 patients with this condition so far. In these cases PIM3 gene (localized in 22q13.33) was considered to be responsible.
We present 7 patients (2 males and 5 females) with Phelan McDermid syndrome. Age at diagnosis ranges from 10 month to 55 years and deletion extent varies from 0.4 Mb to 7.6 Mb. All patients present common features of the syndrome, but they differ in inconstant sypmtoms. Any correlations between phenotype and deletion range haven't been observed.
Patients have been diagnosed using FISH and MLPA, deletion extents were proven by oligonucleotide arrayCGH.
There are 6 patients with deleted PIM3 gene in our group, including two adult patients, but only one of these patients (10-year-old boy with 1.9 Mb long deletion) has developed hepatic failure recently. Therefore simple PIM3 deletion doesn't seem to be the cause of hepatic failure.
The possible mechanism is an alteration of the gene on the other allele, epigenetic factors or mutation in some modifier genes. We discuss potential benefit of whole exome sequencing to further elucidate mechanism in these patients.
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P05.015
2q24.3 deletion involving SCN1A in a patient with profound multiple disabilities and severe epilepsy

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SCN1A haploinsufficiency is associated with Dravet syndrome. We report a microdeletion involving SCN1A in a patient with profound handicap. After uneventfully first months of life, the proband presented at 4 months febrile seizures after a vaccination. Examination showed hypotonia and poor eye contact. In the following weeks, he presented recurrent febrile or afebrile seizures and his neurological status deteriorated with loss of eye contact, worsening hypotonia, stagnation of development. EEG, initially normal, were severely modified after several months. Epilepsy was refractory to drugs. Different types of seizures were reported : generalized, partial, myoclonic, absences, status epilepticus. Seizures were mostly provoked by slight hyperthermia. At 20 years, he was unable to sit without support, had no eye contact, expressed no words, had dysmorphic facial features, microcephaly. He underwent vertebral arthrodesis and gastrostomy. Array-CGH analysis identified a 3.2Mb de novo deletion at 2q24.3 involving SCN1A and 11 adjacent genes. The proband presented several criteria of Dravet syndrome : onset in the first year of life, frequent and intractable seizures of different types, febrile seizures, but his cognitive impairment was more severe than that commonly associated with this disease. 2q24.3 deletions are rare and mostly much larger and are associated with profound disability. The severity of the clinical presentation in this patient shows that haploinsufficiency of other genes on 2q24.3 contributes to the profound disability associated to 2q24.3 deletions. This observation shows that 2q24.3 deletions should be considered in patients presenting severe handicap associated with febrile seizures, a key feature of Dravet syndrome.

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P05.016
2.5 Mb Deletion of 3p25.3

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A 6 years old girl with severe mental retardation was referred to our genetics lab. The first and only child of non- consanguineous healthy parents, she was delivered at age of 9 months by C-section. Her birth weight was 255 grams. At examination she does not walk or crawl. She had seizures when she was 2 months. Her facial features include epicanthal folds, puffiness of eyelids, strabismus, arched eyebrows, synophris, short upturned nose and long philtrum . She had notable hirsutism on her chin, forehead and legs.
Her karyotype was normal. Whole genome BAC Array Comparative Genomic Hybridization was performed using CYTOCHIP genomic BAC array version

3.0 and AGILENT 4x44 whole genome array. Data were analysed using BlueFuse Multi software. A 2.5 Mb deletion of 3p25.3p25.3 was detected.
The deletion spans nucleotides 8.85 Mb to 11.3 Mb covering 23 OMIM genes and 7 OMIM diseases. We compare phenotypic and genotypic findings of other overlapping cases. The deleted region in our patient includes the SRGAP3/MEGAP gene that has been formerly implicated in cognitive impairment in 3p microdeletion syndrome but does not include CAV3 gene.

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P05.017
A pure de novo duplication of 9p in a woman with intellectual disability and dysmorphic features

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Duplication of 9p is a fairly well-described syndrome. In spite of the variations in the size of the duplicated segment, its phenotype is clinically recognizable.
However, there are some reported cases with relatively mild phenotype, without intellectual disability. Due to phenotypic heterogeneity it is essential to present detailed phenotype description to delineate a critical region for the 9p duplication syndrome.
We report on a 20-year-old woman with pure de novo 9p duplication. She was referred to the genetic counseling unit because of intellectual disability and dysmorphic facial features. Moreover hypotonia in early childhood, short stature, scoliosis and hypergonadotropic hypogonadism were noted. The diagnosis of duplication was established by MLPA and delineated by arrayCGH as 46,XX.arr cgh 9p13.1-pter(199,254-38,751,949)x3 dn with the size of 38.55 Mb. Our report is one of the few ones giving the molecular and clinical characteristics of pure 9p duplication. In the literature there are some suggestions concerning the critical regions and phenotype-genotype correlation. However, the genes influencing the individual clinical manifestations of the 9p duplication have not yet been identified.
There is the need for further studies of 9p duplication patients to establish precise genotype-phenotype correlations in this chromosomal region.
The study was supported by MNiSW Grant No. 0605/B/P01/2009/37. Acquiring of the Roche NimbleGen microarray platform was co-financed by ERDF project (EU Structural Funds) POIG.02.01.00-14-059/09.

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P05.018
Implementation of array comparative hybridization technology in detection of chromosomal abnormalities in patients with intellectual disability

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Array Comparative Genomic Hybridization (aCGH) technology is a powerful tool for simultaneous detection of multiple chromosomal abnormalities with much higher resolution than conventional cytogenetics.
The aim of our study has been to implement the aCGH technology in detection of postnatal chromosomal abnormalities in patients with developmental delay/intellectual disabilities, with/without congenital anomalies (DD/ID/CA).
Seven patients with DD/ID/CA and dysmorphic features, aged between 1 to 14 years, previously assessed by conventional cytogenetic analysis, were included in aCGH analysis with NimbleGen ISCA Plus 3x1.4M Platform (Roche).
The aCGH analysis detected clinically relevant chromosomal abnormalities in three of the patients analyzed, with relevant abnormal phenotype.
For the first patient, the aCGH analysis precisely characterized the marker chromosome found by conventional cytogenetic analysis (karyotype: 46,XX der (8)), a duplication of 30 Mb being involved (8q13.3-q22.3 regions).
In the second patient case, a boy of 3 year old with DD/ID, obesity (karyotype: 46,XY,t(6;12)dn), the aCGH analysis identified a 6 Mb deletion on chromosome 6 (6q16.1-q16.2-q16.3 regions), with pathogenic significance.
In the third case patient, a boy aged 14 with DD/ID, facial dysmorphism and skeletal abnormalities (normal karyotype) a microduplication of 1.9 Mb in the 17p12-11.2 region was detected. This chromosomal region contains several important genes, including the dosage-sensitive RAI1 gene, suggesting the recently described Potocki-Lupski syndrome.
Our ongoing study demonstrates that aCGH is very useful in precisely identifying and characterizing chromosome anomalies (previously detected or not by conventional cytogenetics), being a valuable tool for providing an accurate diagnosis and helpful genetic counseling to the family.

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P05.019
Submicroscopic abnormalities in a girl with mental retardation and dysmorphic findings revealed by aCGH analysis

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Specific copy number variations (CNVs) have been found to be associated with susceptibility to various intellectual and developmental disorders. One of those is mental retardation (MR) with developmental delay with an estimated prevalence of 2-3% in the general population. Application of array comparative genomic hybridization (aCGH) techniques to clinical practice, unraveled genetic aberration in MR patients with normal constitutional karyotype. Here we report a five-year-old girl with mental and motor retardation, epilepsy, and dysmorphic findings with normal constitutional karyotype. To determine the genetic contribution in the development of clinical findings, aCGH analysis was performed. Roche NimbleGen Human CGH ISCA Plus 3x1.4M Array was used for aCGH analysis. The data were analyzed by using Nexus6 software (BioDiscovery). As a result of aCGH analysis, 5 chromosomal regions were marked as important regions. Among these regions, one of them has been found to be related with autism spectrum disorder and intellectual disability, previously. Microdeletion in the *PTCHD1* locus on Xp22.11 has been has been found in autism spectrum patients but not in healty controls. However, we found a 24.3kb copy gain in the same locus in our patient. This finding showed that *PTCHD1* locus may not be disrupted with deletion only, copy gain in that region might also have effect on the gene, and its function. Also, other identified copy gains and loss may have contributed to the accompanying dysmorphic findings. As we experienced in the current patient, array CGH analysis is a valuable tool in uncovering submicroscopic chromosomal rearrangements.

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P05.020
Novel changes in the SLC16A2 gene identified by X-exome sequencing in two Finnish families with Allan-Herndon Dudley syndrome.

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Background: Allan-Herndon-Dudley Syndrome (AHDS) is typically characterized by severe psychomotor impairment. Mutations in the TH transporter gene, SLC16A2 (also known as MCT8) have been associated with the AHDS in more than 45 families. SLC16A2 is an essential TH transporter involved in the transport of TH across the blood-brain barrier and the blood-cerebrospinal fluid barrier.
Methods: We performed Agilent SureSelect enrichment of all X chromosome specific exons from the index patients, followed by massively parallel sequencing. All variants were filtered against the publicly available database.
Results: We identified a 5bp insertion in exon 3 of SLC16A2 gene in family D299, which likely caused a frameshift mutation (p.G334PfsX11). In family L107 we identified a C>A missense change (p.R445C) in exon 4. Both changes completely segregated with clinical phenotype in the families they were found and were not present in 100 anonymous Finnish controls. Affected patients in both families suffered from severe intellectual disability, impaired speech, poor head control & movement disorders.
Conclusions: The novel mutations in SLC16A2 confirm the diagnosis of Allan-Herndon-Dudley syndrome and represent, to the best of our knowledge, the first molecularly confirmed cases in the Finnish population.

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P05.021

Allan-Herndon-Dudley syndrome: increased serum triiodothyronine (T3) is a key diagnostic marker

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Allan-Herndon-Dudley syndrome (AHDS) is an X-linked intellectual disability syndrome caused by mutations in SLC16A2 which encodes the monocarboxylate thyroid hormone transporter 8 (MCT8). Clinically, the affected male patients are characterized by severe intellectual disability, initial hypotonia with subsequent spastic paraplegia, dystonic posturing and superimposed paroxysmal dyskinesia. The patients typically have elongated faces, but other dysmorphic features are less consistent. Leukodystrophy and white matter changes are present in early childhood and improve with age. The most specific laboratory finding in AHDS patients is an increase of serum triiodothyronine (T3), while serum thyroxine (T4) is decreased and thyroid-stimulating hormone (TSH) is either normal or mildly increased.
We report on a 17-year old patient with severe intellectual disability and neurological problems in whom increased serum T3 led us to suspect AHDS which was confirmed by the detection of a SLC16A2 mutation (c.812G>A, p.R271H).

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P05.022

Alstrom syndrome: two brothers with two heterozygous *ALMS1* mutations (Y1571X AND L1423X)

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Alstrom syndrome (AS, #203800) is a rare autosomal recessive disease characterized by multiorgan dysfunction. The key features are childhood obesity, blindness due to congenital retinal dystrophy, and sensorineural hearing loss. Associated endocrinologic features include hyperinsulinemia, early-onset type 2 diabetes, and hypertriglyceridemia. Thus, AS shares several features with the common metabolic syndrome, namely obesity, hyperinsulinemia, and hypertriglyceridemia. Mutations in the *ALMS1* gene have been found to be causative for AS. ALMS1 protein localizes to the basal bodies of cilia and plays an unknown role in intracellular trafficking.
CASE REPORT: We describe the case of two brothers of 13-year-old (male) and 11-year-old (female) born to unrelated healthy spanish parents. Both patients were attended in the Paediatric Service with a diagnosis of Alstrom syndrome. Boy had hypothyroidism, acanthosis nigricans and hepatosplenomegaly. He had normoacusia in the limit. The child also gave a history of photophobia with nystagmus along with 100% visual disability. The patient also had secondary metabolic myopathy which involved the proximal muscles and kyphosis. Girl had hypothyroidism, dyslipemia, hypoacusia and dilated caridomiopathy. As her brother, she was blind (100%) and had a history of photophobia with nistagmus. Both brothers had a progressive cronic nephropathy and central obesity. Genetic analysis showed that both were carriers of two heterozygous *ALMS1* mutations: c.5145T>G; p.Tyr1715X and c.4268T>A; p.Leu1423X. Both mutations cause a truncated protein and had been described previously in literature. Diagnosis of Alstrom Syndrome can be difficult because some features begin at birth and others emerge as the child develops and phenotype is family-dependent.

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P05.023

6q25.3 deletion encompassing ARID1B in a patient with intellectual disability and aggressive behavior

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Introduction
Intellectual disability (ID) is one of the most frequent and disabling neurological impairments in school-age children, accounting for 1-3% of the population in the developed countries. The impact of aCGH in the practice of Medical Genetics has been transformative, allowing genome-wide studies in patients with ID.
Patients and Methods
In this work we report a *de novo* deletion at 6q25.3 citoband in a patient with hirsutism, sparse and coarse hair, coarse face, bushy eyebrows, squint, bulbous nose, large mouth, thin upper lip, short stature, brachydactyly, umbilical hernia, borderline hepatosplenomegaly (on US).
The alteration was determined by aCGH analysis (Agilent 180K custom array) and the confirmation studies and inheritance analysis was carried out by qPCR using a fragment designed inside the altered region.
Results and conclusion
aCGH revealed a *de novo* 2.7 Mb deletion at chromosome region 6q25.3 containing 14 genes. This region was found to be deleted in patients with development delay, microcephaly, dysmorphic features and hearing loss together with sacral/anorectal malformations (Nagamani S. et al, 2009; Titomanlio L. et al, 2006). Among the genes affected in the patient, *ARID1B* (AT-RICH INTERACTION DOMAIN-CONTAINING PROTEIN 1B) was recently found to be mutated in Coffin-Siris syndrome patients. The authors also described copy number variations affecting *ARID1B* gene to be associated with the syndrome (Santem GW, et al, 2012). Siris syndrome and the intellectual and speech impairment also featured in the syndrome. Our patient brings new insight to the delineation of the 6q25 microdeletion syndrome and *ARID1B* haploinsufficiency in Coffin-Siris syndrome.

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P05.024

Chromosome Xq11.23 deletion of 846 kb in a 2,5-years old developmentally delayed boy with agammaglobulinemia

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We present a 2,5 years old male from orphanage, examined at the age of 11 months for developmental delay and frequent bronchitis. The boy is the first child of a healthy, consanguineous couple. His cousin died at the age of 3 years and was mentally retarded with immunodeficiency. He was delivered at 37 weeks of gestation, birth weight of 2700 g (pc=25th), length of 48 cm (pc=50th) with normocephaly. Clinical evaluation revealed: short stature (-4,2 SDS), hypotonia, hypotrophy, pectus excavatum, alopecia areata occipital, ventricular septal defect, congenital glaucoma, hypacusis mixta, CD19 negative agammaglobulinemia. Development milestones were delayed, he was lying in bed. For epilepsy and feeding problems we performed screening for metabolic disorders, but results were negative. Cerebral MRI was normal. The aCGH-chip 44 Oligo Microarray Agilent detected a deletion on chr X: 48.635.654-49.481.774 bp 846kb, Xp11.23. Fourty four genes are located in the deleted region including PQBP1 and SYP, which may be good candidates in generating the phenotype. The PQBP1caused Renpenning syndrome, an X-linked mental retardation syndrome with clinically recognizable features in our patient. The SYP is involved in regulation of gene for Wiskott-Aldrich syndrome.

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P05.025

Array-CGH study of chromosomal rearrangements in patients with intellectual disability from Russia and Ukraine

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This research is an extension of the chromosomal rearrangements study in patients with intellectual disability (ID), which was supported by EC FP-7 CHERISH project (no. 223692). The previously obtained results based on array-CGH screening revealed pathogenic causative CNVs in 15.8% of patients with ID (9 of 57). Thereby the high information content of array-CGH for identification of genomic rearrangements associated with ID has been proved. As far as the majority of identified chromosomal deletions and duplications were benign (non-pathogenic) CNVs, it is important to distinguish common CNVs in the observed groups. Therefore we initiated a molecular cytogenetic study in ID-patients of two similar ethnic groups (Slavic Russian and Ukrainian populations).
The first data of array CGH analysis showed that the most frequent CNVs in our groups were dup8p11.23-p11.22, dup/del15q11.2 and dup6p21.32.

The high frequency of these rearrangements suggests that they are benign, but this hypothesis needs to be verified. In contrast, rare probably pathogenic chromosomal rearrangements have been also detected in both groups of patients, some of which are identical in patients from Russia and Ukraine.
Further research will allow us to define the role of novel CNVs in the ID-phenotype manifestation, and determine the frequency of common pathogenic as well as non-pathogenic rearrangements in both populations.
The results of this study will significantly improve information content of molecular cytogenetic screening based on array-CGH in ID-patients from our countries.

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P05.026
A small de novo 16q24.1 duplication in a woman with severe clinical features

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We report here a de novo 16q24.1 interstitial duplication in a woman with a severe phenotype consistent with mental retardation, spastic paraplegia, severe epilepsy, a narrow and arched palate, malar hypoplasia, little subcutaneous fat and arachnodactyly. Although conventional karyotyping was found to be normal, array-CGH detected a small duplication on chromosome 16. Using QFM-PCR, we characterised its proximal and distal breakpoints. The duplication, which is approximately 250 kb, encompasses seven genes (KIAA0182, GINS2, c16orf74, COX4NB, COX4I1, MIR1910 and IRF8). Several reports have previously described large 16q duplications, and some of these overlap with our region in 16q24.1.
Due to the variability of the described phenotypes, the characterisation of small 16q duplications may help to determine critical regions and the genes they contain that are associated with the components of complex phenotypes.

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P05.028
New insights on cognitive and structural brain imaging phenotype in primary microcephaly due to ASPM mutations

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Primary recessive microcephaly (MCPH) caused by ASPM mutations is a model of abnormal brain development linked to neural progenitors proliferation defects. MCPH is defined as a homogenous reduction of cerebral volume principally affecting the cortex. The reduction of brain volume has been correlated to cognitive disabilities. However, correlation between cognitive functions and structural brain phenotype has not yet been well established in genetic microcephalies.
Here, we provide evidence that specific cognitive functions are preserved in ASPM-related patients and this is correlated with their structural brain changes. General intelligence, memory scales and structural brain magnetic resonance imaging scans were acquired from 6 ASPM-related patients.
Using cranial MRI, we measured the volume of the different brain structures and focused on the analyze of the cortical volume, surface and thickness. These microcephalic patients have a normal mnesic functioning and preserved hippocampal volume compared to other cortical areas.
Unlike most previous data that linked microcephaly to mental retardation, these findings suggest i) that these microcephalic ASPM-related patients are able to learn despite their cognitive disabilities and ii) that other master genes than ASPM are necessary for the development of hippocampus formation and function.

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P05.029
ATRX duplication syndrome: two overlapping duplications at Xq13.3-q21.1

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In two unrelated male patients with syndromic intellectual disability (ID) we detected two duplications of 12 and 18 Mb, respectively, by array-CGH. The common overlapping interval spans 6Mb at Xq13.3-q21.1, where only 25 genes are annotated. Interestingly, both patients also share many clinical features, suggesting a major role for any of the genes contained in the common interval. Among these, the most outstanding is ATRX, the causative gene of X-linked alpha-thalassemia/mental retardation syndrome, one of the growing list of genes implied in chromatin remodeling causing ID: MECP2, CBP, RSK2, NSD1, etc. Many of these genes, especially MECP2, which closely interacts with ATRX, are dose-sensitive so that not only haploinsufficiency mutations, but also duplications are related with syndromic ID.
Among the clinical features, both patients share severe ID, absent expressive speech, early hypotonia, behavioral problems (hyperactivity, repetitive self-stimulatory behavior), postnatal growth deficiency,microcephaly,micrognathia, cryptorchidism and several dysmorphic features such as low-set posteriorly rotated ears or downslanting palpebral fissures. These features strikingly resemble the usual findings among patients with loss-of-function mutations of this gene. Furthermore, only one of the carrier mothers of our patients was informative in the analysis of X-chromosome inactivation, and she showed a completely skewed X-chromosome inactivation. This is a constant finding among female carriers of inactivating point mutations of this gene. Taken all together, although the implication of further genes cannot be excluded, we propose that the increased dosage of ATRX is the major pathogenic mechanism of this X-linked phenotype, a duplication syndrome that evokes that of MECP2.

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P05.030
Custom designed CGH array in autism spectrum disorders

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Introduction: Autism spectrum disorders (ASD) have a complex multifactorial etiology. Recently, copy number variants (CNV's) have been shown to contribute to over 10% of ASD cases. Many of the known genes whose mutations cause ASD, have functions related to epigenetic regulation. We have applied a custom-designed oligo array CGH with an exonic overlapping coverage of 450 genes known to cause autism or intellectual disability and autism candidate genes related to epigenetic machinery.
Material and methods: Custom oligonucleotide arrays (formats 4x44 and 8x60) were designed with e-Array Agilent technology. The final array design contains: 36,000 exonic probes for 450 genes considered of high priority (mean distance between probes 25bp), 2,000 probes for regulatory elements and microRNA's and 22,000 whole genome probes (backbone).
Results: We have studied a series of 139 patients including syndromic autism and/or familial cases of idiopathic autism. We have found 24 cases with pathological CNV's. Most are non-recurrent CNV, what allow us to propose new candidate genes involved in autism.
Discussion: Our results confirm the importance of three regions in autism: Xq28 (MECP2), 22q13 (SHANK3) and 16q13, while all others regions are extremely heterogeneous. Most pathological CNV were found in cases with syndromic autism.

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P05.031
The 15q11.2 deletion and autism spectrum disorders

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Autism Spectrum Disorders (ASDs) are frequent genetic neurodevelopmental disorders. Several studies have shown the association of Copy Number Variations, both *de novo* and rare inherited, with ASD. Deletions of chromosome 15q11.2 (BP1-BP2 region) including *CYFIP1, NIPA1, NIPA2* and *TUBGCP5*, are associated with intellectual disability, epilepsy, schizophrenia and autism. *CYFIP1* is an important binding partner of FMRP and *FMR1* mutations cause Fragile X syndrome, often associated with autism (30%). Also, patients with the Prader-Willi phenotype caused by paternal deletions of the 15q11-13 BP1-BP3 region present a more severe behavioral phenotype

(ADHD, autism and OCD) compared to those having the BP2-BP3 deletion. Together, this pinpoints the 15q11.2 deleted region as a risk variant for ASD.
Quantitative Real-Time Polymerase Chain Reaction is used to test the occurrence of deletions of *CYFIP1* in ASD patients with intellectual disability (IQ<70), ASD patients with normal intelligence (IQ>70) and controls. We also performed exome sequencing on two ASD families with the proband carrying the del15q11.2, inherited from a normal parent.
We present the results of an association study of *CYFIP1* deletions in ASD patients. Given the variability of the phenotype of del15q11.2, we hypothesize that additional variants in the other allele, genes of the FMRP network or known ASD genes can contribute to the phenotype (multi-hit model). We present the results of exome sequencing of two ASD families, searching for additional hits.
The 15q11.2 deletion is likely to be a risk factor for ASD with/without mental retardation. A multi-hit model could explain the phenotypic variability.

V. De Wolf: None. **H. Peeters:** None. **K. Devriendt:** None.

P05.032
The genetic basis of autism spectrum disorders: identification and analysis of structural variants

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Background:
The genetic causes of autism spectrum disorders (ASDs) are heterogeneous and still unknown in the majority of cases. Structural copy number variants (CNVs) were found in sufficiently high frequency to suggest that cytogenetic and microarray analyses are considered in routine clinical workup. An interesting paradigm for clinical practice is that each rare CNV may account for only a small proportion of variance in ASD at the population level but may have a large effect in a few families in which it segregates.
Material and methods:
CNV association studies are performed in a family based cohort. The sample contains 161 families ascertained through one or more autistic probands with normal intelligence or mild intellectual disability. The study cohort contains 648 individuals: 227 probands, 324 parents, 97 unaffected siblings belonging to 161 families. All probands, unaffected siblings and parents are genotyped with high-resolution Illumina OMNI 2.5-8v1 microarrays. For all individuals, an extensive list of phenotypic information is collected including IQ, SRS scores, 3DI, clinical genetic examination and family history.
Results:
We present the results of a family based study on the validity of CNV detection in ASD using a high resolution platform. We study the segregation of known and novel rare CNVs with qualitative and quantitative autism phenotypes. Additionally association studies are performed with respect to amongst others gene content and parental origin.
Conclusion:
With this study we aim to contribute to the clinical validation of the current knowledge of ASD risk variants and to the identification of novel variants.

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P05.033
New role for microtubule plus-end tracking proteins: mutation in the CLIP-1 (CLIP-170) gene causes autosomal recessive intellectual disability (ARID)

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In the context of a comprehensive research project, investigating novel ARID genes, linkage analysis based on autozygosity mapping helped us to identify a new ID locus on Chr.12q24.13-q24.31 (LOD =3.7), in an Iranian family. Next generation sequencing following exon enrichment in this genomic interval, detected one nonsense mutation (p.Q1010X) in the *CLIP-1* gene. This gene encodes a member of microtubule plus-end tracking proteins that specifically associates with the ends of growing microtubules. CLIP-170 contributes to kinetochore–microtubule attachments, and has a role in neuronal development. It is also necessary for axon formation and dendrite morphology in rat neurons. The detected mutation completely removes part of the central α-helical coiled coil domain and the two functional C-terminal metal-binding motifs, disturbing CLIP-170 intramolecular conformational changes and its interaction with other proteins. In order to prove the involvement of *CLIP-1* in neurodevelopmental disorders, we studied mutant and normal established lymphoblastoid and skin fibroblast cell lines. Reverse transcriptase PCR confirmed the stop codon in all of the mutant cell lines. Western blotting with antibodies against the N-terminus of CLIP-170 revealed a truncated protein in affected individuals' cell lines; while a C-terminal-specific antibody didn't detect any protein. Immunofluorescence microscopy experiments confirmed the Western blot data, showing microtubule plus-end staining in wild type and mutant *CLIP-1* fibroblasts with N-terminal-specific antibodies, whereas C-terminal-specific CLIP-170 antibody just showed staining in normal fibroblasts. Altogether, our data present the first evidence for the involvement of the CLIP-170 in cognitive function and we propose *CLIP-1(CLIP-170)* as a novel gene for ARID.

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P05.034
De Novo Intragenic Deletion of *AUTS2* in a Patient with Developmental Delay: A Case Report

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Array CGH identified a de novo interstitial deletion of 683-806kb at chromosome 7q11.22 involving multiple exons of the *AUTS2* gene, in a 13 year old male with learning difficulties, developmental delay, short stature, ptosis and facial dysmorphism. This is the second report of an isolated *de novo* intragenic deletion of *AUTS2.*
The proband was referred with a history of proportionate short stature, hypotonia, delay in speech and fine motor skills, ptosis and facial dysmorphism. Array CGH was performed using the BlueGnome Cytochip oligo ISCA 8 x 60K array platform. Array CGH revealed a *de novo* interstitial deletion at chromosome 7q11.22 involving exons 3 - 5 of *AUTS2* and no other copy number variants. His parents and unaffected brother had no deletion.
The *AUTS2* gene spans 1.19Mb of genomic DNA on chromosome 7 contains 19 exons. The molecular function of *AUTS2* is unknown. Previous publications describe a number of individuals with disruptions involving *AUTS2* who present with developmental delay/ intellectual disability, hypotonia and autism. Early cases all resulted from translocations or inversions which disrupted other genes in addition to *AUTS2* and made it difficult to attribute the phenotype to *AUTS2* alone. The patient described in our report (and a recent report of another two patients) who have intragenic deletions of AUTS2 with no other CNVs provides strong evidence that disruption of *AUTS2* alone results in a syndromic neurodevelopmental disorder.

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P05.035
A comprehensive study on Iranian patients with Bardet-Biedl syndrome

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Bardet-Biedl syndrome (BBS) is a rare genetic disorder affecting brain with a wide range of phenotypes, including deterioration of the intellect and neurological functions. BBS is a heterogeneous disorder, typically inherited in an autosomal recessive manner with 16 known genes.
This study was performed to detect responsible genes and mutation spectrum in a cohort of 23 Iranian BBS patients. Initially, the most commonly mutated genes (BBS1, BBS2 and BBS10) accounting for about 50% of BBS patients were screened by direct Sanger sequencing. Final diagnosis was determined for 4 patients in BBS2 gene, including three novel mutations. Next, three of the remaining patients were chosen and whole exome sequencing

was performed. Coverage of known BBS genes was 97% in average at 20x depth of coverage. Whole exome sequencing revealed novel BBS4 mutation in one patient, and other two patients had no mutations in any of the 16 known BBS genes. Finally, six other commonly mutated BBS genes (BBS3, BBS4, BBS6, BBS7, BBS9 and BBS12) accounting about 20% of BBS patients, were selected for screening in the remaining 16 patients, which is currently underway. However, responsible mutations have been identified for 5 other patients in BBS12 and BBS9 genes, including three novel mutations.
Our result shows that despite the reported high prevalence of BBS1 and BBS10 mutations in BBS patients of other populations, these two genes may not play an important role in Iranian patients whilst other genes like BBS2 and BBS12 seem to have a greater role comparing to other populations.

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P05.036
Diagnosis and management of Bohring-Opitz Syndrome with or without ASXL1 mutations

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We define the characteristic features and management for Bohring-Opitz Syndrome (BOS), a rare genetic condition characterized by distinctive facial features, characteristic posture, microcephaly, severe intellectual disability and feeding problems. Since being initially delineated in 1999, there are now approximately 30 published cases. In 2011, Hoischen *et al.* identified *de novo* nonsense mutations in *ASXL1* in 7 out of 13 patients with BOS. Previously, somatic mutations in *ASXL1* have been detected in myeloid malignancies, suggesting *ASXL1* might be involved in tumor suppression. We report natural history from 3 previously unpublished patients with BOS, including the first known case of BOS with bilateral Wilms tumors. All 3 patients had novel *de novo* frameshift mutations in *ASXL1*, and 2 patients are normocephalic with varied feeding issues and distinctive personalities (interactive, happy, and curious). Surviving patients with BOS may have a risk for tumors, and 2 previous patients with BOS had neoplasms. One patient with BOS died at 5 months with bilateral nephroblastomatosis, and another patient with BOS developed medulloblastoma at age 5 years; neither had mutations in *ASXL1*. With a malignancy potential for BOS, tumor surveillance should be considered in patients with and without *ASXL1* mutations for disease monitoring and management. Diagnostic criteria may also need to be broadened to help identify previously undiagnosed cases. We review these 3 new cases and compare them with previous cases of BOS with and without mutations in *ASXL1,* and also with patients with mutations in *ASXL3,* to broaden diagnostic criteria and suggest a tumor surveillance protocol.

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P05.037
Phenotype of the french cohort of Börjeson-Forssman-Lehmann syndrome

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Börjeson-Forssman-Lehmann syndrome (BFLS) is an X-linked mental retardation syndrome first described in 1962. It is characterized by the association of intellectual disability, epilepsy, microcephaly, short stature and obesity, hypogonadism and characteristic craniofacial features. Some heterozygous females have mild clinical manifestations of BFLS.
BFLS is caused by mutations in the gene PHF6 whose function is unknown. The encoded protein might play a role in chromatin remodeling, in transcriptional regulation, cell growth and proliferation.
In a National Clinical Research Project on Intellectual Disabilities linked to the X chromosome, we gathered the medical records of 8 french male patients with a mutation in PHF6 gene and 7 female carriers within 5 families.
In males, intellectual disability, ranging from moderate to severe, characteristic craniofacial morphology, obesity, gynecomastia and hypogonadism were constant fetaures. Deafness and hypothyroidism were two frequent features in our cohort, whilst microcephaly and short stature were less frequent.
Three female carriers with random X-inactivation were symptomatic, with one of them having mild intellectual disability and the other two harboring suggestive craniofacial dysmorphism. Three asymptomatic female carriers had a skewed X-inactivation. Thus, X-inactivation, whether random or skewed, may determine the presence or the absence of mild signs of BFLS in female carriers.
At the molecular level, two PHF6 mutations had been previously reported and three were novel missense mutations. On the whole, 17 different PHF6 mutations were identified in BFLS patients.
Our data are in line with those of the BFLS literature. This ongoing study will focus on neuropsychological phenotyping of BFLS patients.

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P05.038
Mutations in TUBG1 and the MT-dependent Motor Proteins DYNC1H1, KIF5C and KIF2A Cause Malformations of Cortical Development and Microcephaly

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The genetic causes of malformations of cortical development (MCD) remain largely unknown. Here we report the discovery of multiple disease-causing missense mutations in TUBG1, DYNC1H1 and KIF2A, as well as a single germline mosaic mutation in KIF5C. We find a frequent recurrence of mutations in DYNC1H1, implying that this gene is a major locus implicated in unexplained MCD. The mutations in KIF5C, KIF2A and DYNC1H1 drastically affect ATP hydrolysis, productive protein folding or microtubule binding, while suppression of Tubg1 expression in vivo interferes with proper neuronal migration and expression of Tubg1 mutations in S. cerevisiae results in disruption of normal microtubule behaviour. Our data reinforce the importance of centrosome- and MT-related proteins in cortical development and strongly suggest that MTdependent mitotic and post-mitotic processes are major contributors to the pathogenesis of MCD.

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P05.039
Prospective Study of Functional Outcomes in Children with Cerebellar Atrophy

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AIM: To identify clinical and radiological predictors of functional outcome in patients with childhood onset cerebellar atrophy.
METHODS: Over a period of 5 years, we evaluated 44 patients with confirmed cerebellar atrophy on MRI. Patients were grouped according to whether the cerebellar atrophy was isolated or associated with other radiological abnormalities. Patients with posterior fossa malformations or non-genetic

aetiologies of cerebellar atrophy were excluded. MRI (1.5 T) was carried out 73 times on 44 patients. The severity of cerebellar atrophy using qualitative and quantitative scoring systems was recorded. Standardized activities of daily living (ADL) assessment was used to characterize the spectrum of functional outcomes. The characteristics of the participants were analyzed using descriptive statistics.

RESULTS: We evaluated 44 patients, 26 male and 19 female. The mean age of symptom onset was 20 months (range, birth to 10 years). The isolated cerebellar atrophy group had better functional outcomes compared to those with cerebellar atrophy associated with other radiological abnormalities. Age of onset of cerebellar atrophy before 2 years of age, progression of cerebellar atrophy on MRI, presence of seizures, and decreased size of transverse cerebellar hemisphere diameter were associated with worse functional outcomes.

CONCLUSIONS: We present a prospective study of clinical and radiological predictors of functional outcome in patients with childhood onset cerebellar atrophy. This information may be useful in the diagnosis and future management of this complex group of disorders.

A. Al-Maawali: None. **S. Blaser:** None. **X. Zhao:** None. **G. Yoon:** None.

P05.040

A new family with *SLC9A6* mutation expanding the phenotypic spectrum of Christianson syndrome

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High-throughput sequencing can broaden the clinical variability associated to a known gene, by sequencing panels of genes in patients with no precise clinical diagnosis. From a series of 50 patients with undiagnosed ID studied by targeted sequencing of 220 ID-genes, we identified a splicing mutation (c.526-9_526-5del) in the *SLC9A6* gene in a 9-year-old boy with mild ID, microcephaly and social interaction disabilities. This intronic microdeletion leads to skipping of exon 3 and to an inframe deletion of 26 aminoacids in the TM4 domain. It segregates with cognitive impairment or learning difficulties in other members of the family. Mutations in *SLC9A6* have been reported in X-linked Christianson syndrome associating severe to profound intellectual deficiency and an Angelman-like phenotype, with microcephaly, absent speech, ataxia with progressive cerebellar atrophy, ophtalmoplegia, epilepsy, and neurologic regression. Interestingly, the proband and his maternal uncle have an attenuated phenotype with mild intellectual disability, attention deficit disorder, speech difficulties, microcephaly and mild asymptomatic cerebellar atrophy. The mutation cosegregated with learning disabilities and speech difficulties in the carrier females (mother and 3 sisters of the proband). Detailed neuropsychological, speech and occupational therapy investigations revealed a disorder of oral and written language acquisition, with dissociation between verbal and performance IQ. An abnormal phenotype has been described previously in a large proportion of carrier females, ranging from learning disability with predominant speech difficulties to mild intellectual deficiency. In conclusion, besides broadening the clinical variability of *SLC9A6* gene mutations, we show an example of a monogenic origin of mild learning disability.

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P05.041

A Novel 0.34 Mb Microduplication of 9q34.3 in a Patient with a Congenital cardiac defects and Learning Disabilities

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We report a 19- year-old male with a learning disability and congenital heart defects. The patient also had mild dysmorphic features including facial asymmetry and mid-facial hypoplasia, high-arched palate with dental crowding. Chromosomal microarray analysis demonstrated a 0.34 Mb duplication on 9q34.3, confirmed by Fluorescence in situ Hybridization (FISH). The 9q34.3 duplication we report is the smallest duplication among the cases reported thus far and includes the genes EHMT1 and NELF. The clinical manifestations in our patient were similar to those described in other reported cases of larger 9q34 duplications, implying that our small duplication encompasses a critical region for brain, cardiac and craniofacial development. We also compare our patient with its 9q34 deletion counterpart, Kleefstra syndrome, and demonstrate the overlap between the two conditions.

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P05.042

Familial supernumerary chromosome composed of centromeric and pericentromeric regions of chromosome 8 cosegregating with intellectual disability and psychiatric disorder

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We describe three generations of a family showing perfect cosegregation of intellectual disability and psychiatric disorder with a small pericentromeric chromosome 8-derived supernumerary chromosome. Two sisters aged 3 and 7 years were referred to our Genetics Department for delayed psychomotor development, intellectual disability, behaviour disorder and autistic spectrum disorder without associated dysmorphism or malformation. They are the first and third born from unrelated parents. Their mother had learning difficulties in her youth and as an adult suffers from psychiatric disorder as well as severe depression and inadequate social behaviour. Their maternal grand-mother presented the same features, with alcohol and benzodiazepin dependence in addition. Molecular investigation for Fragile-X and Steinert's disease, as well as metabolic screening and cerebral MRI, revealed no anomaly. All 4 have a small supernumerary chromosome, discovered on standard blood chromosome analysis. Fluorescence in situ hybridization identified the marker as derived from the centromeric and pericentromeric regions of chromosome 8. Comparative genomic hybridization array (25kb-resolution) confirmed a 9.1Mb partial trisomy for chromosome 8; The karyotype is: 47,XX,+der(8)(p11.2q11.21).

The two maternal half-sisters of the mother and the sister of the two children, who don't carry the chromosomal anomaly, have neither ID nor psychiatric disturbance. There is little concerning this chromosomal anomaly in the literature. Only two patients have been reported, with psychomotor retardation or mild intellectual disability. It is our opinion that that this supernumerary chromosome is responsible for a phenotype associating intellectual disability and psychiatric disorder without specific facial features nor malformation.

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P05.043

The first familial case with the entire *CNKSR2* gene deletion corroborating its involvement in X-linked intellectual disability and Attention-Deficit/Hyperactivity Disorder (ADHD)

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CNKSR2 (Connector enhancer of Kinase Suppressor of Ras 2) is exclusively expressed in adult and fetal brain and encodes CNK2, a protein involved in signal transduction in mitogen-activated protein kinase (MAPK) pathway downstream from RAS. Until now, this gene has been reported as causing non syndromic intellectual disability only in one sporadic case (*Houge et al. Mol Syndromol. 2012*). We could see two brothers (age of 10 and 11 at the moment of investigation) with delayed psychomotor development (they could seat at 8-9 months and walk at 2 years of age), with especially severe ADHD and speech delay. Furthermore, the youngest brother developed epilepsy. Physical examination did not show any dysmorphic feature, but both brothers present flat and large feet and mild joint hyperlaxity. Whole genome oligonucleotide microarray CGH 105K (Agilent Technologies, Inc. Santa Clara, USA) revealed in both brothers a maternally inherited 513 kb deletion on Xp22.12, which removed the entire sequence of *CNKSR2*. This is the second observation of CNV affecting this gene inherited from unaffected carrier mother, and the first familial case. In the previous case, a 234-kb deletion, removing the major part of *CNKSR2*, was observed. The main clinical features in published and present cases are: developmental delay, especially

speech delay, ADHD and epilepsy. Until now, no point mutation in this gene has been reported. We suggest that the *CNKSR2* gene would be analyzed in male patients with non syndromic intellectual deficiency, when associated with severe ADHD, once the fragile X syndrome has been excluded.

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P05.044

Exome sequencing identifies a novel mutation in X-chromosomal gene in brothers with congenital hypothyreosis, short stature and mental retardation

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The proband is the 3rd child of healthy non-consanguineous Finnish parents. During pregnancy polyhydramnion was noted. Birth measurements were 2982 g/ 48 cm/ 34 cm. The older brother of the proband was diagnosed with congenital hypothyroidism at the age of 6 months, with epilepsy at 3 years of age. He is moderately mentally retarded. His height was 88,1 cm (-2.1 SD) at the age of 3 years. The 2nd child of this family is a healthy girl.
Muscle hypertonia was noted when the proband was 3 months old. Hypothyroidism was diagnosed when he was 6 months old and around the same time his growth started to lag. Proband's height is 95,5 cm (-2.6 SD). He developed his first seizures at age 1 year and 2 months. His global development has been moderately delayed. At the age of 4 years and 3 months he walks independently, but is not able to speak.
The chromosomes (400 bands) and aCGH (44K) were studied because of short stature and mental retardation. Both of these results were normal. Because the family history fitted X-chromosomal inheritance and clinical findings resembled Allan-Herndon-Dudley, MCT8 gene was analysed with normal results. Exome sequencing (NimbleGen SeqCap EZ Human Exome Library v2.0) identified a novel 3 bp deletion in a X-chromosomal gene causing a known syndrome. Affected boys were hemizygous and their mother is a carrier of the mutation. The mother is healthy, but her height is 150 cm (-2.5 SD).

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P05.045

The wide spectrum of alpha and beta-Tubulinopathies: Key features of phenotypic heterogeneity and approach for diagnostic and molecular orientation

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Complex cortical malformations associated with mutations in tubulin genes TUBA1A, TUBB2B, TUBB3 and TUBB5, commonly referred to as « Tubulinopathies », are a heterogeneous group of conditions which include a wide spectrum of clinical severity. Of the 125 patients selected as having complex cortical malformations, 41 (32.8%) were found to carry TUBA1A, 18 (14.4%) TUBB2B mutations, 11 (8.8%) TUBB3 and 3 (2.4%) TUBB5 mutations. Here, we describe their occurrence together as the two major findings in a group of at least five cortical malformation syndromes: (i) lissencephaly (16) (ii) microlissencephaly (10), (iii) central pachygyria (13), (iv) polymicrogyria-like cortical dysplasia either diffuse (6), central (11) or multifocal (10) and (v) simplified gyral subtype (7). Dysmorphic basal ganglia (45; 61.6%) are hallmark of tubulinopathies more visible in central pachygyria, polymicrogyria, and simplified gyral malformations. Tubulinopathies are also characterized by a high prevalence of corpus callosum agenesis (35.6%), and mild to severe cerebellar hypoplasia and dysplasia (79.4%). Foetal cases (23) represent the severe end of the spectrum and show specific abnormalities (i.e. enlarged germinative zones, neuronal heterotopia and disorganised corticospinal tracts) that provide insights in the understanding of pathophysiology.
Overlapping phenotypes may exist between tubulinopathies. TUBA1A mutations account for lissencephaly (15 ; 93.7%), central pachygyria (13 ; 100%), microlissencephaly (7 ; 70%), but less frequently for PMG (6 ; 22%). By contrast, β-tubulin (TUBB2B, TUBB3B and TUBB5) mutations account for a higher prevalence of polymicrogyria (21;77.8%) and simplified gyral pattern (7;100%). However, phenotype genotype correlations with specific mutations are found.

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P05.046

Haploinsufficiency of *CTNND2* on 5p15.2 is associated with intellectual disability

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Deletions of the short arm of chromosome 5 is associated with Cri-du-chat syndrome (CdCs), a condition characterized by a high-pitched cat-like cry in newborns. Patients usually present with microcephaly, round face, hypertelorism, epicanthal folds, micrognathia, low-set ears, hypotonia, and severe developmental delay. CdCs is a contiguous gene syndrome, and even though it is a well defined clinical condition, the exact genotype-phenotype correlations remain to be established. However, the critical region for the cat-like cry has been located to 5p15.3, and the critical region for the intellectual disability has been suggested to be located within 5p15.2. We describe two patients with deletions of 73 kb and 3.2 Mb, respectively, that overlap part of the CdCs critical region in 5p15.2. Clinically our patients lack many characteristics of CdCs, but both have intellectual disability. Only *CTNND2*, encoding delta-catenin, is included in the deletion overlap between our two patients. *CTNND2* is predominantly expressed in brain and has been suggested to be involved in neuronal cell adhesion. The deletion of *CTNND2* in our two patients is likely causing their intellectual disability.

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P05.047

KBP-cytoskeleton interactions underlie developmental anomalies in Goldberg-Shprintzen Syndrome

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Goldberg-Shprintzen syndrome (GOSHS, MIM #609460) is an autosomal recessive disorder of intellectual disability, specific facial gestalt, and Hirschsprung disease. In 2005, homozygosity mapping in a large consanguineous family identified KIAA1279 as the disease-causing gene. KIAA1279 encodes KIF-binding protein (KBP), whose function is incompletely understood. Studies have identified either the mitochondria or the cytoskeleton as the site of KBP localization and interactions. To better delineate the KIAA1279-related clinical spectrum and the molecular mechanisms involved in GOSHS, we studied five new patients from three different families. The ho-

mozygous KIAA1279 mutations in these patients (p.Arg90X, Ser200X, or p.Arg202IlefsX2) led to nonsense-mediated mRNA decay and loss of KBP function. Despite the absence of KBP function, respiratory chain complex activity in patient fibroblasts was normal. KBP did not co-localize with mitochondria in human fibroblasts but interacted with the actin and tubulin cytoskeleton. KBP expression directly affected neurite growth in a neuron-like cell line (human neuroblastoma SH-SY5Y), in keeping with the central (polymicrogyria) and enteric (Hirschsprung disease) neuronal developmental defects seen in GOSHS patients. The KBP interactions with actin filaments and microtubules demonstrated in our study constitute the first evidence that an actin microtubule cross-link protein is involved in neuronal development in humans.

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P05.048

Analysis of the phenotypes in the rett syndrome networked database.

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The Rett syndrome networked database (RSND) is a repository of clinical and molecular data for patients affected by Rett syndrome. Although it was initially targeting the european population of RTT patients, it is now open to countries outside of Europe. RSND is different from existing repository for Rett syndrome clinical and molecular data. First, RSND records are curated by experienced clinicians. This is important to exclude potential bias existing when clinical data is gathered using questionnaires sent out by mail to families. Second, RSND is currently the largest RTT database in the world with 1900 patients on file. This number could significantly increase in the next few months with the addition of cases from large countries such as Russia or India. Third, RSND is an open access initiative and data can be retrieved directly through a web-based search engine by all interested professionals. Records are de-identified but access to the invidiual patient files can be granted through the participating clinicians in each country.
We are providing here a description of the first 1900 records contained in the networked database and are discussing the content of RSND in the light of published guidelines for RTT, the development of clinical trials and with respect to other RTT cohorts.

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P05.049

A mutation in the DOPA decarboxylase gene (DDC) causes a new syndromic form of Intellectual Disability

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Intellectual disability (ID) is characterized by a substantial below-average score on tests of mental ability, and limitations in functions related to areas of daily life. Two male sibs and a male first cousin, born of two double consanguineous marriages (first cousins once removed and second cousins), presented developmental delay and moderate mental retardation, craniofacial dysmorphisms and chronic diarrhea with or without recurrent hypoglycemia, gastro-esophageal reflux and progressive kyphoscoliosis.
SNP-array analysis (Affymetrix 6.0 platform) revealed no causative CNV. ROH analysis identified a shared region of homozygosity corresponding to the genomic coordinates chr7: 45,716,212-50,602,470.
Whole exome analysis identified a homozygous variant g.50,531,015 G>A in both affected sibs in the gene DDC (DOPA decarboxylase; aromatic L-amino acid decarboxylase). This variant causes a non-synonymous substitution p.Arg453Cys, predicted damaging by PolyPhen-2. Moreover, it co-segregates in the affected first cousin and is absent in 500 control Italian chromosomes. It therefore seems causative of the clinical phenotype in the patients.
Mutations in DDC have been reported in autosomal recessive DOPA-decarboxylase syndrome (OMIM 608643), but the clinical characteristics of our patients appear different from those previously described. Current analyses are ongoing to verify dopamine and serotonin levels in blood and liquor of the affected sibs, as well as functional studies on wild-type and mutant isoforms, for a better definition of the clinical features and etiology of this DCC variant. This work was supported by the EU-funded grant CHERISH (FP7 Health -2007).

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P05.050

A further patient with a deletion 14q32.2, clinical features of maternal uniparental disomy 14 and pronounced intellectual disability: evidence for a novel microdeletion syndrome

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We report on a female patient with a upd(14)mat-like phenotype. The patient is the second child of non- consanguineous Tunisian-Lebanese parents. Pregnancy started as a twin pregnancy. Recurrent bleedings and reduced fetal movements were observed. The patient was born by cesarean section with normal birth measurements at 36 weeks of gestation. Muscular hypotonia and poor sucking led to tube and PEG feeding during the first year of life. She is severely delayed, walking with 2 ½ years and talking with 4 years. Precocious puberty occurred with 5 years. With 3 years all her body measurements lay below the 3rd centile, with 13 years she was short, but weight and head circumference were normal. She shows facial dysmorphism with long, narrow face and hypertelorism, behavioral problems started with 12 years.
Karyotyping was normal. SNP-array hybridization and quantitative real-time PCR for the *MEG3* gene revealed a 1.1 Mb *de novo* deletion, including most of the imprinted genes of the region and 14 not imprinted genes (ar r[hg19]14q32.2q32.31(100.405.409-101.504.293)x1). Using MS-MLPA we detected hypomethylation of the *MEG3* promotor region, indicating that the deletion is of paternal origin.
Our patient carries the recurrent microdeletion 14q32.2 with breakpoints inside TGG repeats, as described for two patients by Bena et al in 2010, and two further patients, reported in 2012 by Ballif et al. She shows all clinical features of the previously described upd(14)mat-like phenotype, except for normal body measurements at birth. Haploinsufficiency of additional, non-imprinted genes may cause the intellectual disability of this novel microdeletion 14q32.2 syndrome.

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P05.051

Clinical and genetic: A study of 30 new cases with duplication of 15q11-q13 region

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Duplication of 15q11-q13 region results either from a supernumerary marker (SMC) derived of chromosome 15 or from an interstitial duplication (ID).

Duplications are characterized by hypotonia, developmental delay, speech delay, seizure, minor dysmorphic features and autism. A retrospective study of 30 unrelated patients with microduplication of the 15q11-q13 region showed 15 cases have a SMC15. In 14 the SMC was of large size, encompassing PWS/ASCR and one was paternal in origin. Furthermore, 15 cases with ID: 9 with typical duplication BP2-BP3, 1 with large duplication BP2-BP5 region and 4 with atypical duplication; one case had overlaps between BP1-BP2 and BP2-BP3 regions and 3 with duplication limited to three paternally expressed genes located in BP2-BP3 region. One case with duplication BP1-BP2. Four cases were inherited from normal parents (3 maternal and 1 paternal). Phenotypic features were highly variable and 74% presented with autism. Twelve patients showed cerebral anomalies and 14 patients had an unusual EEG pattern of diffuse fast rhythms even in absence of epilepsy that appears to be characteristic for duplication 15q11-q13 syndrome which could help in early diagnosis of this syndrome.

E.I. Al Ageeli: None. **S. Drunat:** None. **C. Delanoe:** None. **L. Perrin:** None. **C. Baumann:** None. **Y. Capri:** None. **J. Fabre-Teste:** None. **A. Aboura:** None. **C. Dupont:** None. **L. El Khattabi:** None. **D. Chantereau:** None. **J. Gallard:** None. **A. Tabet:** None. **A. Verloes:** None.

P05.052

Clinical performance and diagnostic yield assessment of the Affymetrix CytoScan® Dx cytogenetic microarray system

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Cytogenetic microarrays have demonstrated a significant increase in the diagnostic yield assessment by identifying pathogenic copy number changes as compared to traditional techniques such as karyotyping and fluorescence in-situ hybridization. The diagnostic yield of CytoScan® Dx was assessed in a cohort of individuals exhibiting intellectual disability, congenital anomalies, and/or dysmorphic features. Consecutive IRB-approved, DNA samples were de-identified and collected at three clinical laboratory sites. Routine patient care (RPC) comprised of one or more of traditional cytogenetic methods, Fragile X testing and microarray (excluding Affymetrix array) for determining genomic alterations/rearrangements. Samples were run at one site on CytoScan® Dx and the data were interpreted by an independent cytogeneticist blinded to any patient information.
In total, 960 samples were analyzed. The diagnostic yield from RPC (excluding all microarray platforms) was 14.5% (95%CI = 9.6-21.3%). On the same sample set, the diagnostic yield from CytoScan® Dx was 23.9% (95%CI = 17.6-31.7%), indicating a net 9.4% incremental yield provided by CytoScan® Dx. Notably, 20 pathogenic alterations were identified by CytoScan® Dx that were not detected by RPC methodologies.
This is the largest study to date assessing the diagnostic yield of CytoScan® Dx as compared to RPC and confirms previous results that whole genome microarrays provide a clinically significant increase in diagnostic yield.

A. Chaubey: None. **K. Kwiatkowski:** A. Employment (full or part-time); Modest; Affymetrix. **A. Roter:** None. **S. Close:** None. **R. Pfundt:** None. **E. Thorland:** None. **B.R. DuPont:** None. **J. Reiswig:** None. **R. Hockett:** None. **E.T. Fung:** None.

P05.053

Small deletion detected by MPLA in the 22q11 region in a patient with attention deficit and behavioral disorders

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DiGeorge syndrome (DGS, #188400) is actually a well-defined primary immunodeficiency disorder classically associated with congenital heart defects, and hypoparathyroidism with hypocalcaemia. The facial appearance of patients with DGS is characterized by hypertelorism, micrognathia, short philtrum with "fish-mouth", antimongoloid slant, and telecanthus with short palpebral fissures. It has been reported that children and adults with DGS have high rates of behavioral, psychiatric and communication disorders. CASE REPORT: Our case report was a 13.8-year-old boy born to unrelated healthy parents. Initially, he was admitted to the Neuropaediatric Department with attention deficit and socialization problems. At physical examination, mild facial dysmorphism was noted. The laboratory investigations revealed normal parathormone levels, but the presence of hypocalcemia and hyperphosphatemia suggested a possible hypoparathyroidism. Patient had no cardiac defects, no immunodeficiency and normal thyroid hormone levels. MLPA analysis was performed using P-245 B1 kit. A deletion in 22q11 region was detected located in the AB region (*CLDN5* and *GP1BB* gene). Deletions in 22q11 are the most frequent cause of DGS, the majority include the AB, BC and CD regions, though some deletions are smaller (AB only as our case) or larger. Patients with chromosome 22q11.2 deletion do not always show all components of DGS. Hypoparathyroidism can be the only abnormality and may exist with no accompanying cardiac or immunologic defects. DGS patients do not always have the typical dysmorphic features and may not be diagnosed until adulthood. DGS is relatively common and this diagnosis should be considered in patients with mental, behavioral, or attention disorder.

F. Gonzalez: None. **A. Zuñiga:** None. **L. Pedrola:** None.

P05.054

Global transcriptome profiling reveals involvement of genes localized across different chromosomes in pathogenesis of Down Syndrome

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Down syndrome (DS), the most frequent genetic disorder leading to mental retardation is caused by partial or complete triplication of human chromosome 21. The differential expression of genes located on extra chromosome 21 is generally assumed responsible for phenotypic abnormalities but this gene dosage hypothesis has not been fully assessed on genome-wide basis. The expression patterns of genes related to phenotypic abnormalities may provide insights into their roles in pathogenesis of DS. To analyze the differential gene expression and understand the molecular mechanism underlying pathogenesis of DS, we performed global gene expression profiling in blood samples of 9 DS and 1 normal subjects using human whole transcriptome microarray. The microarray analysis revealed total of 22 genes present on chromosome-21 showing differential expression. Genes involved in physiological pathways such as apoptosis regulation, cell cycle regulation, signal transduction, cell maturation, and immunity showed dysregulation. Several genes localized on chromosome-21 such as APP, SOD1, DYRK1A, COL6A1 showed similar expression levels across all DS subjects. Interestingly, several non chromosome-21 genes such as RCAN3 (chromosome 1), ANK3 (chromosome 10), CDK17 (chromosome 12) etc., having roles in cardiogenesis, signal transduction and differentiation of neurons showed conserved levels of expression across the DS subjects. The gene dosage hypothesis on chromosome-21 may partially explain the neurological and other symptoms but our results substantiate the involvement of genes localized across different chromosomes in pathogenesis of DS. Our data may provide the basis for a more systematic and improved understanding of molecular mechanism underlying the pathogenesis of the disease.

A. Pathak: None. **S.R. Phadke:** None.

P05.055

A complex chromosomal rearrangement with X-autosome translocation and PCDH19 gene duplication in a girl with drug resistant epileptic encephalopathy

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The advent of CGH microarray technique helps to define abnormalities in chromosome structure very precisely and therefore seems to be a perfect tool for medical genetics.
We describe a case of a 6 year old girl with profound mental retardation and drug resistant epileptic encephalopathy. Derivative chromosome X, observed in karyotype was further characterized by CGH microarray technique. Three pathogenic aberrations were identified: 34Mb duplication in chromosome 2 (2p25.3-2p22.3), 57Mb interstitial duplication in chromosome X (Xq21.33-Xq28) and 2.7Mb duplication in chromosome X (Xq28), with a translocation of a fragment of the short arm of chromosome 2 onto the long arm of chromosome X. More than 600 genes have been characterized in the above mentioned regions. The 57Mb duplication encompassed protocadherin 19 (PCDH19) gene, involved in female-restricted epilepsy-mental retardation syndrome (EFMR). So far, PCDH19 mutations have been identified in affected heterozygous females and in asymptomatic hemizygous males. A random X inactivation is expected to disturb cell-to-cell interactions leading to the phenotype of mutated females. The influence of the X-autosome translocation in the patient on the X-inactivation pattern and the phenotype

is discussed in detail.

M. Piechota: None. **M. Wysocki:** None. **M. Badura-Stronka:** None. **A. Wilczewska-Wiktor:** None. **B. Steinborn:** None. **A. Latos-Bieleńska:** None.

P05.056

Case report of a de novo 17q12q21.32 duplication of 9.2 Mb in a girl with hypotonia, facial dysmorphism, and cardiac abnormalities

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Background: Duplication in 17q21 region are rare. Microduplication of the region 17q21.31 (OMIM 613533) reciprocal to the classical 17q21.31 deletion of 500.000 bp has been reported in 6 cases with variable facial dysmorphism, psychomotor delay, hypotonia, abnormal digits, hirsutism (decipher and literature). We report a patient with a large duplication of 9.2Mb (17q12-q21.32) encompassing this microduplication.

Method: The duplication was diagnosed by classical cytogenetics showing a longer 17q arm and mapped with array-CGH (Agilent 180k). 46,XX,dup(17)(q12q21.32).arr 17q12q21.32(36,473,175-45,620,194)x3 dn. Parental karyotypes were normal.

Clinical report: We report a female patient born at 35 weeks of gestational age with weight 2.6 kg(M), OFC 32 cm(M). She was referred for severe hypotonia, feeding difficulties, facial dysmorphism with large frontal bossing, hypertelorism, asymmetrical eyeballs, strabism, synophris, anteverted nostrils, everted lower lip, micrognathia, plagiocephaly, hirsutism. On cardiac ultrasound persistent foramen ovale, apical ventricular septum defect, persistent left superior venous cava were found. At 16 months of age she could sit but not walk, she had feeding difficulties and growth delay with weight 7020 g (-3SD), length 78.5 cm (M) and OFC 45cm (-1SD).

Conclusion: To our knowledge, this case is the first reported with a de novo 17q12-q21.32 duplication. Compared to the reported 17q21.31 duplication syndrome, in the present case, the common symptoms are : hypotonia, psychomotor retardation, synophris, hirsutism, micrognathia, high-vaulted palate. The discrepant features are : ophthalmic asymmetry, hyperlaxity, facial dysmorphism, and cardiac abnormalities.

T. Ait Mouhoub: None. **M. Doco-Fenzy:** None. **E. Landais:** None. **C. Leroy:** None. **A. Lannoy:** None. **P. Mauran:** None. **P. Sabouraud:** None. **P. Morville:** None. **P. Feucher:** None. **S. Clomes:** None. **N. Gruson:** None. **C. Mangeonjean:** None. **D. Gaillard:** None.

P05.057

On the lookout for links between genetic variants and the occurrence of Dyslexia in Spanish Children

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Reading is one of the pillars of education and a central skill to succeed in our information-based society. Dyslexia is a reading disorder with a prevalence of approximately 10% of the population. The aim of this study is to identify genetic variants associated with Dyslexia and to integrate genetic and cognitive-behavioural markers to establish causal links and promote early diagnosis. To that end, we performed a genetic association study to identify patterns of DNA-polymorphisms that vary between individuals with different cognitive phenotypes following two strategies. On the one hand, we are carrying out a Genome-Wide-Association-Study (GWAS), analyzing 730000 Single Nucleotide Polymorphisms (SNPs) along the genome in a subset of dyslexics and controls. Preliminary analyses have identified 11 SNPs associated with Dyslexia with a P-value of 10-6, which should be conveniently validated. On the other hand, we are following a candidate gene association approach focusing on individual polymorphisms that have been reported to be implicated in cognitive disorders. We have genotyped variants of KIAA0319, DCDC2, FOXP2, DYX1C1, COMT1, DBH, MAOA, DRD4 and DAT1 genes in 4700 individuals. The missense SNP rs4504469 located within the KIAA0319 gene shows the highest association with Dyslexia in the studied population (p<10-2). In addition, we will present the relationships between the genotype of these candidate genes and cognitive-behavioural variables such as Intelligence quotient and reading ability among the general Spanish child population.

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P05.058

STXBP1 and KCNQ2 are mutated in a large number of patients having early onset epileptic encephalopathy.

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Early onset epileptic encephalopathies (EOEE) are rare and severe disorders in which patients have impaired cognitive development. A part of EOEEs, known as Ohtahara syndrome (OS), is characterised by the association of frequent motor seizures occurring before three months of age, with an EEG showing a "suppression-burst" pattern. The prognosis is classically poor with drug resistant epilepsy and a majority of patients evolving into West Syndrome.

Cases related to brain structural or metabolic abnormalities have been discarded, to consider genetic causes. Several genes have been described in the pathology ARX, SLC25A22, STXBP1 and KCNQ2.

Here, we have collected a cohort of 105 patients with EOEE. In order to identify key clinical features and perform genotype-phenotype correlations, EOEE patients are screened for the known genes. We found 8 patients mutated for STXBP1 and 15 patients mutated for KCNQ2. Interestingly, the two groups evolve in the same feature with a remission of the epilepsy in the majority of patients. We have already shown that the course of the epilepsy was unexpected in patients having a mutation of STXBP1. KCNQ2 patients all had a neonatal onset of the epilepsy and have heterogeneous evolution, with mild phenotype in some cases or dramatic outcome in other cases. In both groups, very few patients evolved into West syndrome, although this has classically been described for OS patients.

In conclusion, this study confirms that KCNQ2 and STXBP1 are mutated in EOEE and associated with variable outcome.

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P05.059

Early infantile epileptic encephalopathy caused by mutations in PCDH19 and CDKL5 genes

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Early infantile epileptic encephalopathy (EIEE) is a severe form of epilepsy characterized by frequent tonic spasms with onset in the first months of life. The disease course is severe with early death or marked psychomotor retardation.

Here we report three female EIEE patients. All of them suffered by tonic seizures in clusters, appearing soon after birth, at 5th and 6th month of age respectively.

In both patients with onset between 5th and 6th month and the seizure type, suggested screening for mutations along the PCDH19 gene as a first step. In contrast, the very early onset of the tonic seizures in the last patient supposed CDKL5 related epileptic encephalopathy. The molecular-genetic

testing of the PCDH19 gene showed the following mutations: c.2705dupA p.(Asp902Lysfs*6) and c.1091delC; p.(Pro364Argfs*4). Although the mutations are situated in different protein regions (extracellular domain 4 and cytoplasmic domain) the clinical features are almost the same. The possible explanation is the nonsense-mediated mRNA decay.
The last patient was found to be a heterozygous carrier of a de novo CDKL5 missense mutation c.539C>T; p.(Pro180Leu). This mutation affects the catalytic domain of the protein and thus causes a typical clinical phenotype with very early onset.
The EIEE syndromes are one of the main groups of severe neonatal epilepsies with suppression-burst pattern. Although, the phenotypes vary, some clinical features and the seizure onset could be used to guide the molecular genetic testing algorithm.
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P05.060
Novel compound heterozygous mutations in TBC1D24 cause familial Malignant Migrating Partial Seizures of Infancy.

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Early-onset epileptic encephalopathies (EOEEs) are a group of rare devastating epileptic syndromes of infancy characterized by severe drug resistant seizures and electroencephalographic abnormalities. The current study aims to determine the genetic etiology of a familial form of EOEE fulfilling the diagnosis criteria for malignant migrating partial seizures in infancy (MMPSI). We identified two inherited novel mutations in TBC1D24 in two affected siblings. Mutations severely impaired TBC1D24 expression and function, which is critical for maturation of neuronal circuits. The screening of TBC1D24 in an additional set of 8 MMPSI patients was negative. TBC1D24 loss of function has been associated to idiopathic infantile myoclonic epilepsy, as well as to drug resistant early onset epilepsy with intellectual disability. Here we describe a familial form of MMPSI due to mutation in TBC1D24, revealing a devastating epileptic phenotype associated with TBC1D24 dysfunction.

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P05.061
Disruption of estrogen receptor beta gene in a three generation family with mild intellectual disability

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We report an apparently balanced reciprocal translocation with breakpoints at 14q22 and 18q21 segregating with mild intellectual disability, speech delay and dysmorphic features in a three generation family. This translocation interrupts the estrogen receptor beta gene (*ESR2)*.
Routine cytogenetic analysis (>500 band resolution) showed an apparently balanced translocation: 46,XY,t(14;18)(q22;q21) in the affected family members. No significant copy number variations were detected on chromosomal microarray.
The translocation breakpoints were defined using LR PCR. The breakpoint region on chromosome 18 does not contain any annotated genes and the region on chromosome 14 contains only *ESR2*. Disruption of this gene was confirmed by FISH.
Estrogen receptor beta is part of the nuclear receptor superfamily, but pools of this receptor are also localized to the plasma membrane and mitochondria. Estrogen receptor beta is expressed in mammary glands, ovaries, bones and brain and has been implicated in cardiovascular and brain functioning as well as in inflammation, bone physiology and various types of cancer.
Evidence for the pathogenicity of *ESR2* come from animal studies where *Esr2* knockout mice display morphologic brain abnormalities and disruption of the gene impairs memory, learning and mood. This three-generation family illustrates the first human disease arising from mutation of *ESR2*. Better understanding of the molecular and physiologic basis of the disease in this family will increase understanding of the genetic causes of intellectual impairment and the complex role of estrogen in human physiology.

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P05.062
Whole-exome sequencing study in patients with intellectual disability.

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Whole-exome sequencing is a powerful tool in discovery of disease causing mutations. Nevertheless the etiology of intellectual disability (ID) remains unknown in about 50% of the cases. Affecting 1–3% of the general population, the cause of ID is very heterogeneous and can be involve environmental and genetic factors.
We carried out within the framework of EU FP-7 CHERISH project (#223692) whole-exome sequencing analysis in 21 patients with idiopathic ID from 12 families, where two of the families were known to be consanguineous. The patients were expected to have X-linked, recessive or dominant inheritance pattern. For exome enrichment we used Illumina's TruSeq 62Mb Enrichment Kit and next-generation sequencing experiment was performed using Illumina's HiSeq 2000. The average read depth for sequenced exomes was 70-fold.
For the analysis we used BWA to map the data to reference human genome version 19; GATK for local realignment, removing PCR duplicates and base quality recalibration; and ANNOVAR for annotating variants.
We found variants in autosomal and X-linked genes, on average about 93 000 variants per patient. After filtration, we found possibly clinically relevant changes in upto six genes per family, that are important in development of nervous system. All the candidate variants were confirmed by Sanger sequencing and segregation within the family were checked. The further analysis of these variants is currently in progress.
For additional analysis we plan to implement two split read–based approaches Splitread and Pindel for discovery of medium size indels in these exome data sets, that are too long for BWA.

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P05.063
Role of CTCF protein in regulating FMR1 locus transcription

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Fragile X syndrome (FXS), the leading cause of inherited intellectual disability, is caused by expansion and methylation of a CGG triplet repeat at FMR1 gene. While DNA methylation of the promoter is the major cause responsible for FMR1 silencing, other factors may be implicated. Several nuclear proteins bind to the promoter of FMR1, like the insulator protein CTCF. Here we show that CTCF does not bind to FXS alleles, whereas it binds equally to wild type (WT) and to unmethylated full mutation (UFM) alleles. In FXS cells CTCF binding cannot be restored by drug-induced demethylation of the DNA. In UFM and WT cells CTCF binds to the methylation boundary upstream the promoter, even though it does not act as insulator of the FMR1 locus. CTCF knock-down results in FMR1 transcription reduction and heterochromatic histone configuration of the locus, which however is not accompanied by spreading of DNA methylation towards the FMR1 promoter. A CTCF binding site is present in intron 2 of FMR1, where one of the transcriptional start sites of antisense transcript is located. CTCF depletion is also associated with antisense FMR1 transcript reduction. The antisense transcript is upregulated in UFM cells, its expression and splicing is closely paralleled by that of the sense transcript. We conclude that CTCF has a complex role in regulating FMR1 expression, probably through the organization of chromatin loops between sense/antisense transcriptional regulatory regions, as suggested by a bioinformatics analysis.
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P05.064

Congenital RTT variant in a Bulgarian patient, caused by mutation in FOXG1 gene

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Rett syndrome (RTT, MIM #312750) is mostly caused by mutations in the X-linked MECP2 gene and mutations have been identified in 95-97% of typical RTT patients, but only in 50-70% of atypical cases. This indicates both genetic and clinical heterogeneity of the RTT variants. Over the last years, mutations in the FOXG1 genes have been associated with congenital RTT variants. Here we report a 4 years old girl who has been clinically diagnosed as RTT. She had normal occipitofrontal head circumference (OFC) at birth and deceleration of head growth from birth, current OFC centile 45 cm, microcephaly (standard deviation score, SDS - 3,43). The patient suffered from sleep disturbances, mood lability and inconsolable crying. The regression started at 5 months of age. The seizure onset was after regression - 11 months of age. Additional features were: severe intellectual disability, severely impaired language, hypotonia, stereotypic hand movements, no ability to walk, bruxism and convergent strabismus. The molecular genetic analysis for mutations in MECP2 gene was negative. The specific phenotype classified the patient as congenital RTT variant and screening for FOXG1 mutations was performed. A novel heterozygous nonsense mutation c.406G>T, p.Glu136* was detected. The detected mutation is situated in the N-terminal protein domain, in the region mostly affected by frameshift disruptions. Our results show how important the strict clinical criteria for distinguishing RTT variants are, in order to be able to offer the family adequate molecular genetic testing.

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P05.065

Behavioral phenotype of 8 patients with fragile X syndrome

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Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability due to an expansion in the full mutation range (>200 CGG repeats) of the promoter region of the FMR1 gene leading to gene silencing. Lack of the corresponding protein FMRP will cause FXS which is characterized by intellectual deficiency, behavioral disorders, facial dysmorphy and macroorchidism. Here we report on the behavioral profile of eight patients showing a confirmed FXS. The parents' interview revealed that all patients expressed symptoms of autism spectrum disorder (ASD). Aggression and self-injury were found in 6 patients, stereotyped patterns of behaviors, interests, and activities were noted in 4 patients and attention issues were observed in 4 patients. Only one patient fulfilled the criteria for infantile autism. Other behavioral anomalies were also seen such hyperactivity (5 patients/8) and psychotic disharmony (1patient/8). These results confirm the heterogeneity of behavioral profile in FXS and the common association between FXS and autistic disorders. Such study, considering the behavioral phenotype of genetic syndromes, like FXS, allows us to explain associated psychiatric disorders, to adjust the assessment of intellectual disability level, to adapt educational guidance and rehabilitative plans and to update patient screening in the future.

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P05.066

Reactivation of the FMR1 gene in fragile X lymphoblastoid cells by 5-aza-2-deoxycytydine does not cause random genomic DNA demethylation

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Fragile X syndrome (FXS) is caused by CGG expansion over 200 repeats at the 5' UTR of the FMR1 gene and subsequent DNA methylation of both the expanded sequence and the CpGs of the promoter region. This epigenetic change causes transcriptional silencing of the gene. We have previously demonstrated that 5-aza-2-deoxycytydine (5-azadC) treatment of FXS lymphoblastoid cells reactivates the silent gene, allowing CpG sites demethylation, increased acetylation of histones H3 and H4 and methylation of lysine 4 on histone 3. Recently we observed that reactivation of FMR1 transcription is long lasting, up to a month after a 7-days treatment with 1 μM 5-azadC, and that maximum level of transcription are reached 10-15 days after 5-azadC last administration. In order to check the specificity of the 5-azadC-induced DNA demethylation, we performed bisulphite sequencing of the entire methylation boundary upstream the FMR1 promoter region, which is preserved in WT cells [Naumann et al., 2009]. We did not observe any modification of the methylation boundary after the treatment. Furthermore, methylation analysis by MS-MLPA of PWS/AS and BWS/SRS loci demonstrated that 5-azadC treatment has no demethylating effect on these regions. Taken together these data show that 5-azadC has a long lasting reactivating effect on the mutant FMR1 gene and that its demethylating effect on genomic DNA is not random but rather restricted to specific regions. This specificity of action may open new perspectives for a drug-based epigenetic therapy of FXS.

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P05.067

BKCa channels: a new therapeutic target in Fragile X Syndrome treatment

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The most common cause of inherited mental deficiency (MD) associated with autistic features, Fragile X Syndrome (FXS), results from the *FMR1* gene silencing and loss of Fragile X Mental Retardation Protein (FMRP). Among synaptic proteins deregulated by the absence of Fmrp in *fmr1* knock-out (KO) mice (murine model for FXS), Kcnma1 (α-subunit of large conductance Ca^{2+}-activated potassium channels, BKCa) seems to be the most significant deregulated one. Since several papers showed an involvement of BKCa channels in MD, this channel could be a new therapeutic target for FXS.

The objectives of this study were: 1) to characterize the molecular BKCa channel anomaly in *fmr1* KO mouse model and 2) to evaluate the therapeutic effect of a BKCa channel opener molecule (BCOM), BMS-204352, on dendritic spines maturation of *fmr1* KO neurons and on social behavior of *fmr1* KO mice. In *fmr1* KO mice, Kcnma1 protein quantity was lower in cerebral structures and neurons, and associated with a reduced BKCa whole cell current. *In vitro* addition of BMS-204352 induced dendrite spines maturation of *fmr1* KO neurons. *In vivo*, using a behavioral test of direct social interaction, a single injection of BMS-204352 rescued affiliative behaviors to wild-type level.

In conclusion, we demonstrated 1) a Kcnma1 protein anomaly in *fmr1* KO mice inducing a decrease in BKCa whole cell current, and 2) that a BCOM, BMS-204352, restored a regular phenotype of dendrite spines and social interaction in *fmr1* KO mice. Therefore, BKCa channel could be a new therapeutic target in FXS treatment.

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P05.068

Detection of full mutation FMR1 and AFF2 alleles by Methylation-Specific Multiplex Ligation-dependent Probe Amplification method in male patients with intellectual disability

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Mutations in genes FMR1 and AFF2 on X chromosome cause Fragile X (FXS) and Fragile XE (FRAXE) syndromes respectively, two common forms of inherited intellectual disability in men. The most frequent alteration in these genes is the abnormal extension of trinucleotide repeats in exon 1 and abnormal gene methylation. Based on the number of trinucleotide repeats, there are four types of the alleles: normal, intermediate, premutation and full mutation. Patients with full mutation alleles usually display classic form of FXS.

Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) method enables detection of deletions/duplications, and also analysis of methylation status of promoter of FMR1/AFF2 gene.

We have analyzed 17 male patients with FXS and one male patient with FRAXE by MS-MLPA method previously tested by Expand-long PCR method; 12

with FMR1-full mutation, three mosaics with FMR1-full mutation and normal /premutation alleles, two with FMR1-normal allele and FXS phenotype and one with AFF2-full mutation. MS-MLPA analysis has shown hypermethylation pattern of full mutation in all but one subject which has normal FMR1 allele. In subject in whom karyotype analysis showed 36% of cells with fragile X chromosome and Expand-long PCR one normal allele, MS MLPA revealed FMR1- full mutation allele.
MS-MLPA method enables clear and reliable distinction between normal/premutation and full mutation FMR1/AFF2 alleles in male subjects that makes it suitable for a screening method. Also, it is highly sensitive and therefore useful in clarification of FXS mosaic cases which Expand-long PCR method was unable to detect.

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P05.069

Genetic dissection of Intellectual Disability using SNP-arrays in Polish cohort.

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The goal of the CHERISH project was to establish an interdisciplinary Eastern Europe and Central Asia consortium of experts to perform a research program of clinical, scientific and public activities for generation of new knowledge about genetic causes of Intellectual Disability (ID). One of the main objectives was to identify genomic rearrangements responsible for ID using SNP-arrays. To screen the CNVs in 24 patients, we used the Genome-Wide Human SNP Array 6.0 (Affymetrix, 1M). In 36 patients, the InfiniumHD whole-genome genotyping assay with the HumanCytoSNP-12 BeadChips (Illumina Inc., 300k) was performed. Twelve potentially pathogenic (9 de novo) rearrangements were found in 10 out of 60 investigated individuals (16,7%), with all rearrangements confirmed by qPCR or MLPA. In Table 1 the most interesting findings have been listed.
Table 1. Selected genomic rearrangements in Polish patients identified by SNP-arrays within the CHERISH project.

Patient number	Rearrangement	Genomic position	Comment
309-POL-001-045	dup11q13.3q13.4	70,308,218-70,478,809	autism susceptibility locus (*SHANK2*)
652-POL-002-123	delXq21.1 in a female patient	76,907,922-77,078,030	deletion within *ATR-X* gene for alpha talasemia mental retardation syndrome
655-POL-001-124	del11q13.1	65,952,841-66,768,341	encompassing genes that are involved or may be involved in ID: *NPAS4, BBS1*

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P05.070

Study of a large Spanish cohort of 22q13 deletion syndrome´s patients. A genotype-phenotype correlation?

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Phelan-McDermid Syndrome (PMS) is a neurodevelopmental disorder caused by the deletion or disruption of a 22q13 region containing the post-synaptic protein gene *SHANK3*. The clinical features of PMS (also known as 22q13 deletion syndrome) are highly variable and include hypotonia, speech and otherdevelopmental delays, autistic traits and mildly dysmorphic features. Other less frequent features include seizures, brain, renal and cardiac malformations, motor deficits, and lymphedema Patient deletion sizes are also highly variable, Clinical expression also varies widely, even among patients with small deletions, and no correlation between deletion size and phenotype has been found. A role for additional genetic factors, in addition to *SHANK3* haploinsufficiency, is likely. Because PMS is considered to be underdiagnosed, the true prevalence is unknown.
Aims. We aim to define a possible genotype phenotype comparison to use high-resolution deletion breakpoint mapping on a large cohort of patients, addressed the hypothesis that additional genes or regions of chromosome 22q13 besides SHANK3 may contribute to the PMS phenotype.
Results. Terminal deletion breakpoints were identified for more than 50 individuals in a patient cohort using a custom-designed high-resolution oligonucleotide array comparative genomic hybridisation platform, prompting this genotype phenotype association study. Most of them have been studied by FISH, MLPA and array-CGH and their clinical features have been defined. Interestingly, a great genetic variability, with deletion sizes between 10 Kb and as much as 9 MB was found.
Conclusions. Preliminary data from this large cohort (first, with Spanish patients) were presented and may it provide additional data on genotype-phenotype hypothesis.

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P05.071

A novel recessive intellectual disability syndrome caused by GPI-anchor deficiency

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Syndromes with a phenotype that include intellectual disability and/or seizures are clinically and etiologically heterogeneous, rendering diagnostic challenges. In the present study, we used whole exome sequencing (WES) to delineate the molecular basis for an autosomal recessive syndrome. We examined four patients in a consanguineous kindred with a strikingly similar phenotype, characterized by distinct facial features, intellectual disability, hypotonia and seizures, in combination with skeletal and ophthalmologic findings. WES identified a homozygous mutation in a gene previously not reported in any human disease, and Sanger sequencing of additional family members confirmed segregation with the disease. The gene encodes a protein in the GPI-anchor pathway, and by flow-cytometry we found that leukocytes from the patients had significantly reduced levels of the protein, strongly supporting the pathogenicity of the mutation. In addition, loss of function of the gene in a morpholino-mediated zebra-fish knock-down model led to a gastrulation defect. Our results demonstrate a new pathogenic mechanism in the GPI-anchor pathway and expand the spectrum of disorders belonging to the emerging group of GPI-anchor deficiencies.

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P05.072

HDAC1: a novel cause of autism, developmental delay and epilepsy

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Autism and epilepsy are clinically and genetically heterogeneous disorders. Epilepsy occurs in 25% of patients with autism. Chromosomal Microarray Analysis (CMA) and massively parallel sequencing have identified a large number of genomic regions as well as specific genes which confer susceptibility to autism. Six pathogenic copy number variants (4 deletions and 2 duplications) which ranged from 1.3 -17.2 Mb and overlapping the HDAC1 gene had been reported. HDAC1, a histone deacetylase, directly interacts with MeCP2 through the transcription co-repressor Sin3A/HDAC complex. Mutations in MECP2 cause Rett syndrome; however, no mutations have been described in HDAC1 so far. In a 10 year old boy with non-syndromic autism, developmental delay and epilepsy, routine karyotype, CMA, metabolic, and neuroimaging studies were normal. Whole exome sequencing of

gDNA from the boy and his parents identified a de novo, novel variant in HDAC1 (c.154N>S) which was not detected in public or 1100 private exome databases. Expression of HDAC1 was reduced by 40% while expression of HDAC4, RAI1 and MBD5 in the proband's peripheral blood lymphocytes was normal.
Taken together, these data suggest that this novel HDAC1 mutation prevents transcription repression leading to autism, developmental delay and epilepsy in this patient. Direct DNA sequencing of gDNA from additional patients with autism +/- epilepsy and normal copy number state of HDAC1 is underway.

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P05.073

Identification of a novel missense mutation in *HUWE1 (Xp11.2)* segregating with intellectual disability in a large family, by Targeted High-Throughput Sequencing.

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Genetic diagnosis in Intellectual Disability (ID) remains difficult because of the extreme genetic heterogeneity of this frequent condition. We report the case of a large 4-generation family with suggestive X-linked inheritance of ID (XLID), in which 20 members are affected, with moderate to severe ID and mild dysmorphic features in males, and behavioral phenotype in females. Standard cytogenetic and molecular investigations did not provide a diagnosis. A young patient of this family was included in a strategy of targeted high-throughput sequencing of 220 ID genes. It led to the identification of a novel missense mutation (c.6437C>G; p.Thr2146Arg) affecting a highly conserved residue in the *HUWE1* gene. We then showed its co-segregation with ID in 3 affected males and 3 obligate carrier females while it was absent in 3 unaffected males. Analysis of available DNA for others family members is ongoing. This missense predicted to be damaging (SIFT, Polyphen2), is absent from 10,563 chromosomes in the EVS database. Overlapping duplications including *HUWE1* have been found to segregate with ID in 12 families (Froyen et al.2012). Three missense mutations were also shown to segregate with ID in 3 families with probable or possible XLID, while a *de novo* missense was recently observed in a boy with autism spectrum disorder but not in his more mildly affected brother. It is therefore probable that the p.Thr2146Arg missense is causative of ID in this large family. A functional test of *HUWE1* activity on DNA repair is currently performed on patient's cells to confirm its pathogenicity.

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P05.074

Homozygosity mapping and exome sequencing approachs to find new gene(s) of recessive hydrocephalus

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Background: Hydrocephalus is one of the commonest brain malformations. Its incidence is about 3 cases per 1000 live births. It is estimated that 40% of hydrocephalus would be of genetic origin. However, very few molecular causes are known. We recruit families with multiple cases of congenital hydrocephalus compatible with a recessive inheritance.
Here, we report a large family in which a consanguineous couple had five female fetuses with hydrocephalus identified during the second trimester of pregnancy. Affected fetuses presented with uni- to triventricular hydrocephalus and small cerebellum. Histology showed an opened aqueduct of Sylvius, ciliated ependymal cells and normal cortex architecture.
Material and methods: We perform homozygosity mapping with Affymetrix 250K Nsp chips, exome capture using NimbleGen SeqCap EZ Exome 60Mb and then we made an exome sequencing using Illumina HiSeq 2000. Mutations and family segregation are validated by Sanger sequencing.
Results: Homozygosity mapping defined three regions of interest. By exome sequencing, we identified a previously unreported homozygous two base-pair deletion in the last exon of the CCDC88C gene. This frameshift mutation p.E1949GfsX26 results in a premature stop codon. The mutation was homozygous in the affected foetuses and heterozygous in the parents and in both healthy sisters.
Conclusions: These results confirm that combination of homozygosity mapping and exome sequencing is a valuable tool to discover new genes. The development of next-generation sequencing will help to better understand brain malformations by uncovering the multiple genetic mechanisms.
Others patients are needed to find new genes.

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P05.075

Hyperphosphatasia with Intellectual Disability (Mabry) Syndrome - two adult patients

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The combination of persistent hyperphosphatasia, intellectual disability, neurologic deficits and epilepsy, and variable morphological anomalies was first described in 1970 by Mabry et al (OMIM #239300). It is caused by deficient synthesis of the glycosylphosphatidylinositol (GPI) anchor required for the attachment of cell-surface proteins to the plasma membrane and is inherited as an autosomal recessive trait. Causative mutations in two genes, *PIGV* and *PIGO*, have been identified. So far only children and adolescents have been reported in the literature.
We report here on two adult siblings of Austrian descent, a 34-year-old man (patient 1) and a 28-year-old woman (patient 2), who showed severe intellectual disability with very little speech development and hyperphosphatasia. Craniofacial features included macrocephaly, coarse facial features, arched eyebrows, up-slanting palpebral fissures, a broad nasal root, downturned corners of the mouth, a tented upper lip, and a bifid uvula. Their thumbs were broad and their fingers short, in particular the distal phalanges. Patient 2 had a short perineum. At the age of 8 months a febrile seizure was suspected in patient 2 and EEG abnormalities were detected; she did not have antiepileptic treatment in years and no further seizures occurred. Patient 1 never had seizures. Both patients showed a pronounced change in facial phenotype with age which lead to a coarsening of facial features.
The parents are distantly related and SNP-array analysis in patient 2 showed several regions of autozygosity, one of which included *PIGV* on chromosome 1. Results of molecular analyses are pending.

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P05.076

A patient with cryptic interstitial 0.72-Mb deletion in the 22q13.2 region

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The 22q13 deletion syndrome is characterized by intellectual disability (ID), developmental delay, speech deficit and hypotonia. Almost all of the published 22q13 deletions have been described as terminal. It is estimated that *SHANK3* gene, which maps to 22q13 region, is the major candidate gene for the neurologic features of the syndrome. Four cases with interstitial deletions of 22q13 are reported previously.
We report on a 7-year-old boy with ID, autistic behavior; abnormal EEG, spasticity and mild dysmorphic features included ptosis, facial asymmetry, prominent ears, clinodactyly of F5, T2-3 syndactyly. Patient also suffers from persistent urticaria with eosinophilia and elevated IgE level (2140 KU/L, normal<90). Chromosomal microarray analysis (HumanCytoSNP-12 BeadChip; Illumina Inc.) revealed a small interstitional *de novo* 0.72 Mb deletion in chromosomal region 22q13.2. This region harbors several known genes with different functions (*SREBF2, CYP2D6, NFAM1, TNFRSF13C*), but not *SHANK3* gene.Our patient has the smallest deletion among reported 22q13 region deletions and two copies of *SHANK3* gene. Our patient phenotype showed some differences in comparison with other patients with 22q13 de-

letion. Although he has ID and early childhood speech delay, he has normal growth parameters. In addition he has immune system dysfunction, which may be caused by a deletion of *TNFRSF13C* gene (related to immune system dysfunction) or *NFAM1* gene (cause atopic dermatitis and high IgE levels). It has been previously supposed that 22q13 deletion syndrome may be associated with immune system dysfunction in addition to neuropsychiatric disorders.

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P05.078

Homozygous intragenic duplication of TRAPPC9 associated with intellectual deficiency, postnatal microcephaly, and brain anomalies.

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Intellectual deficiency (ID) is a public health problem concerning 1 to 3% of children. ID is clinically and genetically heterogeneous, and can be divided into non-syndromic- (isolated) and syndromic forms when associated with other features. We report a 5-year-9-month-old girl born to first-cousin parents after an uneventful pregnancy. She presented with psychomotor delay (walking achieved at 2.5 years), ID, behavioural troubles (hyperactivity, stereotypies, sleep disorders), speech defect, postnatal microcephaly, and spasticity of lower limbs. Brain MRI showed corpus callosum and vermis hypoplasia, and moderate bilateral symetric leucopathy. Microarray analysis revealed an intragenic duplication of the TRAPPC9 gene in the 8q24.3 region. qPCR identified 4 copies of the microrearrangement as well as an heterozygous status for the duplication in both parents. TRAPPC9 encodes for a protein involved in intracytoplasmic vesicular transport with a wide tissular expression. TRAPPC9 mutations have been reported in non-syndromic ID patients from consanguineous families, with features similar to those observed in our patient. These mutations could represent a frequent cause of autosomal-recessive ID when associated with postnatal microcephaly, behavioural troubles, and corpus callosum anomalies. Although point mutations have often been published, to our knowledge, this is only the second report of a microrearrangement of the TRAPPC9 gene, which may be responsible for autosomal-recessive ID (first case: homozygous 8q24.3 microdeletion). In a attempt to delineate a specific phenotype suggesting a TRAPPC9 alteration, we reviewed the patients reported in the literature.

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P05.079

Molecular characterization of a patient suffering Intellectual Disability.

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Introduction: Intellectual disability is of major concern troughout the world. In 40% of the cases etiology remains unknown.
Here we present a four years old girl showing psychomotor retardation, language difficulty and dismorphic features. Parents were non consanguineous and healthy. In all of them, kariotype was normal, at 550 level banding GTG. We performed MLPA analysis in order to detect possible subtelomeric rearrangements, and confirmed our results by FISH.
Methods: MLPA analysis were performed using the SALSA P036 Human Telomere-3 containing one probe for each subtelomeric region from chromosome 1-22 and the two X/Y pseudoautosomal regions and SALSA P286 containing eight probes specific for the 10q26.3 region (MRC Holland, Amsterdam, The Netherlands). Fluorescence in situ hybridization (FISH) with subtelomeric probes (Kreatech) were carried out.
Results: MLPA analysis with SALSA P036 showed evidence of a deletion in the terminal region of chromosome 10q (probe PAOX gene in 10q26.3). Further analysis with SALSA P286 showed a deletion from at least 5.44Mb in 10q26.3 involving all eight probes located in this region. FISH using probes 10pter (D10S2488) and 10qter (D10S2290) confirmed this deletion detected by MLPA in the index case and showed normal patterns in her parents, determining it was a *de novo* alteration.
Conclusion: This study confirms that the combination of MLPA analysis and FISH still allows to resolve cases, where the genetic causes of the clinical features remain unexplained.

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P05.080

Distal 16p11.2 duplication in two patients with intellectual disability

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Several recurrent rearrangements have been described at 16p11.2 locus. Deletions and duplications of a proximal 600 kb region (29.5-30.1 Mb) have been associated with autism, intellectual disability, schizophrenia, and mirror metabolic phenotypes. In addition, deletions of an adjacent, distal 200 kb region (28.7-28.9 Mb) have been associated with obesity and developmental delay. Here we describe two male patients presenting with mental retardation and carrying the reciprocal distal 16p11.2 duplication, identified by whole genome SNP array (HumanCytoSNP-12, Illumina) in one case and CGH-array (Agilent, 180K) in the other one.
The first patient displayed intellectual disability, motor delay, attention deficit, dysmorphic features and dextrocardia. He had average height and weight. The duplication (200 kb, 28.7-28.9 Mb) was inherited from his father. The second patient presented with intellectual disability, severe language delay, mild hand tremor but without dysmorphic features. His height was average, and his weight within low normal range (10th percentile). The duplication (285 kb, 28.7-29.0 Mb) was inherited from his mother, while there was a marked history of language disorders for his father and paternal family.
These two observations suggest that the distal 16p11.2 duplication may be a risk factor for intellectual disability. One or few genes included in the duplicated region could be dosage sensitive. However, it is likely that undetermined, additional hits contribute to the phenotype of these patients, as indicated by the transmission of the duplication by an unaffected parent, the informative family history of the second patient, and the phenotypic differences between the two patients.

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P05.081

Clinical and cytogenetic features of Smith Magenis and Potocki-Lupski syndromes: about two Tunisian cases

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Smith Magenis (SMS) and Potocki Lupski (PTLS) syndromes have long been known to display specific neurobehavioral traits. These syndromes are two examples of Contiguous Gene Syndrome that are associated with a microdeletion and microduplication respectively within chromosome 17 band p11.2. The dosage sensitive gene responsible for most phenotypes in SMS has been identified: the Retinoic Acid Induced 1 (*RAI1*).
Studies on mouse models and humans suggest that *RAI1* is likely the dosage sensitive gene responsible for clinical features in PTLS.
We carried out a complete cytogenetic analysis for two Tunisian patients screened for craniofacial anomalies, intellectual disability and behavioral disorders with a diagnosis of SMS and PTLS syndromes. RHG banding analyses were normal. Whole genome analysis with the Agilent Human Genome CGH Microarray Kit 180K proceeded on our patients' DNAs revealed de *novo* 3,5 Mb microdeletion and 7,5 Mb microduplication on chromosome 17p11.2 encompassing OMIM genes especially Retinoic Acid Induced 1 Gene (*RAI1*). The confirmation and parents'analysis were performed by fluorescent *in Situ* hybridation (FISH).
In the present study, the karyotype's level of resolution did not achieve to detect these aberrations. However, the whole genome coverage provided by high resolution level technique as array CGH has displayed these micro imbalances and achieved to delineate the breakpoints. This highlights the effectiveness of this microarray which overcomes the standard karyotype.
So, our results enable us to discuss the role of the microarrays' spot in cytogenetic laboratories and whether it can replace the standard karyotype.

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P05.082

Contribution of copy number variants (CNVs) in congenital unexplained intellectual and developmental disabilities in 149 patients: the first Lebanese study leading to new findings in CNVs

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Purpose: Molecular karyotyping is nowadays the most adopted clinical test for patients with unexplained intellectual disability (ID) and developmental delay (DD). This study exposes the strategy and the conditions of analysis leading to the determination of causative copy number variants (CNVs). It also presents de novo pathogenic CNVs and reviews for several described ones.
Methods: We have applied whole-genome technique to a cohort of 149 lebanese patients with ID/DD using the 2.7M array of Affymetrix. Confirmation of array findings was performed using quantitative PCR. Bioinformatical and statistical analysis were used in order to reduce false positive CNVs.
Results: Criteria were set to improve the reliability of CNVs. When the latter is greater than 62 Kb and contains at least 49 markers it is then considered as certainly existing.
14.8% abnormalities in 149 Lebanese ID/DD patients were detected, of which 15 aberrations overlapped known causative CNVs and 7 potentially pathogenic.
A database of copy number polymorphisms was constructed displaying CNVs carried by 30 healthy individuals.
Conclusion: This is the first Lebanese whole genome study of ID/DD patients. It has shown the importance of uncovering genomic imbalances in the diagnostic and research approaches. All CNVs found will be a guide to clinicians, helping them in the diagnosis of further cases.

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P05.083

EPHA1 C1475T and C1891T polymorphisms in Ukrainian patients with idiopathic intellectual disability

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Exome analysis of an Ukrainian family with healthy unrelated parents and two brothers affected by Intellectual Disability (ID) revealed several variants in either homozygous or compound heterozygous state in five genes. Among these genes, we decided to concentrate on the *EPHA1* gene, where C1475T (maternal) and C1891T (paternal) substitutions were detected in both patients. *EPHA1* is an ephrin receptor gene, highly expressed in the human brain, and it seemed a good candidate for ID.
The proband is a 12 year old boy with ID (IQ=43) and hyperactivity. His brother is a 4 year old boy with febrile convulsions, ID and multiple congenital anomalies. Standard karyotype, molecular analysis of *FMR1* gene and array-CGH analysis (400K) were normal in both patients.
We further investigated the possible role of *EPHA1* gene variants in severe ID. Case-control study included 51 patients with severe ID and 186 individuals as a population control. We performed a screening of the two variants through PCR-RFLP (C1475T) and allele-specific PCR (C1891T). The *EPHA1* 1475T allele frequency was significantly higher in ID patients (2,9%) as compared to controls (0,5%). *EPHA1* 1891T variant was found neither in the control group, nor in ID patients. These preliminary results indicate a possible association between *EPHA1* variants and ID among Ukrainian patients. Further studies (especially the complete sequencing of *EPHA1* coding exons) will be conducted to confirm the involvement of *EPHA1* mutations in ID development. This study was supported by EC FP-7, CHERISH project no. 223692.

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P05.084

Loss of FMR2 further emphasises the link between deregulation of immediate early response genes FOS and JUN and intellectual disability

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Loss of FMR2 causes Fragile X E (FRAXE) site associated intellectual disability (ID). FMR2 regulates transcription, promotes alternative splicing with preference for G-quartet structure harbouring exons, and is localised to the nuclear speckles. In primary skin fibroblasts from FRAXE patients (n=8), we found a significant reduction in the number but a significant increase in the size of nuclear speckles, when compared to the controls (n=4). Since nuclear speckles are enriched with factors involved in pre-mRNA processing, we explored the consequence of these defects and the loss of FMR2 on the transcriptome. We performed whole genome expression profiling using total RNA extracted from these cell lines and found 27 genes significantly deregulated by at least 2-fold at $P < 0.05$ in the patients. Among these genes, FOS was significantly upregulated and was further investigated due to its established role in neuronal cell function. We showed that i) 30% depletion of Fmr2 in mouse primary cortical neurons led to a 2-fold increase in Fos expression, ii) overexpression of FMR2 significantly decreased FOS promoter activity in luciferase assays, and iii) as FOS promoter contains a serum response element, we found that not FOS, but JUN, which encodes for a protein that forms a transcriptional activator complex with FOS, was significantly upregulated in the patients' cell lines upon mitogen stimulation. These results suggest that FMR2 is an upstream regulator of FOS and JUN, and further link deregulation of the immediate early response genes to the pathology of ID and FRAXE associated ID in particular.

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P05.085

Familial intellectual disability in Brazilian institutions

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We present the results of the first part of a project whose main purpose is to understand the relevance of familial intellectual disability among the population of individuals with moderate to severe intellectual disability who attend special day care institutions.
For this purpose we developed a survey instrument which was applied by members of our team to the guardians of 800 familial intellectual disability individuals in SC, Brazil, to select the families where two or more individuals in three generations have familial intellectual disability. Possible familial cases were selected to proceed to the second part of this project (collect family history to validate the data, try to clarify diagnostics).
Findings of our survey indicate:
1) Etiology: 14% with Down Syndrome; 3% due to infectious diseases (meningitis, rubella, toxoplasmosis, cytalomegalovirus and others; 1.5% due to known monogenic causes, 0.8% with known microdeletion/duplication syndromes and about 1% with known syndromes of unclear cause. Cerebral palsy was reported for 11% of the cases, most of them attributed to neonatal oxygen deprivation.
2) Other cases in the family: 52.5% reported other cases, 8% of which were obviously not related, leaving 39.5% of potential familial cases to proceed to the next part of our project. Of those, 16% had 2 cases; 10% had 3 cases; 14% had 4 or more cases (20% of those with 7 to 14 affected individuals). Even if many cases may show to be unrelated, the numbers are astonishing. This is the first survey of this kind we know.

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P05.086

Analytical & clinical performance assessment of the Affymetrix CytoScan® Dx cytogenetic microarray system

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Identification of pathogenic copy number variants (CNVs) by cytogenetic microarrays has demonstrated a higher diagnostic yield as compared to conventional methods, such as karyotyping and FISH. This study's objective was to characterize analytical and clinical accuracy of CytoScan Dx compared to routine patient care (RPC) in surplus samples from a post-natal population exhibiting developmental delay, intellectual disability, congenital anomalies/dysmorphisms. Samples were collected consecutively at 3 sites where RPC comprised of one or more methods (karyotyping/FISH/microarray) for establishing a copy number call (excluding Affymetrix arrays). An independent cytogeneticist (with no previous patient information) interpreted the samples processed at one site on CytoScan Dx.

960 samples were analyzed. RPC reported 425 CNVs. Analytical accuracy was calculated as the percentage of RPC-identified regions that were corroborated by CytoScan Dx. Excluding 12 RPC-reported regions below the reported resolution of CytoScan Dx, the analytical accuracy of CytoScan Dx as compared to RPC was 97.3% (95% CI = 95.3-98.5%, 402/413 CNVs). The average percent CNV overlap between RPC and CytoScan Dx was 92.8%±11.23. Of the 402 samples that agreed analytically, 67.4% had the same interpretation.

To date, this data represents the largest clinical study assessing both the analytical and clinical accuracy of CytoScan® Dx as compared to RPC and validates the high analytical accuracy of CytoScan® Dx. Differences in clinical interpretation are expected as they are influenced by many factors including availability of clinical information, parental results, and the time period of evaluation, since the body of literature regarding the pathogenicity of CNVs continues to evolve.

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P05.087

Clinical description of a female patient with a mutation in *WRD45*, a gene involved in a new X-linked NBIA (neurodegeneration with brain iron accumulation)

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Neurodegeneration with brain iron accumulation (NBIA) is a group of diseases characterized by the accumulation of iron in the basal ganglia. Six genes are presently involved in NBIA presenting in childhood/adolescence, each of them having particular clinical and/or radiological features. We report the clinical history of a young adult patient with a disease history suggestive of SENDA syndrome (static encephalopathy with neurodegeneration in adulthood), now called BPAN syndrome.

A diagnosis of epilepsy and global developmental delay was made at first examination at 22 months. The evolution was characterized by: 1) easy epilepsy control with anti-epileptic drug, 2) severe intellectual deficiency that became obvious during the first years of life (independent walking achieved at 2 years, absence of language, limited communication skills) associated with stereotypies and unmotivated laughters, 3) facial dysmorphism with body asymmetry, 4) the apparition of pyramidal signs with walking difficulties after puberty, 5) an OFC growing on the -2SD curve.

Brain MRI showed at the age of 16 years a heterogeneous signal of the pallidum on T1-weighted images and a hyposignal of the pallidum and locus niger on T2-FLAIR-weighted images. *PLA2G6* mutations were ruled out. The c.56-1G>A splice-site mutation not found in the proband's mother was identified in *WDR45.*

Clinical and radiological characteristics in our patients are consistent with the diagnosis of SENDA/BPAN syndrome and with those of other patients with mutation in *WDR45*. Though the initial clinical picture suggested an atypical form of Rett syndrome, brain MRI features only guided the etiogogical diagnosis.

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P05.088

Phenomic clues to genomic variation in patients with developmental delay or intellectual disability

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Background and aims. Unless high-resolution microarray technologies have been recommended as the first-tier cytogenetic diagnostic test for patients with developmental delay/intellectual disability (DD/ID), still not every laboratory has possibilities to perform this analysis to every patient with DD/ID. Our aim was to determine whether patients with pathogenic copy number variants (pCNVs) are confident to a distinct clinical subgroup.
Method. Retrospective review of clinical and molecular karyotyping data of 211 patients was performed. Clinical data of patients with chromosomal alterations (n=29) were compared with those without pCNVs (n=182).
Results. The findings indicate the increased frequency of pCNVs in patients with syndromic DD/ID (p=0.018), at least one congenital anomaly (p=0.005), three and more minor anomalies (p=0.016), congenital malformations of CNS (p=0.026), congenital malformations of musculosceletal system (p=0.037) and minor anomalies of eye, ear, face and neck subgroup (p=0.003). Statistically significantly several phenotypic traits were more frequent in patients with pCNVs, as hydrocephalus (p=0.023), congenital malformations of corpus callosum (p=0.014), downward slanting palpebral fissures (p=0.008), minor anomalies of ear (p=0.002), micrognathia (p=0.004), brachydactyly (p=0.005), umbilical hernia (p=0.008). *Café au lait* spots more frequently were in patients without pCNVs (p=0.023). A multivariate logistic regression analysis was performed to determine the predictors of pCNVs. Three independent predictors of pCNVs were determined: congenital malformations of corpus callosum, minor anomalies of ear and brachydactyly, increasing the risk of pCNVs by 0.1, 3.3 and 7.5 times respectively.
Conclusions. Our results suggest, that clinical features, as syndromic DD/ID, specific congenital and minor anomalies might be possible indicators for pCNVs.

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P05.089

A wide spectrum of intellectual disability in two siblings with a 6p22.3-p23 microdeletion encompassing JARID2

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Interstitial deletions of 6p22-6p24 are associated with a variable phenotype including intellectual disability, autistic spectrum disorder, hypotonia and congenital abnormalities. There are many potential disease-causing genes in this region, including JARID2, DTNBP1, ATXN1, and MYLIP, and delineating genotype-phenotype correlations has been a challenge. It has been suggested that deletions of JARID2, a DNA-binding transcriptional repressor expressed in embryonic and adult neurones, are associated with a characteristic facial appearance as well as intellectual disability.

Our first patient presented with mild to moderate learning disability and some Marfanoid systemic features, scoring 6/20 on the revised Ghent criteria. Cardiac and ophthalmic investigations were normal. CGH microarray identified a 1.2Mb microdeletion on chromosome 6p22.3-p23 involving JARID2 and DTNBP1 only; targeted microarray and FISH studies did not identify this deletion or a predisposing rearrangement in either parent. The patient's younger brother presented with much more severe learning disabilities and autistic spectrum disorder. He had no similar skeletal features but both boys had similar prominent supraorbital ridges. Investigation with CGH microarray found the same microdeletion, suggesting low level somatic-gonadal mosaicism or true gonadal mosaicism as a cause for the two affected siblings.

This case illustrates the wide spectrum of learning difficulties and behavioural difficulties that may be seen even in siblings with identical small microdeletions in this region. This may represent a challenge to the establishment of a clinically recognisable phenotype for JARID2 deletions and highlights the complexity of the genetic aetiology of intellectual disability.

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P05.090

Further delineation of medical and behavioural aspects of the KBG syndrome caused by *ANKRD11* mutations

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Introduction Recently *ANKRD11* mutations were identified as the cause of KBG syndrome, an autosomal dominant intellectual disability (ID) syndrome with specific dental, craniofacial and skeletal anomalies. To further expand the phenotypic spectrum and knowledge on medical and behavioural management, we present a comprehensive overview of clinical and molecular characteristics of nine so far unreported cases from five different families with *ANKRD11* aberrations.
Methods Clinical features of nine cases with KBG syndrome were evaluated and a review of the existing literature on KBG syndrome was performed. Sequence analysis of the *ANKRD11* gene was performed using Sanger sequencing. An Affymetrix CytoScan HD array was used according to the manufacturer's protocol.
Results In the first family a heterozygous frameshift mutation in *ANKRD11* was identified in four affected children as well as their mother. Three other isolated KBG syndrome patients all harboured frameshift mutations in *ANKRD11*. Microarray analysis revealed a 100 kb deletion encompassing exons 1-3 of the *ANKRD11* gene in one patient.
Discussion Hallmark characteristics of KBG syndrome are mild to moderate ID, macrodontia and other dental anomalies, skeletal anomalies, short stature and typical dysmorphic features. Less common features are heart defects, palatal defects, hearing loss, ophtalmologic abnormalities and central nervous system involvement. Neurobehavioural problems, especially autism spectrum disorder and attention deficit hyperactivity disorder, are often present but gained less attention so far in medical literature. However, we observed that these can be severe and require adequate management. Better recognition, a multidisciplinary approach and early intervention are essential to patients and their families.

C.W. Ockeloen: None. **M.H. Willemsen:** None. **S. de Munnik:** None. **B.B. van Bon:** None. **A. Verrips:** None. **H. IJntema:** None. **N. de Leeuw:** None. **C.C. van Heumen:** None. **C.E. Carels:** None. **T. Kleefstra:** None.

P05.091

Exome Sequencing assesses the role of the KIAA2022 gene in the aetiology of X-linked intellectual disability

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Here we report on the clinical and molecular characterisation of an X-linked intellectual disability (XLID) family ascertained by next-generation sequencing (NGS) of most of the X-chromosome transcripts. Two brothers were referred for genetic work up because of intellectual disability. The youngest presented with severe intellectual disability, seizures and hyperactivity. The elder brother was highly social, communicated verbally with good sentence structure and was able to read and write. His IQ was 50. Their 38 years old maternal uncle was unemployed, unable to read and write and his IQ was 46. All of them had normal neurological examination and no remarkable facial features were present. The two affected sibs were tested using the XSeq ™ Research Screening Panel developed by Raindance Technologies. The two brothers shared only 3 previously unreported variants, two misseense variants in DMD and PLP2 gene and one frameshift variant in KIAA2022 gene. Additional analysis showed that only the KIAA2022 mutation segregated with known carrier status and in all affected individuals. Interestingly, disruption of this gene have previously been identified in two mentally boys and this gene is highly expressed in fetal brain and the adult cerebral cortex. Our data confirm the role of the KIAA2022 gene in the aetiology of intellectual disability and the interest of NGS in small XLID families.

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P05.092

The common polymorphism of the KIBRA gene is associated with academic achievement

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The KIBRA protein is assumed to play an important role in synaptic plasticity. Genetic variation at the KIBRA gene rs17070145 polymorphism has been linked to episodic memory and executive function. T allele carriers of SNP rs17070145 have been reported to outperform individuals that are homozygous for the C allele in episodic and working memory tasks. Since academic achievement is directly related to the memory, we hypothesized that the KIBRA T allele could be associated with the ability to get a higher education and a PhD degree. We have tested this hypothesis in three groups of the Russian population (n=98; all Caucasians): primary school-aged children, students of universities and PhD degree holders. The frequencies of the T allele and TT genotype in the Russian children were similar with those reported in the European populations (T allele: 29.0% and 31.9%; TT genotype: 9.7% and 10.6%, respectively). We found an increasing linear trend of T allele with increasing academic level of individuals (children - 29.0%, students - 37.7%, PhD degree holders - 64.3%; P=0.0032). Interestingly, the frequency of the T allele in students willing to apply for a PhD in future was higher (54.5% vs. 33.3%; P=0.08) in comparison with unwilling students. Compared with CC homozygotes, the odds ratio of being a PhD holder in TT homozygotes was 12.44 [95% CI: 1.995-77.632, P=0.0108]. In conclusion, our results suggest that the rs17070145 C/T polymorphism of the KIBRA gene seems to be associated with academic achievement, with the T allele exerting a beneficial effect.

I.A. Alexeev: None. **L.M. Sungatullina:** None. **E.S. Nasibulina:** None. **A.S. Kruchkov:** None. **I.I. Ahmetov:** None.

P05.093

Neuroanatomical correlates of Klinefelter syndrome studied in relation to the neuropsychological profile

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Brain imaging in Klinefelter syndrome (47, XXY)(KS), a genetic disorder characterized by the presence of an extra X chromosome, may contribute to understanding the relationship between gene expression, brain structure, and subsequent cognitive disabilities and psychiatric disorders.
We conducted the largest to date voxel-based morphometry study of 65 KS males and 65 age- and educational-matched male controls and correlated these data to neuropsychological test scores. The KS males had significantly smaller total brain volume (TBV), total gray matter volume (GMV) and total white matter volume (WMV) compared to male controls, whereas no volumetric difference in cerebral spinal fluid (CSF) was found. There were no differences in TBV, GMV, WMV or CSF between testosterone treated KS (T-KS) and untreated KS (U-KS) males. Compared to male controls, KS males had significantly decreased GMV bilaterally in insula, putamen, caudate, hippocampus, amygdala, temporal pole and frontal inferior orbita. Additionally, right parahippocampal region and cerebellum was reduced in KS males. KS males had significantly larger volumes in right postcentral gyrus, precuneus and parietal regions. Multivariate classification analysis discriminated KS males from male controls with 96.9 % ($p<0.001$) accuracy. Regression analyses, however, revealed no significant association between GMV differences and cognitive and psychological factors within the KS males and male controls or the groups combined. These results show that although gene dosage effect of having and extra X-chromosome may lead to large scale alterations of brain morphometry and extended cognitive disabilities no simple correspondence links these measures.

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P05.094

Transcriptome sequencing in patients with Koolen-de Vries syndrome

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The Koolen-de Vries syndrome is a multisystem disorder characterized by intellectual disability (ID), hypotonia, epilepsy, distinctive facial features, and congenital malformations of the heart, urogenital tract and central nervous system. The syndrome is also known as 17q21.31 microdeletion syndrome, due to typical microdeletions that can cause the phenotype. Recently, we and others showed that haploinsufficiency of *KANSL1* is sufficient to cause the syndrome. The *KANSL1*-gene encodes a chromatin-modifying protein that is a member of the nonspecific lethal complex. This complex contains, among other proteins, the acetyltransferase KAT8, which influences gene expression through acetylation of H4K16 and p53.

To investigate the effect of *KANSL1* mutations on genome-wide expression levels, we performed transcriptome sequencing of twelve different samples from patients with Koolen-de Vries syndrome. The mutations in these samples comprise six classical 17q21.31 microdeletions, two atypical deletions, three *KANSL1* point mutations and one so far unidentified mutation. Enrichment of mRNA was done using the MicroPoly(A)Purist Kit (Ambion), whole transcriptome library preparation was performed using the SOLiD Total RNA-Seq Kit (STaR Kit, Life Technologies) and paired-end sequencing was performed on a 5500XL sequencer (Life Technologies). The expression levels of these trancriptomes were compared to expression levels of eight healthy controls and functional annotation clustering of differentially expressed genes was done using the Database for Annotation, Visualization and Integrated Discovery (DAVID).

We have detected new genes that are differently regulated in the 17q patients compared to controls. Further investigation of these genes might give new insights into the pathways that are underlying ID.

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P05.095

Hemizygous deletion of LRFN2/SALM1 is responsible for a selective working memory deficit.

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Learning disabilities (LD) are a clinically and genetically heterogeneous group of disease. The identification of familial cases of clinically homogeneous endophenotypes of LD might help the management of the patients, and precise the genetic counselling, Array-CGH and high-throughput sequencing dramatically expand the number of genes implicated in isolated ID, highlighting the implication of neuron specific post-mitotic transcription factors and synaptic proteins as candidate genes. We report on a unique family diagnosed with a 6p21 microdeletion segregating in 3 patients with learning disability of autosomal dominant heredity. Neuropsychological assessment identified a selective deficit of working memory, without ID in the patients. Further investigations identified a defect in the executive functions, and auditoro-verbal processes. These data were consistent with brain MRI and FDG-PET functional brain imaging that revealed atrophy and hypometabolism of cerebral region strongly implicated in working memory processes when compared to controls. The 870kb microdeletion encompassed 3 genes with only one brain expressed gene encoding for a postsynaptic protein named LRFN2/SALM1. We performed an immuno-colocalization with electronic microscopy demonstrating the tight co-localization of LRFN2/SALM1 with the NMDA (N-Methyl-D-Aspartate) receptors in the lateral part of the postsynaptic density of cerebellar and hippocampal rat neurons. Altogether, the combined approaches highlighted the implication of LRFN2/SALM1 in LD, specifically by its implication in working memory processes and executive functions. A medication by NMDA modulator could be discussed because of the interaction of LRFN2/SALM1 with the NMDA receptors.

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P05.096

KCNT1 is the major gene causing malignant migrating partial seizures in infancy

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Migrating partial seizures of infancy (MPSI) is a rare epileptic encephalopathy characterized by i) seizure starting during the first months, ii) focal seizures randomly migrating from one cortical region to another, iii) arrest of psychomotor development with a severe cognitive and motor outcome and acquired microcephaly. We identified de novo *KCNT1* mutations in 6/12 patients with MPSI. *KCNT1* encodes a sodium-activated potassium channel. We showed that MPSI-associated mutations are located in the C-terminal domain of KCNT1 and cause a gain of function.

We analyzed *KCNT1* sequence in 16 additional patients with MPSI and in a cohort of 14 patients with infantile multifocal epilepsy (IME), a rare epilepsy syndrome of infancy sharing with MPSI

early onset, drug-resistance and severe cognitive outcome.

We found four novel de novo mutations in MPSI patients, all mapping within the C-terminal domain. In two MPSI brothers, an inherited mutation from the unaffected father raises the issue of genetic counseling. Genotype-phenotype correlation revealed no differences between *KCNT1* positive and negative MMPSI cases regarding age of onset, pharmacoresistance, cognitive or motor outcome. *KCNT1* sequencing revealed no mutations in patients with IME.*KCNT1* mutations are found in 43% of cases (12/26) of

MPSI confirming *KCNT1* as the major disease-causing gene in this syndrome. Absence of KCNT1 mutation in IME may suggest a specific link between KCNT1 and the «EEG migrating pattern »

phenotype and confer to the genetic testing of *KCNT1* a diagnostic value in the context of neonatal pharmacoresistant epilepsy with severe delay.

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P05.097

Characterization of supernumerary marker chromosomes by high resolution array CGH

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Background: Small supernumerary marker chromosomes (sSMC) are described as small structurally abnormal chromosomes that occur in addition to the normal 46 chromosomes. The phenotypes associated with the presence of a marker vary from normal to severely abnormal. The rate of sSMC among patients with mental retardation (MR) is estimated to be about 0.288%. In a series of 165 patients with MR/developmental delay we found 3 with de novo sSMC by conventional GTG karyotyping.
Aim: The aim of the study was to clarify the chromosome origin of the supernumerary markers and to map the chromosome regions with aberrant copy number.
Materials & methods: Array CGH analysis was performed using custom designed whole-genome oligonucleotide arrays (OGT, UK) with a median probe spacing of about 2.5 kb and median resolution of about 10 kb. CytoSure Interpret (OGT, UK) software was used for CNV detection.
Results & discussion: The supernumerary marker chromosomes are found to originate from chromosomes 18 (arr 18p11.32p11.21(123,155-15-,065,191)x5), 15 (arr 15q11.2q13.3(18,410,710-30,678,185)x4) and 20 (arr 20q13.33(60,446,113-62,382,429)x4), respectively. Clinical presentation of the patients and some specific symptoms correlated well to the chromosomal abnormalities found. We concluded that molecular techniques such as arrayCGH, associated to cytogenetic methods can help in detecting genomic imbalances and unraveling the genes involved in the phenotype variability of patients with supernumerary marker chromosomes. Furthermore, the molecular characterization of the alterations found provides valuable information for the follow-up of the patients and genetic counseling of their families.

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P05.098
MBD5 alteration by multiple mutational mechanisms leads to variable phenotypic outcomes

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Just recently, partial or complete deletion of methyl-CpG-binding domain 5 (MBD5) gene has been implicated as causative in the phenotype associated with 2q23.1 microdeletion syndrome. The core phenotype observed includes intellectual disability (ID), seizures, significant speech impairment and autistic-like symptoms. In the course of systematic whole-genome screening of individuals with unexplained ID by array-based comparative genomic hybridization, we identified de novo intragenic deletions of only the 5'-non-coding region of MBD5 in three patients leading, as previously documented, to haploinsufficiency of MBD5. In addition, we described two patients with two different unreported MBD5 intragenic duplications. The first one leads to the presence of numerous aberrant transcripts with premature termination codon. The second one is inherited from a healthy parent suggesting either this duplication is unrelated to the phenotype or this mutated allele in not fully penetrant. To further elucidate the involvement of MBD5 in ID, we sequenced coding and non-coding exons in a selected cohort of 78 subjects with a phenotype reminiscent of 2q23.1 microdeletion syndrome. We identified for the first time a de novo nonsense mutation associated with a much more damaging phenotype without walking and verbal speech at the age of 10 years. Besides this fully penetrant mutation, we identified four missense variants most often inherited from a healthy parent. One of these, the p.Gly79Glu variant, has been recently described as an allele risk for autism spectrum disorders. Taken together, our findings suggest MBD5 gene is sensitive to perturbation by multiple mutational mechanisms, leading to variable phenotypic outcomes.

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P05.099
Identification of a complex *MECP2* mutation in a 11 year-old male with myoclonic encephalopathy and severe cognitive impairment detected by targeted next-generation sequencing.

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To date, the complexity of genetic causes of Intellectual Disability (ID) makes difficult the molecular diagnostic of patients who do not present with clinical features evoking a specific syndrome. We report here the case of a severely affected young boy for whom molecular, cytogenetic and biochemical investigations had failed to identify the etiology of its ID. During the targeted high-throughput resequencing of 220 ID genes, a complex anomaly was detected in exon 4 of *MECP2* gene in this patient. This mutation is a deletion of 139bp of exon 4 additioned by two inverted insertions of intron 2 sequences, which leads to the replacement of 41 amino acids but keeps the reading frame downstream of the rearrangement. This mutation was inherited from the mother who presents with mild cognitive impairment, but no Rett syndrome phenotype despite an unbiased X-inactivation in blood. We review and discuss all the other *MECP2* rearrangements and mutations reported in males in literature and the associated clinical phenotypes.

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P05.100
Impact of the overexpression and deficiency of Mecp2 in mice: a balance always to maintain.

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The X-linked methyl-CpG binding protein 2 gene (Mecp2) plays a role in the regulation of chromatin architecture, gene expression and fine tune of adult neurons. An abnormal dosage of this protein causes a large group of neurological disorders. Mutations or deletions in MeCP2 gene cause Rett syndrome in females, whereas its overexpression causes the Mecp2 duplication syndrome in boys. A fine control of the amount of MeCP2 protein is necessary for a normal function of neurons. Several mouse models were generated to mimic the Mecp2-pathies. Here, we studied mice with a double expression of Mecp2 (Mecp2Tg1) (Collins et al, 2004) to get closer to the human case of duplication. We used a battery of behavioral tests: grip strength, rotarod, open field and the respiratory profile of the Mecp2Tg1 mice during postnatal development. In parallel, in vivo studies were made in different parts of the nervous system, on a set of transcripts regulated by Mecp2. Our results show that Mecp2Tg1 mice exhibit behavioral disorders: At 5 weeks, these mice appear hyperactive and anxious. Much later (from 35 weeks), they develop spasticity and seizures. This is followed by a period of hypoactivity and kyphosis. The clinical implications of our study are twofold: first we seek to understand the phenotypic effects of a double dose of Mecp2. Second, gene therapy projects are being evaluated on MeCP2-deficient mice. Since the level of MeCP2 must be tightly regulated, a good control of Mecp2 dosage will be necessary in order to prevent overdosage of the protein.

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GABA and Glutamate metabolisms are affected in the brain of Mecp2-deficient mouse.

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Rett syndrome (RTT) is a severe neurological disorder that affects female patients. RTT is caused by mutations in the X-linked methyl-CpG binding protein 2 (MECP2) with an incidence of 1/15000. RTT patients develop normally until 6-18 months of age, before the onset of deficits in autonomic, cognitive, motor functions (stereotypic hand movements, impaired locomotion) and autistic features. Studies on Mecp2 mouse models revealed a severe deregulation in the neurotransmission. In particular, bioamine de-

ficits have been primarily and extensively studied in Mecp2 deficient mice. Dysfunction of the gabaergic and glutamatergic systems has been also pointed out since the balance between brain excitation and inhibition appears affected in the brain of the Mecp2 deficient mouse. However, the results are divergent due to the differences in the age and tissues studied. To date few projects have been dedicated to the follow GABA and Glutamate metabolism in eight different brain areas of the Mecp2-deficient mice. In the present study we used real-time PCR, western blotting and HPLC to compare the GABA and Glutamate metabolism in several key areas of the Mecp2 mutant mice brain at different developmental stages (early and late symptomatic). Key enzymes (Kcc2, Nkcc1, Vglut1/2, Gad1/2) will be considered. In conclusion, our results revealed a spatial and temporal deregulation of the glutamatergic and gabaergic systems for all levels of regulations studied. Moreover, we found that pharmacological stimulation of the gabaergic system is efficient to improve the lifespan of Mecp2 deficient mice.

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The French cohort of *MECP2* duplication patients: clinical delineation of 45 affected patients

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Duplication of Xq28 including the *MECP2* gene has been primarily described in male patients with severe developmental delay, constipation, epilepsy, hand stereotypies and recurrent infections. In the first part of this work, we carried out an epidemiological study that permitted to identify 95 patients in France carrying a *MECP2* duplication of less than 4 Mb (86 males and 9 females ranging from 0 to 40 years) since the implementation of MLPA and array-CGH. 84 patients carried a pure interstitial duplication of various size and 11 patients a complex rearrangement. In the second part of the study, we report on the clinical manifestations of 45 patients of the series. 27 patients were examined by the same physician and a standardised form was sent in the remaining 18 patients. Besides to the classical clinical features, we describe frequent manifestations insufficiently reported in the literature including tapering fingers, flessum attitude due to progressive spasticity and translucent skin with prominent veins. 77% of patients had brain abnormalities consisting mainly in corpus callosum malformations and ventricular dilatation. We particularly focus on the affected females phenotype that can be similar to that observed in males, especially when the *MECP2* duplication results from an unbalanced X-autosome translocation. Careful genetic counselling should be given to couples at risk, given the risk of abnormal phenotype in females. The third part of the study consisting in searching genotype-phenotype correlations is in progress, in order to check if the different sizes of the duplication can be associated to variable clinical features.

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Cytogenetic Microarray for Delineation of Cryptic Genomic Rearrangements in Mental Retardation

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Mental retardation (MR) is a variable, heterogeneous manifestation of central nervous system dysfunction, present in 1-3% of general population. Many environmental and genetic factors can cause MR, including premature birth, prenatal infections, chromosomal abnormalities, and single-gene mutations. Though an etiology can be established in 60-75% of cases of severe MR, it is only 38-55% in mild cases. Moreover, genetically determined MR accounts by for 17 to 41% of cases, depending on use of the different techniques. In the present study, we report the cryptic genomic rearrangements identified MR cases by selective use of chromosomal microarray.
Methodology: Two cases of unexplained MR after initial biochemical, radiological and chromosomal evaluation were studied. DNA was extracted from whole blood and analysed on Illumina HumanCytoSNP-12 array. Raw data obtained was analysed with KaryoStudio software. The rearrangements were checked in DECIPHER database.
Results and Conclusion: Both the samples showed a male karyotype and some cryptic genomic rearrangements. First case had multiple rearrangements-duplications in the chromosome 7q11.21 (398kb) and 17q12 (186kb), and 1.6Mb hemizygous deletion at cytoband 14q13.3-q21.1. Second case was observed to have a duplication of 549Kb at 7q36.2, region associated with autism spectrum disorder and amyotrophic lateral sclerosis; a 2.6Mb hemizygous deletion at 22q11.21 q11.22 region. All these regions (non-recurrent genomic aberrations) have been implicated as pathogenic in several other patients as per DECIPHER database. Thus, chromosomal microarray analysis can help in identifying underlying cause of MR in some apparently unexplained cases, and should be a diagnostic tool for evaluation of MR.

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Plasma levels of leptin and adiponectin in Fragile X syndrome

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Fragile X syndrome (FXS) is the most common form of familial mental retardation and one of the leading known causes of autism. The mutation responsible for FXS is a large expansion of the CGG repeats in the promoter region of the *FMR1* gene resulting in its transcriptional silencing. Leptin may be considered a cytokine-like hormone with pleiotropic actions since it may be involved in the regulation of neuroendocrine functions, immune system, in addition to its role in development. Leptin and adiponectin may act in parallel as opposing metabolic counterparts. Thus, we hypothesized on involvement of adiponectin in the pathophysiology of autism.
Material and methods
23 male patients, mean age 19,3 ± 6,6, affected by Fragile X syndrome (full mutation in the *FMR1* gene), were enrolled into the study. Controls were 24 healthy males, mean age 21,8 ± 5,8. Plasma leptin and adiponectin levels were determined by ELISA method.
Results
Plasma leptin levels in Fragile X syndrome patients were similar as in control group (11,53 ± 10,24 ng/ml) vs (5,73 ± 4,52) were not significantly different from those found in controls (p = 0,5).
While plasma adiponectin levels in Fragile X syndrome patients (6,68 ± 3,44 ng/ml) were significantly lower in controls (8,95 ± 4,24) (p< 0,04).
Conclusion
Adiponectin may be involved in autistic features observed in FXS patients in light of its anti-inflammatory properties. Further investigations are necessary to evaluate the role of adipokines in FXS.
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Clinical utility of the chromosomal microarray analysis in patients with neurodevelopmental disorders.

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Developmental delay/intellectual disability (DD/ID) affects 1-3% of the population. The study of the DD/ID is one of the most complex fields of human genetics because of their high clinical and genetic heterogeneity. In the last decade, the application of genome analysis technologies to the study of chromosomes has enabled dramatic progress in the field of clinical genetics.
The objective of this work is the study of these anomalies, which not only helps to determine the genetic causes of these disorders, but also allows to correlate genotype-phenotype and to define the clinical and behavioral characteristics of these microduplication and microdeletion syndromes. An accurate diagnosis is the key providing genetic counseling that helps the family to take reproductive decisions.
We have applied array-CGH (44,000 60 mer probes and/or 6,000 BAC clones) in a cohort of 246 patients with DD/ID, congenital anomalies (CA), facial dysmorphysm and/or positive family history for ID, CA or miscarriages. We have collected 109 different clinical variables for each patient.
We have found a pathogenic or potentially pathogenic chromosomal imbalance (pCNVs) in 29.7% of patients. Comparison of the clinical data showed that hands and/or feet abnormalities were the most frequent congenital anomaly (55%) and was statistically more frequent in children with pCNVs (63%; p-value 0.05). Further clinical criteria indicate a higher probability of a chromosomal aberration causative for DD/ID was skeletal defects, microcephaly, genital abnormalities and congenital heart defects.
Our results reinforce the importance of clinical criteria in the application of array-CGH in patients with neurodevelopmental disorders.

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Searching for microcephaly genes amongst an endogamous population

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Irish Travellers are an endogamous population numbering 26,000 in the Irish Republic. First cousin marriage is common and they operate a clan within a clan structure. This project focuses on two clans with microcephaly (<3SD) and normal MRI scans. Microcephaly is common and proving it is homogeneous is difficult clinically. The first family (A) consist of 3 affected children. One child is more severely disabled than his siblings raising the possibility of sibship heterogeneity. The second family (B) consist one affected child and 4 affected maternal grandaunts with a similar microcephaly phenotype.
SNP homozygosity mapping showed that the affected individuals from both families share 12 and 6 regions of homozygosity (ROH) respectively.
Comparison of the homozygosity patterns across the families identified a single shared ROH (5.6 Mb) on chromosome 11. However, exome sequencing did not identify a shared recessive mutation within the candidate locus, suggesting that (i) the two families may have different disease genes or (ii) the shared disease mutation is regulatory/non-coding. One affected from each clan share mutations in a candidate gene, but these mutations are not found in other affecteds from either family.
Exomic sequencing unexpectedly revealed that 1 affected individual each from family A and B had McArdles disease confirming that they are from the same wider clan. Neither had signs of this disease.
Our analytic approach has been to accommodate heterogeneity both between and within families. This study highlights the complexities of this analysis & the unexpected identification of disease genes through exomic sequencing.

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Genome-wide expression studies in 14 patients with microdeletion 5q14.3 syndrome - A novel tool for the systematic study of functional interactions and pathways

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Many novel candidate genes for intellectual disability (ID) have been identified in microdeletions. However, our etiological understanding of these genes often remains unclear. So far, only targeted expression experiments provided evidence e.g. for functional interactions between novel and well-established ID genes or for the haploinsufficiency of deleted candidate genes. However, genome-wide and hypothesis-free expression analyses have not yet been applied to non-recurrent microdeletion syndromes to systematically study functional interactions and pathways.
We performed genome-wide expression studies on 14 patients with non-recurrent 5q14.3-q15 microdeletions using Illumina HT12 expression arrays and whole blood RNA. Adapting an established analysis protocol, we first compared all transcripts with reliably detectable signals (detection p-value / DPV<0.01) between patients and controls and demonstrated significantly reduced TRABD expression in patients (verified by qPCR). However, this protocol may exclude those transcripts with the largest expression difference between patients and controls. Thus, we developed a novel strategy to identify transcripts that met the above-mentioned DPV threshold EITHER in controls OR in patients. We identified several additional genes. Due to limited RNA, only one of these, COX7B, was verified by qPCR. Both the significant correlation of the transcript levels of TRABD / COX7B with MEF2C, the recently identified causative gene in 5q14.3 microdeletion syndrome, and the fact that TRABD and COX7B were connected to MEF2C over only two „nodes" in a network analysis pointed to a biological significance of the interactions.
Thus, both analysis protocols yielded verifiable and biologically significant results, demonstrating the applicability of genome-wide expression studies to non-recurrent microdeletions.

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P05.108

An emerging phenotype of microdeletion Xq22 involving *PLP1* gene in females. Case reports of four independent patients

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Pelizaeus-Merzbacher Disease commonly results from duplications of the PLP1 gene at Xq22, with males being affected and females usually healthy carriers. Microdeletions encompassing PLP1 in females with severe intellectual disability have not been described previously. Here we present four females with deletions at Xq22.1-q22.3 involving PLP1.
The four patients of British, South Asian, Swedish and Japanese origin are aged 19, 8, 7 and 3 years respectively. All presented with severe intellectual disability and cerebral abnormalities including generalized white matter and corpus callosum hypoplasia, atrophy and delayed myelination. The two eldest patients walked independently from 6 and 7 years, but have no speech

and impaired comprehension. Additional features seen in one or more cases include: polyhydramnios, distinct sleeping disturbances, purposeless hand movements, mutilation, hearing loss, overgrowth/delayed growth, slow growing hair and deep palmar creases. Neurological features include severe hypotonia, seizures and cortical blindness. Skeletal features include advanced bone age, proximal placed thumbs, severe pes equinovarus, extremely short feet and overriding toes. No patient showed developmental regression or periventricular leucomalacia. There is no distinctive dysmorphology.
Array analysis detected de novo Xq22 deletions 3Mb, 85kb, 4.5Mb and 4.9Mb in size, all encompassing PLP1. X-inactivation studies in blood were unquantifiable but skewed in two, 79:21 skewed and non-informative respectively. Clinical and molecular features of all four females with Xq22 deletions suggest a new microdeletion syndrome separate from Pelizaeus-Merzbacher disease. The smallest deletion contains only PLP1, along with transmembrane protein 31 (TMEM31) and glycine receptor, alpha 4 (GLRA4) about which we have very limited information.

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P05.109

20 ans après: a second family with mutation in X-linked monoamine oxidase A gene affecting cognition and behavior

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Intellectual disability (ID) is characterized by an extraordinary genetic heterogeneity, with more than 200 genes implicated in monogenic forms. This complexity precluded systematic testing for mutations and because clinical features are often non-specific, for some of these genes only few cases or families have been unambiguously documented. It is the case of the the X-linked gene encoding monoamine oxidase A (MAOA), for which only one nonsense mutation has been identified in Brunner syndrome, characterized in one unique family by borderline ID and impulsive and aggressive behaviors. We have developed targeted NGS sequencing of 220 ID-genes in patients with undiagnosed ID. We identified a p.C266F missense mutation in MAOA in a boy with autistic features and behavioral disturbances. He has good verbal comprehension but IQ could not be measured due to attention deficit and heterogeneous results in WISC-IV test. Two maternal uncles carry the mutation and have severe ID, with a history of maltreatment in early childhood. The phenotype overlaps with Brunner syndrome. This novel missense mutation decreases MAOA activity, as shown by abnormal levels of urinary monoamines and by measurement of enzymatic activity. The identification of this point mutation confirms, for the first time since 1993, the monogenic implication of the MAOA gene in ID, autistic features and behavioral abnormalities. Variable expressivity might be due to environmental factors. We will discuss possible therapeutic options and hypotheses that could account for the very long time lag between the initial and highly publicized report and the finding of a second family.

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Microduplication disrupting the *MYT1L* gene in a familial case of intellectual disability, and neuropsychiatric disorder.

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MYT1L is a member of the myelin transcription factor 1 gene family and is highly expressed in mouse embryonic brain. MYT1L interacts directly with DISC1, which is involved in schizophrenia. We describe a family in which the father, his son and his daughter have a 549 kb interstitial duplication in the 2p25.3 region detected by array comparative genomic hybridization and confirmed by quantitative PCR. This duplication encompasses the five first exons and is predicted to disrupt the *MYT1L* gene. The phenotype includes: slight facial features including bulbous nasal tip, wide mouth, overfolded helix, strabismus, moderate intellectual disability (ID) with speech impediment, hypotonia, hyperactivity, mood disorder with aggressive behaviour, neonatal feeding difficulties followed by a tendency to hyperphagia with overweight and a prominent abdomen with thick panniculus adiposus. Partial deletions of chromosome band 2p25.3 encompassing the *MYT1L* gene have been reported in patients presenting with ID and overweight whereas duplications disrupting the *MYT1L* gene have been reported in patients presenting with autism and schizophrenia. Moreover, single-nucleotide polymorphisms (SNPs) of *MYT1L* contribute to major depressive disorder and schizophrenia. Recently, de Ligt et al. reported a „de novo" splice site mutation of the *MYT1L* gene identified by exome sequencing in a patient with severe ID, autistic features, neonatal hypotonia and feeding difficulties, as well as mild facial features.
This report and previous publications strongly suggest that alterations of the *MYT1L* gene are responsible for diverse neuropsychiatric disorders with intellectual disability and a tendency to overweight.

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P05.111

Zooming into the chromosomal breakpoints by genome paired-end tag sequencing: application in patients with intellectual disabilities

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Structural variations are common genetic hallmarks in patients with developmental delay or congenital malformations. Although SVs are present in relatively healthy individuals (10^{-4} mutation rate) and have been annotated comprehensively in 1000 Genome Project, however, when arise *de novo* in the germline, it has been shown to be an informative path towards identifying causative mutations in human diseases. We apply genome-wide paired end tag sequencing in 8 patients carrying *de novo* or familial segregating chromosomal aberrations with intellectual disability (ID). For one *de novo* patient, we included the parents for genome sequencing investigation, and applied variants filtering to exclude parental SVs. The ratio of paternal and maternal inheritance rate is relatively balanced (52% : 46 %) and after applying variants filtering, we obtained four *de novo* SVs, in which 2 SVs are balanced translocation disrupting *GTDC1* gene at chromosome 2 and two other SVs did not coincide within a coding region/TF-binding sites from recent ENCODE release dataset. The transcript level of all *GTDC1* isoforms is reduced in the patient's lymphoblasts compared to the control. The expression profile of *GTDC1* is ubiquitous across different tissues, including the brain. We further investigated the loss of function effect of *GTDC1* in neural precursor cells and neurons derived from mouse embryonic stem cells, and found reduced number of neurons in the *GTDC1* depleted cells. This implicates *GTDC1* as an eligible candidate for ID and screening validation in larger cohort of patients is required to provide a stronger evidence for its involvement in ID.

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Two brothers with mental retardation and microduplication Xp22.32-p22.31

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Neuroligins (NL) are synaptic cell adhesion molecules that bind specifically

to a set of synaptic membrane proteins called neurexins. In humans, this protein family is encoded by five genes, NLGN1, -2, -3, -4 and -4Y. Several recent studies have implicated NL in autistic disorders and mental retardation. The functional role predicts that mutations and/or polymorphisms in NLGN genes may be implicated in the development of neuropsychiatric disorders.
We describe two brothers born to non-consanguineous parents, with a family history of mental retardation (elder brother 16 years-old). Both had showed delayed psychomotor development and language disorders; phenotypic examination were negative except for long and asymmetric face; clinodactyly of the 5th finger of hands were present only in the second brother. Brain MRI were normal. The karyotype analysis and genetic analysis for X-fragile syndrome were negative. Both have performed array-CGH which showed a microduplication Xp22.32-p22.31 (5,842,988-6,106,308)x2 mat. The mother was heterozygous unaffected. Our observation helps to confirm the pathogenic role of this microriarrangements. The microduplication highlighted our patients is in the short arm of the X chromosome that includes NLGN4 gene. Our cases and the data in literature suggest that neurodevelopmental defects in autism spectrum disorders and mental retardation (MR) could be related to a reduced function of this protein.

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Alteration in synaptic genes *NRXN1* and *NLGN4* may predispose to neurological syndromes

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Neurexins and neuroligins are proteins functionally connected by interaction in synaptic network formation and maitenance. According to recent studies alteration in *NRXN1* (2p16.3) and *NLGN4* (Xp22.3) genes can predispose to the wide spectrum of neurodevelopmental disorders including autistic spectrum disorders, intellectual disability, speech and language delay, hypotonia and schizophrenia. *NRXN1* as one of the largest known human genes (1,1 Mb) tends to frequent intragenic alterations. Whole genome genotyping was performed using the Illumina HumanCytoSNP 12v2.1.
We present 3 children of comparable age (5-6 year old), having different size of microdeletion in isoform alpha1 *NRXN1* gene (40 - 360 kb) and slightly different phenotype. All children had mild to moderate mental retardation, two of them had speech delay and anxiosity, hypotonia and facial dysmorphism. Two of detected deletions were intronic, the third deletion covered exones 1 to 11 of *NRXN1*. As the deletion in the *NRXN1* may not be fully penetrant, we examine also parental DNA.
Microduplication (300 kb) spanning the exones 1,2 and 3 of *NLGN4* gene was found in 22 year old patient with hyperkinetic syndrome, attention failure, but without mental retardation. The same duplication was detected in clinically normal mother and maternal cousin with Asperger syndrome. Thanks to this family examination prenatal array diagnosis in a new pregnancy could be provided. Consequently proband´s brother without duplication in *NLGN4* was born.

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P05.114
Genotype-phenotype correlations of *NRXN1* deletions

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Chromosomal microarray is now a key diagnostic test for patients with developmental disabilities. This analysis led us to the identification of intragenic deletions of the *NRXN1* (neurexin 1) gene, in eight unrelated patients with intellectual disability (8/8), speech delay (8/8), autism spectrum disorders (5/8) and epilepsy (2/8). The size of these deletions varies from 50 to 600 kb.
The *NRXN1* gene encodes a protein that belongs to a family of cell adhesion molecules and plays a fundamental role in synaptic function. *NRXN1* transcription results in two isoforms α and β. The α neurexins are transcribed from a promoter located upstream of exon 1, while the β neurexins are transcribed from an intragenic downstream promoter.
The main majority of our deletions (7/8) alter the α isoform only, and five of them involve exonic sequences whereas three deleted intronic regions only. One case occurred *de novo*. Two cases are familial ones and the exonic deletion is inherited from one parent who presented learning difficulties. The five other cases are inherited from an asymptomatic parent or do not segregate with the phenotype in the siblings, even if the deletion involves exonic regions.
This heterogeneity of genotype-phenotype correlations of intragenic *NRXN1* deletions implies that the greatest care must be taken in the cytogenetic report, and that deletions of this gene may participate in the phenotype but are probably not sufficient to give rise to the neurodevelopmental disorder.

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P05.115
More or Less: reverse phenotypes as a consequence of reciprocal 5q35 deletions and duplications

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The recent implementation of array techniques in research and clinical practice has revealed the existence of recurrent reciprocal deletions and duplications in several genome loci. The most intriguing feature is that some reciprocal genomic events can result in opposite phenotypic outcome. One of such examples is 5q35.2-q35.3. Deletions in this locus, encompassing NSD1 gene, lead to Sotos syndrome (Sos) characterized by childhood overgrowth with advanced bone age, craniofacial dysmorphic features including macrocephaly, and learning difficulties; while duplications have been proposed to manifest in opposite phenotype related to growth. Short stature since the birth, microcephaly, brachydactyly, delayed bone age, mild to moderate intellectual disability and mild facial dysmorphism seem to be characteristic features of 5q35.2-q35.3 duplication. It has been proposed that NSD1 plays a role in regulation of somatic growth in humans. Obviously, gene expression studies might be the first step towards the understanding the exact mechanisms of the influence of 5q35.2-q35.3 structural changes in general and NSD1 particularly on human growth.
We aimed to figure out the gene expression pattern differences in patients with 5q35.2-q35.3 deletion and duplication. The expression of 16 genes from Sos critical region and flanking regions was studied by RT-qPCR. Most of the genes showed correlation between their copy number and expression level. In addition, whole-genome expression was analyzed using HumanHT-12 v4 Expression BeadChip (Illumina Inc.) to estimate the global influence of these chromosomal aberrations. Although, some expected changes were observed, more patients are needed to make any statistically significant conclusions.

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Molecular diagnosis of deletions and duplications associated with syndromic obesity

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Some rare genetic syndromes lead to obesity, often associated with intellectual disability, abnormal development, dysmorphic features and congenital malformations. Prader-Willi syndrome (PWS) is the most common syndromic form of obesity, caused more specially by deletions in the paternally derived 15q11q13 region. Other genomic disorders associated with copy number variants (CNVs), such as chromosomal deletions of 1p36, 2q37, 6q16, 9q34, 11p13 and 17p11.2, have an increased prevalence of obesity. Moreover, an increasing number of CNVs linked to obesity are being identified in recent years. Nearly 300 patients with syndromic obesity were evaluated for chromosomal imbalances by MLPA after DNA methylation analysis ruled out PWS. Half of these patients were further investigated with different array platforms. Among the pathogenic CNVs, we identified syndromic genomic disorders, such as deletions of 1p36 (7 cases), 2q37 (HDAC4; 5 cases), 6q16 (SIM1; 2 cases), 9p24 (3 cases), 9q34 (EHMT1; 1 case), and 17p11.2 (RAI1; 5 cases), as well as specific CNVs already linked to obesity, such as 1p21.3

and 2p25.3 deletions. Additionally, we identified 22q11.2 deletion causing DiGeorge syndrome (6 cases), 22q11.2 duplication syndrome (1 case), and 22q11.2 distal deletion syndrome (2 cases). Known genomic disorders with incomplete penetrance and variable expressivity included 15q11.2 deletion of NIPA1 (2 cases), 16p11.2 deletion of SH2B1 (1 case), 16p11.2 duplication of TBX6 (1 case), 16p13 deletion of MYH11 (1 case), 17q11.2 duplication of NF1 (1 case), and 17q21.31 duplication of MAPT (1 case). Clinical variability in well-known syndromes may facilitate the identification of disease genes.
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P05.117
Pallister-Killian syndrome: an under-diagnosed syndrome?

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Pallister-Killian syndrome (PKS) is usually described as a rare syndrome caused by the presence of a supernumerary mosaic isochromosome 12p (i(12p)) in patients presented with multiple congenital anomalies, characteristic dysmorphic features, pigmentary skin anomalies, seizures and severe developmental delay. The gold standard diagnostic requires skin biopsy establishing the karyotype in normal and abnormal pigmented skin. Nevertheless, PKS can be associated with mild or unusual phenotype making the diagnosis and the genetic counselling difficult.
Here we report two cases; the first one is a 1-year-old patient with severe developmental delay, atypical facial features, cardiac malformation, hirustism and ulnar drift. Chromosome analysis on peripheral lymphocytes showed a normal karyotype. The PKS diagnosis was suspected because the assessment of subtelomeric rearrangements by MLPA technique showed a trisomy 12pter. The PKS was confirmed by FISH using D12Z1 probe on jugal and peripheral blood smears. The second case is a patient with prenatal diagnosis of tetrasomy 12p. Despite a reserved counselling, a boy was born at term. At 5 years old, he presented with minor pigmentary skin anomalies, no dysmorphic features and very mild developmental delay.
The first case highlights the difficulty of the diagnosis in absence of typical phenotype. It also confirms that the diagnosis can be performed by buccal and/or peripherical blood smears using FISH techniques without skin biopsy. The second observation highlights the variability of the phenotype leading to a difficult prenatal genetic counselling. Together, these observations suggest that PKS is a probably under-diagnosed syndrome.

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P05.118
Partial deletion of TCF4 in two sisters of whom only one had typical Pitt-Hopkins syndrome

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Haploinsufficiency of the TCF4 gene is known to cause Pitt-Hopkins syndrome, characterized by severe intellectual disability, epilepsy, hyperventilation, microcephaly and facial dysmorphism.
The proband is a 23-year-old female with psychomotor retardation since birth. She walked without support at the age of three years and has never had any meaningful speech. She walked somewhat ataxic with flexed elbows. She has brachycephaly, a broad nose, a large mouth with full, everted lips, laxity of finger joints and flat feet. Her nine years younger sister also has intellectual disability. She walked independently at the age of two years. Speech development was retarded, but eventually she learned to speak well. She visited a normal primary school for two years followed by special education. She can read and write a little and plays computer games. She has obesity (BMI=32), mildly upslanting palpebral fissures, otherwise no dysmorphism.
Array CGH analysis (Agilent 180K oligo-array; AMADID 023363) revealed an interstitial deletion of 67 kb at chromosome band 18q21.2 comprising part of the TCF4 gene in both sisters. Array CGH analysis and FISH examination in the phenotypically normal father showed the same deletion in about one third of his lymphocytes.
In conclusion, the same partial deletion of TCF4 can cause typical Pitt-Hopkins syndrome and aspecific intellectual disability, respectively, in two sisters. Apparently, the phenotype of TCF4 disruption is broader than previously thought. As far as we know, this is the second report of recurrence in sibs and somatic mosaicism in the parent, which is important for genetic counseling.

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P05.119
Three novel WDR62 mutations in a consanguineous Turkish family and in a non-cansanguineous European patient: further molecular investigations and clinical manifestations

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Autosomal recessive primary microcephaly (MCPH) is a genetic disorder of brain growth characterized by small brain, variable degree of mental retardation and occasionally mild seizures. It is genetically heterogeneous with nine genes involved so far.
WDR62, coding for a centrosomal protein, is the second most commonly implicated gene, preceded by *ASPM.* Cortical malformations, not described in classical MCPH phenotype, are often present in *WDR62* mutated patients. We report on 2 families with three novel mutations in *WDR62*. The first proband is the only child of a non-consanguineous European couple. She presents with mild microcephaly (-2.5SD), developmental delay, moderate mental retardation and epilepsy. Cerebral MRI shows mild pachygyria and simplified cerebral cortex. Sanger sequencing of *WDR62* identified compound heterozygous mutations: a nonsense mutation c.2788C>T resulting in a premature stop codon (p.Gln930X) and a synonymous variant c.1521G>A (p.Leu507Leu). Functional studies were performed on cDNA and an aberrant splicing of exon 11 confirms the pathogenicity of this unknown variant. Both parents are healthy heterozygous carriers of one of the mutations.
The second proband, third child of consanguineous Turkish parents, presents with moderate microcephaly (-4SD) and mental retardation. Cerebral MRI does not reveal any brain malformation, except microcephaly. Sequencing identified a homozygous missense mutation c.1526C>T (p.Ser509Leu) in *WDR62.*
These three mutations have not been reported before. We discuss the molecular results, the phenotypic heterogeneity and the prevalence of this gene in MCPH patients from variable ethnicity, even in non-consanguineous populations, and in patients with mild phenotype and no obvious cortical malformations.

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Revealing the complexity of a monogenic disease: Rett Syndrome exome sequencing

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Rett syndrome (RTT) is a monogenic disorder manifesting as a large variety of phenotypes ranging from very severe to mild disease. Since there is a weak correlation between the mutation type in the Xq28 disease gene MECP2/X-inactivation status and phenotypic variability, we used this disease as a model to unveil the complex nature of a monogenic disorder. Exome sequencing was used to analyze the functional portion of the genome of two pairs of sisters with RTT. Although each pair had the same MECP2 mutation and balanced X-inactivation, one individual from each pair could not speak or walk, and had a profound ID (classical RTT), while the other could speak and walk, and had a moderate ID (Zappella variant). In addition to the MECP2 mutation, each patient has a group of variants predicted to impair protein function. The classical RTT girls, but not their milder affected sisters, have an enrichment of variants in genes related to oxidative stress, muscle impairment and ID and/or autism. On the other hand, a subgroup

of variants related to immune system modulation, exclusive to the Zappella variant are driving toward a milder phenotype. Togheter our results indicate that the final phenotype is likely the result of a combination of MECP2 mutation, X-inactivation and around 40 disrupting variants in other genes. We demonstrate that exome analysis has the potential to provide insights into disease pathophysiology. Most importantly, hereby identified genetic modifiers of speaking, walking and intellectual capabilities may represent targets for new therapies (accepted by PLOS ONE).

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P05.121

Bio-distribution and efficacy of a self-complementary AAV9 construct expressing a codon-optimized Mecp2 transgene for further use in a preclinical model of Rett Syndrome

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Rett syndrome (RTT) is an X-linked neurodevelopmental disorder primarily affecting CNS functions but also peripheral functions. There is currently no cure for the disease and treatments available are aimed at improving RTT symptoms. Most RTT cases are due to mutation in methyl CpG binding protein 2 (MECP2), whose main function is that of a global transcriptional repressor.
The recent findings that reactivation of Mecp2 rescued adult diseased RTT mice not only indicates that MECP2 is needed for normal adult function (Robinson et al, Brain 2012) but also that gene therapy might be beneficial for RTT patients, even after the disease has started.
AAV9 has been shown to cross the blood brain barrier (BBB) and infect brain cells after intravenous injection in both rodent and primates (Foust et al, Nat Biotech 2009; Duque et al, Mol Ther 2009; Gray et al, Mol Ther 2011).
We generated an AAV9 expressing GFP (control virus) or a codon-optimized version of Mecp2 under the regulation of the mouse Mecp2 promoter (named scAAV9-MCO) and confirmed their efficacy in vitro by infecting NIH/3T3 mouse fibroblasts and primary mixed neurons/glia cultures. Preliminary experiments investigating the efficacy and bio-distribution of these viruses in vivo after intravenous injection show that scAAV9 crosses the BBB, which is in agreement with results from other groups. Our next experiments will be aimed at the in vivo testing of both control and scAAV9-MCO virus in the RTT mouse model and the determination of a potential rescuing effect in these mice.
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MECP2 mutations in girls with intellectual disabilities.

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Rett syndrome (RTT,OMIM 312750) is a progressive neurologic developmental disorder mainly affecting females with an incidence of 1:10000. Clinical presentation appears very heterogeneous. "Classical" phenotype includes impaired communication and hand skills, stereotypic hand movements, and a gradual decrease in acquired gross motor abilities over time; among atypical variants the "Zappella variant shows more moderate traits, in presence of crucial diagnostic criteria. Mutations in the MECP2 gene is the most common cause of RTT. Out of a cohort of 138 girls with MECP2 mutation, we distinguished three mentally impaired patients with "high-functioning" phenotype, preserved hand skills and able to articulate even complex sentences; no microcephaly and clear regression were observed but occurrence of hand stereotypies or dyspraxic gait were suggestive of RTT. Molecular testing revealed two MECP2 mutations already described in Zappella-variant or mild phenotypes, while the third is a mutation reported in carriers males showing learning impairment and spasticity, who inherited the mutation from their unaffected XCI balanced mothers and in a mildly affected sporadic mental retardation- Non Rett syndrome female with completely skewed XCI. For two out of three patient we could perform mRNA investigation to confirm preferential expression of a single MECP2 allele. Our data underline the broad phenotypic spectrum of the MECP2 mutations and suggest to screen it in female patients with mental impairment and some features reminding of Rett syndrome

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P05.123

GluD1 is a Common altered player in Rett syndrome due to *MECP2* or *CDKL5* mutations

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Rett syndrome is a monogenic disease due to de novo mutations in either *MECP2* or *CDKL5* genes on the X chromosome. In spite of their involvement in the same disease, a functional interaction between the two genes has not been proven. MeCP2 is a transcriptional regulator; *CDKL5* encodes for a kinase protein that might be involved in the regulation of gene expression. Therefore, we hypothesized that the two genes may lead to similar phenotypes by dys-regulating the expression of common genes. To test this hypothesis we used induced pluripotent stem (iPS) cells derived from fibroblasts of one Rett patient mutated in *MECP2* and 2 patients with mutations in *CDKL5*. Expression profiling was performed in *CDKL5*-mutated cells and genes of interest were confirmed by real-time RT-PCR in both *CDKL5* and *MECP2* mutated cells. The only major change in gene expression common to *MECP2*-mutated and *CDKL5*-mutated cells was for *GRID1*, encoding for glutamate d1 receptor (GluD1), a member of the delta family of ionotropic glutamate receptors. GluD1 ability to form functional AMPA or NMDA glutamate receptors has not been demonstrated. It acts like an adhesion molecule by linking the postsynaptic and presynaptic compartments and inducing excitatory and inhibitory presynaptic differentiation. Our results demonstrate that *GRID1* gene is down-regulated in both *MECP2*-mutated and *CDKL5*-mutated iPS cells and up-regulated in neuronal precursors, providing the first functional link between the two genes. These data provide novel insights into disease pathophysiology and identify possible new targets for therapeutic treatment of Rett syndrome.

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Mutation analysis of MECP2, CDKL5, and FOXG1 genes in Czech patients with Rett syndrome and Rett-like phenotype

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Rett syndrome is a severe X-linked dominant neurodevelopmental disorder affecting mostly females and primarily caused by de novo mutations in the MECP2 gene. It is characterized by developmental regression, acquired microcephaly, loss of purposeful hand use and speech, stereotypic hand movements, autistic features, gait apraxia, breathing anomalies, and seizures. Mutations in other genes have been identified in early-onset seizure variant (CDKL5) and congenital variant (FOXG1) of Rett syndrome. We report the results of molecular analysis of MECP2, CDKL5, and FOXG1 genes in patients with Rett syndrome and mental retardation with Rett-like features from Czech Republic.
MECP2 and CDKL5 were analyzed by high-resolution melting analysis and DNA sequencing of coding regions and exon/intron boundaries. FOXG1 was analyzed by DNA sequencing. Large deletions in all three genes were analyzed by multiplex ligation-dependent probe amplification.
We identified MECP2 mutations in 65 out of 86 patients with classic Rett syndrome (76%). 20 patients with Rett syndrome variants were initially screened for MECP2 mutations and 6 MECP2 mutations were identified (30%). The subsequent analysis of CDKL5 gene in patients with early-onset seizure variant revealed mutations in 2 cases (6,7%). No mutation has been identified in FOXG1 gene in the patients with congenital variant of Rett syndrome.
We confirmed the high frequency of MECP2 mutations in classic Rett syn-

drome. On the other hand, MECP2 mutations are not a frequent cause of early-onset seizure and congenital variant of Rett syndrome or mental retardation with Rett-like phenotype.
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Astrocyte transcriptome from Mecp2-deficient mice: relevance to the pathogenesis of Rett syndrome

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Mutations in the *MECP2* gene cause the neurodevelopmental disorder Rett syndrome which is one of the most frequent causes of intellectual disability with genetic origin in women. Although MeCP2 levels are roughly five-fold lower in astrocytes than in neurons, recent studies showed that Mecp2 loss in astrocytes contributes to Rett-like symptoms and restoration of Mecp2 can rescue some of these defects. Moreover, mutant astrocytes have a non-cell autonomous effect on neuronal properties, probably resulting from aberrant secretion of soluble factor(s). To identify these soluble factors, we have compared the gene expression profiles of wild type and mutant astrocytes from Mecp2 $^{308/y}$ mice using Affymetrix mouse 2.0 microarrays. The results obtained were confirmed by quantitative real-time RT-PCR. A false discovery rate (FDR) of 0.05 was applied to the lists of differentially expressed genes between wild-type and mutated samples. 2152 genes passed the stringent FDR filter of 0.05 including 1784 coding transcripts. However, only 80 also had an expression fold change >1.25 in Mecp2 $^{308/y}$ cells versus Mecp2 $^{+/y}$ cells. Interestingly, among these genes, several genes encode secreted proteins such as myocilin, lipocalin 2, SHH or Wnt7b, implicated in neuronal maturation. Others contribute to major astrocytic functions such as the exocytotic release of glutamate (Slc17a8), glutamate metabolism(GAD1) and glucose metabolism (Adcy8, Acot4). We need to confirm these data at the proteomic level in astrocyte and conditional medium from symptomatic Mecp2-deficient mice. Therefore, insights into astrocyte secretion are critically important for understanding physiological responses and pathological mechanisms in Rett syndrome.

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Phenotypic spectrum and GPC3 mutations in a series of 42 patients with Simpson-Golabi-Behmel syndrome.

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Simpson-Golabi-Behmel syndrome (SGBS), a rare X-linked MCA/ID syndrome, is characterized by pre- and postnatal overgrowth, distinctive craniofacial features, congenital malformations, visceromegaly, an increased risk of tumour and mild/moderate intellectual deficiency in some cases. In 1996, causative mutations were identified in the GPC3 gene mapping at Xq26 (major gene of SGBS). Since the first description, many other cases have been reported with a wide clinical range of symptoms and severity. Among them, a few cases were significantly different as they had atypical clinical presentation with more severe prognosis and/or numerous congenital malformations. However, these descriptions occurred before the availability of molecular analysis. Moreover the mutation detection rate in SGBS is only 37% to 70%, suggesting that some patients could have different disorders. Finally, in the family reported by Golabi and Rosen, a duplication of GPC4, mapping close to GPC3, was recently identified, suggesting that GPC4 could be the second gene for SGBS.
In order to better delineate the phenotypic spectrum of SGBS caused by GPC3 mutations, and to try to define specific clinical criteria for GPC3 molecular testing, we reviewed the clinical features of all male cases with a GPC3 mutation identified in the two molecular laboratories providing this test in France. A clinical questionnaire based on literature data was send to the referring physicians in order to collect relevant information i.e. family history, prenatal and postnatal data.
We present here the results of the clinical and molecular analysis of 42 patients including 8 foetuses or stillborn babies.

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P05.127

Characterization of novel "de novo" mutation in the *SLC6A8* gene

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Introduction: Cerebral creatine deficiency is an X-linked disorder caused by mutations in the *SLC6A8* gene (Xq28). It consists of 13 exons and encodes a protein of 635 amino acids. This disorder causes an increase of creatine levels in urine and plasma, and urinary creatine/creatinine ratio. The clinical presentation of males affected is mental retardation, expressive speech and language delay, epilepsy, developmental delay and autistic behaviour.
Subjects and Methods: An 8 year-old male patient presents learning difficulties, behaviour disorder and epilepsy. An increase of creatine/creatinine ratio, guanidineacetate and glycine levels (plasma and urine) was detected. The 13 exons of the *SLC6A8* gene, including the intron-exon junctions, were analyzed by PCR and direct sequencing reaction.
Results: We detected a 34 pb deletion (c.1016+11_1017-52del34) in intron 6, that was predicted to cause an aberrant splicing into acceptor splice-site. At the cDNA level, this mutation produces a 30 pb deletion in exon 7 and a single amino acid insertion (p. K339_S349delinsN). This variant was not detected in the mother and is therefore considered *de novo.*
Discussion: DNA and RNA analysis were necessary to characterize this novel pathogenic mutation. The detected change in the creatine transporter could be responsible for a lower and/or no activity of the protein. Functional studies in cultured skin fibroblasts will be necessary to show a deficiency of the creatine transporter function.

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P05.128

Characterisation of de novo dicentric supernumerary marker chromosome 15 (Prader-Willi / Angelman critical region included) in a boy with subtle phenotype

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Although it is estimated almost 3 million of people of the world population are carriers of supernumerary marker chromosomes (SMCs), only less than 5000 SMCs are reported in their largest database, Liehr´s SMC-homepage assembled in Universitätsklinikum Jena. More than every fifth gathered SMC is derived from chromosome 15, most of them in isochromosome 15 syn-

drome patients.
SMC(15)s containing Prader-Willi/Angelman critical region (PWACR, 15q11-q13) are usually associated with severe phenotype, according to N.R.Dennis et al. (2006): hypotonia, motor and speech delay, seizures, moderate to severe learning disability, and autism while dysmorphic features are absent or subtle, growth is usually normal.
We report a case of a 4-year-old boy with de novo dicentric SMC(15). The FISH analysis of this abnormal chromosome revealed two copies of PWACR region and only one signal of the acro-p-arm probe. The patient has short stature and suffers from moderate intellectual disability. He has no history of seizures and his muscle tone is evaluated as slightly decreased. J.A.Crolla et al. (2005) pointed out that SMC(15)s are the only SMCs, which incidence could be related to increased maternal age - our proband was born to 36-years-old mother.
Other analyses are to be done to define the exact range of this abnormal chromosome and to explain parental origin and DNA-methylation pattern of every individual copy of the critical region. Their results will be presented and discussed on the poster.
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P05.129

SRS-like phenotype associated with mono-allelic expression of the IGF-I receptor gene and NF1 gene microduplication

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Silver-Russell syndrome (SRS) is a clinically and genetically heterogeneous syndrome characterized by intrauterine growth restriction, body asymmetry, relative macrocephaly and a triangular face. SRS is mostly caused by hypomethylation of the imprinting control region 1 (ICR1) on chromosome 11p15.5, but cytogenetic aberrations have also been described in sporadic cases. Microdeletions 15q26 including the *IGF1R* gene with clinical features overlapping with SRS have been reported few times in the literature.
Here, we report clinical and molecular findings in a 12-year-old boy with features suggestive of SRS. The MS-MLPA method did not reveal ICR1 hypomethylation. On the other hand array-CGH identified 3.37 Mb deletion in 15q26.3 causing *IGF1R* gene haploinsufficiency and 1.21 Mb duplication in 17q11.2 including region of the Neurofibromatosis 1 with intellectual disabilities with *NF1* gene.
Clinical features suggestive of SRS included small anthropometric parameters at birth (SGA) and lack of postnatal catch-up growth, feeding problems, gastro-esophageal reflux, dysmorphy including triangular and asymmetric face and micrognathia, as well as minor skeletal abnormalities. The discriminating feature was microcephaly, which has been described in cases with del15q26.3, carrying also characteristics overlapping with SRS, such as SGA, postnatal growth retardation and the triangular face. The del15q26.3, along with 17q11.2 duplication, could also contribute to motor and speech delay, as well as intellectual disability.
The presented case proves the array-CGH method is a powerful diagnostic tool confirming genetic etiology in patients with ambiguous phenotypes.
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P05.130

Early epileptic encephalopathy associated with STXBP1 mutations: could we better define the phenotype?

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STXBP1 (Munc18.1), encoding syntaxin binding protein1, is a new gene causing epileptic encephalopathy. Mutations in STXBP1 have been first reported in early onset epileptic encephalopathy with suppression-bursts, then in infantile spasms and, more recently, in patients with non syndromic mental retardation without epilepsy.
To better define the clinical phenotype and to determine the occurrence of specific neuroradiological abnormalities in patients with STXBP1 mutations, we analyzed clinical and neuroradiological findings in a series of patients having STXBP1 mutations.
We included patients with STXBP1 abnormalities followed at our institution and who underwent a high resolution brain MRI.
We identified 7 unrelated children (6 females, 1 male) with STXBP1 mutations. Age at seizure onset ranged from 1 day to 3 months. All children presented with infantile spasms and 5 had a suppression-burst pattern on EEG. Although all patients were seizure free before the age of 2 years, they showed global and severe developmental delay. Neuroimaging analysis showed a peculiar aspect of thin and dysmorphic corpus callosum associated with cortical frontal atrophy.
STXBP1 mutations are detected only in a minority of patients with early epileptic encephalopathy. We suggest that patients with thin corpus callosum and frontal cortical atrophy on brain MRI, especially if seizures are controlled in the first years, could represent the best candidate to genetic analysis of STXBP1.

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P05.131

Expanding the phenotype of *IQSEC2* mutations: truncating mutations in severe intellectual disability

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Intellectual disability (ID) is frequent in the general population, with 1 in 50 individuals directly affected worldwide. The multiple etiologies include X-linked ID (XLID). Among syndromic XLID, few syndromes present severe ID associated with postnatal microcephaly and midline stereotypic hand movements. We report on two male patients with ID, non inherited and postnatal onset microcephaly, midline stereotypic hand movements, hypotonia, hyperkinesia, strabismus, as well as seizures for one of the patients. Using array-CGH and exome sequencing we characterized two truncating mutations in IQSEC2, namely a de novo intragenic duplication mapped to the Xp11.22 region and a nonsense mutation in exon 7. We propose that truncating mutations in IQSEC2 are responsible for syndromic severe ID in male patients and should be screened in patients without mutations in MECP2, FOXG1, CDKL5 and MEF2C.

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P05.132

A new disorder of neurotransmission associated with SYT1 mutation

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SYT1 is an essential regulator of fast, synchronous vesicle exocytosis. We report the first case of a human disorder associated with SYT1 mutation. The clinical features are severe dyskinetic movement disorder and profound cognitive impairment. Epileptic seizures have not been observed and clinical MRI is normal. EEG demonstrates extensive low-frequency oscillations, abnormal paired-pulse depression of visual evoked potentials, and immatu-

re auditory evoked potentials. Whole exome sequence analysis revealed a de novo missense mutation (I368T) at a functionally-critical residue in SYT1. This case suggests that activity-dependent regulation of vesicle release is necessary for motor control and for emergence of higher cognition.

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P05.133

Differential expression of the Ubiquitin-conjugating genes (E2) during the differentiation of hippocampal neurons and after stimulation by NMDA

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The Ubiquitin pathway controls protein stability and signalling during neuronal development, maturation and plasticity. Mutations in genes encoding Ubiquitin-conjugating enzymes (E2) and Ubiquitin-ligases (E3) have been described in neurodevelopmental diseases (i.e. UBE2A in XLMR, UBE3A in Angelman syndrome for example).
We identified 38 E2 genes in the Human genome, 39 in the Mouse genome (Michelle et al., 2009). To better understand their role during brain development and function we analyzed their expression during the differentiation of hippocampal neurons in vitro and after stimulation. RT-QPCR analyses were performed on RNA extracted from primary cultures of hippocampal neurons from mouse embryos (E17). A large majority of the 39 E2 genes were expressed during the differentiation of hippocampal neurons, and interestingly their expression varied with the stage of differentiation and maturation. Moreover, we observed important variations of expression for several E2 genes after N-methyl-D-aspartate (NMDA) stimulation. Using in silico analysis, we identified several potential binding sites for neural transcription factors, such as Creb1, Pdx1, Runx1, in the promoters of many E2 genes.
The variations of expression coupled with the presence of potential binding sites for transcription factors important during brain development gave insight into the regulation and the roles of E2 genes in neurons. These results support further studies on E2 genes in neurodevelopmental diseases such as intellectual disability.

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P05.134

West syndrome : molecular screening of 73 patients with infantile spasms

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West syndrome is characterized by the association of infantile spasms with an age onset between 3 and 7 months, characteristic EEG pattern (hypsarrythmia) and developmental delay. Incidence is 1/6000 and unfavourable outcome is observed in 80% of patients. Etiologies are numerous, acquired or congenital, including genetic anomalies. Genetic causes include Down syndrome, 1p36 deletion, tuberous sclerosis and mutations in ARX or CDKL5 genes. However, 50% of the patients remain without etiological diagnosis. Knowledge of etiological diagnosis is critical because it represents a major prognostic factor and underlies the possibility for genetic counselling.
We present the molecular analysis of 73 patients with West syndrome or infantile spasms without etiological diagnosis. aCGH was performed and 5 genes were screened : CDKL5, STXBP1 and KCNQ2 for their implication in early epileptic encephalopathy, GRIN2A and MAGI2, candidate genes from the literature.
We found 2 mutations in CDKL5 including an atypical case of 2 mosaic populations and 3 mutations in STXBP1. All of these were de novo. Among other anomalies, aCGH found 3 deletions (one of 10Mb in 2q24.3, one of 3,24Mb in 5q14.3 including region up to MEF2C, one of 256 kb in 9q34 corresponding to the Kleefstra syndrome), and 2 duplications (one of 671 kb including SCN2A gene and one of 11.93 Mb in Xq28).
So, 13.6% of our cohort presented pathogenic mutations. These concerned the most severe patients, most of which presented a history of neonatal seizures. These results stress the importance of genetic screening for patients with West syndrome.

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P05.135

William's neural stem cells: microRNA deregulation

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Williams Syndrome (WS) is a developmental neurogenetic disorder caused by hemizygous deletion at 7q11.23, distinguised by a characteristic neurocognitive profile wich include intellectual disability (ID). Haploinsufficiency of some genes in the region has been associated with the cognitive phenotype. ID is difficult to address because of multiples etiologies, intrinsic complexity in neural tissue and high degree of regulation in neural development and function. The aim of this work is to determine if there are differences in the expression of microRNAs in neural cells derived from olfactory neuroepithelium of patients with WS in regards to controls, and to assess its possible involvement in neurodevelopment or neural function. Relative expression was assessed by Real-time PCR, using the TLDA Human MicroRNA Panel v2.0 (Applied) and prediction for potential neural targets and pathways involved, by in silico analysis (software TargetScan, miRecords and PITA Top Targets). Results show 15 deregulated microRNAs (3 over-expressed and 12 under-expressed), wich themselves include brain rich miRNAs as miR-32, miR-125b, let-7c and miR200, and others previously reported with specific neural functions or deregulated in brain diseases. For the majority potential targets were found associated with neural developmental pathways and function or other entities that present with intellectual disabilities. These data suggest miRNAs contribute greatly in regulating intrinsic neurodevelopmental processes and that specific miRNAs could be involved in various molecular pathways in the neurobiology of WS.

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P05.136

Exploration of Williams-Beuren syndrome-like cases

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Background: Williams-Beuren syndrome (WBS; OMIM#194050) is a rare genetic disorder characterized by an abnormal development expressed by congenital heart defects, suggestive facial dysmorphy, as well as cognitive and behavioural impairments. About 95% of WBS cases are due to a 1.5 to 1.8 microdeletion of chromosomal region 7q11.23, containing 26 to 28 genes. The remaining 5% of cases, which cannot be explained by a contiguous-genedeletion syndrome, may be classified as WBS-like cases.
Purpose: We focused on patients with a WBS-like disorder to determine the molecular cause of these atypical forms.
Method: Seven individuals of three different WBS-like families were tested by whole-exome sequencing: two trios composed by a male patient and his asymptomatic parents, and a third female patient. *De novo*, X-linked and recessive mutations were searched in patients of trios, and their variants were compared to the ones observed in the third patient. A fourth WBS-patient was subsequently tested by Sanger sequencing for mutations in candidate genes.
Results: Predicted deleterious mutations very likely related to their disorder were found in the two patients from trios, yet none in a common gene. No

potentially pathogenic variant were found in the third and fourth patients in inferred candidate genes.
Conclusions: Our study points to the genetic heterogeneity of Willams-Beuren-like syndrome. It highlights however strong candidate genes, which might contribute to a better understanding of WBS pathogenesis. Additional cases would need to be tested to confirm our present observations.

S. Bezieau: None. **S. Küry:** None. **B. Leheup:** None. **B. Isidor:** None.

P05.137

Partial monosomy 1q23 associated with severe seizures and profound mental retardation due to a mosaic translocation (1q,16q) (q23; qter)

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Reciprocal translocations are common cytogenetic anomalies occurring with frequency of about 1 in 500 births. Mosaic state of the translocation is a rare event which indicates that the breakage occurred in the first mitotic divisions of the zygote. Sometimes a certain amount of DNA within the breakage region could be lost and determines clinical presentation depending on the lost genes. A newborn girl have been evaluated because of poor sucking and dysmorphism. It was the first child in the family. Diminished fetal movements and intrauterine growth retardation were noticed. The baby was full-term neonate with birth weight and length under 3rd percentile. Dysmorphic features were present - narrow forehead, hypotelorism, ptosis, telecantus, micrognathia, low-set simple ears. Hemangioma of lumbosacral region was present. Hypotonia, brisk reflexes were present, primitive reflexes persist after 6 months. At the age of 3 months she developed refractory generalized seizures. MRI showed global cortical atrophy, especially in frontal and temporal areas. Anticonvulsive drugs had poor effect. At the age of 1 year the girl have profound mental retardation. Chromosomal finding showed translocation between chromosomes 1q and 16q in 50% of the analyzed mitoses; the rest of the cells showed normal karyogram. FISH for subtelomeric ragions on 1q and 16 q were present on marker chromosome 16. The breakpoint was on 1q23 with probable microdeletion in that region. There are several genes within the region that could be disrupted by the deletion, therefore further investigation is needed to acquire the exact cause of disorganized cortex.

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P05.138

CGH analysis in a cohort of 54 males with unexplained X-linked intellectual deficiency.

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In order to assess the presence of submicroscopic deletions/insertions in X-linked genes, we screened a cohort of 54 male individuals suffering from moderate to severe intellectual disability using custom-designed Roche Nimblegen 720K oligonuclotide array CGH. These individuals were selected in families were the phenotype is transmitted through unaffected (or mildly affected) carrier females. Our custom arrays contain 720,000 probes with average probe spacing across the X chromosome of 50bp in genic regions and 20kb and intergenic regions.
We found 70 copy number variations (CNVs) affecting the X chromosome in this cohort. The CNVs were all confirmed using Q-PCR. 42 CNVs are known in the control population, including 20 CNVs located in the pseudoautosomal region. 28 CNVs are unknown in variation databases (17 deletions, 8 duplications, 3 found both deleted and duplicated in our cohort). Among these later cases, 8 CNVs are located in the pseudoautosomal region. For the 20 remaining CNVs, 15 are located in introns and 5 affect exons of 17 different X-linked genes. Two CNVs affecting already known XLID genes (IL1RAPL1 and OPHN1) were considered to be pathogenic. We identified 4 « potentially pathogenic » aberrations, involving genes not yet implicated in XLID but considered as new candidate genes for intellectual disability.

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P05.139

Difficulties in screening small families with X linked Intellectual disability

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Intellectual disability (ID) is characterized by significant limitations in cognitive abilities and social/behavioural adaptive skills. It is estimated that 1-3% of the general population is affected with ID. Intellectual disability is one of the primary reasons for paediatric, neurologic, and genetic referrals. We investigated 3 families with mentally retarded males, clinical examination, cytogenetic and molecular studies and basic metabolic screening were performed.
We identified in each family a mutation in X-linked genes. The mutations were a splice mutation in PAK3 gene, a frame shift mutation in PQBP1 gene and a missense one in NHS gene. All the founded mutations that segregate with intellectual disability were described for the first time.
Despite the fact that our families were small, a mutation was identified each time. This is due to the fact that a good clinical examination was performed first followed by good literature search which was essential to compare the clinical features of our families to those reported in the literature. This highlights the importance of clinical data in genetic linkage studies. Our study shows that in a lack of large families, small families like ours can be interesting to study.

I. Rejeb: None. **L. Ben jemaa:** None. **L. Kraoua:** None. **M. Chograni:** None. **H. Jilani:** None. **F. Maazoul:** None. **J. Chelly:** None. **H. Chaabouni:** None.

P05.140

X-exome analysis detects novel mutations in XLID genes in a cohort of male patients with intellectual disability

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Intellectual disability (ID) has a prevalence of 1-3 % and is a major medical problem. Mutations in X-chromosomal genes are estimated to account for approximately 10 % of male ID patients. Apart from fragile X syndrome, which is at cause in about 25 % of X-linked ID (XLID) and which has been part of the routine diagnostic work-up for many years, more than 90 other XLID genes are known to date. The low prevalence of mutations in each individual gene has, however, rendered routine testing of these genes impractical in patients with unspecific clinical features.
The advent of new sequencing technologies has enabled us to establish a platform combining in-solution enrichment of the coding regions of all XLID genes and subsequent next-generation sequencing (NGS). We have employed this XLID panel for analyzing a cohort of more than 120 unselected male ID patients in whom chromosome aberrations and fragile X syndrome had already been excluded. As a result, we found unambiguously disease-causing mutations in genes such as ATRX, MED12, CUL4B, DLG3, SLC9A6, KDM5C and UBE2A in more than 5% of the patients, and variants of unclear pathogenicity were present in additional patients. Considering the high recurrence risks for X-linked disorders, XLID panel analysis has thus been shown to be a valuable diagnostic tool in male patients with non-syndromic or atypical syndromic ID.

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P05.141

The Kaiso gene region is implicated in X-linked mental retardation.

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X-linked mental retardation (XLMR) is the most common form of mental retardation in males. Syndromic MR encompasses intellectual disability deficits associated with other medical and behavioral symptoms. Recently, a large Sardinian family with a novel form of syndromic XLMR was characterized. Eight of 24 members of the family are male and have MR, short stature, aphasia, skeletal abnormalities and dysmorphic features. Previous linkage studies identified a region of 16 cM around DXS1001 in Xq24 with

a maximum Lod score of 3.61. Whole-exome sequencing was performed on the parents, three affected sons and one unaffected daughter. One SNP was found in the 3'-UTR of the ZBTB33 (Kaiso) gene which maps within the previously associated region of linkage and segregates with the disease phenotype in the family. This SNP was not found in 30 unrelated control samples. Kaiso binds to methylated DNA, represses target genes in the Wnt signaling pathway and is highly expressed in the brain. The SNP creates a binding site for miR-4999 and miR-4774; however, luciferase expression assays failed to validate increased targeting of these miRs to the variant 3'-UTR. This SNP may affect 3'-UTR structure leading to decreased mRNA stability, or the SNP may be closely linked to the disease-causing mutation, which is not located in an exon. While the functional consequences of this base change are not yet known, our results implicate this region as a new locus for XLMR.

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P05.142

Non-syndromic X-linked intellectual disability caused by a missense mutation in *RPS6KA3*, the gene responsible for Coffin-Lowry syndrome

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Coffin-Lowry syndrome (CLS) is a rare X-linked intellectual disability disorder caused by mutations in *RPS6KA3*, which encodes RSK2, a growth factor-regulated protein kinase acting in the MAPK signaling pathway. Affected males usually present with severe intellectual disability and characteristic facial and hand features allowing a gestalt diagnosis. The face coarsens over time and typically includes a prominent forehead, hypertelorism, narrow downward slanting palpebral fissures, anteverted nares, thickened alae nasi and nasal septum, and wide mouth with thick everted lips. The hands are broad and soft with stubby tapering fingers. Additional CLS features include short stature and progressive scoliosis.
We describe three boys with non-syndromic intellectual disability in a family with a pattern compatible with X-linked transmission. Sequencing of all exons on the X-chromosome identified a missense mutation, c.646A>G (p.Lys216Glu), in *RPS6KA3* (NM_004586.2) that segregated with the phenotype in the family. Two of the patients were reassessed a posteriori and found to have subtle facial CLS features (narrow palpebral fissures, broad based nose with thick septum and alae nasi, and slightly everted lower lip), uncharacteristic digits, normal growth and no spinal deformity. Several clinical geneticists agreed that the diagnosis of CLS would not easily be considered on clinical grounds. A few other such cases have been reported, also caused by proximal missense mutations or small in frame deletions. Together these patients illustrate the potential spectrum of clinical variability in CLS and the power of massive parallel sequencing to produce etiological diagnosis in situations of wide genetic heterogeneity.

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P05.143

'A frameshift mutation in the ubiquitinylation-related gene KLHL15 causes a novel X-linked intellectual disability syndrome associated with hypogenitalism, short feet and hypoplastic alae nasi'

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X-linked intellectual disability (XLID) is a clinically complex and genetically heterogeneous group of disorders with a growing number of disease-associated proteins implicated in ubiquitin-mediated proteolysis. To date, three XLID genes involved in the ubiquitination pathway have been identified on the X-chromosome (*CUL4B*, *UBE2A*, *HUWE1*). We report a four-generation family with 7 affected males with mild to moderate intellectual disability, small penis and testes, very short, flat feet, and relatively mild dysmorphic features including flat philtrum, thin upper and prominent lower lip, straight nose with hypoplastic alae nasi, micrognathia and deep-set eyes. Massively parallel sequencing of all X-chromosome specific exons in the index patient identified a frameshift mutation in *KLHL15* which resulted in a premature stop codon. This mutation co-segregated with XLID in the family. These findings were concordant with previous linkage results showing a significant LOD Score in the *KLHL15* containing region Xp22.22-Xp22.11. X-inactivation studies in female carriers, who are all asymptomatic, revealed an extremely skewed X-inactivation profile in their blood lymphocytes. *KLHL15* encodes an E3 ubiquitin ligase adaptor, which has been recently shown to promote the turnover of a brain-specific PP2A regulatory subunit by ubiquitylation and proteasomal degradation. Our results endorse further need for focusing on ubiquitination as an important contributor to intellectual disability.

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P05.144

Investigation of the interstitial microduplication of Xp22.31 with array-CGH

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The pathogenicity of the Xp22.31 duplication is a debatable subject amongst several authors. Some describe the microduplication as pathogenic; others as a normal variant whilst others remain unclear about its clinical significance. The region contains 7 genes: STS, VCX, VCX3A, VCX2, MIR4767, HDHD1A, PNPLA4. Here we present our findings after investigation of two cases where the same microduplication of Xp22.31 region was detected. The first case involves a 12 year old boy with Intellectual Disability (ID). Array-CGH was performed using Cytochip BAC array (BlueGnome) and revealed a duplication of approximately 2 Mb on the region of Xp22.31. The duplication was suspected to be causative for the phenotype. The duplication was present in the patient's mother and grandmother. The second case was a female carrier of the same duplication, detected using Cytochip ISCA array (BlueGnome). QRT-PCR revealed the same duplication in the carrier's mother.
Both have normal phenotype. X-inactivation was determined by methylation analysis for the androgen receptor (AR) locus and revealed skewed X-inactivation pattern for the mother and a normal pattern for the carrier. All male family members tested did not have any copy number gain. To further define the breakpoints, Chromosome X exon specific array, designed by Oxford Gene Technology (OGT) was used. Based on the current literature, there is a possibility that this duplication is predisposing to the onset of developmental delay and ID, possibly having an additive effect or represents a very rare population variant. Therefore genetic counselling for the Xp22.31 microduplication poses great challenges.

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P05.145

A *de novo* loss of function mutation in an individual with short stature, microcephaly and ID establishes *ZNF238* as a candidate gene for the 1q43q44 microdeletion syndrome and Floating-Harbor like syndrome

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Floating-Harbor syndrome is an autosomal dominant disorder caused by mutations in *SRCAP*, and is characterized by short stature, speech delay, intellectual disability, and distinctive facial features. At present, mutations in *SRCAP* have been identified in nineteen individuals with Floating-Harbor syndrome. The phenotype of individuals with a chromosome 1q43q44 microdeletion consists of intellectual disability with limited or no speech, growth retardation, microcephaly, recognizable facial features, seizures, and agenesis of the corpus callosum. Several candidate genes for microcephaly, agenesis of the corpus callosum, and/or seizures have been proposed in literature, including *ZNF238*. So far, however, no mutations in this gene have been described.
We performed exome sequencing in an individual with a phenotype resembling Floating-Harbor syndrome, and her parents. The phenotype of

the girl comprised intellectual disability with speech delay, short stature, microcephaly, and dysmorphic facial features. A *de novo* nonsense mutation in *ZNF238* was detected: 397G>T (p.(Glu133)).
Sequencing in ten individuals with a similar phenotype revealed no additional mutations in *ZNF238*.
We present the first individual with a mutation in *ZNF238* with phenotypic overlap with the 1q43q44 microdeletion syndrome and Floating-Harbor syndrome. We propose *ZNF238* as the candidate gene for the phenotype of the 1q43q44 microdeletion syndrome and Floating-Harbor-like syndrome. More individuals with mutations in *ZNF238* need to be identified to support our findings and further delineate the clinical phenotype. *s.demunnik@gen.umcn.nl*

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P06.01

Familial segregation of 15q11.3-13.3 duplication: phenotypic variability and lack of association with depression.

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We report a 3 generation family segregating a 15q11-q13 duplication with variable expressivity and lack of association with depression.
The proband presented with severe clinical depression, aggression and intellectual impairment. Comparative genomic hybridization showed a duplication at 15q11.2-q13.1. A similarly affected sister (autism spectrum disorder, anxiety and intellectual impairment) and their clinically depressed mother also carried the duplication. The mother has normal intellect. Another clinically depressed sibling with normal intellect did not carry the duplication. Methylation studies showed that the duplication had been inherited from the maternal grandfather who had normal intellect and absence of psychiatric disease.
Duplication of 15q11-q13 has previously been associated with cognitive impairment, autism, and psychiatric disease. Furthermore, the maternally inherited duplication most likely confers this phenotype whereas paternally derived duplications primarily show normal phenotypes.
Our family provides additional evidence that autism, psychiatric disease and intellectual disability segregate with a maternally inherited duplication at 15q11-q13 although with variable presentation. The presence of depression in the non carrier sister however, suggests a lack of association between clinical depression and the chromosomal duplication in this family, providing evidence that this duplication does not play a strong role in depression.

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P06.02

3q27.3 microdeletional syndrome: a recognizable clinical entity associating dysmorphic features, developmental delay with psychotic bipolar troubles and a marfanoid habitus.

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Since the advent of array-CGH, numerous new microdeletional syndromes have been delineated. We report on the first clinical delineation of 7 patients with interstitial deletions restricted to 3q28q27.3 gathered through the Decipher database and suggest this existence of a new microdeletional syndrome. The patients shared a recognisable facial dysmorphism psychiatric troubles and intellectual disability of variable intensity, associated with marfanoid habitus in the majority of patients. Delay in gross psychomotor acquisition was not systematically noticed; contrasting with severely impaired communicative skills with speech delay and psychiatric troubles. Skeletal manifestations, when present, included scoliosis, arachnodactyly, or feet anomalies, pectus excavatum, flat feet, long and thin habitus with low BMI. Two smallest regions of overlap were defined. The first one, on the 3q27.3 locus, was common to all patients and associated with bipolar troubles with psychotic manifestations and facial dysmorphism. It includes several candidate genes (MASP1, ADIPOQ, and SST), and SST seemed especially relevant because of its implication in interneurons migration and differentiation, frequently altered in schizophrenia. A familial case with a smaller deletion permitted to define the second region outside their interval, associated with severe intellectual disability, marfanoid habitus, long and thin habitus, and localised on the 3q27.2 locus, notably containing the AHSG gene. The AHSG gene encodes a secreted protein implicated in bone maturation, TGFb signalling pathway, and was associated to leanness in several studies. In conclusion, we report on a putative new microdeletional syndrome associating intellectual disability, bipolar disorder with psychosis, a recognisable facial dysmorphism and marfanoid habitus.

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P06.03

Genetic bases of Attention Deficit Hyperactivity Disorder (ADHD) in 300 Spanish patients: Preliminary results.

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Aim: The purpose of this study is to preliminary describe the information available on the main group of genes that have been related with a susceptibility to Attention Deficit Hyperactivity Disorder.
Patients and Methods: 304 Spanish patients (between 6 and 17 years old) with clinical diagnosis of ADHD were evaluated over the following scales: ADHD Rating Scale-IV, Apgar Family function test, Parents-Rated Strengths and Difficulties Questionnaire, Children´s Depression Inventory, Children´s Global Assessment Scale and Clinical Global Impression.
Patients were genotyped for 7 genetic variants in genes in the dopaminergic system (DRD4: Exon 3-48bp VNTR, 5´120bp duplication; DAT1: 3´UTR 40bp VNTR; and DRD2: rs1800497), in the serotonergic system (SLC6A4: VNTR intron 2) and the latrophilin 3 gene (LPHN3: rs1397548, rs2305339). Allelic frequencies were assessed and compared to data reported in Caucasians, using chi-squared test.
Results: For all the above-mentioned variants, allelic association analysis showed no significant differences between Spanish patients with ADHD and control population: Exon 3-48bp VNTR (p=0.846), 5´120bp duplication (p=0.09), rs1800497 (p=0.559), 3´UTR 40bp VNTR (p=0.147), VNTR intron 2 (p=0.862), rs1397548 (p=0.076) and rs2305339 (p=0.699).
Conclusions: Although in recent years there has been an increase in the number of genetic studies conducted on ADHD, findings differ significantly from one study to another. Our preliminary results suggest the need of: 1) examination of refined phenotypes; 2) recruitment of control population matched for sex, age, and in which ADHD symptoms have been specifically excluded. Therefore, these considerations may reduce the heterogeneity and help to provide enough statistical power to these association

C. Gomez-Sanchez: None. **R. Riveiro-Alvarez:** None. **P. Tirado Requero:** None. **V. Soto Insuga:** None. **M. Rodrigo Moreno:** None. **M. Prados Alvarez:** None. **A. Segura Frontelo:** None. **J. Carballo Belloso:** None. **C. Ayuso:** None.

P06.04

Genetics of Attention-deficit/hyperactivity disorder in the Portuguese population: candidate genes from GABAergic system

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Attention-deficit/hyperactivity disorder (ADHD) is a frequent childhood-onset psychiatric disorder and despite its high prevalence and heritability

the identification of ADHD risk genes has been challenging. Recent evidences suggest that dysfunctions in the GABAergic neurotransmission might be implicated in the etiology of ADHD, namely through genes encoding the GABA A receptors. Since no genetic studies have addressed this hypothesis, the current study aimed to investigate whether polymorphisms in GABRG2 (G3145A) and GABRA1 (A15G) genes are associated with ADHD in a sample of the Portuguese population. Blood samples were acquired from parents-offspring trios consisting of two parents and respective offspring, diagnosed with ADHD according to DSM-IV-TR, and genomic DNA was extracted from leukocytes through an enzymatic procedure. The GABRG2 G3145A and GABRA1 A15G polymorphisms were investigated by PCR-RFLP. We used either haplotype relative risk (HRR) and transmission disequilibrium test (TDT) and both strategies found no biased transmission of the alleles of GABRG2 G3145A polymorphism (HRR: $\chi2 = 0.104$, P = 0.747; TDT: $\chi2 = 0.047$, P = 0.829) and GABRA1 A15G polymorphism (HRR: $\chi2 = 0.426$, P = 0.514; TDT: $\chi2 = 0.275$, P = 0.600). The results seem to contradict the role of GABRG2 G3145A and GABRA1 A15G polymorphisms in the pathophysiology of ADHD in the Portuguese population. However, additional studies using a larger sample are being carried out to strengthen these preliminary results. This work was supported by the Portuguese Science and Technology Foundation (PhD grant SFRH/BD/69270/2010).

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P06.05
The association analysis of ABHD11 gene (rs2293484 and rs10279013 with autism in a South African population.

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Background : Autism is genetically inherited neurodevelopmental disorder characterized by significant impairments of social interaction, difficulties in communication and displays of restrictive or repetitive behaviors activities and interests. Genome scan data have pointed towards the long arm of chromosome 7 as a strong candidate region. Various association and linkage studies are being undertaken in order to screen candidate genes mapping to the long arm of chromosome 7 as susceptibility loci. The anhydrolase domain containing 11 gene (ABHD11) is located on 7q11.23 which is a hotspot region for autism. This gene has not been investigated for its association to autism. Aims : The aim of this study was to investigate the association of two SNPs from genes ABHD11 (rs2293484 and rs10279013) in South African autistic population.Methods : A total of 435 individuals were recruited including 217 autistic and 218 control subjects. The Taqman ®Real-Time PCR and geno typing assay was utilized to determine the genotypes. Results: A significant association of SNP rs10279013 but not for SNP rs229348 with autism in the South African (SA) population is observed. Conclusion: There might be a possible role of ABHD11 in autism especially for SA populations. The present study represents the first report on genetic association studies on ABHD11 gene in SA population

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P06.06
CNV characterization, inheritance and phenotypic correlations in families with autism

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In this study, we characterized potentially pathogenic CNVs in a sample of 342 Portuguese children with Autism Spectrum Disorders (ASDs), genotyped by the Autism Genome Project whole genome CNV analysis. We selected high confidence CNVs (detected by 3 algorithms) not overlapping more than 20% with CNVs in 8000 controls.
We identified 165 CNVs in 132 individuals, 78% with only one CNV. CNVs ranged from 5 kb to 3 Mb, and 67% were deletions. However, genic CNVs, which represented 53% of all CNVs, were more frequently duplications (65%). Genic CNVs integrated between 1 and 17 genes, and included genes implicated in ASD etiology like NRXN1 and CHD2, as well as novel genes. CNVs were mainly inherited, with only 10% de novo.
We further evaluated the presence of autistic traits in parents, using appropriate questionnaires, and the type of inheritance of the CNV (inherited vs de novo). We observed a significant excess of autistic traits in the fathers that transmitted CNVs, mainly for the "aloof" personality, defined as lakking interest in social interaction. Analysis of familial correlation data from parents and probands showed a significant correlation between parents. We therefore show evidence for an excess of subthreshold autistic traits in CNV transmitting parents of children with ASD, particularly for paternal transmission. We also observed a tendency for assortative mating in families of affected individuals, in particular for inherited CNVs.
We conclude that a large fraction of CNVs are inherited and correlate with autistic traits in parents in the studied sample.

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P06.07
Missing heritability of Korean patient with autism spectrum disorder(ASD)

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Genome-wide association studies (GWAS) have been identified SNPs associated with complex traits. However, a great deal of the heritable variation associated with common traits remains unaccounted for within the genome. The objective of this study is to discover large parts of additive genetic variance not typically associated with autism spectrum disorder (ASD) through GWAS in Korean population and estimates of ASD heritability.
Subjects with ASD were recruited through the Korean Autism Genetic Study Consortium. Each child was diagnosed as ASD by using the Korean versions of ADOS and ADI-R (Lord et al. 1994; Lord et al. 1997).
Total 146 patients were genotyped on the Affymetrix 5.0 platform. Genotype data for n=858 sex-matched controls were drawn from the Korea Association Resource database. Genotype data were filtered for <5% missingness/sample or /SNP; MAF >0.01; HWE p>0.0001. Cases were verified as unrelated using identity-by-descent estimation in PLINK v1.07. In order to examine the missing heritability in ASD, we applied genome-wide complex trait analysis (GCTA) to 142 case and 842 controls after data filtering.
The results identified phenotypic variants of 84.5% (±0.313 SE) with affected status of ASD, 7.0% (±0.027 SE) with topGWAS SNPs (± 1mb region) and 81.0%(± 0.313 SE) with nonGWAS SNPs. Increase from the genetic variance identified by top GWAS hits region indicates there might be more risk loci to be identified. Our results suggest that many common variants of small effect remain to be discovered, although GWAS is useful in identifying the most common variants associated with ASD.

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P06.08
A rare microdeletion disrupting KLHL23 may influence the autism phenotype

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Autism spectrum disorder (ASD) is a severe neurodevelopmental disorder with highly complex genetic predisposition.
Rare copy number variants (CNVs) are known to play a role in the genetic etiology of ASD. The follow-up of these rare events, by targeted gene sequencing and functional studies, has proven to be a powerful tool for the identi-

fication of novel candidate genetic loci.
In a genome wide CNVs screening performed by the AGP, a rare inherited microdeletion, disrupting two genes transcribed in the same direction, KLHL23 and SSB, was identified in an Italian ASD individual. This deletion, potentially resulting in a gene-fusion transcript, causes a frameshift with the introduction of a premature stop codon. No fusion transcript was detected by RT-PCR, suggesting that it is subject to "nonsense-mediated mRNA decay". KLHL23 encodes for a brain expressed Kelch-repeat protein of unknown function. While several CNVs affecting SSB are reported in controls making it an unikely dosege-sensitive gene, no CNVs have been previously described in KLHL23. KLHL23 expression analysis in peripheral blood of the CNV carriers did not show reduction of RNA levels, although we can't exclude that the deletion could have an effect on the brain.
In a mutation screening of KLHL23 in 85 multiplex familis with ASD we identified a damaging variant (M65V), absent in controls, and segregating with the ASD in an affected sibling pair.
Taken together our results suggest that rare variants altering KLHL23 function in the brain, could contribute to ASD risk in a small number of cases.

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P06.09
Identification of MicroRNA -486-3p and miR-361-5p molecular signatures associated with autism in whole blood samples as a promising candidate biomarkers

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For molecular diagnostic purposes, it would be desirable to develop peripheral blood-based biomarkers which effectively identify disease state. As autism is one of the most debilitating neurocognitive disorders and only clinical criteria are available, some more effective and not so subjective signs are needed. To verify that pooled miRNA expression, reproduce the average of single subject values, we chose 3 micro RNAs (mir-766-3p, mir-486-3p and mir-361-5p), that were found to be dysregulated in autism patients whole blood in previous our work. Concordance between pooled and single subject results were demonstrated for 2 of the selected miRNAs by qRT-PCR analyses, identifying significant difference only for down-regulated mir-361 -5p (p<0,0001; mean RQ: - 0,38) and up-regulated mir-486-3p (p<0,0001; mean RQ: 8,003), but not for miR-766-3p (p=0,995) in autistic group. The expression changes of the miRNA signature were then evaluated for their correlation with the patients' clinical symptoms measured by Gilliam Autism Rating Scale (GARS) which reveals correlation of the communication and stereotype subscales with mir-361-5p (p=0,028/ p=0,043). The results indicated that the whole blood-based miRNA profiling is a promising way to identify candidate biomarkers for autism, and the identified miRNA signature warrants further investigation. Further molecular analysis on miRNA gene expression changes will give a more detailed picture about the miRNA associated mechanism in autism.

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P06.10
Undestending MicroRNA gene expression signiture in peripheral blood of children with autism

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MicroRNAs are a class of sophisticated regulators of gene expression, acting as post-transcriptional negative regulators of gene expression that recognize their target mRNAs molecules through complementary base pairing. Due to the dynamic nature of the whole blood transcriptome, understanding miRNAs expression profile by examining mature miRNA signature in autism is a promising tool for discovery of novel disease-related biomarkers. To perform large-scale miRNA profiling we employed a pooled-RNA technique of 30 autistic patients' and 25 healthy controls' whole blood. Using µParafloTM miRNA microarray assay based on the Sanger miRBase v18.0 database, we found a set of 77 differentially expressed miRNAs (p<0,05) including miR-766-3p, log2 -1,10; miR-128, log2 - 0,97; miR-29a-3p, log2 0,67; miR-361-5p, log2 -0,70; miR-148a-3p, log2 0,96; miR-663, log2 -0,10; miR-486-3p, log2 -1,14. To validate the results of the microRNA microarray assay, we examined the expression of these miRNAs, by stem-loop qRT-PCR using pooled assay. All tested miRNAs showed a significant changes in accordance with microarray data (p<0,01), but miR-486-3p.
Finally, to study the modulation in protein-coding gene expression that may be associated with specific miRNAs changes, target studies were carried out using publically available database, miRWalk (http://mirwalk.uni-hd.de). We identified some genes connected with neuronal development as RELN and PTEN, other involved in epigenetic processes: DNMT and DICER, as downstream targets of the dysregulated miRNAs. All these findings clearly support the integrative genomic hypothesis of heterogeneous neurodevelopmental disorder, where specific changes in gene function contribute to disease phenotype.

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P06.11
Runs of homozygosity associated with speech delay in autism in a Taiwanese Han population

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Background: Runs of homozygosity (ROH) may play a role in schizophrenia and Alzheimer disease. Here, we aimed to test if ROHs are linked to autism.
Methods: A total of 315 Han Chinese affected with autism and 1,115 controls were genotyped with the Affymetrix SNP 6.0 array. ROH was defined as an extended homozygous haplotype spanning at least 500 kb. We also used Affymetrix GeneChip HG U133_Plus 2.0 to perform gene expression analysis.
Results: The case-control analysis yielded no genome-wide significant associations. An ROH region on 11q22.3 was significantly associated with speech delay (p = 2.51 x 10^{-11}). Among these genes, NPAT (nuclear protein, ataxia-telangiectasia locus) and ATM (ataxia-telangiectasia mutated) are linked to ataxia telangiectasia characterized by language impairment; CUL5 (culin 5) may regulate neuronal migration to influence cortical layering associated with language development. Our gene expression analysis also show that the NPAT gene was less expressed in cases with language impairment than cases without language impairment (p = 5.67 x 10^{-10}). We further obtained nominal evidence for the association between speech delay and an ROH region on 11q22.3 in another independent sample of 1,387 subjects (p = 0.037). The ROH on 11q22.3 remained to be significantly associated with speech delay in combined samples (Stouffer's z trend = 0.0005).
Conclusions: Our findings suggest that extended recessive loci on 11q22.3 may play a larger role in in language impairment than susceptibility to autism. More research is warranted to investigate if these genes, especially NPAT, influence speech pathology by perturbing cerebellar functions.

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P06.12
Large deletion 11q13.3 encompassing SHANK2 in a patient with autism

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Autism Spectrum Disorders (ASD) are characterized by deficits in social interaction, communication, and repetitive behaviour. Recent studies have highlighted the involvement of rare copy-number variations in the genetic etiology of ASD particularly those affecting genes involved in the neuronal synaptic complex. Among them, the SHANK gene family consists of three members: SHANK1, SHANK2 and SHANK3, which encode scaffolding proteins required for formation and function of neuronal synapses. SHANK2 mutations have been associated with ASD and mild intellectual disability. Recently, three patients with autism carrying a de novo deletion of SHANK2 associated with a duplication of the alpha 7 nicotinic receptor CHRNA7 were reported. Based on these observations, a „multiple hit model" for ASD has been proposed for SHANK2.
Here we reported a patient with autism, minor dysmorphic features, hypotony, and multiple ear infections. The whole genome SNP array (HumanCytoSNP-12, Illumina) analysis revealed a de novo 11q13.3q13.4 deletion (69,789,180-71,783,240) of 1.9 Mb encompassing 6 referenced OMIM genes including SHANK2. In addition, the patient carried an intermediate CGG re-

peat length at the Fragile X mental retardation (FMR1) locus inherited from his mother.
We discuss the genotype-phenotype correlation of the 11q13.3 deletion and the co-occurrence of the 11q13.3 deletion and the FRAXA intermediate allele.

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P06.13

Mosaic copy number variation in the autism and schizophrenia brain: providing a basis for a new (mitotic) theory of neuropsychiatric diseases

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A plethora of data on germ-line genomic rearrangements in brain disorders including autism and schizophrenia does exist. On the other hand, the contribution of non-heritable genomic alterations to the pathogenesis is hardly appreciated. Here, we have assessed post-zygotic autosomal aneuploidy in prefrontal cortex of the diseased and normal brain by molecular cytogenetic techniques (FISH and interphase chromosome-specific multicolor banding with DNA probes for chromosomes 1, 7, 8, 9, 10, 11, 16, 17 and 18). Twelve autistic, 19 schizophrenic and 27 control samples provided by the Brain and Tissue Bank for Developmental disorders, University of Maryland and bank of our institute were analyzed. Aneuploidy rate per autosome was 0.3-0.8% in all three cohorts analyzed (0.46%, 0.62% and 0.59%). However, we observed statistically significant increase of chromosome 9 aneuploidy in the autistic brain. In schizophrenia, chromosome 1 and 18 aneuploidy rates were significantly increased. Additionally, we performed array CGH and in silico analysis of our data on genomic rearrangements in childhood neurodevelopmental diseases (124 individuals) and found that CNV burdens in these individuals typically affect two pathways specific for brain cells: cell cycle regulation and programmed cell death. We concluded that somatic genome variation (chromosome-specific mosaic aneuploidy) is a non-heritable genetic factor contributing to pathogenesis of brain disorders, that is, however, a result of specific CNV burdens. Our data provide a basis for a new (mitotic) theory of neuropsychiatric diseases. Supported by the President of the Russian Federation Grant MD-4401.2013.7 and RFBR 12-04-00215-a.

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P06.14

Microdeletion in the 4p16.3 region in a case of autism

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A large body of evidence suggests that autism is a multifactorial disease with a strong genetic component; however the underlying mechanisms are not yet clear. We report a patient with a normal development up to the third year of life. At this age the child presented impairment of reciprocal social interaction, deficit in verbal communication ability, stereotyped behavior with head banging, brief attention span, poor eye contact. The diagnosis of autism was done according to the "Autism diagnostic Observation Scale". Actually the child is 10 years old. Brain NMR and vision are normal. Electroencephalogram shows spike/wave complexes at 2-3Hz over the right frontal region. Conventional karyotype is 46, XY. Array-based comparative genomic hybridization (aCGH) reveals a 647 Kb microdeletion of 4p16.3. The microdeletion found in our analysis is closed to but does not include the critical region associated with the Wolf-Hirschorn syndrome (WHS); accordingly, the child exhibited mild dysmorphic features, which included a prominent forehead and glabella, but not the typical WHS appearance. Evaluation of both parents demonstrates that the microdeletion of the child is a "de novo" mutation. Only limited number of coding genes (namely ZNF595, ZNF718, PDE6B, ZNF732, ZNF141, ZNF721, PIGG, MYL5) are located in the deleted chromosomal area. Since a different patient with autism and a similar microdeletion in the 4p16.3 region has been recently described (Velinov et al., Annual Meeting of American Society of Human Genetics, 2008), we hypothesize that such genome alteration might be associated with the autism spectrum disorders.

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P06.15

Synaptic transmission: looking for clues to Autism Spectrum Disorders (ASD) etiology in Copy Number Variants containing synaptic genes

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Copy Number Variants (CNVs) play an important role in susceptibility to ASD, often mediated by the deletion or duplication of genes involved in synaptic structure and function. In this study we tested the hypothesis that there is an enrichment in CNVs encompassing synaptic transmission genes in ASD.
To obtain a list of genes involved in synaptic structure and function, we query pathway databases for synaptic pathways (KEGG and GO). By intersecting this list with the results of a large CNV genomic screening in ASD patients (performed by the Autism Genome Project), and CNV information on the Database of Genomic Variants (DGV), we found a significantly increased burden of CNVs encompassing synaptic genes in ASD subjects compared to DGV controls (Fisher exact test P=2.2X10^{-16}, OR=1.47).
Detailed analysis of the frequencies of each synaptic gene duplicated or deleted by CNVs in ASD and control subjects identified 39 genes that were significantly more frequent in ASD (P<0.05). Closer inspection of CNV overlap between the ASD sample and additional control databases (*eg* SAGE, PopGene) highlighted synaptic structure or function genes (eg *NF1*, *GABRG1*, *CHRNA7*) that warrant further investigation. Additionally, 5 synaptic genes, *PLA2G1B, PPP2R3C, KCNK7, ADCY7* and *RAPSN*, were disrupted by CNVs exclusively in the ASD dataset, and were also absent from the additional control CNV databases queried.
The present results show an excess of structural alterations encompassing synaptic genes in ASD and highlight candidate genes for sequencing and functional studies.

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P06.16

A *NCAM2* deletion in a patient with autism

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An 8-year-old boy with autism spectrum disorder, speech delay, behavioural problems, disturbed sleep and macrocephaly presented in our genetics clinic. He is the first child of non-consanguineous parents. Chromosomal analysis had revealed a normal male karyotype 46,XY, and testing for fragile X syndrome and *PTEN* sequencing was inconspicuous.
Array-CGH analysis showed a microdeletion of 1.6Mb: arr 21q21.1-q21.2(22444986-24047363)x1. This part of the chromosome contains the entire *NCAM2* gene and no other functional genes. His mother also carries this microdeletion. She has no obvious behavioural features of autism, but also is macrocephalic. Three maternal blood relatives are reported to have speech problems. Results of psychological assessments of the mother and the other affected individuals are pending.
Autism spectrum disorder (ASD, OMIM 209850) encompasses different forms of autism with a broader phenotype. Two-thirds of all patients with ASD suffer from mental retardation. Among the genes involved, *NCAM2* has been assumed to play a role in the development of ASD because of its function in neurites (outgrowth, bundling). In the literature, there is one report of an autistic boy with an 8.8 MB-microdeletion involving 19 genes including *NCAM2* and another autism-related candidate gene, *GRIK1*. Our case supports the assumption that *NCAM2* deficiency, as other cell adhesion pro-

tein defects, plays a role in the development of autism, speech development and macrocephaly, perhaps with variable expressivity.

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P06.17

Targeted high-throughput gene sequencing of the NMDA receptor multi-protein complex and high-resolution genomic study of 100 families with autism spectrum disorder

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Autism spectrum disorder (ASD) is an early childhood neurodevelopmental disorder characterized by a significant genetic aetiology and a prevalence currently estimated at 1/100. Epidemiological studies described that ASDs are heterogeneous at both clinical and genetic levels, which has been further illustrated by the large number of loci and genes that have been identified to date (>100). Several biological pathways have been highlighted, particularly the NMDA receptor multiprotein (NRC) complex, affected by copy number variations (CNV) and mutations enriched in NRC proteins encoding genes.
We performed a global genetic study of 100 French families including at least 1 individual with ASD, which was included in the research project "Neurophysiological Molecular and Developmental Analysis of the Glutamate Synapse in Autism (NMDA-Autism)" (ClinicalTrials.gov NCT01770548).
For each family, we firstly performed a high-resolution pangenomic comparative genomic hybridization (CGH) analysis with 1M CGH Agilent Array format to identify rare or de novo CNVs; Secondly, a high-throughput targeted sequencing of 216 genes mostly belonging to the NRC complex was carried out with the SureSelect Agilent strategy in order to assess the contribution of gene mutations in our cohort.
Preliminary results on CGH experiments led us to identify small rare CNVs in several known regions (20p12.1 MACROD2; 18q21.1 KATNAL2; 11p13.11) or novel potential candidates such as FRMPD4, GUCY2F and NXPH3. We have also observed several large genomics variations previously described (1,2Mb amplification at 16p13.11 and 5,6 Mb deletion at 11q14.2-q14.3). The results from the high-throughput sequencing analysis, which is currently being performed, will be also presented.

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P06.18

Homozygous exonic deletion in CTNNA3 suggests a role for alfaT-catenin in susceptibility to Autism Spectrum Disorders (ASDs)

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Autism spectrum disorders (ASDs) are highly heritable, neurodevelopmental conditions, showing extreme genetic heterogeneity. While it is well established that de novo genetic variation plays an important role in ASD risk, recent studies have demonstrated a convincing evidence of a rare recessive contribution to the heritability of ASDs.
In the genome-wide CNV study carried out by the Autism Genetic Consortium (AGP), we identified one homozygous deletion intersecting the CTNNA3 gene in an Italian proband with ASD and moderate intellectual disability (PIQ=50). This deletion includes a coding exon, leading to a putative frameshift and premature stop codon, inherited from both parents, each heterozygote for a deletion of slightly different length. The unaffected sister is also heterozygote for the deletion.
CTNNA3 encodes for the alfaT-catenin protein that has a crucial role in cellular adherence showing suggestive association in GWAS and a de novo deletion.
Even if the frequency of exonic deletions of CTNNA3 is not significantly different between ASDs cases and controls (17/2446 cases and 35/5097 controls, P=.88), no homozygous exonic deletions were found in a sample of 5097 controls, suggesting that only the complete knockout of CTNNA3 could cause or confer susceptibility for ASDs. Expression analysis of alfaT-catenin and alfaN-catenin proteins in mouse cortex and hippocampus (P0-P90), provided further support of a role of CTNNA3 in early mid-fetal development of the brain.
While the exact biological significance of CTNNA3 homozygous deletion is yet to be determined, we hypothesize that it could have clinical relevance to the ASDs phenotype in this Italian patient.

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P06.19

Copy number variant analysis using SNP microarrays identified novel candidate genes in patients with autism spectrum disorders

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Copy number variants (CNVs) have repeatedly been found to cause or predispose to Autism Spectrum Disorders (ASDs). For diagnostic purposes, we screened 194 patients with ASDs for CNVs using Illumina SNP arrays. In several patients, we also analyzed candidate genes located in inherited deletions to unmask autosomal recessive variants. Three CNVs, a *de novo* triplication of chromosome 15q11-q12 of paternal origin, a deletion on chromosome 9p24 and a *de novo* 3q29 deletion, were identified as the cause of the disorder in one patient each. We also identified multiple private or recurrent CNVs, the majority of which were inherited from asymptomatic parents. Finally, an autosomal recessive cause was considered in two patients: a homozygous 1p31.1 deletion encompassing *PTGER3* was identified in a patient and a rare missense variant in *DOCK10* was found in a second patient in association with an inherited deletion that deleted the entire gene. Although highly penetrant CNVs or variants inherited in an autosomal recessive fashion were detected in rare cases, our results mainly support the hypothesis that CNVs contribute to ASDs in association with other CNVs or point variants located elsewhere in the genome. Identification of these genetic interactions in individuals with ASDs constitutes a formidable challenge.

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P06.20

0.12Mb microdeletion at 19p13.2 including NFIX in an individual with autism spectrum disorder.

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Marshall-Smith syndrome (MSS) is the overgrowth syndrome, characterized by tall stature, macrocephaly, distinctive facial appearance, and intellectual disability. Recently, Sotos-like and MSS were found to be caused by mutations of Nuclear factor I-X (NFIX). We describe an additional male patient with microdeletion at 19p13.2 encompassing NFIX. He was born to nonconsanguineous parents at 40 weeks of gestation. His birth weight was 2570 g, and height 46 cm. At the age of 2 years and 8 months, he was referred to our clinic for a diagnostic evaluation because of developmental delay and growth retardation. His weight was 10.6 kg (-1.8 S.D.), height 86.7 cm (-1.2 S.D.), and OFC 48.0 cm (-0.7 S.D.). He could crawl, but could not walk alone and speak a significant word. He had some autistic traits and stereotypes.He was

medicated with antiepileptics for recurrent febrile convulsions. Array CGH revealed 0.12 Mb deletion at 19p13.2 encompassing NFIX. Further analysis with quantitative PCR confirmed the deletion of NFIX as a de novo event. Our patient had a relative macrocephaly, but did not present overgrowth and characteristic facial features as MSS. The autistic traits are the most prominent clinical feature in our patient. Haploinsufficiency and mutations in the DNA-binding /dimerization domain of NFIX causes Sotos-like syndrome, whereas the truncated mutations of NFIX represent MSS [Malon et al., 2010; Yoneda et al., 2012; Priolo et al., 2012]. The present report provides insight into consideration of genotype-phenotype correlation in NFIX mutations.

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P06.21

Analysis of the hexonucleotide repeat expansion and founder haplotype at C9ORF72 in an Irish psychosis case-control sample.

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A hexonucleotide repeat expansion 'GGGGCC' in an intronic region of the C9ORF72 gene has been found to account for up to 70% of amyotrophic lateral sclerosis (ALS), and this locus also being implicated in the pathogenesis of frontotemporal dementia (FTD). Study of an independent FTD sample showed a strong association between C9ORF72 mutations and psychotic symptoms. We sought to screen a large Irish psychosis case-control sample for evidence of association between the repeat expansion and psychosis. We carried out haplotype analysis on this region, due to reports of a founder haplotype. Our sample included 1,165 cases and 1,283 controls. We used a reverse-primed PCR method to amplify the hexonucleotide repeat expansion. Haplotype analysis was carried out using available GWAS data for these samples. The distribution of repeat numbers was very similar for cases and controls. We identified four samples that carried a repeat number approaching the pathogenic range >30. There were two controls samples (26 and 25 repeats respectively) and two schizophrenia cases (27 and 28 repeats). Haplotype analysis found that for the 512 samples that carried more than 7 repeats, 482 (94%) carried the founder haplotype. The significance of the intermediate number of repeats (between 24 and 29) is still unclear. Haplotype analysis showed a clear association between repeat number and the founder haplotype. It would appear that this haplotype is not unique to ALS-FTD cases, but its presence predisposes this region to the repeat expansion, and in turn this may lead to a greater risk of molecular instability.

C.A. Fahey: None.

P06.22

Effects of cocaine use on the expression of psychotic symptoms, with respect to the age at onset and COMT polymorphism

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Objective: To determine if the age at onset of cocaine use affects the manifestation of psychotic symptoms and if there are differences on the age at onset of cocaine use that are dependent on the expressed polymorphism of the Catecol-O-Metil Transferase (COMT).
Methodology: The sample consists of 138 cocaine users. In order to evaluate the presence of psychotic symptoms we used the Community Assessment Psychic Experience (C.A.P.E.) test. This test evaluates the expression of both positive (paranoia, deliriums, hallucinations…) and negative (lack of social and emotional skills…) symptoms, and the associated discomfort.
Results: The results show differences among the cocaine users in the expression of psychotic symptoms, depending on age. The individuals that started using cocaine when they were 17 or younger scored higher in expression of positive psychotic symptoms (p=0.078) and associated discomfort (0.047) when compared with individuals that started using cocaine when they were 18 or older. In addition we have found significant differences in the age at onset of cocaine use associated to the expressed COMT polymorphism. Cocaine users with Met-Met polymorphism had an earlier age at onset (17.5 DT=2.6) than those with Val-Val (18.1 DT=2.1) o Met-Val (19.5 DT=3.7) polymorphisms.

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P06.23

Genome-wide association study identifies a potent locus associated with human opioid sensitivity

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Opioids, such as morphine and fentanyl, are widely used as effective analgesics for the treatment of acute and chronic pain. In addition, the opioid system has a key role in the rewarding effects of morphine, ethanol, cocaine and various other drugs. Although opioid sensitivity is well known to vary widely among individual subjects, several candidate genetic polymorphisms reported so far are insufficient for fully understanding the wide range of interindividual differences in human opioid sensitivity. By conducting a multistage genome-wide association study (GWAS) in healthy subjects, we found that genetic polymorphisms within a linkage disequilibrium block that spans 2q33.3-2q34 were strongly associated with the requirements for postoperative opioid analgesics after painful cosmetic surgery. The C allele of the best candidate single-nucleotide polymorphism (SNP), rs2952768, was associated with more analgesic requirements, and consistent results were obtained in patients who underwent abdominal surgery. In addition, carriers of the C allele in this SNP exhibited less vulnerability to severe drug dependence in patients with methamphetamine dependence, alcohol dependence, and eating disorders and a lower 'Reward Dependence' score on a personality questionnaire in healthy subjects. Furthermore, the C/C genotype of this SNP was significantly associated with the elevated expression of a neighboring gene, *CREB1*. These results show that SNPs in this locus are the most potent genetic factors associated with human opioid sensitivity known to date, affecting both the efficacy of opioid analgesics and liability to severe substance dependence. Our findings provide valuable information for the personalized treatment of pain and drug dependence.

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P06.24

Genetics of the development of heroin addiction and the pharmacogenetics of the substitution therapy

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Primary goal of this study was to clear the role of the dopaminerg genes in the pathophysiology of heroin addiction and also expanding the research, using modern molecular biology technics in order to investigate gene variants in the shared genetics of common addicitons.
In addition to our case-control study, we carried out a TaqMan® OpenArray® study on *in silico* selected addiction candidate genes. Investigated

polymorphisms of the case-control study were: the dopamine D4 receptor gene (DRD4) III.exon VNTR; rs1800955, rs747302, rs936462 polymorphisms and the 120bp duplication; the dopamin trasporter 3' and VIII.intron VNTRs; rs1800497 of the ANKK1 and rs1079597, rs1800498 of the dopamine D2 gene (DRD2). Further goal was to determine environmental and genetic factors effecting the efficiency of the substitution therapy of heroin dependence. Previously in our lab two polymorphisms of the dopamine D4 receptor gene showed association with the therapeutic response of methadone-treated patients.
The results of the case-control study showed significant association between the ANKK1 rs1800497 (p=0.009), the DRD2 rs1079597 (p=0.003) and the DRD4 rs1800955 (p=0.007) and heroin dependence. Additional bioinformatic analyses revealed an indirect effect of the DRD4 rs936462 (p=0.0013). The OpenArray analysis found 5 nominally significant associations, from which only one SNP in the ALDH2 gene (aldehyde dehydrogenase 2) stayed significant after multiple correction. ALDH2 is thought to be involved in the metabolism of dopamine so probably has a role in the pathophysiology of other addictions. This study reinforces the results of a previous Chinese association study between the ALDH2 and heroin dependence.

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P06.25

Expression of neurotransmitter regulatory and neurodevelopment genes in peripheral blood of patients at the first episode of psychosis

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Schizophrenia is a severe mental health disorder with a high heritability. The study of gene expression levels in blood of patients in the beginning of the disease, such as first-episode of psychosis (FEP) may be useful to detect changes in gene expression despite treatment effects. In this study we aimed to compare the expression levels of genes related to neurotransmission and neurodevelopment, in blood of FEP patients (*N*=30) and healthy controls (*N*=29). Whole blood was collected from each participant during clinical assessments. Expression levels of 40 genes related to neurotransmission and neurodevelopment were quantified with a customized RT^2 Profiler™ PCR Array, which is based on SYBR Green detection of cDNA amplification. For data analysis, we compared $2^{-\Delta Ct}$ values of FEP patients and healthy controls using t-test. Twenty-eight of 40 genes presented undetectable expression levels in whole blood and, hence were excluded from the analyses. Significant downregulation of two genes was observed: *GCH1* (Fold regulation (FR)= -1.32, *p*=0.005); and *TACR2* (FR= -1.32, *p*=0.017). *GCH1* codes for GTP cyclohydrolase I, an enzyme involved in the synthesis of BH4, which is an essential cofactor for tyrosine, serotonin and L-Dopa. Moreover, it was previously suggested as a candidate gene in a linkage analysis in bipolar disorder. Although no study had investigated *TACR2* gene (Tachykinin receptor 2) in psychosis, its antagonists exhibited anti-depressant-like activity. Therefore, both genes seem to have a role in the genesis of psychosis leading towards a better understanding of illness. Funding for this study was provided by FAPESP 2010/08968-6 and 2011/50740-5.

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P06.26

Investigating the first genomic response to stress - insights into the genetics of major depression

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Exposure to adverse life events, which is associated with an activation of the stress hormone system, is an important risk factor for the development of major depressive disorder (MDD). A main effector of this system is the glucocorticoid receptor (GR), a nuclear receptor that functions as a transcription factor. We characterize genetic variants that moderate short-term effects of GR activation on mRNA transcription in peripheral blood cells. We demonstrate that these functional variants are significantly enriched among variants associated with MDD in a large case-control study and interact with early adversity to predict amygdala reactivity to threat. The transcripts regulated by these functional MDD risk variants in human blood are also regulated by GR activation and chronic adolescent stress in mouse brain and point to ubiquitination and proteasome degradation and their impact on neurite outgrowth as important stress-sensitive systems influencing the risk to suffer from MDD.

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P06.27

Upregulation of GCHFR gene expression in peripheral blood of first episode psychosis patients before and after antipsychotic treatment

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Schizophrenia is a severe mental health disorder with a high heritability. The study of gene expression levels in peripheral blood, screening exclusively drug-naïve patients at two time points, may be useful to detect changes across disease progression and treatment. In this study we aimed to compare the expression levels of neurotransmission genes in peripheral blood of 30 drug-naïve first episode psychosis patients (FEP) before and after antipsychotic treatment with risperidone. All patients were evaluated by a psychiatrist twice (at admission and 8 weeks after treatment). Expression levels of 40 genes were quantified with a customized PCR Array which is based on SYBR Green detection of cDNA amplification. We compared 2-ΔCt values of FEP patients before and after treatment using paired T-test, considering p<0.05. GTPcyclohydrolase I feedback regulator gene (GCHFR) was significantly upregulated in FEP patients after risperidone treatment (p=0.025). GCHFR is an important modulator of GTPcyclohydrolase I (GCH1), their interaction influence directly the synthesis of tetrahydrobiopterin (BH4), which is a vital cofactor maintaining availability of some monoamine neurotransmitters. These data agree with other study in literature that described a deficiency of BH4 in patients compared to controls. Interestingly, in another study of our group that investigated the same FEP individuals, we found a downregulation in GCH1 gene compared to healthy controls. In this way, we suggest this upregulation of GCHFR after treatment could be related to risperidone effectiveness, by altering BH4 synthesis. Our study may contribute to a better understand the BH4 and GTPcyclohydroxylase system in psychosis and antipsychotic treatment.

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P06.28

A *de novo* 16p11.2 microdeletion encompassing *SRCAP* gene identified by array-CGH in a patient with Floating-Harbor syndrome.

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We describe a patient referred to our diagnostic centre for speech delay, global developmental delay and behavioural problems with normal standard karyotype, as indications for array-CGH analysis.
Molecular karyotyping revealed a 186 kb *de novo* microdeletion in 16p11.2 that encompasses 9 RefSeq genes including *SRCAP* gene (Snf2-related CREBBP activator protein, MIM #611421).
Recently, mutations in the *SRCAP* gene have been shown to cause Floating-Harbor syndrome (FHS, MIM#136140), a rare disorder characterized by peculiar facial features, short stature with delayed osseous maturation and speech impairment. To date, 19 out of 22 patients with clinical diagnosis of FHS and heterozygous truncating mutations in the final exon (34th) of *SRCAP* have been reported. The absence of mutations in 3 patients suggests the genetic heterogeneity of the syndrome. After the arrayCGH result, a new clinical evaluation was performed to evaluate the possibility of clinical overlapping between our patient and reported patients with a clinical and molecular diagnosis of FHS. On the basis of this second examination, it appears evident that the patient fulfils the diagnostic criteria for FHS. This is the first report of an heterozygous deletion of *SRCAP* gene indicating that in addition to truncating mutations reported so far partial or whole-gene deletion may be present. Therefore, it could be suggested to perform copy number analy-

sis in patients whose clinical examination is strongly suggestive of FHS and which are negative for point mutations in the final exon of *SRCAP.*

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P06.29

Genetic syndromes are frequently found in patients manifesting with primary neuropsychiatric disorders and developmental co-morbidities

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Background: Many genetic syndromes present with psychiatric disorders. We aim to determine the role and prevalence of hidden genetic conditions in patients with psychiatric disorders and comorbidities. This has the potential to significantly impact the standard of care and treatment of selected individuals with psychiatric impairment. Methods: We prospectively recruit patients ≥16 years of age with psychiatric disorder(s) and at least one of 1) neurologic abnormality, 2) developmental delay (DD), autism spectrum disorder (ASD) or pervasive DD (PDD), 3) dysmorphic features, 4) congenital anomalies or 5) family history of DD, ASD or PDD. A clinical database of phenotypic correlates is being established to delineate the highest yield data that lead to the most effective and efficient diagnosis of genetic syndromes in patients with neuropsychiatric disorders. Results: Of the initial 62 patients recruited, 15 (24.2%) have been diagnosed with genetic conditions. This includes seven patients with six different single gene disorders, six patients with six distinct chromosomal variants and two patients with different metabolic disorders. Discussion: Our preliminary results have begun to demonstrate that genetic syndromes are common in patients with primary psychiatric disorders. Many of the genetic conditions identified in this cohort have implications for medical surveillance, management and, in some cases, treatment. The high diagnostic yield in patients with "psychiatric plus" phenotypes demonstrates the significance of psychiatric disorders in genetic syndromes and vice versa. Identifying phenotypic 'red flags' will enable development of algorithms to make important, possibly treatable, genetic diagnoses in psychiatric patients in a more efficient manner.

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P06.30

DNA methylation profiles of paediatric obsessive-compulsive disorder (OCD)

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Obsessive-Compulsive disorder (OCD) is a complex, common and debilitating psychiatric disorder. Heritability estimates range from 40% to 65% suggesting that genetic factors play a role in the etiology of this disorder. However, there are other potential important determinants, including epigenetics. Epigenetic mechanisms, such as DNA methylation, have increasingly been shown to be important in the etiology of complex disorders. We hypothesized that there are alterations in DNA methylation patterns in children with OCD compared to controls. We used a novel genome-wide approach to identify epigenetic variants. Analysis of blood DNA methylation patterns in 10 OCD patients compared to 10 controls identified both gain and loss of methylation at a number of CpG sites. Candidate DNA methylation alterations identified in this initial investigation were highly relevant to the OCD phenotype and include altered DNA methylation in promoters of genes that function in glutamate signaling, myelin synthesis and lipid transport. We then obtained DNA methylation profiles using the Illumina Infinium Methylation450 array in an expanded cohort of 30 OCD patients compared to 30 tissue, age and sex-matched controls based on saliva collected using Oragene•Dx saliva kits (DNA Genotek). Data were analyzed using the IMA package in R and Genome Studio software from Illumina. The identification of epigenetic modifications in children with OCD will lead to an improved understanding of the pathophysiology and molecular mechanisms leading to this disorder. These epigenetic alterations could provide early diagnostic biomarkers for OCD which may influence treatment strategies, including both pharmacologic and cognitive behavioural therapy.

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P06.31

‚Association of *ADARB1* gene with major psychiatric disorders'

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Adenosine to inosine RNA editing, catalyzed by ADARs, is a major mechanism for transcriptome diversification in the nervous system with an important role in neurotransmission, neuronal outgrowth and plasticity. Changes in the editing of a few transcripts have been reported in postmortem studies on brains of individuals with psychiatric disorders, with the most consistent results obtained for suicide victims. Moreover, studies on mice suggest antipsychotics may reverse altered editing. To test hypothesis that variants in *ADAR* genes may contribute to these phenomena, we study association between *ADARB1* gene and major psychiatric disorders. A population based case-control study included 128 individuals with schizophrenia (SCZD), 140 with major depressive disorder (MDD) and 77 with bipolar disorder (BD), all diagnosed according to DSM-IV criteria, and 163 controls. Twelve tagSNPs in *ADARB1* gene were selected using HapMap database and HaploView. Genotyping was performed by TaqMan probes and data were analyzed by PLINK.
We show association of rs1051385 with SCZD (p=0.04, Pearson chi-square test). rs1051385 is located in the 3'UTR and may affect *ADARB1* expression. This may be consistent with previously reported increased expression of ADARB1 variants with reduced catalytic activity in SCZD. Furthermore, we show specific *ADARB1* haplotypes association with attempted suicide in MDD (p=0.0002, Pearson chi-square test) and SCZD (p=0.009, Pearson chi-square test) patients, and with SSRIs response in MDD patients (p=0.001, Pearson chi-square test). To our knowledge, this is the first study reporting association of *ADARB1* gene with psychiatric disorders, and it further underscores the importance of RNA editing in psychiatric pathology.

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P06.32

Interrater reliability of hypernasality amongst psychiatrists

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22q11.2 Deletion Syndrome is a significant risk factor for the development of psychiatric disorders. Although 22q11.2DS is commonly detected by other specialties in infancy, individuals are not screened if the anomalies do not warrant medical or surgical interventions. Facial dysmorphisms in 22q11.2DS are not clinically reliably detected. If hypernasality, found in ~90% of adult probands, is accurately and reliably detected by psychiatrists untrained in voice resonance abnormalities, it may increase the detection of 22q11.2DS. **Objective:** To determine if psychiatrists can accurately and reliably detect hypernasality. **Methods:** 40 adult psychiatrists from the Ottawa Hospital or the Royal Ottawa Mental Health Center listened to 20 voice samples (10 male, 10 female) repeated three times. Psychiatrists characterized each voice sample according to a 4-point Likert scale (1 = normal, 2 = mildly hypernasal, 3 = moderately hypernasal, 4 = very hypernasal). **Results:** Sensitivity and specificity of hypernasality detection (95% CI) were as follows: 88.5% (86-90) and 64.4% (61-67). Using logistic regression analysis, sensitivity and specificity were not affected by psychiatrist gender (p = 0.803; p = 0.520); native language (p = 0.637; p = 0.542); general vs. subspecialty psychiatry (p = 0.487; p = 0.550); gender of voice sample (p = 0.887; p = 0.416). Repeated exposure significantly affected the sensitivity (p = 0.003) of hypernasality detection but not specificity (p = 0.877). **Conclusions:** With no prior training, psychiatrists accurately and reliably detect hypernasal speech; detection of hypernasality is not affected by native language and

psychiatrist gender, voice sample gender, and psychiatric subspecialty.

T.S. Eapen: None. **N.I. Erdmann:** None.

P06.33
Duplications of RB1CC1 on 8q11.23 in neuropsychiatric disorders: presentation of a further patient.

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Duplications of RB1CC1 have been associated with various neurodevelopmental and psychiatric disorders such as psychomotor delay, epilepsy, autism spectrum disorder (ASD), and schizophrenia. A damaging de novo mutation in this gene has recently been identified in a schizophrenia patient. However, duplications of this locus have also been described in healthy individuals, thus complicating interpretation of such findings. Altogether, there is growing evidence suggesting a possibly incomplete penetrance as well as an important phenotypic variability of RB1CC1-associated disorders.
We report on a 5-year-old male, referred for initial genetic evaluation because of psychomotor delay and ASD. On clinical examination the boy presented with marked truncal obesity and mild dysmorphic features. The proband underwent several neurologic evaluations for his delay as well as for an episode of seizure, occurring in the context of a probable benign occipital epilepsy. At 9 years, he was reported to have severe anxiety and psychotic symptoms, notably auditory hallucinations, with poor response to risperidone.
Array-CGH analysis showed a maternally inherited 444 kb duplication of 8q11.23 encompassing RB1CC1 and FAM150.
Re-evaluation of the family history revealed possible psychiatric symptoms in his mother, as well as a maternal uncle suffering from chronic depression with psychotic episodes.
Our case demonstrates the phenotypic variability of duplications involving RB1CC1 and underscores the need to carefully assess the phenotype and relevant family history of such patients.

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P06.34
Investigation of a non-synonymous variant at ABCA13 in family with bipolar disorder

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Bipolar disorder (BD) is a major mental illness across all populations, with an estimated heritability of 80%, indicating a large genetic component. However, it is unclear how genetic variation such as rare single nucleotide variants (SNVs) contributes to the risk of the disease. Genome-wide association studies (GWAS) have shown support for the role of common genetic variation in increasing risk for bipolar disorder albeit, only modestly. Hence, the contribution of rare genetic variants that may exert a strong effect has been proposed. Complex diseases such as bipolar disorder are potentially caused by a combined effect of rare and common genetic alterations that would result in the disruption of gene networks. In this study, we investigated the effect of a potentially functional non-synonymous SNV (rs74859514) in the gene ABCA13 located in chromosome 7p21.3, which cause the amino acid substitution (Ala2223Pro) .This SNV was one of several uncommon functional variants shared by members of an extended family with BD who were exome-sequenced on Illumina arrays. Expression analysis using RT-qPCR was performed on patient-derived fibroblasts from two siblings carrying the mutation and diagnosed with BD. ABCA13 gene was found expressed in very low levels in fibroblasts as well as in other cell lines we tested previously, such as SH5Y5 and HeLa. We did not find significant differences in expression levels between heterozygous carriers of the ABCA13 variant when compared to a wild type fibroblast cell line. Sequencing of both genomic DNA (gDNA) and complementary DNA (cDNA) was performed to confirm each individual's genotype.

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P06.35
Genetic association of RGS2 gene polymorphic locus with schizophrenia and typical antipsychotics response.

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Several lines of evidence indicate that Regulator of G Protein Signaling 2 (RGS2) contributes to schizophrenia (SZ) vulnerability because it modulates signal transduction of neurotransmitter receptors that play a role in the pathogenesis of SZ. DNA samples of 258 patients with paranoid schizophrenia and of 263 healthy controls of Russian and Tatar ethnic group living in the Republic of Bashkortostan (RB) of Volga-Ural region of Russia were involved into the present study. All patients met the ICD10 criteria for SZ. Clinical response was determined by administering PANSS at base line and at days 21 and 45. The severity of extrapyramidal symptoms was assessed using The Simpson-Angus Scale (SAS). Genomic DNA was isolated from peripheral blood using the standard procedure. Differences between groups were tested by using unpaired t-test, analysis of variance (ANOVA), and chi-square test.
The present study is aimed at exploring whether rs2746071 of RGS2 gene could be associated with SZ and whether it could predict clinical outcomes in Russians and Tatars from RB.
In the result of the present study SZ high risk genetic markers RGS2*G/*G (rs2746071) in Russians (OR=4.08) and in Tatars (OR=4.88); allele RGS2*G in Russians (OR=2,37) and Tatars (OR=2,51), genetic markers of treatment efficacy in Tatars RGS2*G/*G (rs2746071) were obtained in individuals from the RB; considerable inter-ethnic diversity of genetic risk factors for this disease was revealed The results of this study support the hypothesis that RGS2 gene polymorphism contributes to interindividual variability in therapeutic effects and are involved in SZ pathway.

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P06.36
Association between variants of candidate genes in 22q11.2 region and schizophrenia and refractoriness to antipsychotic treatment

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Schizophrenia is a mental disorder arising from a complex interaction of genetic and environmental factors. One of the strongest genetic risk factors is the 22q11 deletion and it has been suggested that genes located in this region might contribute to susceptibility to the disease. Our aim was to investigate the association between polymorphisms in *COMT*, *ZDHHC8, UFD1L* and *PRODH* genes, located at 22q11.2 region, with schizophrenia and treatment resistance schizophrenia (TRS). A total of 260 patients with schizophrenia was compared with 192 healthy controls. The patients were genotyped for rs4680, rs737865 and rs165599 for *COMT*, rs175174 for *ZDHHC8*, rs5746744, rs5992403 and rs1547931 for *UFD1L*, rs4819756, rs137852934, rs16983466, rs2238731, rs2904551, rs2904552, rs3970559, rs2238730, rs2870984, rs2870983, rs4550046 and rs372055 for *PRODH* polymorphisms using TaqMan® PCR assay, PCR-RFLP or sequencing. Chi-square test and logistic regression were used to verify Hardy-Weinberg equilibrium and to investigate the association of polymorphisms and disease or TRS respectively. All polymorphisms were in Hardy-Weinberg equilibrium except for *COMT* rs737865 in patient group. Significant associations were observed between schizophrenia and *PRODH* rs2904552 polymorphism (p=0.004, OR=2.52, 95%CI=1.33-4.76) and *COMT* rs737865 (p=0.034, OR=0.20, 95%CI=0.05-0.88). Higher *PRODH* GG frequencies and lower *COMT* CC frequencies were observed in patient group compared to control group. None polymorphism analyzed was associated with TRS. No functionality data of *COMT* rs737865 is available to this variant. *PRODH* rs2904552 is a functional polymorphism, which changes an aminoacid and modifies the protein structure, supporting an association between this gene and schizophrenia pathogenesis. Funding support: FAPESP 2011/50740-5, 2012/12669-0.

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P06.38

Association study of candidate gene polymorphisms with paranoid schizophrenia susceptibility in Russian population

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Schizophrenia is a common, chronic and complex psychiatric disorder, affecting 1.0% of the worldwide population. The data collected from family, twin, and adoption studies show unequivocally that schizophrenia is a predominantly genetic disorder with heritability ranged from 70-80%. Traditionally, genetic research of schizophrenia was focused on identifying linkage regions or on candidate genes and polymorphisms. Based on previous molecular genetic studies the following polymorphisms: CACNA1C (rs1006737), ANK3 (rs10761482), TPH1 (rs1800532), PLAA (rs7045881), SNAP25 (rs3746544, rs1051312), PLXNA2 (rs1327175) have been chosen and analyzed in 189 patients with paranoid schizophrenia and 195 healthy individuals. The genotyping procedure included multiplex PCR with fluorescently labeled nucleotides and allele-specific hybridization of labeled PCR products with a biochip. The statistically significant association between AA genotype of rs1800532 (TPH1) and risk of paranoid schizophrenia was found (p = 0.027). Also it was shown that C allele of rs1327175 (PLXNA2) was associated with family history in schizophrenic patients (p = 0.0001). The work was supported by Russian Foundation for Basic Research (grant #11-04-01998).

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P06.39

Gender specific epigenetic profiles in patients with schizophrenia

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Background: Schizophrenia is one of the major psychiatric disorders in the world, characterized by severely debilitating behavioral abnormalities. Although it affects men and women equally, the illness has varying expression between the sexes. DNA methylation is a dynamic process that can be influenced by environmental factors. It is a major epigenetic modification that stably alters gene expression pattern in cells and is important for normal organismal development and cellular differentiation. This modification can be inherited through cell division. Materials and methods: We analyzed age-matched pools of 110 female, 110 male schizophrenia patients and corresponding healthy controls. We have performed high-resolution genome-wide methylation array analysis (Agilent 1x244K). We analyzed the methylation status of 27,627 CpG islands of all groups to identify methylation profile differences. Results: Our experiments show difference in the methylation profile between patients and controls. Comparing patients to healthy controls we established 69 differentially methylated genes (43 in female group and 26 in male group). Hypermethylated in the gene promoter region are 22 genes (13 in females and 9 in males). They play a role in neural system functioning (*PYGO2, GABBR1*), cell adhesion, cell division and signal transduction (*LRFN3, FXYD5, RMND5B u ARHGEF19*), transcription and translation (*FOXH1, ZNF488, EIF4E3, HEXIM1*) etc. Conclusions: Our data suggest that there is a major differences in methylation profile between males and females patients and controls. This dysregulation can play a critical role in schizophrenia etiopathogenesis. Acknowledgements: funded by project DMY 03-36/2011, Ministry of Education and Science.

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P06.40

Relationship between LSAMP gene and schizophrenia

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Schizophrenia is a devastating psychiatric syndrome with a median lifetime prevalence of 4.0 per 1000 and a lifetime morbid risk of 7.2 per 1000 persons and genetic heritability 80 %. It is now generally
accepted that the origins of the disorder lie early in neurodevelopment and that synaptic dysfunction and altered neural connectivity are likely to be important in its pathogenesis. The limbic system-associated membrane protein (LSAMP) is a 64-68-kDa glycoprotein that is found on the somata and dendrites of
neurons of cortical and subcortical regions comprising the adult mammalian limbic system, which is involved in the mediation of emotional behaviour, learning, and memory. We studied the relationship between single-nucleotide polymorphisms (SNPs) of LSAMP gene from chromosomal region 3q13.2-q21 and schizophrenia. The association study design was used: 22
SNPs covering the LSAMP gene were analyzed in 127
unrelated patients and in 171 healthy control subjects. All
subjects were individuals of Caucasian origin living in Estonia. The most common diagnose of patients was paranoid schizophrenia. The SNPlex Genotyping System and tetra-primer ARMS-PCR method were applied for genotyping, following association and haplotype
analyses with Haploview program. Association analysis revealed the most prominent associations with SNPs rs16824691 and rs9874470 (corrected allelic p values 0.0176 and 0.0033, respectively). Haplotype analysis revealed six haplotype blocks. Significant
haplotypic associations confirmed allelic associations. These results suggest that LSAMP gene from 3q13.2-q21 chromosomal region may possibly be related to schizophrenia.

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P06.41

Transcriptomic profile of microRNAs and protein coding genes in peripheral blood of schizophrenia patients

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MicroRNAs (miRNAs) are non-coding RNA molecules that are now thought to regulate the expression of many mRNAs. There is strong evidence that in schizophrenia inadequate or mistimed expression of a functional protein may occur due to post-transcriptional events such as abnormal miRNA regulation of a normal gene expression.
The present study aims to shade a light on the epigenetic aspect of schizophrenia pathophysiology. Therefore, we explore the dysregulation of miRNA expression levels and profile the transcriptom of protein coding genes of peripheral blood pooled samples from 30 schizophrenia patients and 25 healthy matched controls.
From microarray expression of 1898 miRNA in schizophrenia and control groups, 25 miRNAs were differentially expressed (p>0.05), where 15 are up-regulated and 10 down-regulated.
Performed digital gene expression tag profiling showed 1012 up-regulated and 2582 down-regulated genes. By qRT-PCR, 8 of the dysregulated miRNAs were tested and the analysis validated 7 of them (down-regulated hsa-mir-320c, hsa-mir-320a, hsa-mir-3173-5p, hsa-mir-421 and up-regulated mir-106a-5p, hsa-mir-192-5p and hsa-mir-222-3p).
Web based tool was used to assess potential miRNA-mRNA interactions. Among the predicted targets of dysregulated miRNAs, we discovered specific differentially expressed genes in periferal blood transcriptome, that were previously associated with schizophrenia (STAT3, BCL2L11, RAD9A, ERBB2, MYB and TFRC genes). Findings from this study strongly suggest that dysregulation of miRNA expression in periferal blood in patients with schizophrenia: 1) can contribute to the observed alterations in protein coding gene expression; 2) may lead to the pathophysiological conditions underlying schizophrenia; 3) provide an empirical source of non-invasive biomarkers for schizophrenia.

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P06.42

Sequencing of the 22q11.2 deletion in VeloCardioFacial syndrome to identify genetic variants predisposing to Schizophrenia

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Hemizygous deletions of a 3Mb region of chr22q11.2 region result in the Velo-cardio-facial syndrome (VCFS). The incidence of schizophrenia in VCFS

patients is 30x higher than in the general population making the del22q11.2 one of the most important risk factor for schizophrenia. The 22q11.2 deletion reduces the normal diploid to an haploid state and therefore can result in the unmasking of otherwise recessive alleles that may remain on the intact homolog. By sequencing the remaining copy of the chr22q11.2 in VCFS patients with and without psychosis/schizophrenia, we expect to identify variations in genes or functional elements that could increase the risk for this psychiatric phenotype.
We have selected 67 VCFS patients; 37 of them showed no psychiatric symptoms, and the rest (n=30) showed psychosis, schizoaffective disorder or schizophrenia. The whole 3Mb region on chr22q11.2 was captured and sequenced using Agilent and Illumina technologies, respectively. Genetic variants were called using an pipeline based on current best practices. The complete haplotype of the targeted 22q11.2 region was created for each subjects and compared between the two diagnostic groups. Two different loci showed a clustering of positive signals (pvalue < 0.001); a locus containing the TXNRD2, COMT and ARVCF genes which are involved in regulation of redox environment, degradation of catecholamine transmitters and adherens junction complexes, respectively and a locus containing the RTN4R gene which plays a role in axonal regeneration and plasticity. The sequencing of the remaining allele of microdeletion syndrome provides an excellent opportunity to identify risk variation for complex phenotypes.

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P06.43

Resequencing of *TBX1* Gene as a Candidate of Schizophrenia in Han Chinese Population

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Recent studies substantiate a higher-than-expected frequency of schizophrenia in patients with 22q11.2 deletion syndrome (22q11DS), suggesting that chromosome 22q11.2 harbors the susceptible genes related to the pathophysiology of schizophrenia. TBX1 (T-box 1), a member of the T-box transcription factor family, is of particular interest for the identification of a family with a 23-bp frameshift deletion of *TBX1* and schizophrenia. We used the systemic mutation detection approach to identify any disruption or rare mutations in *TBX1* among 500 healthy controls and 500 non-22q11DS schizophrenic patients. A total of 39 genetic variants of the *TBX1* were identified in this study, including 16 known SNPs and 23 rare mutations. Six known SNPs in which minor allele frequencies were above 5 % were selected to analyze the association between the genotype and allele frequencies of these SNPs and schizophrenia. Twenty three rare variants including five missense variants were identified. Three missense mutations (p.Asp151Glu, p.Glu257Ala, p.Arg342Gln) were only detected in schizophrenic patients, and one (p.Asp155Asn) was only detected in control group. The mutation of p.Ala393Thr was found in both groups. There was no increasing burden of these missense mutations being found in the patient group (p= 0.4812). Missense mutations were examined by the amino acid analysis programs Polyphen-2 and SIFT to identify those predicted to be possibly or probably deleterious to protein function. Our study suggests some private genetic variations might occur in *TBX1* gene, and their relationship to the pathogenesis of schizophrenia needs further investigation.

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P06.44

Genome-wide copy number variant analysis in a large sample of suicide attempters

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Suicidal behaviour is influenced both by environmental and genetic factors. Based on twin studies, its heritability was estimated to be ~30%. A small number of potential suicide susceptibility genes have been identified. In order to unravel the underlying genetic architecture, additional studies are warranted. It is reasonable that these will include the analysis of copy number variants (CNVs). Rare CNVs have been identified as a risk factor for psychiatric disorders such as schizophrenia (SCZ).
Recently, the first CNV analysis in 189 patients with major depressive disorder (MDD) who attempted suicide was published (Perlis et al. 2012). The aim of our study was to further elucidate the potential role of CNVs as a risk factor for suicidal behaviour in a larger sample.
We used genome-wide SNP array data from: 1637 patients with SCZ (~27% attempted suicide), 882 patients with bipolar disorder (BPD;~35% attempted suicide), and 575 patients with MDD (~30% attempted suicide). All individuals were genotyped on HumanHap550v3, Human610-Quadv1, or Human660W-Quad arrays (Illumina, USA). CNVs were identified using QuantiSNP and PennCNV.
Two separate analyses were performed: genome-wide CNV analysis and CNV screening in genes reported by Perlis et al. (2012). The genome-wide CNV analysis is still ongoing and the results will be presented at the conference. In LOC339822 (Perlis et al.), duplications in five patients were detected. Three patients (two SCZ and one BPD) attempted suicide while one of the two affected MDD patients reported severe suicidal ideation. LOC339822 is in close proximity to SNTG2, a gene implicated in psychiatric disorders.

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P06.45

Effect of antipsychotics drugs in gene expression and promoter methylation of neurotransmisson genes in brain of Spontaneously Hypertensive Rats (SHR)

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Recently our group suggested the SHR strain as an animal model to study schizophrenia aspects. Our aim was to verify treatment effect with antipsychotics drugs (APD) on the expression of 84 genes and the promoter region methylation of differentially expressed genes in the prefrontal cortex (PFC) and nucleus accumbens (NAC). Each group was composed of 8 rats treated 30 days with saline, risperidone, clozapine (CL) or haloperidol (HA). To expression analysis we used the PCRarray and t-test to investigate the significance, considering p≤0.001. Four genes were downregulated in PFC: Brs3 (CL: p=0.001; HA: p=0.0007), Drd2 and Drd3 in CL (p=0.001, p=0.0004) and Glra1 in HA (p=0.0008). Only Glra1 promoter showed hypermethylation in treated group (p=0.027). Drd2 and Drd3 downregulation demonstrate APD are playing a role in dopaminergic pathway of SHR. Brs3 is a bombesin neuropeptide receptor modulating pathways including dopaminergic. Studies found bombesin reduction in cerebrospinal fluid of schizophrenia patients and decreased social interaction in Brs3 knockdown mice. Our results suggest Brs3 dowrexpression may be correlated to elevated social interaction after APD, described previously in SHR. There is no study involving Glra1 and APD, however, the downregulation in expression and hypermethylation of promoter in the treated group suggest this drug can change the Glra1 expression by an epigenetic mechanism. In conclusion, our study indicated two genes related to APD action, pointed new pathways whereby APD may be acting and, that changes in the pattern of Glra1 expression could be modulated by hypermethylation of its promoter region. Financial Support: Fapesp 2010/0968-6.

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P06.46

Candidate genes for sleepwalking from exome sequencing in an autosomal-dominant sleepwalking family

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Sleepwalking is a common childhood parasomnia that seldom persists into adulthood. Twin studies have shown higher concordance in monozygotic twins and relatives of sleepwalkers have a 10-fold increase in disease risk. A linkage study has suggested a candidate region on chromosome 20, but no responsible gene has been identified. We performed exome sequencing in an autosomal-dominant sleepwalking family. We sequenced two affected siblings and the unaffected mother using Agilent SureSelect All Exome Kit for enrichment and Illumina 100bp paired-end reads for sequencing. We generated on average 14.9Gb of sequence with 89% covered >= 20x. For candidate variant identification, we used an autosomal-dominant disease model and looked for heterozygous missense, nonsense, splice-site, stoploss and frameshift variants shared by the affected siblings. We excluded variants present in dbSNP135, the 1000genomes data, and an in-house exome database of unrelated phenotypes with a MAF > 1%. Subsequently, we excluded those variants shared by the two siblings but also found in the unaffected mother. This approach left 47 variants for co-segregation analysis in the pedigree. Of these 47 variants, 23 were compatible with the disease segregation pattern observed in the pedigree. The respective candidate genes include *C1R*, a component of the complement system, and *PZP*, which has been implicated in Alzheimer's disease. Sequencing of the variant-containing regions of the candidate genes in a cohort of 30 parasomnia cases detected neither the candidate nor additional variants in these genes. We are currently expanding the sleepwalking cohort in order to pinpoint the causal variant in the family.

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P06.47

Increased prevalence of sex chromosome aneuploidies in Specific Language Impairment and Dyslexia.

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Specific language impairment (SLI) and dyslexia are developmental disorders exhibiting deficits of spoken (SLI) or written (dyslexia) language in the absence of comorbid neurological deficits, despite adequate intelligence and education. Sex chromosome aneuploidies increase the risk of spoken or written language disorders but, compared to other developmental disorders, e.g. autism, individuals with SLI or dyslexia do not routinely undergo cytogenetic analysis.
To assess the frequency of sex chromosome aneuploidies within individuals with SLI or dyslexia, genome-wide single nucleotide polymorphism genotyping was performed in three sample sets: a clinical cohort of individuals with SLI referred to a child development centre (87 probands), a replication cohort of individuals with SLI, from both clinical and epidemiological samples (209 probands) and a set of individuals with dyslexia (310 probands).
In the clinical SLI cohort, three abnormal karyotypic results were identified in probands, representing a proband yield of 3.4%. In the SLI replication cohort six abnormalities were identified providing a consistent proband yield (2.9%). In the sample of individuals with dyslexia, two sex chromosome aberrations were found giving a lower proband yield of 0.6%. In total two XYY, four XXY (Klinefelter syndrome), three XXX, one XO (Turner syndrome) and one proposed XO/XY mosaic karyotype were identified.
The frequency of sex chromosome aneuploidies within each of the three cohorts was increased over the expected population frequency (approximately 0.1%) suggesting that genetic testing may prove worthwhile for individuals with language and literacy problems, enabling therapies associated with these sex chromosome abnormalities to be implemented more promptly.

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P06.48

Identification of a genomic homozygous deletion of *ZNF277* in a child with SLI

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Specific language impairment (SLI) is a common neurodevelopmental disorder in which language abilities are below age expectations, in the absence of explanatory environmental or medical conditions, such as hearing loss, intellectual disability or autism. SLI affects 3-7% of English-speaking preschool children. During a genome-wide CNV scan using a multi-algorithm approach, we identified a homozygous deletion of 21,379bp in the *ZNF277* gene, overlapping exon 5, in an individual with severe receptive and expressive SLI. This deletion was of particular interest as it falls within the AUTS1 region of linkage to autism. *ZNF277* flanks the *DOCK4* and *IMMP2L* genes, which have been suggested to play a role in autistic spectrum disorders (ASD). We therefore screened cohorts of children with SLI or ASD and control subjects for the presence of *ZNF277* deletions. We observed an increased frequency of *ZNF277* deletions in probands with SLI (6/318, 1.9%) compared to both probands with ASD (1/253, 0.4%) and independent controls (2/224, 0.8%). We performed quantitative PCR analyses of the expression of *IMMP2L*, *DOCK4* and *ZNF277* in lymphoblastoid cell lines carrying either a *DOCK4* microdeletion or a *ZNF277* microdeletion. We found that, while *ZNF277* microdeletions affect the expression of *ZNF277*, they do not alter the levels of *DOCK4* or *IMMP2L* transcripts. Similarly, *DOCK4* microdeletions do not affect the expression levels of *ZNF277*. Given these findings, we postulate that *ZNF277* microdeletions may contribute to the risk of speech and language impairments in a manner that is independent of the autism risks previously described in this region.

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P06.49

A balanced translocation t(3;9)(q25.1;q34.3) leading to *OLFM1* fusion transcripts in a patient with Tourette syndrome and comorbidity disorders

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Karyotype analysis of a male with Tourette syndrome (TS), obsessive-compulsive-disorder and attention-deficit-hyperactivity-disorder revealed an apparently balanced and maternally inherited translocation, t(3;9)(q25.1;q34.3). The mother had tics, but no TS-diagnose. Using mate-pair sequencing we mapped the translocation breakpoints. The 9q breakpoint truncated the *OLFM1* gene, while the 3q breakpoint was within a region without known protein coding genes. However, *in silico* analyses revealed that this breakpoint truncated two
transcripts of unknown coding potential. Since translocations can unmask recessive mutations, sequencing of the *OLFM1* gene and the unknown transcripts was performed; however, no mutations were identified. Reverse transcription of RNA from blood of both individuals identified two fusion transcripts including the 5'-end of *OLFM1* and the 3'-end of either of the two

unknown transcripts, respectively. *In silico* analysis revealed open reading frames for both transcripts and resulting protein products predicted to encode a truncated OLFM1 protein lacking the olfactomedin domain.
Although widely expressed in neuronal tissues, the function of OLFM1 is relatively unknown. OLFM1 has not been directly associated with any neuropsychiatric disorders, but has been shown to interact with several proteins encoded by schizophrenia susceptibility genes. We therefore suggest that *OLFM1* could be involved in the TS/tic-symptoms of the patient and his mother, either through haploinsufficiency of *OLFM1* or presence of fusion transcripts/proteins. *OLFM1* expression levels will be measured in both individuals and *in situ* hybridization will be used to investigate whether the unknown transcripts show brain specific expression.

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P07.01
R521C mutation in the FUS gene in a large Italian family

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FUS/TLS gene mutations were identified in familial and sporadic amyotrophic lateral sclerosis (ALS). Genotype-phenotype correlation for this gene is mostly characterized by early age at onset, high rate of progression, early involvement of neck and axial muscles and predominant lower motor neuron involvement, but a high variability has been described.
We studied a four-generation family in which eight individuals of both sexes were affected by motor neuron disease. The pedigree showed a dominant pattern of inheritance. Neurological examination was performed and these patients were clinically diagnosed as ALS. We performed mutational analysis of FUS exons 5, 6, 12, 14 and 15 in the proband, resulted negative for mutation in *SOD1, ANG, VAPB* and *TARDBP* genes, identifying the known heterozygous missense mutation c.1561C>T (p.R521C) in exon 15. Further analysis demonstrated the same mutation in the DNAs from the proband's affected mother and the paucisymptomatic aunt, confirming segregation of the mutation with disease in this family.
R521C is the most common dominant missense mutation in familial ALS related to FUS gene. However this is the first report of a large four-generation Italian ALS family with this mutation. The genotype-phenotype correlation in this family confirmed that the expression of FUS R521C mutation extends beyond classical ALS. Early involvement of neck and proximal limb muscles and a predominant LMN phenotype were confirmed as typical features, but the phenotypic expression may be characterized by a high variability in terms of age at onset and site of onset of the disease also within the same family.

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P07.02
Mitochondrial network genes in skeletal muscle of amyotrophic lateral sclerosis patients

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Recent evidences suggested that muscle degeneration might lead and/or contribute to neurodegeneration and play a key role in the etiopathogenesis and progression of amyotrophic lateral sclerosis (ALS) (Dobrowolny et al, J Cell Biol 2005; Wong et al Hum Mol Genet 2010). To test this hypothesis, this study attempted to categorize the functionally relevant genes within the genome-wide expression profile in the skeletal muscle of ALS patients, using microarray technology and gene regulatory network analysis. The correlation network structures significantly change between patients and controls, indicating an increased inter-gene connection in patients compared to controls.
Looking at the biological functions of the strongly connected genes of the ALS patients, a large number of mitochondrial genes belonging to the oxidative phosphorylation pathway were represented.
On the whole the perturbation of gene expression occurring in the muscle of affected individuals involves genes that share relevant functional connections. The network observed in the ALS muscles includes genes (PRKR1A, FOXO1, TRIM32, ACTN3, among others), whose functions connect the sarcomere integrity to mitochondrial oxidative metabolism. In particular connection between PRKAR1A, FOXO1 and the ubiquitin ligase TRIM32 provide some hints towards the delineation of the molecular events associated to human muscle atrophy.
In conclusion, the results obtained in this study, supported by some of the most recent literature data, could pave the way to future targeted studies focusing on the functional link between genes involved in metabolic pathways and muscle contractility.

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P07.03
Deletion of UBE3A gene in brothers with Angelman syndrome close to breakpoint of inversion at 15q11.2 and 15q26.1

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Angelman syndrome (AS) is characterized by severe intellectual disability with major speech impairment, ataxia and behavioral uniqueness. The underlining molecular deficit is the failure of maternal copy of the imprinted UBE3A gene due to maternal deletions, the most common present in 70-75% of case, which is detected by FISH of the UBE3A region. Only a small number of intragenic or whole genomic microdeletions of UBE3A have been reported. We report on two brothers with AS with microdeletion of 15q11.2-q12 emcompassing UBE3A gene, close to breakpoint of inversion at 15q11.2 and 15q26.1. The brothers were the first and second children of unrelated and healthy parents. They were born at term after uneventful pregnancy. Patient 1 (older brother) had developmental delay, walked with ataxic gait, and did not speak word at 6 years old. He developed seizure at 4 years-old. Patient 2 (younger brother) had also developmental delay and started sitting at 15 months. They had mild phenotype and behavior of AS. Karyotype revealed pericentric inversion of 15q and FISH test revealed deletion of UBE3A region. Array CGH revealed 467kb deletion at 15q11.2-q12, only emcompassing UBE3A and a part of SNORD115, and 53kb deletion at 15q26.1. Their mother revealed normal karyoptype and no deletion of 15q11.2-q12 with array CGH, so we assumed germline mosaicism. This report is a rare familial case with AS detected by routine FISH test. We suggest that array CGH can detect atypical submicroscopic deletion of UBE3A in patients with mild AS phenotype.

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P07.04
Altered splicing of the BIN1 muscle-specific exon in human and dogs with highly progressive centronuclear myopathies

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We have identified a splice mutation of the muscle-specific exon 11 of BIN1 as the genetic cause of the Inherited Myopathy of Great Danes (IMGD). This severe canine muscle disorder typically starts before 10 months of age with severe debilitating muscle weakness. BIN1 codes for amphiphysin 2, a key factor in muscular membrane remodeling implicated in excitation-contraction coupling (ECC). The muscle-specific exon 11 codes for a phosphoinositide-binding domain, which is essential for the membrane-deforming properties of amphiphysin 2. In humans, BIN1 mutations have been associated with centronuclear myopathy, showing an abnormal internalization of nuclei on muscle biopsies. All mutations were found in the ubiquitous exons and together with the IMGD model we present the first mutation in humans affecting the muscle-specific exon 11. Both human and canine mutations have a strong impact on the splicing of exon 11 and patients and dogs have a very similar etiopathology. Comparative histological and ultrastructural analyses demonstrated striking similarities between the human and the canine conditions and immunolabeling revealed a defective structure of the triad, harboring the ECC machinery. Myotubes transfected with the exon 11 containing isoform showed massive tubulation, whereas the construct

without exon 11 did not have this effect. Our data suggest that the amphiphysin 2 muscle isoform plays an important role in triad buildup and/or maintenance and that defective excitation-contraction coupling is a primary cause of the progressive human and dog diseases. The identification and characterization of a spontaneous canine model represents a faithful model for preclinical trials of potential therapies.

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P07.05

First French family of cerebello-cerebral atrophy with new SEPSECS mutations

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Progressive cerebello-cerebral atrophy is an extremely rare entity described in 2003 in less than 10 patients (Ben-Zeev *et al.*). Clinical findings consist of severe spasticity, profound mental retardation with progressive microcephaly. Brain imaging shows progressive cerebellar atrophy followed by severe cerebral atrophy. The disease is caused by mutations in the *SEPSECS* gene. The Sepsecs protein needs a cofactor, pyridoxal phosphate (PLP), and is involved in the syntheses of Selenocysteine, an essential component of the active sites of some enzymes (selenoenzymes). We describe a family with 2 affected children. Patient A is the 2nd child of non-consanguineous parents. The boy was born with normal parameters, after an uneventful pregnancy. Bilateral adductus thumb were noticed at birth and generalized stiffness appeared rapidly with tetra spastic paraplegia. He developed with a profound mental retardation. His best milestones were smiling at 4,5 months and head control at 24 months. Spontaneous movements were very poor. He developed severe postnatal microcephaly (-3SD at 6 months and -7SD at 7 years). Successive brain MRI found progressive cerebellar atrophy, rapidly followed by severe cerebral atrophy. Patient B is the 4th child of the family. Recurrence of the disease was suspected in the young sister of patient A because she displayed dystonic movements, stiffness of the trunk at 3 months and progressive microcephaly. *SEPSECS* sequencing revealed in the affected children, 2 novel mutations: a splice mutation predicted to affect splicing of exon 1, and a missense predicted to disrupt folding of the protein or affect binding with PLP.

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P07.06

Phenotypic spectrum of *COL4A1* mutations: porencephaly to schizencephaly

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Recently, *COL4A1* mutations have been reported in porencephaly and other cerebral vascular diseases, often associated with ocular, renal and muscular features. In this study, we aimed to clarify phenotypic spectrum and incidence of *COL4A1* mutations.
We screened for *COL4A1* mutations in 61 patients with porencephaly and 10 patients with schizencephaly, which may be similarly caused by disturbed vascular supply leading to cerebral degeneration, but can be distinguished depending on time of insult.
COL4A1 mutations were identified in 15 patients (21%, 10 mutations in porencephaly and 5 mutations in schizencephaly), who showed a variety of associated findings including intracranial calcification, focal cortical dysplasia, pontocerebellar atrophy, ocular abnormalities, myopathy, hyper-CK-nemia, and hemolytic anemia. Mutations include 10 missense, a nonsense, a frameshift, and three splice site mutations. Five mutations were confirmed as de novo events. One mutation was co-segregated with familial porencephaly, and two mutations were inherited from asymptomatic parents. Aberrant splicing was demonstrated by reverse transcriptase PCR analyses in two patients with splice site mutations.
Our study first confirmed that *COL4A1* mutations are associated with schizencephaly and hemolytic anemia. Based on the fact that COL4A1 mutations were frequent in patients with porencephaly and schizencephaly, genetic testing for *COL4A1* should be considered for children with these conditions.

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P07.07

A novel mutation in the FRMD7 gene in an Iranian pedigree exhibiting congenital Nystagmus: A case report

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Congenital nystagmus is a genetically heterogeneous disorder characterized by repetitive, involuntary, to and for oscillation of the eyes, which may be inherited with the pattern of X-linked, Autosomal dominant and rarely Autosomal recessive. To date, three X-linked types of congenital nystagmus (NYS1, 5 and 6) have been introduced, which the most prevalent form (NYS1) results from mutations in the FERM domain-containing-7 gene (FRMD7). Here, we report a large 5-generation Iranian pedigree consistent with the pattern of recessive inheritance. Twelve individuals in the pedigree were suffering from congenital nystagmus and all of them presented involuntary, pendular movement of the eyes, and decreased visual acuity without any neurologic abnormalities. STR markers' analysis did not show any linkage with known autosomal recessive gene. Due to large locus heterogeneity and absence of net inheritance pattern in this family, we performed exome sequencing to elucidate the pathogenic mutation.
A novel truncating mutation (Q13X) in exon 1 of FRMD7 was detected in the proband, which was confirmed by direct sequencing. This mutation co-segregated with the disease in the family and we could not find this mutation in 100 chromosomes from ethnically matched healthy controls by restriction fragment length polymorphism (RFLP) technique.

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P07.08

Salbutamol-responsive limb-girdle congenital myasthenic syndrome due to a novel missense mutation and heteroallelic deletion in MUSK

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Congenital myasthenic syndromes (CMS) are clinically and genetically heterogeneous disorders characterized by a neuromuscular transmission defect. In recent years, causative mutations have been identified in at least 15 genes encoding proteins of the neuromuscular junction. Mutations in MUSK are known as a very rare genetic cause of CMS and have been described in only 3 families, world-wide. Consequently, the knowledge about efficient drug therapy is very limited. We identified a novel missense mutation (p.Asp38Glu)

heteroallelic to a genomic deletion affecting exons 2-3 of MUSK as cause of a limb-girdle CMS in two brothers of Turkish origin. Clinical symptoms included fatigable limb weakness from early childhood on. Upon diagnosis of a MUSK-related CMS at the age of 16 and 13 years, respectively, treatment with salbutamol was initiated leading to an impressive improvement of clinical symptoms, while treatment with esterase inhibitors did not show any benefit.
Our findings highlight the importance of a molecular diagnosis in CMS and demonstrate considerable similarities between patients with MUSK and DOK7-related CMS in terms of clinical phenotype and treatment options.

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P07.09
Combined linkage analysis and exome sequencing in a large Italian family with distal myopathy

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Distal myopathies (DM) belong to a larger group of rare progressive genetic disorders characterized by atrophy and weakness of the voluntary distal muscles of the upper and lower limbs. Based on clinical and molecular findings, more than 20 different genetic entities have been reported to date and for 14 of them, the causative gene has been identified. We studied a large Italian family affected by an autosomal dominant form of DM characterized by adult onset of the disease and a more prominent involvement of hand muscles. After exclusion of known DM-causing genes, we performed a genome-wide linkage analysis with high density SNP-array and identified 3 regions co-segregating with the disease in all affected subjects. Two patients were then subjected to whole exome sequencing and the obtained sequence variants were prioritized in accordance with linkage information. We identified a heterozygous mutation in the CHRNE gene (L241F) which encodes for the ε-subunit of muscle nicotinic acetylcholine receptors (AChR). This mutation has been already associated to slow-channel congenital myasthenic syndrome (SCCMS), a disease clinically characterized by the selective weakness of cervical, scapular, and finger extensor muscles. Interestingly, all patients share a second functional relevant mutation in a gene located in the same linkage region. Despite the recognized power of combining exome sequencing and linkage information for studies of mendelian disorders, we cannot rule out the possibility that the phenotype in our family may result from the contribution of multiple rare mutations.

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P07.10
Novel phenotypic variant of MYH7 mutation associated with dominant distal myopathy in a Roma family

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We describe a Hungarian Roma family, in which the clinical picture originally raised the possibility of a novel form of autosomal dominant atypical spinal muscular atrophy. To identify the causative genetic abnormality, whole-exome capture and next-generation sequencing was performed in two affected girls and their unaffected father. The analysis unexpectedly revealed the *MYH7* mutation c.4849_4851delAAG (p.K1617del) in both affected girls, a mutation previously reported to be causative for Laing distal myopathy. Subsequent dideoxy sequencing confirmed the same mutation in the affected mother and her third affected daughter. In contrast to reported cases of Laing distal myopathy, our patients presented a more severe phenotype, with proximal and distal muscle involvement, and loss of ambulation at the age of 27 years, in the case of the mother. Furthermore, all three daughters presented with steppage gait at the age of 3 years. Progression of symptoms is variable among the affected family members. Muscle biopsies were non-specific, showing both myogenic and neurogenic lesions. Our family further underlines the importance of whole exome sequencing - as an unbiased strategy - to uncover the causes of often phenotypically indistinguishable myogenic and neurogenic diseases.

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P07.11
Occurrence of a duplication-inverted triplication-duplication (DUP-TRP/INV-DUP) rearrangement mediated by inverted repeats at the DMD locus

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The implementation of newly developed dose-sensitive methods of quantitative analysis results in wide interpretation of complex genomic rearrangements as a cause of hereditary diseases. Sophisticated models of multiple-step pathways for their generation were proposed but still remain controversial. We report a case of complex duplication-triplication rearrangement involving exons 45 to 60 of the DMD gene. These copy number changes were detected by conventional diagnostic methods, and lately confirmed by a custom-designed array-CGH. Successful sequencing of the breakpoint junctions disclosed that a 690-kb region comprising exons from 45 to 60 was duplicated in tandem, and another 46-kb segment containing exon 51 was inserted in between them in inverted orientation, leading to the DUP-TRP/INV-DUP structure recently identified at the PLP1 and MECP2 loci. For the first time in DMD, the elucidation at the nucleotide level of the junctions of such a complex rearrangement evidences that the inverted repeat-mediated mechanism takes place at the DMD locus as two inverted homologous LINE 1 repeated elements were found to be involved in the formation of one of the junctions. The identification of a 13-bp consensus motif CCnCCnTnnCCnC, which specifically binds to PRDM9, a well-established trans determinator of allelic meiotic recombination hotspots in the vicinity of the repeat-junction suggests that the double strand break that initiated the complex DMD recombination may be indeed catalysed by meiotic-specific proteins. While microhomology-mediated processes prevail in simple DMD deletions and duplications, we show that the formation of complex rearrangements follows a different model.

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P07.12
Modelling and severity prediction in Moldavian DMD/DMB patients

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The main task of our study was to evaluate the relationship between the progress and/or severity of DMD/DMB and the four different genetic polymorphisms *MTHFR*C677T and A1298C, *MTR*A2756G, *MTRR*A66G as the main participants in the folat/homocysteine metabolism (FHMG).
Methods: A retrospective long-term follow-up study was carried out in DMD/DMB patients included in the NRHD. The genotyping of dystrophin gene were performed by the MPCR to detect the long deletion in dystrophin gene and the PCR-RFLP to identify the *MTHFR*C677T and A1298C, *MTR*A2756G, *MTRR*A66G polymorphisms using HinfI, MboII, HaeIII and NdeI enzymes respectively. Statistical analysis were included 179 corticosteroids-free DMD/DMB patients using software SPSS 20.0 for Windows 15.0.
Results: **Model A** - dependent variables was wheelchair up 9 years and factors-4 mutation of FHMG - general model fitting is not significant, but -2log-likelihood of reduced model gave the significant parameters estimates for *MTHFR*C677T (p=0.011) and two variants with heterozygous compound polymorphisms of 3 loci (*MTHFR* C677T, *MTHFR*A1298C and *MTR*A2756G (β=33.7) and (*MTHFR*C677T, *MTRR*A66G and *MTR*A2756G (β=34,7) showed as the highest category of dependent variables, respectively, have a high degree of influence on wheelchair up 9 years. **Model C** for appreciating the role of in/out of frame mutation in DMD gene and "modifier genes" and diagno-

sis (DMD/DMB) on up 9 years wheelchair is significant.
Conclusion:Prognosis frequencies that the MDD patient will be in wheelchair up to 9 years if he has out of frame deletion in DMD gene and heterozygous compound of MTR, MTRR and MTFHR1298 and homozygous MTHFRT677T is 75.4%.

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P07.13

Pharmacokinetic studies of 2'-O-methyl phosphorothioate antisense oligonucleotides in mdx mice

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The exon skipping approach is a promising therapy for Duchenne muscular dystrophy (DMD), aiming to correct the underlying genetic defect, which is currently being evaluated in phase III clinical trials. DMD is characterized by the absence of functional dystrophin, an important protein for maintaining muscle stability during contractions and protection against damage, due to frame-shifting mutations and/or premature stop codons. The exon skipping therapy aims to restore the DMD transcript's open reading frame by skipping a specific exon during pre-mRNA splicing with antisense oligonucleotides (AONs). This allows production of partly functional proteins, similar to those found in the typically milder BMD patients. To support clinical studies pre-clinical experiments on optimization of treatment regimens in dystrophin-deficient animal models, i.e. mdx mice, are highly informative. We studied the pharmacokinetic (PK) and pharmacodynamic (PD) profile of 2'-O-methyl phosphorothioate AONs in mdx mice. First, we tested the effect of different dosing regimens, i.e. the same total dosage (200 mg/kg/wk for eight weeks) divided over one, two or seven injections per week. Multiple smaller doses showed increased effects at PK, mRNA and protein level, compared to other regimens. Furthermore, we studied the effect of different maintenance regimens on the preservation of AON-effects, the time-course of the turn-over of the compound and its effect on RNA and protein level. This revealed that the AON and its induced exon skipping could still be detected at least 12 weeks after the last injections and dystrophin protein even up to 24 weeks.

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P07.14

The carrier frequency in the mothers of 158 Japanese cases with Duchenne/Becker muscular dystrophy

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Duchenne/Becker muscular dystrophy (DMD/BMD) is an X-linked recessive muscular disease caused by mutations in the dystrophin gene. Theoretically, two-thirds of the DMD patients are inherited through carrier mothers, however the carrier frequency seems to be different with the type of mutations. The aim of this study was to clarify the carrier frequency in the mothers of DMD patients based on the type of mutation, and to compare with that of BMD. 139 DMD cases and 19 BMD cases were included. Only one patient was included within a same family. Mutations in the dystrophin gene were detected in probands in Kobe University Hospital, and carrier status was determined by molecular analysis in their mothers. 113 cases (99 DMD/14 BMD) and 13 cases (12/1) had deletions or duplications of one or more exons, respectively. Small mutations including nonsense mutation, small deletion/insertion, and splice site mutation, were identified in 32 cases (28/4). In DMD cases, the carrier frequency was 53.5% in the cases with deletion mutations, which was lower than duplication cases (66.7%) or small mutation cases (67.9%). This result suggests that the carrier frequency is different depending on the type of mutations. Deletion mutations seemed to occur more often as de novo mutation. On the other hand, the carrier frequency was 57.6% in DMD cases including all types of mutations, which was significantly lower than that in BMD cases (89.5%) ($p<0.05$). BMD cases can leave offsprings, which results in the higher carrier frequency.

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P07.15

Fragile X syndrome, Duchenne muscular dystrophy and X-linked ichthyosis in a single carrier. How these pathologies are transmitted to the next generation?

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The human X chromosome is characterized by genomic instability and rearrangements, associated with X-linked disorders. Deletions in the Xp22.31 region, involving steroid sulfatase gene (STS) cause X-linked ichthyosis. Rearrangements in the Xp21.2 region are associated with Duchenne/Becker muscular dystrophy (DMD/BMD). The Xq27.3 unstable region, containing the (CGG)n repeated expansion in the FMR1 gene is associated with fragile X syndrome. We report a family with two affected boys, the elder diagnosed as fragile X syndrome, the younger as DMD, and both suffering from severe ichthyosis. The family members were analyzed by PCR, MLPA and genotyping analysis. The mother was proved to be a nonsymptomatic carrier of all three noncontiguous mutational events, involving STS gene, DMD gene and FMR1 expansion. The boy with fragile X syndrome has inherited a recombinant maternal X chromosome, this way inheriting FMR1 expansion and ichthyosis, originating from different X chromosomes and overcoming the DMD gene deletion. The most probable explanation of this finding is DMD gene conversion between the deleted and nondeleted X chromosome and double-strand-break model for recombination with two Holliday joints formation, resulting in recombination of the flanking chromosomal regions. In our opinion, this is the first description of a nonsymptomatic carrier of three different X-linked pathologies, involving severe genetic rearrangements on both long and short arms of the X chromosomes. The transmission of these extremely defective chromosomes in the next generation involves genetic events like gene conversion and recombination, as an attempt to escape severe rearrangements and to try to restore genetic information.

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P07.16

Recovery of Dysferlin Function after Exon-Skipping

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Dysferlinopathies are disabling muscular dystrophies including LGMD2B and Miyoshi myopathy as the main phenotypes. They are associated with molecular defects in DYSF, encoding dysferlin, a key player of sarcolemmal homeostasis. Previous investigations suggest that exon-skipping is a promising therapy for a subset of patients with dysferlinopathies. Such an approach aims to rescue almost functional proteins, when targeting modular proteins and specific tissues. This has been well documented in another myopathy, Duchenne Muscular Dystrophy, for which exon-skipping recently led to encouraging results in phase II/III clinical trials.
We intended to translate into clinics our pre-clinical proof of principle of exon-skipping feasibility for dysferlinopathies, and evaluated the dysferlin function recovery following exon 32 skipping in muscle cells obtained from patients. Exon-skipping efficacy was characterized at the protein level, and by use of new in vitro myotubes formation assays and quantitative membrane repair and recovery tests. Data obtained confirm that dysferlin function is rescued by a quasi-dysferlin expression in treated patients' cells, paving the way towards an antisense based trial in a subset of dysferlin deficient patients.

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P07.17

Clinical and genetic diversity of Emery-Dreifuss muscular dystrophy in Russia

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Emery-Dreifuss muscular dystrophy (EDMD) is a rare genetically heterogeneous muscular disease. In EDMD, 6 causative genes have been identified, including EMD, LMNA, SYNE-1, SYNE-2, FHL1, and TMEM43. Despite the identification of mutations in these genes, no genetic mutation was confirmed in >60% of patients with EDMD, indicating the existence of other causative genes.

We herein present the results of EDMD diagnosing in patients from Russia. We have confirmed the diagnosis in 30 families of the 89 examined. 13 mutations identified in EMD gene, 16 mutations in LMNA gene, and one mutation in FHL1 gene.

In probands with mutations in EMD and LMNA genes onset (1-45 years), severity, and progression of disease are highly variable. Some patients were followed at the cardiomyopathy, and contractures and /or myopathy were later, sometimes a random finding. Creatine kinase (CK) level ranged from normal to 1800 U/L. Only in two probands skeletal myopathy was very heavy, deeply debilitating. In most cases, the lead was cardiac disease. There is no clear genotype-phenotype correlation for LMNA and EMD mutations.

Mutation in FHL1 gene was identified in family with five male patients in four generations. The clinical picture in the examined 35-year-old proband included proximal myopathic syndrome, „rigidity" of the cervical spine, moderate contraction ankles, the high activity of CK (1550 U/L). Significant pathology of heart was not found.

Our observations support the hypothesis of the existence of other causative genes and emphasize the need for further search of candidate genes.

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P07.18

FSHD 1 and 2 testing - a clinical diagnostic service perspective

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FSHD is the third most common muscular dystrophy, characterized by progressive wasting of upper body muscles. ~95% of cases are associated with contraction of D4Z4 tandem repeat in the subtelomere region of 4q35 (FSHD1, OMIM 158900).

Bristol Genetics Laboratory offers a UKGTN specialist diagnostic FSHD service. First level testing for FSHD1 by Southern blotting uses EcoRI/BlnI/ApoI digests and the probe p13E-11 (to determine chromosome of origin and size contraction); where appropriate the permissive haplotype is confirmed. Patients with an extended deletion of the p13-E11 region (~2%) are identified using the D4Z4 1kb probe. The remaining ~3% of patients have clinically indistinguishable FSHD2 (OMIM 158901).

A five year audit of 1190 diagnostic FSHD referrals indicated 37% showed a contraction of the D4Z4 repeat at 4q35 including 6 individuals with two pathogenic 4qA fragments, where further haplotyping was required. 87 patients were referred for extended testing and a deletion was confirmed in 4 patients. 91% of patients showed no deletion of the p13E-11 probe region; for these patients (6.6% of diagnostic referrals) a diagnosis remains on clinical grounds.

Lemmers *et al* (2012) showed 80% of patients with a negative FSHD1 result and D4Z4 hypomethylation have a mutation *SMCHD1* (18p11.23) indicating digenic mode of inheritance for FSHD2; giving a recurrence risk for FSHD2 between 25-50%.

We present a pilot study of FSHD2 testing on 20 clinically typical FSHD1 negative patients (assessed by clinical proforma), a five year audit, and interesting cases highlighting the clinical utility and complexity of FSHD genetic testing.

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P07.19

Genetic epidemiology of Charcot-Marie-Tooth disease in the Voronezh region of Russia

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Charcot-Marie-Tooth (CMT) neuropathy is one of the most common hereditary diseases of the nervous system. The thesis provides information on more than twenty years experience of clinical, electromyographic and molecular-genetic survey the disease in the Voronezh region.

We included 436 patients from 210 unrelated families with CMT to the register for the last period. Prevalence of the disease was established it amounted to 17.4 per 100 000 population. To share CMT1 and CMT2 are 73% and 27%, respectively. Diagnoses of 323 patients from 129 families were verified by molecular-genetic tests. We studied PMP22, CX32, MPZ, MFN2 and HSPB1 genes. Mutations in this genes were found in 61% of all families. The most frequent CMT1 cause in is a duplication of the PMP22 gene (71% of all cases). The frequency of Cx32, MPZ and MFN2 mutation are 13%, 7% and 3% respectively. At CMT2 families mutations were detected in MFN2 (3%) and HSPB1 (7%) genes. Frequent occurrence of the S135F mutation in HSPB1 gene is very interesting. For the first time CMT2F has been mapped by us in the Voronezh family (MIM 606595). We have proved the presence of the founder effect for this mutation in the region. Moreover we identified mutations in GDAP1 (two families) and NDRG1 (one family) in ARCMT.

Analysis of clinical, electromyographic and molecular genetic correlations in CMT-disease allows optimize molecular diagnosis of this group and increase the effectiveness of genetic counseling.

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P07.20

Limb Girdle Muscular Dystrophy in the Czech Republic

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Limb girdle muscular dystrophy (LGMD) is a group of disorders characterised by atrophy and weakness of proximal limb girdle.We perform analysis of the most common LGMD types (LGMD2A, LGMD2D, LGMD2I and LGMD2L) in a cohort of patients with preliminary diagnoses of LGMD at both the mRNA level using reverse transcription-PCR or at the DNA level using PCR and direct sequencing.

LGMD2A is an autosomal recessive disorder caused by mutations in the *CAPN3* gene (15q15) that encodes the muscle specific protein, calpain-3 (p94). LGMD2A is the most frequent form of LGMD in many European countries.LGMD2D is caused by mutations in the α-sarcoglycan gene (*SGCA*). *SGCA* (17q21) is an integral membrane glycoprotein that forms part of the dystrophin associated glycoprotein complex.LGMD2I is caused by mutations in the *FKRP* gene (19q13.3) that encodes a protein which participates in the glycosylation of α-dystroglycan in the muscle fibre. LGMD2L is caused by mutations in the by *ANO5 gene* (11p14.3), which encodes a putative calcium-activated chloride channel possibly involved in membrane repair mechanism in muscular dystrophies.

Using this conventional molecular diagnosis based on a gene-by-gene approach the molecular causes remain unknown for approximately 65 % of patients.

At present, we are introducing next-generation sequencing to accelerate patient diagnosis. We designed capture library to target the coding and all exon-intron boundaries of genes responsible for all known types of LGMD and genes responsible for muscular dystrophy with similar phenotype to LGMD.

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P07.21

Limb-girdle muscle dystrophies mutation analysis in Latvian and Lithuanian patients using Illumina's VeraCode GoldenGate Genotyping Assay

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Limb-girdle muscular dystrophies (LGMDs) is a group of muscular dystrophies characterized by a predominant involvement of the scapula, pelvic girdle and trunk muscles without affecting the facial muscles. Different autosomal recessive LGMDs (>10) have been identified as distinct entities with a similar phenotype and clear clinical overlap that makes their differential diagnosis difficult. To date, several hundreds different mutations have been

described with their biological relevance remaining unclear.
We have developed genotyping microarray of 96 mutations - insertions/deletions and SNP, within different genes related to LGMD-2 (SGCA, SGCB, SGCD, SGCG, CAPN3, DYSF), several polymorphism and gender controls using Illumina's VeraCode GoldenGate Genotyping Assay.
Here we report study analysing six patients from Latvia and 25 patients from Lithuania with clinical symptoms of LGMD, elevated creatine kinase values, EMG data of myopathy and suggestive/putative autosomal recessive inheritance. 204 healthy unrelated randomly selected individuals as control group from the Genome Database of Latvian Population were analysed. After data cleaning was completed, the data analysis revealed mutation CAPN3 550delA in homozygous position in eight cases within Lithuanian group (32%). Mutation was confirmed by direct sequencing in all cases. Controls showed no 550delA, but four other SNPs: DYSF_00178, CAPN3_00119, rs2287717 (one heterozygous allele each) and rs2306942 (four heterozygous individuals).
Our data suggest that CAPN3 550delA, previously referred as Slavic origin, is the most frequent mutation in LGMD patients of Baltic populations as well. Thus, we suggest direct sequencing of 550delA as the first step of LGDM molecular diagnostics before using other high throughput genotyping assays.

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P07.22

Identification and confirmation of homozygous RYR1 mutation status in Malignant hyperthermia individuals.

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Background:
Malignant hyperthermia (MH), linked to the ryanodine receptor 1 gene (RYR1) on chromosome 19, is a potentially fatal autosomal dominant disorder which may lead to a disturbance of intracellular calcium homeostasis when susceptible individuals are exposed to volatile anesthetics.
Methods and results:
Fourteen members of the large pedigree were investigated, with a clinical examination, a caffeine halothane contracture test on the muscle biopsies, and mutational analysis on the genomic DNA isolated from family blood samples. Fifteen RYR1 amplicons covering major hotspots were screened for mutations in the RYR1 by direct sequencing. Two siblings homozygous for the missense mutation R614C were observed. There was no evidence of consanguinity parents. The presence of only mutated allele and the absence of wt allele were confirmed using melting point analysis on real-time PCR. Haplotype analysis using microsatellite markers linked to the RYR1 gene and qHRM were performed to exclude the loss of heterozygosity. The loss of a functional allele at any heterozygous locus can be due to multi locus chromosomal events like deletions and mitotic recombinations or gene conversions at a particular locus. We performed further analysis to exactly define the change in the genome of siblings homozygous for the affected RYR1 allele, e.g.MLPA and Amplicon-Based Ultra-Deep Next-Generation Sequencing.
Conclusion:
We here demonstrate methodical assays and results of confirmation the presence of two MHS RYR1 alleles in the homozygotes.
Genetic characterization was necessary because the sensitivity of muscle sample to caffeine halothane contracture test no clearly distinguished two homozygotes from other heterozygous individuals.

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P07.23

Clinical and genetic study of hereditary motor-sensory neuropathy patients from Republic Bashkortostan with novel mutations in MFN2 gene

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Hereditary motor-sensory neuropathy 2A occurs in 3 % of all HMSN cases in the Republic of Bashkortostan. We found four missense mutations in MFN2 gene, one of them was previously described: c.2113G>A (p.Val705Ile). Mutations c.775C>T(p.Arg259Cys); c.776G>A(p.Arg259His); c.2171T>C (p.Leu724Pro) were novel. These mutations were found in 8 patients from four unrelated families of different ethnic groups. The family with c.2171T>C (p.Leu724Pro) mutation in the MFN2 gene demonstrated is an autosomal-dominant pattern of inheritance. Three patients had apparently sporadic disease. In the families with novel mutations the disease began during the first or the second decade of patient's life. The initial symptoms were muscle weakness and wasting of distal extremities, gait disorder. The clinical picture was presented by progressive weakness and wasting of distal extremities, distal sensory loss, bilateral pes cavus deformity. The postural tremor in the hands was the most common additional symptom. Scoliosis and vasomotor troubles were seen in three of the patients. One female patient with (c.776G>A(p.Arg259His) mutation in the MFN2 gene had dysphagia and dysphonia. Neurophysiological data were available from three patients. All of patients had median motor nerve conduction velocities greater than 38 m/s and reduced compound motor action potential.

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P07.24

VRK1 mutations associated with complex motor and sensory axonal neuropathy plus microcephaly

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Here we report three children from two unrelated families with a previously uncharacterized complex axonal motor and sensory neuropathy accompanied by severe non-progressive microcephaly and cerebral dysgnesis. The patients did not meet clinical criteria for any known hereditary neuro-developmental disorder, and extensive molecular investigations that included sequencing of known hereditary peripheral neuropathy genes failed to delineate the molecular cause of the disease. We performed whole-genome and targeted whole-exome sequencing in these three affected subjects. Using genome-wide sequence analysis we identified compound heterozygous mutations in two affected siblings from one family and a homozygous nonsense mutation in the vaccinia-related kinase 1 (*VRK1*) gene. *VRK1* encodes a serine/threonine kinase that is crucial for cell cycle progression and cell division and is proposed to be involved in nervous system development and maintenance.
It is an early response gene that directly phosphorylates and regulates p53 and has crucial roles throughout the cell cycle. We hypothesise that the VRK1 mutations lead to abnormal neural apoptosis and secondary brain dysgenesis and axonal neuropathy.

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P07.25

In search of genetic modifiers for Emery-Dreifuss muscular dystrophies and related striated muscle laminopathies

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Emery-Dreifuss muscular dystrophy (EDMD) is characterised by early onset joint contractures, humero-peroneal muscle wasting/weakness then and cardiac disease within adulthood. Mutations in the *LMNA* gene, encoding nuclear envelop proteins lamins A and C, are responsible for autosomal dominant EDMD forms. Important intra-familial clinical variability in terms of age at onset, severity and progression of skeletal muscle and cardiac involvements has been reported in several EDMD families with *LMNA* mutations. Modifier genes have been suggested to explain such variability. The p.Glu6Stop *LMNA* mutation was previously identified in a large French family associated with a wide range of age at onset of myopathic symptoms (AOMS) (Becane et al. PACE, 2000). Two modifier loci that could contribute to that variability have been mapped on 2q36-q37 and 6q25-q27 (Granger et al. Hum Genetics, 2011).We have designed a sequence capture array that includes the entire genes and intergenic regions of the 28Mb covering those two loci (198 genes on chr2 and 61 genes on chr6). DNA samples from 19 members of the family carrying the *LMNA* mutation with various ages

at onset of myopathic symptoms (AOMS) have been sequenced using next generation sequencing (NGS). The mean coverage depth was 113x and only 3% regions was not covered. Three phenotypic subgroups were considered including those with AOMS before 20 years, those with AOMS after 30 years and finally those with isolated cardiac disease and without musculoskeletal symptoms. The analysis of sequenced variants will be presented.

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P07.26

Progressive muscular dystrophies with life-threatening arrhythmias

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Cardiomyopathy (CMP) is typical for many muscular dystrophies (MD). A special place takes MD in which CMP characterized by impaired intracardiac conduction with arrhythmias, causing severe complications, including sudden death.

In our work we perfomed clinical and molecular-genetic diagnosis of X-linked Emery-Dreifuss MD (EDMD), associated with EMD gene, autosomal dominant EDMD and limb-girdle MD 1B, associated with LMNA gene, myofibrillar myopathy 1, caused by mutations in DES gene, in MD patients with arrhythmic CMP. Mutations in studied genes were found in 30 families: 13 mutations in EMD gene (13 families), 14 mutations in LMNA gene (16 families), one mutation in DES gene. It is revealed clinically interfamilial diversity and in 6 family cases intrafamilial diversity, especially in families with mutations in LMNA. There is no clear genotype-phenotype correlation for LMNA and EMD mutations. Onset (1-45 years), severity, and progression of disease highly variable. Of the total sample, only one patient with EDMD had a very severe myopathy, in other cases the state is mainly determined by the severity of the CMP. Early, including pre-clinical detection of these MD with molecular genetics verification is important not only for the prevention of new cases in families, but especially for the prevention of fatal cardiac events in patients.

Given the clinical diversity of MD with life-threatening arrhythmias, the indications for DNA-diagnosis should not limit the „classic" phenotype of some forms. On pre-laboratory stage of examination of patients with MD should make greater use of daily holter monitoring.

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Carrier frequency of the c.525delT mutation in gamma-sarcoglycan gene and estimated prevalence of Limb Girdle Muscular Dystrophy type 2C among the Moroccan population

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Autosomal recessive Limb-Girdle Muscular Dystrophies (AR-LGMD) are characterized by clinical and genetic heterogeneity. LGMD type 2C or γ-sarcoglycanopathy is the most frequent in North-African populations, as a result of the founder c.525delT mutation in SGGC gene. Its epidemiology is poorly known in Morocco and its prevalence among the Moroccan population has never been evaluated.

26 patients with a LGMD2C and 45 patients with an AR-LGMD phenotype were screened for the c.525delT mutation. DNA extracted from umbilical cord blood samples of 250 newborns was tested for the same mutation. We used molecular epidemiological methods to calculate the frequency of heterozygotes for this mutation in Moroccan newborns and estimate the prevalence of LGMD2C in the Moroccan population.

The carrier frequency was estimated to be 1/250 which would imply that the prevalence of LGMD2C would be approximately 1/20 492 considering the effect of consanguinity. The homozygous c.525delT mutation was found in 65% of all AR-LGMD.

These findings suggest that AR-LGMD are prevalent in Moroccan population and LGMD2C is one of their most common forms. This might be useful for the development of diagnosis strategies in a large scale for a better management of patients with AR-LGMD and genetic counseling of families.

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P07.28

Next-generation sequencing identifies the causative gene for limb-girdle muscular dystrophy 1F

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Limb girdle muscular dystrophies (LGMDs) are genetically heterogeneous disorders, characterized by a progressive weakness that begins from the proximal limb muscles. In addition to the genetic heterogeneity, the different forms are clinically heterogeneous, with different ages of onset and diverse distribution of the muscle damage. The primary distinction is made between the autosomal dominant (LGMD1) and the autosomal recessive forms (LGMD2). LGMD1F is very puzzling disease. It is characterized by muscle weakness affecting earlier the pelvic girdle. The critical interval was mapped to a 3.68-Mb interval on chromosome 7q32.1-7q32.2. We studied the same linked family with additional family members by exome sequencing using two different NGS platforms. We sequenced four affected individuals and identified a number of new variations: only one of which was completely new, shared by all affected subjects, and mapped to 7q32. They all shared heterozygous frame-shift variant in the Transportin 3 (TNPO3) gene, encoding a member of the importin-β super-family. In addition, we identified an isolated case of LGMD with a new missense mutation in the same gene. The TNPO3 gene encodes a 923-amino acid product that is also expressed in skeletal muscle. We localized the mutant TNPO3 around the nucleus, but not inside. Our present data indicate that TNPO3 is the gene mutated in LGMD1F. The involvement of gene related to the nuclear transport suggests a novel disease mechanism leading to muscular dystrophy.

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Identified novel mutations in two families with limb girdle muscular dystrophy using whole exome sequencing

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Introduction: Neuromuscular disorders are a progressive heterogeneous group with considerable heterogeneity. The frequency of these diseases is one in 3000 to 4000 live births. The most common form of these disorders is muscular dystrophies of which Limb girdle muscular dystrophy (LGMD) has a higher prevalence.

LGMDs are generally characterized by progressive muscle weakness and wasting which are restricted to the limb musculature and caused by autosomal dominant or recessive gene mutations. There are 25 known genetic loci including 21 genes for LGMDs that makes genetic testing for this group of disorders somehow impractical using conventional Sanger sequencing method; however, the availability of next generation sequencing technology has provided an opportunity for identifying the causal mutations in these patients with screening all the known genes together using disease specific gene panel sequencing or whole exome sequencing (WES).

Materials and methods: We ascertained two families with autosomal recessive LGMD with unknown genetic mutations. To identify the causal mutations in these families, WES was performed on affected individuals using Agilent SureSelect V4 exome enrichment kit combined with Illumina Hiseq2000 sequencing.

Results: We identified two novel recessive mutations in CAPN3 gene (c.795_800delCATTGA/ p.266_267delIleAsp and c.1967G>A/p.Arg656Gln) in the germline DNA of the two tested individuals.

Discussion: By using WES for identifying mutations responsible for LGMD in these families, we can obtain better understanding of the genotype-phenotype correlation and provide more focused approach for molecular diagnosis of other families who suffered from this disease.

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P07.30

VAV1 and BAFF polymorphisms predispose to early-onset myasthenia gravis

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Myasthenia gravis (MG) is a heterogeneous autoimmune disease, but patients with early-onset MG (before age 50; EOMG) are a particularly well defined subgroup. Their MG is clearly mediated by autoantibodies against the acetylcholine receptor at the neuromuscular junction. With the goal to identify genetic loci that predispose to EOMG, a two stage genetic association study was performed on 384 SNPs in 35 candidate genes selected based on MG pathophysio-logy. Top hits were replicated in about 1200 EOMG patients and 1000 matched controls collected from nine European centres. First, significant frequency differences were found for 11 SNPs in 6 genes: HLA-DRA, tumor necrosis factor alpha (TNF-α), CD86, AKAP12, VAV1, and B-cell activating factor (BAFF). Second, haplotype analysis supported the SNP association results identifying several haplotypes with a frequency significantly different between MG patients and controls. Both HLA-DRA and TNFα have been previously associated with EOMG, but the association with VAV1 and BAFF are novel. VAV1 is a key signal transducer that is essential for the development and activation of T- and B-cells, and BAFF is a cytokine that plays important roles in the proliferation and differentiation of B-cells. TNF loci showed the largest and most consistent differences, with 20% difference in both allele and haplotype frequencies in each of 9 patient cohorts genotyped. We identified a statistically significant interaction between VAV1 and BAFF, suggesting shared functional pathways, that, if perturbed, might contribute to EOMG predisposition.

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P07.31

Molecular, electrophysiological and behavioural evidence of cerebellar dysfunction in a mouse model of myotonic dystrophy type I

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Myotonic dystrophy type I (DM1) is caused by a non-coding CTG trinucleotide repeat expansion in the DMPK gene. Disease pathogenesis is mainly mediated by a trans-dominant role of toxic DMPK transcripts: CUG-containing RNA accumulates in nuclear foci and perturbs important developmental splicing regulators, resulting in missplicing of downstream genes. The DMSXL transgenic mice generated in our laboratory express expanded DMPK transcripts in multiple tissues, notably in the central nervous system (CNS), recreate important aspects of the disease and provide a useful tool to study DM1 molecular pathogenesis.
Clinical, neuropsychological and imaging evidence have demonstrated the involvement of the CNS in DM1, however, the molecular pathways, neuronal circuits and brain regions preferentially affected remain largely unknown. During the behavioural phenotyping of DMSXL mice, transgenic animals showed impaired motor coordination in the elevated runway test, as well as electrophysiological abnormalities in the cerebellum, suggestive of changes in the firing rate and rhythmicity of Purkinje cells. In addition, we found signs of RNA toxicity in DMSXL cerebellum (nuclear foci, MBNL sequestration, missplicing of downstream genes). Interestingly, RNA toxicity appears to be more pronounced in glial cell subpopulations of the cerebellum, suggesting cells-specific susceptibility.
We have confirmed RNA toxicity in human DM1 cerebellum samples, which show abundant nuclear foci, missplicing events and deregulation of splicing regulators. Taken together, the mouse and human data open the possibility of cerebellar dysfunction in DM1, which may contribute to the neurological manifestations of the disease.

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P07.32

Myotonic dystrophy type 2 - healthy range, premutation range and mutation range alleles in the general population

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Myotonic dystrophy type 2 (DM2) is a multisystemic disorder caused by expansion of the $(CCTG)_n$ repeat tract in the *CNBP* gene (also called *ZNF9*). The CCTG tract is generally interrupted in healthy range alleles and is uninterrupted in pathologically expanded alleles. Our study reports the variability of the healthy range CCTG tracts of the *CNBP* gene in a population of Slovakia. Using repeat-primed PCR, we identified wider range and higher frequency of healthy range alleles containing uninterrupted CCTG tracts, than it was previously reported. Altogether, we identified 12 alleles with uninterrupted tracts, in a range of 12 CCTG repeats up to 70 CCTG repeats and 2 additional alleles exceeding the sizing limit of our assay (>60 CCTG repeats). As uninterrupted alleles were so far reported mainly on larger alleles, they have been considered as possible DM2 premutations. Our findings, however, suggest that uninterrupted CCTG parts are not restricted to large alleles and can be found continuously throughout the whole range of healthy range alleles. In 6 cases we were able to track the inheritance of the alleles. The rs1871922 marker was also genotyped in all of the uninterrupted alleles. Our results suggest that uninterrupted alleles with less than approximately 30 CCTG repeats are likely stable during transmission, while instability increases with increasing length of uninterrupted tracts above this approximate threshold. Unstable DM2 premutation alleles likely contain from approximately 30 to 55 CCTG repeats, as the smallest pathogenic alleles identified so far had 55 and 75 repeats.

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P07.33

Molecular, physiological and motor performance defects in DMSXL mice carrying >1000 CTG repeats from the human DM1 locus

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Myotonic dystrophy type I (DM1) is an autosomal dominant multisystemic disease caused by the expansion of an unstable CTG repeat in the 3' non-coding region of the DMPK gene. DMPK transcripts carrying CUG expansions form nuclear foci and affect splicing regulation of various RNA transcripts. Furthermore, bidirectional transcription over the DMPK gene and non- conventional RNA translation of repeated transcripts have been described in DM1. It is clear now that this disease may involve multiple pathogenic pathways including changes in gene expression, RNA stability and splicing regulation, protein translation and micro-RNA metabolism. We previously generated transgenic mice with 45-kb of the DM1 locus and > 300 CTG repeats (DM300 mice). Recently, mice carrying over 1000 CTG (DMSXL) were obtained due to CTG instability over successive generations. In the present study, we described for the first time, the expression pattern

of the DMPK sense transcripts in DMSXL and human tissues. Interestingly, we also demonstrate that DMPK antisense transcripts are expressed in various DMSXL and human tissues. Molecular features of DM1-associated RNA toxicity in DMSXL mice (such as foci accumulation and mild missplicing) were associated with high mortality and growth retardation. In addition, significant changes in functional properties and morphology of the skeletal muscle were measured in these mice.
These data demonstrate that the human DM1 locus carrying very large expansions induced a variety of molecular and physiological defects in transgenic mice. As a result, DMSXL mice provide an animal tool to decipher various aspects of DM1 mechanisms and for preclinical assay.

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P07.34

New methods for nemaline myopathy diagnostics - NM-CGH microarray and exome sequencing

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Nemaline myopathy (NM) constitutes a heterogeneous group of disorders among the congenital myopathies. Mutations in the nebulin gene (*NEB)* are a main cause of recessively inherited NM. We have identified compound heterozygous *NEB* mutations in 91 families, and homozygous mutations in 14 NM families. The mutations are located all along the 183 exons of *NEB.* Novel mutation detection methods are needed as there still remain dozens of families in whom the causative mutations have not yet been identified. We have approached this issue by designing a custom NM-CGH microarray to detect copy number variations in the currently known seven NM genes. To date we have studied 160 samples from 130 families and identified four novel disease-causing aberrations in *NEB* in four different families. Two of these aberrations are the largest deletions characterized in *NEB* to date (53 and 88 kb) encompassing 24 and 68 exons, respectively. Copy number variation, both deletions and duplications, have also been identified in the triplicate region of *NEB* in 13 different families. Further study is ongoing to elucidate the potential pathogenicity of these variants. In addition to these, one potential mutation is currently being verified in another NM gene besides *NEB*.
We have recently launched an exome sequencing pilot study with the aim to sequence 50 NM samples to find the causative mutations. We believe that the combination of exome sequencing and NM-CGH microarray analysis will accelerate mutation analysis and improve the diagnostics of nemaline myopathy and related disorders.

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P07.35

Genetic findings and detection rate in patients tested for neurofibromatosis over a six years period.

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Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominant disorders, with a worldwide incidence of 1 in 3000 individuals. The diagnosis of NF1 show high variation in expressivity and is usually based on clinical findings. The diagnostic criteria are met in a patient who has two or more of the following main characteristics: six or more café au lait spots, neurofibromas, freckling, optic glioma, iris Lisch nodules, distinctive bone lesion and first-degree relative with NF1. NF1 is characterized by lack of neurofibromin, a GTPase activating protein encoded by the NF1-gene.
Department of Pathology and Medical Genetics, St. Olavs University Hospital has performed genetic testing for NF1 over a six years period. Mutation detection in the NF1 gene is complex due to size of the gene (58 exons), existence of several pseudogenes, lack of clustering of the mutations and great variety of possible changes.
We have performed mutation analysis of the entire NF1 gene in a total of 287 patients since 2006, using different techniques such as high resolution melt (HRM) analysis, multiplex ligation-dependent probe amplification (MLPA), Sanger-sequencing and RNA-analysis.
This study presents the mutation detection rate in NF1 found in our laboratory, both for individuals fulfilling the diagnostic criteria of NF1, and in those with more diffuse symptoms (uncertain NF1 clinical diagnosis). The most common mutations reported found in NF1 patients are nonsense mutations, frameshift mutations and splice mutations. We will present an overview of frequencies of different types of mutations found in our population.

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P07.36

NMD-Chips: a new tool meant to resolve diagnostic enigmas in the post Sanger era

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Duchenne/Becker, limb-girdle and congenital muscular dystrophies represent a wide range of conditions caused by various types of mutations, including point mutations, small indels, and large deletions/duplications. During the two past decades, diagnostic laboratories have been faced with an ever-increasing range of disease genes that could be tested individually by Sanger sequencing. However, since the advent of NGS technologies, it appears to represent a limited approach for those heterogeneous genetic disorders.
The EU-funded NMD-Chip project was meant to design, develop and validate new sensitive high throughput tools to efficiently diagnose patients carrying mutations in genes already known to be involved in neuromuscular disorders (NMDs), but also to identify new disease-causing mutations using a candidate-gene approach. A specific custom in-solution sequence-capture DNA library including 820 genes was developed. Among them, 50 are known to be involved in common NMDs, the others being candidate genes selected from experimental and published data. To validate this approach, we have selected 10 patients: 5 with one known mutation either at hemizygous or heterozygous states, including point mutations and large rearrangements, and 5 with no molecular diagnosis. Sequencing of the captured sequences was performed using HiSeq2000.
Mutations in control DNAs were correctly detected, and we identified the causal mutations in *TRIM32*, *RYR1*, *GNE* and *DMD* genes in 4/5 patients without previous diagnosis. We show that this approach is technically robust and accurate, and also that NGS is likely to represent a time and cost-reducing alternative to the Sanger process, that will accelerate molecular diagnostics of NMDs.

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P07.37

Molecular genetic analysis of eighty eight patients with leukodystrophy reveals eight new mutations in the PLP1 gene

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Introduction. Pelizaeus-Merzbacher disease and its allelic disorder, spastic paraplegia type 2 are X-linked recessive dysmyelinating disorders affecting the central nervous system. Pelizaeus-Merzbacher disease is caused in most cases by either duplication or point mutations in the *PLP1* gene. Less frequently it has been reported the presence of large deletions of the gene. This disease has a wide clinical spectrum and its causing mutations act through different molecular mechanisms. Methods. Eighty-eight male patients from non-related families with leukodystrophy were studied. *PLP1* gene analysis was performed by the Multiplex Ligation-dependent Probe Amplification technique (MLPA) and DNA sequencing, and in duplicated cases of PLP1 do-

sage were completed by using a custom oligonucleotide (8x15K) array-CGH. Results. We have identified 21 patients with mutations in the *PLP1* gene, including duplications, short and large deletions and several point mutations in our cohort. To address the extension of *PLP1* duplications we used a customized array-CGH within PLP1 region at the Xq22.2 area, identifying several duplications and triplications including *PLP1* gene, with sizes ranging from 181 Kb to 5,17 Mb. Conclusions. Mutations found in the *PLP1* gene are the cause of Pelizaeus-Merzbacher disease in around 20% of the patients in this series. Interestingly, we described eight new mutations. Array-CGH results demonstrate that there is not a correlation between the extension of the duplication and the severity of the illness.

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P07.38

A nonsense mutation in the Acid α-Glucosidase gene causes Pompe disease in both humans and dogs

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Pompe disease is a recessively inherited and often fatal disorder caused by the deficiency of acid α-glucosidase, an enzyme encoded by the *GAA* gene and needed to break down glycogen in lysosomes. This glycogen storage disease type II has been reported also in Swedish Lapphund dogs. Here we describe the genetic defect in canine Pompe disease and show that three related breeds from Scandinavia carry the same mutation. The affected dogs are homozygous for the *GAA* c.2237G>A mutation leading to a premature stop codon at amino acid position 746. The corresponding mutation has previously been reported in humans and causes infantile Pompe disease in combination with a second fully deleterious mutation. The affected dogs from both the Finnish as well as the Swedish breed mimic infantile-onset Pompe disease genetically, but also clinico-pathologically. Therefore this canine model provides a valuable tool for preclinical studies aimed at the development of gene therapy in Pompe disease.

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P07.39

Twenty years of experience in the diagnosis of Spinal Muscular Atrophy in Hungary

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Spinal Muscular Atrophy (SMA) represents the second most common autosomal recessive disorder, characterised by degeneration of spinal motoneurons.
Since 1993 blood samples have been collected from 452 Hungarian patients with all types of SMA. Clinical diagnosis of all probands were estabilished by using the inclusion and exclusion criterias. Direct mutation analyses have been performed in the SMN1 gene by using PCR-RFLP and real-time PCR techniques. Homozygous deletions of exon 7 (and 8) of the SMN1 gene were detected in 159 of SMA I patients, 82 of SMA II patients and in 105 of SMA III patients. In 106 (23,5 %) of the patients no homozygouse deletion in the SMN1 gene has been found so we defined them as uncertain cases. Out of the 106,
55 uncertain patients were analysed for the SMN1 exon 7 copy number by quantitative real-time PCR and 21 patients were detected as compound heterozygouse. The second mutation has to be established later on by direct sequencing. For carrier screening, SMN1 copies were determined in 246 related family members and 131 carriers were detected. Prenatal diagnosis for all families with confirmed genetic diagnosis were offered during these twenty years. The outcome of the prenatal testing resulted in 59 affected and 153 unaffected fetuses. Additionally, SMN2 copy number were estimated in 164 patients and good correlation was found between copy number and severity of the disease.
SMA is a common and fatal disorder, therefore carrier detection and prenatal testing is essential for prevention and proper genetic counselling.

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P07.40

Spinal muscular atrophy associated with progressive myoclonic epilepsy is caused by mutations in ASAH1

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Spinal muscular atrophy (SMA) is a clinically and genetically heterogeneous disease characterized by degeneration of lower motor neurons. The most frequent form is linked to mutations of the SMN1. Childhood SMA associated with progressive myoclonic epilepsy (SMA-PME) has been reported as a rare autosomal recessive condition unlinked to SMN1 mutation. Through linkage analysis, homozygosity mapping and exome sequencing in three unrelated SMA-PME families, we identified a homozygous missense mutation (c.125C>T; p.Thr42Met) in the ASAH1 exon 2 in affected children of two families and the same mutation associated with a deletion of the whole gene in the third one. Expression studies of the c.125C>T mutant cDNA in Farber fibroblasts showed an acid ceramidase activity deficiency of 32% of that generated by normal cDNA. More recently, two additional living patients were analyzed: identical *ASAH1* mutation was found leading to reduction of acid ceramidase activity (17-20 % of the control value*)* in patient fibroblasts. Ceramide content was normal. These *in vivo* results confirmed *in vitr*o data. Morpholino knockdown of ASAH1 ortholog in zebrafish led to a marked loss of motor neuron axonal branching associated with increased apoptosis in the spinal cord. Our results reveal a wide phenotypic spectrum associated with ASAH1 mutations. An acid ceramidase activity below 10% results in Farber disease, an early onset disease starting with subcutaneous lipogranulomata, joint pains and hoarseness of the voice whereas a higher residual activity may be responsible for SMA-PME, a later onset phenotype restricted to the CNS and starting with lower motor neuron disease.

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P07.41

Re-interpretation of mutations in the Tudor domain of SMN1 as splicing mutations

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Spinal muscular atrophy, an autosomal recessive neuromuscular disorder, is a frequent cause of death in early childhood. In 95% of the cases, the disease is due to homozygous deletion of the survival motor neuron gene (*SMN1*). In rare cases (about 5%), patients are compound heterozygous with a *SMN1* deletion in one chromosome and a subtle *SMN1* mutation in the other. This study focused on mutations identified in the highly conserved Tudor domain of the SMN protein, a domain encoded by the exon 3 of the *SMN1* gene and where numerous *SMN1* mutations have been detected (n=17). We hypothesized that, besides altering the coding sequence of the gene, a subset of these mutations could also affect the splicing pattern of the SMN1 transcripts. To address this question, we performed *ex vivo* splicing assays using representative minigenes. Our results showed that 5 out of the 17 mutations altered the splicing pattern of exon 3: four by probably altering splicing regulatory elements and one by creating an internal splice site leading to a deletion of part of the exon. Because the effect of this latter mutation was very drastic in the minigene assay and confirmed on the RNA from a patient, we propose that it should be classified not as a missense substitution but as a deleterious splicing mutation. This study brings clues into the splicing regulation of exon 3 and contributes to a better understanding of the structure-function features of the Tudor domain of the SMN protein.

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P07.42

Functional prediction analysis of coding and non-coding regions of TTR gene: insights on phenotype expression of transthyretin-related amyloidosis

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Pathogenesis of transthyretin-related amyloidosis (ATTR) is focused on mutations of TTR protein. Significant heterogeneity in the genotype-phenotype correlation has been reported and other genetic and epigenetic factors may interact on phenotype and penetrance of this autosomal dominant trait. In particular, additional genetic variants in the TTR gene and its surrounding regions may influence the phenotype of the disease. In order to explore this hypothesis, we provided a functional prediction analysis of coding and non-coding variants in TTR gene.
Using databases of 1,000 Genomes project and of Mutations in Hereditary Amyloidosis, we provided a deep analysis of TTR gene. Several bioinformatic tools were used to identify the functional impact of the identified variants, analyzing their potential involvement in disease phenotypes.
Our analysis highlighted the presence of different non-coding variants potentially associated with large impact in TTR gene function. In particular, genetic variation in promoter region and in untranslated regions seems to be involved in the regulation of gene expression. Furthermore, investigation on coding variants (disease-causing mutations, and polymorphisms) suggested that amyloid aggregation variability mong coding variants is present. Finally, combining functional effect of coding and non-coding, we predicted the disease phenotypes associated with different haplotypes.
In conclusion, our data support the hypothesis that genetic variation in cis-regulatory regions affects ATTR phenotypes. Confirmation of these in silico outcomes in ATTR patients may strongly improve the disease genetic testing, enhancing our capacity to assist individuals carries of TTR mutation.

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P08.01

Kohlschutter-Tonz Syndrome*:* Clinical and Genetic Insights gained from sixteen cases deriving from a close knit village in Northern Israel.

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Kohlschutter-Tonz
syndrome (KTZS) (MIM 22675) is a rare autosomal recessive disorder, characterized by intellectual impairment, spasticity, epilepsy and amelogenesis imperfecta. Here we report on 16 cases of KTZS (8 females, 8 males), from 7 families (5 kindreds), originating from the same Druze village in Northern Israel and show that the extent of the convulsive disorder, as regards age of onset, frequency, severity and response to treatment predicts the magnitude of mental and motor deficiencies and that amelogenesis imperfecta and deficient speech occur in all cases. We have recently identified the causative gene and mutation underlying KTZS in our cohort of patients, namely, p.R157X corresponding to *ROGDI*
c.571C>T that creates a premature stop codon in ROGDI homolog (Drosophila), a gene of unknown function. A screen of the healthy Druze population inhabiting the village, revealed a carrier frequency of 1:10. The unique KTZS phenotype that compiles mental and verbal deficiency, motor deterioration, seizures and amelogenesis
imperfecta is common to all affected individuals. However, age at onset and severity of convulsions parallel the progression of mental and motor disability and invariably vary among affected sibs. By late adolescence and early twenties KTZS individuals are bedridden, fed by gastrostomy, spastic, practically with no cognitive and language perception.

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P08.02

POLR3A and POLR3B mutations in 4H syndrome.

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Hypomyelinating leukoencephalopathies form a major group among inherited white matter disorders. Additional clinical findings sometimes allow making a definitive diagnosis. The 4H syndrome has as characteristic features, in addition to Hypomyelination, Hypodontia and Hypogonadotropic Hypogonadism (4H), but dental and hormonal anomalies are not obligatory. Cerebral MRI shows hypomyelination, additionally cerebellar atrophy and relative T2-hypointensity of the lateral thalami, allowing a tentative diagnosis of this disease also in the absence of all clinical features. Recessive mutations in two genes coding for subunits of the RNA polymerase III, POLR3A and POLR3B, were shown to cause this disease.
We sequenced the entire coding region and splice junctions of POLR3A and POLR3B in 28 patients (age 2 - 40 years) with hypomyelination and clinical and/or MRI features suggesting 4H syndrome. All patients were found to carry mutations in POLR3A (7 patients) or POLR3B (21 patients). Eleven new mutations were identified in POLR3A: 10 missense and 1 nonsense mutation. In POLR3B, 17 new mutations were identified: 5 splice site, 3 frameshift, 2 nonsense and 7 missense mutations. Nineteen of the 21 patients with POLR3B mutations shared the common exon 15 mutation (p.Val523Glu), but none was homozygous for this mutation. These results further support POLR3A and POLR3B mutations as the major cause of 4H syndrome and stress that if patients fulfill clinical and/or MRI criteria, the chance of confirming mutations in either of the 2 genes is high. It also makes 4H syndrome the second most common entity after Pelizaeus-Merzbacher disease among hypomyelinating white matter disorders.

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P08.03

Analysis of candidate genes with non-coding hexanucleotide GGGGCC-repeats in French patients with Amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the loss of motor neurons in brain and spinal cord. Large expansions of the non-coding hexanucleotide GGGGCC-repeat in the first intron of the *C9ORF72* gene have been demonstrated to be a major genetic cause of ALS. Interestingly this GGGGCC-repeat could form RNA G-quadruplexes affecting transcription and splicing by sequestration of various proteins, and/or could produce a pathogenic poly-(Gly-Ala) dipeptide-repeat protein. We hypothesized that other genes expressed in brain and spinal cord, and carrying GGGGCC-repeats may be involved in ALS.
Using *in silico* analysis we identified 65 genes in the Human genome (genome browser UCSC) carrying 3 or more GGGGCC-repeats. The repeats were located in the first intron for 28 of them. Some of these genes were expressed in brain and spinal cord (bioGPS.org) and participate in cellular or physiological pathways implicated in neurodegenerative diseases. For example, *NUB1* gene encodes an ubiquitin-related protein which accumulates in neuronal and glial inclusions in various neurodegenerative diseases. Another example is *MARCKSL1*, a gene highly expressed in spinal cord and encoding the myristoylated alanine-rich C kinase substrate implicated in cytoskeletal plasticity during nerve degeneration.
We next developed repeat-primed PCR and Genescan analyses to study the hexanucleotide repeats in these genes in French patients with ALS. Our results on *C9ORF72* show a frequency of pathogenic repeats (>30) in 33% of familial ALS and 3% of sporadic ALS. Preliminary results indicated that no amplification of the repeat was present in *NUB1* and *MARCKSL1* genes in ALS patients.

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P08.04

Mutational analysis of the major Amyotrophic lateral sclerosis genes in French ALS patients

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the selective death of motor neurons in the brain and spinal cord. The aim of our study was to quantify the overall contribution of mutations in the major ALS genes in a cohort of French ALS patients and to perform functional analysis on novel mutations.

We screened the *C9ORF72* hexanucleotide expansion, and *SOD1*, *TARDBP*, *FUS* genes in 118 familial ALS cases (FALS) and 602 sporadic ALS cases (SALS). The frequency of mutations in FALS patients was 33% (17/52) for *C9ORF72*, 11.9% (14/118) for *SOD1*, and 10.5% (2/19) for *TARBP*. In the SALS group, the frequency of mutations was 3% (4/115) for *C9ORF72*, 3.3% (20/602) for *SOD1*, 1.5% (3/195) for *TARBP*, and 0.9% (1/109) for *FUS*. We identified five novel mutations in the *SOD1* gene, three heterozygous nonsens mutations and two heterozygous missense mutations, $SOD1^{V31A}$ and SOD^{E121G}. Motor neurons-like cells NSC-34 over-expressing $SOD1^{31A}$ showed cytoplasmic aggregates and decreased cell viability in presence of oxidative stress (H_2O_2 1mM for 3h).

These results confirmed the major roles of the *C9ORF72* hexanucleotide expansion and *SOD1* gene mutation in the etiology of FALS, and consequently have relevant implications in clinical practice. We described novel mutations in *SOD1* and performed functional analysis to support their implication in the disease. We are currently analyzing whether or not the groups of patients with mutation have differences with regard to age and site of onset, clinical phenotypes, and survival.

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P08.05

Genetic interaction between the *Grm1^{crv4}* and the *SOD1^{G93A}* mutations protects from neurodegeneration, prolongs survival and ameliorates disease progression in the *SOD1^{G93A}* mouse model of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (*ALS*) is a late-onset neurodegenerative disease affecting upper and lower motor-neurons. Different genes and cellular events, including glutamate-mediated excitotoxicity, are though to contribute to *ALS*. Mutations in the superoxide dismutase-1 (*SOD1*) accounts for 20% of familial *ALS*. Our recent studies with mice expressing human *SOD1* carrying the G93A mutation (*SOD1^{G93A}*) indicate that glutamate release is abnormally increased in the spinal cord neurons by mechanisms involving presynaptic metabotropic glutamate type 1 (mGlu1R) and type 5 (mGlu5R) autoreceptors activation and mGlu5R overexpression.

mGlu1R and mGlu5R are the only members of group I mGlu receptors, G protein-coupled receptors implicated in synaptic plasticity, feed-back control of glutamate release and excitotoxicity mechanisms of neurodegeneration.

We thought to explore whether the excessive mGlu1R activity plays a role in the pathogenesis of *ALS*. To this end, we generated mice carrying half dosage of mGlu1R in the *SOD1^{G93A}* background by crossing the *SOD1^{G93A}* mouse with the *Grm1^{crv4}* mouse, which lacks mGlu1R because of a spontaneous recessive mutation, thus providing a genetic tool to evaluate the mGlu1R role in *ALS*. *SOD1^{G93A}Grm1$^{crv4/+}$* mice showed prolonged survival and amelioration in motor skills. Histological studies revealed as mGlu1R half dosage in the *SOD1^{G93A}* background protects motor neurons from degeneration, preserves integrity of mitochondria and reverts astroglia activation. Over-expression of mGlu5 receptors and abnormal glutamate release were also normalized.

These results demonstrate that mGlu1R deletion has a significant impact in *ALS* mice, thus providing the rationale for pharmacological approaches to *ALS* by blocking Group I metabotropic receptors.

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P08.06

Association of ApoE polymorphisms in Bulgarian patients with Alzheimer disease and Frontotemporal dementia.

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Background: Alzheimer disease (AD) is characterized by cognitive impairment and is the most common form of dementia caused by degeneration of the brain neurons resulting in memory and personality disorders. Apolipoprotein E plays important role in cholesterol metabolism, immune regulation, synaptic function, intracellular signaling and Aβ-trafficking. ApoE4 is known risk factor for AD and other diseases.

Materials and methods: In this study 197 patients with AD, 32 patients with Frontotemporal dementia (FTD) and 84 healthy control individuals (HC) were included. ApoE alleles (E2, E3, and E4) and genotypes were determined using restriction fragment length polymorphisms (RFLP) after simultaneous digestion of an amplified segment by AflIII and HaeII. Statistical analysis was done using chi square test.

Results and discussion: The ApoE4 allele frequency showed significant difference between the AD group (22%) and HC group (12%). It was associated with increased risk of AD (P=0.0047, OR=2.11), compared to E2 and E3 alleles. ApoE2 allele was found in 4% of AD and in 9% of HC group and was associated with decreased risk of AD (P=0.012, OR=0.38). ApoE4 allele in FTD (16%) compared to HC group (12%) shows difference as well as ApoE2 allele in FTD (3%) compared to HC group (9%) but did not reach significance due to the small sample size.

Conclusions: Our findings confirm the role of ApoE4 allele as risk factor for AD in Bulgarian patients. The study adds to the understanding of the molecular basis of neurodegenerative diseases and may have implications for diagnostic testing and genetic counseling.

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P08.07

Genome-wide association study of CSF biomarkers for identifying quantitative trait loci influencing the progression from MCI to AD

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Background: CSF biomarkers of t-tau and p-tau_{181p} are predictive of future conversion from mild cognitive impairment (MCI) to Alzheimer disease (AD). Other common genetic risk factors associated with CSF biomarkers that influence progression are still unknown. **Method**: Quality controlled data from 51,4932 SNPs and 177 Caucasian subjects from the ADNI cohort was included in the analysis. The main effect of SNP and SNP/progression interaction was assessed on the CSF biomarkers of t-tau, p-tau181, and two ratios t-tau/Aβ1-42 and p-tau181 /Aβ1-42, respectively. General linear regression models were performed with adjustment for age, sex and APOε4 status.

Results: The two genetic marker rs1445093 and rs12327358 in high linkage disequilibrium (LD) (r^2 =0.9) reached genome-wide significance for association with both endophenotypes t-tau and t-tau/Aβ1-42. These two SNPs are located in the upstream of the gene Netrin receptor DCC for which amyloid precursor protein (APP) functionally acts as a co-receptor to mediate axon guidance [1]. Further investigation is needed to see if these SNPs belong to the promoter or regulatory elements of DCC. We also discovered that SNP rs1983298, in the gene encoding the receptor-type tyrosine-protein phosphatase-like N (PTPRN), differentially affected the CSF ptau levels (p= 6.73E-07) in the MCI to AD converter group and MCI stable group. In situ *hybridization* (*ISH*) showed that PTPRN was specifically expressed in the neurons but that severely degenerated neurons with aggregated ptau are associated with low or no mRNA expression of PTPRN. These genetic markers might be potential prognostic biomarkers and biomarkers for patient segmentation.

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P08.08

Expression Profiling of Medial Temporal Gyrus in Alzheimer's Disease Patients Reveals an Enrichment in Neurotransmission Signaling Pathways

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We performed whole genome differential expression analysis on medial temporal gyrus (MTG) tissue from clinically characterized and neuropathologically verified Alzheimer's disease (AD) cases and controls (ND, n=100 in each group). Quantile normalization followed by moderated t-statistic for differential analysis on data from Illumina HumanHT-12 BeadChip was performed. After P-value (FDR<0.05) and Fold Change (>|0.8|) filtering, we identified 143 DEGs in the AD/ND comparison. The DEGs were enriched for two groups of genes (both underexpressed in AD) belonging to pathways labeled "Synaptic Vesicle Trafficking" (SVT) and "Muscarinic Acetylcholine Receptor Signaling" (MARS, Table 1). The AD/ND comparison when analyzed by gender showed 45 and 328 DEGs for males and females, respectively. SVT was significantly enriched in both gender groups while MARS was only significant in the female group suggesting a potential gender difference in the pathways affected by AD. The major finding in the AD/ND group comparison was the decreased expression of several SNARE complex components involved in synaptic vesicle docking at the presynaptic membrane.
Our data replicates prior findings in AD that include a decreased expression of SVT genes in frontal cortex and a demonstrable SNARE complex protein expression loss in the hippocampus and entorhinal cortex. In conclusion, in the MTG of AD patients we demonstrate a decreased expression of SNARE complex members and highlight that MARS gene expression changes are only found in females.

Tables

Pathway and associated genes with Fold change values	Total Sample	Males	Females
Synaptic_vesicle_trafficking	*P = 1.43E-05*	*P = 1.87E-08*	*P = 1.04E-04*
VAMP2 - Vesicle-associated membrane protein 2	-0.982	-0.987	-0.974
SYT1 - Synaptotagmin-1	-1.418	-1.037	-1.810
SYN1 - Synapsin-1	-0.877	-0.872	-0.881
STX1A - Syntaxin-1A	-0.850	-0.811	-0.887
STXBP1 - Syntaxin-binding protein 1	-1.264	-1.091	-1.440
NSF - Vesicle-fusing ATPase	-1.075	-0.830	-1.327
SYP - Synaptophysin	-0.954	-0.850	-1.058
Muscarinic acetylcholine receptor 1 and 3 signaling pathway	*P = 5.02E-03*	*P = 1.000*	*P = 2.67E-02*
PAK1 - Serine/threonine-protein kinase N1	-0.912	-	-1.137
VAMP2 - Vesicle-associated membrane protein 2	-0.982	-0.987	-0.974
PRKCB - Protein kinase C beta type	-0.994	-	-1.255
STX1A - Syntaxin-1A	-0.850	-0.811	-0.947
GNG2 - Guanine nucleotide-binding protein G subunit gamma-2	-0.874	-	-1.128

Table 1: *Results for pathway analysis for different AD/ND comparisons. Results of Fold Change > |0.8| for associated genes in the pathways are shown. P values for pathway analysis are multiple-testing corrected.*

J. Krate: None. **I.S. Piras:** None. **E.R. Delvaux:** None. **J.D. Nolz:** None. **T.G. Beach:** None. **P.D. Coleman:** None. **A.M. Persico:** None. **M.J. Huentelman:** None.

P08.09

Comparison of amplicon sequencing on Ion Torrent PGM with Sanger- and whole exome-sequencing

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Background: At the Department of Geriatric Medicine, patients with family-history of dementia that indicates autosomal dominant inheritance are referred to mutation screening to identify causative mutations. Generally, Alzheimer patients are screened by Sanger-sequencing for variations in APP, PSEN1 and PSEN2, and patients with frontotemporal dementia are screened for variations in MAPT, GRN, and C9orf72. A less discriminative and a more time-efficient strategy of mutational screening, is to employ massive parallel-sequencing on all possible dementia causing genes in a single analysis.
Aim: To compare the detection of genetic variations between different method/platforms of sequencing and to validate the usefulness of custom AmpliSeq panel for screening of dementia causing genes.
Material: DNA was collected from 10 patients at the Memory Clinic, Karolinska University Hospital, Stockholm Sweden, with an informed consent.
Method: Sanger sequencing was performed on ABI 3100-instrument and massive-parallel sequencing was done on an Ion Torrent PGM with a custom made AmpliSeq panel amplifying 196 different targets in 17 known dementia genes.
Results and Discussion: Sanger-sequencing of the patient DNA identified 134 genetic variations in subsets of the genes. Sequencing on PGM detected 775 variants in all of the genes. Further analysis will compare the correlation between Sanger- and PGM-sequencing to validate the use of massive-parallel sequencing in a clinical setting. The analysis will also compare variants detected by whole-exome sequencing and PGM. Data from the comparison will be presented and compared at the ESHG, 2013 conference.

H. Thonberg: None. **L. Lilius:** None. **C. Forsell:** None. **C. Graff:** None.

P08.10

Positive association of C677T MTHFR polymorphism in Alzheimer Disease

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Alzheimer's disease (AD) is a progressive neurological disorder characterized by loss of memory and cognition. In the last years, many genes were identified as a risk factor for AD through Genome-Wide Association Studies. However, only few of them showed association in case controls studies. Although a meta analysis for C677T *MTHFR* polymorphism to suggest risk for AD, case control studies show discordants results in different populations. The confirmation of positive association of specific polymorphisms in different population can help us to identify the contribution of the particular genes in the etiology of the AD according to the ethnic background. The objective of this work was to analyze through case control study the contribution of C677T polymorphism as a risk factor for AD in population from Vitória (Southeast of Brazil). Eighty-two patients, selected by NINCDS-ADRDA clinical parameters and 182 controls matched by gender, age and ethnicity were genotyped through PCR-RFLP. Positive association was found in CT genotype (OR=1.852; 95% IC.: 1,084- 3,162; p=0.0243), but not in other genotypes or alleles. The very small number of T allele observed in studied population can explain the absence of positive results for TT genotype. This research suggests that carries of C677T *MTHFR* polymorphism has 1,8 more chance to develop AD than other in studied population. This work may improve our knowledge about alleles of risk in AD and could help in future providing a profile of genetic susceptibility for AD in specific populations. Supported by FAPES, FACITEC, and MCTI/CNPQ/MEC/Capes.

F. Paula: None. **D. Camporez:** None. **L. Belcavello:** None. **R.L. Morelato:** None. **I.D. Louro:** None. **M.C.P. Batittuci:** None.

P08.11

UBQLN-2 and *PFN1* mutation screening in French FTLD and FTLD-ALS patients

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Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Lobar Degeneration (FTLD) are two adult onset neurological disorders with overlapping symptoms and clinical characteristics. It is well established that they share a common pathological and genetic background. The most common mutated gene in both conditions is *C9ORF72* but there are a number of other genes responsible in different percentages of a limited number of sporadic and familial cases. Ubiquilin-2 (*UBQLN*-2) is at the moment the only ALS-related gene mapping on the X-chromosome, with mutations in the PXX domain first described in ALS patients with a mutational frequency of 2.6% in familial ALS cases with no evidence of male-to-male transmission. Recently, mutations in profilin 1 gene (*PFN1*) have been identified in patients with familial ALS, suggesting a role for this gene in the pathogenesis of the disease. To determine the genetic contribution of *UBQLN-2* and *PFN1* in FTLD and FTLD-ALS, we screened a cohort of 136 French patients. We did not find any variant in *PFN1* gene, while we identified a novel missense variant (c.1006A>G, p.T336A) in *UBQLN-2* gene in one FTLD patient (0.7%). The variant is not present in dbSNP137, 1000 Genomes database and Exome Variant Server. Its pathogenicity is suggested by the high conservation of Thr336 residue through the evolution but its role is still unclear. We conclude that *UBQLN-2* and *PFN1* mutations are extremely rare in a French cohort of FTLD and FTLD-ALS patients and should not be analyzed systematically.

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P08.12

Mutations in the 3′ Untranslated Region of *FUS* causing FUS overexpression in primary fibroblast coltures are associated with Amyotrophic Lateral Sclerosis.

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Amyotrophic Lateral Sclerosis (ALS) is a severe neurodegenerative disorder involving upper and lower motor neurons. Mutations in the genes encoding FUS have been identified in a subset of sporadic and familial ALS patients.
We studied whether mutations in the 3' Untranslated region (3'UTR) of *FUS* are associated with ALS. Sequencing the whole 3' UTR region of *FUS* in 555 ALS patients revealed 4 variants (c.*48 G>A, c.*59 G>A, c.*108 C>T and c.*110 G>T) in 4 sporadic ALS patients. Identified mutations were not detected in 400 ethnically matched controls. Primary fibroblast coltures were studied in patients harbouring the c.*59 G>A, c.*108 C>T and c.*110 G>T variants, and results were compared with those of four controls, of one patient with the *FUS* R521C variant , and of two sporadic ALS patients without mutations in ALS genes. By immunostaining, patients with mutation in 3'UTR of *FUS* showed huge FUS deposits in the cytoplasm. The patient with the R521C mutation showed a slight increase of cytoplasmic FUS associated with a loss of detection in nuclei. Compartmental fractionation of fibroblasts and immunoblotting of these fractions disclosed a large amount of FUS in the cytosol of cells from patients with mutated 3'UTR and, in a lesser extent, from the R521C patient.
Our findings indicate that mutations in 3'UTR of *FUS* lead to translation deregulation and suggest that FUS deposits in the wild type configuration is the underlying cause of the disease in these cases.

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P08.13

Somatic instability in sporadic amyotrophic lateral sclerosis ?

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Amyotrophic lateral sclerosis (ALS) is a devastating neurological disease characterized by motor neuron degeneration of motor cortex, brainstem and spinal cord. The majority of ALS cases are sporadic (sALS) and only 5-10% have a family history (fALS). Whereas 60-80% of fALS cases are explained by germinal mutations, the genetic etiology of sALS remains unclear. Nonetheless, it's important to keep in mind that current genetic evidences for sALS come from genetic examinations mainly made using blood DNA. Here, we hypothesize that somatic mutations appear early during the development of embryonic spinal cord and could later trigger the development of sALS. Given ALS is a motor neuron disease affecting the corticospinal tract, we are in the process of preparing 1000 sections using flash frozen spinal cords obtained from sALS patients. These sections will be individually screened for the presence of mutations in the most commonly observed ALS genes. At first, we will look for repeat expansion in C9ORF72 by repeat-primed pcr (RP-PCR), since repeat mutations have been shown to be unstable during replication and DNA repair. Following this, we will look for point mutations in SOD1, TARDBP and FUS using a targeted deep sequencing method specifically designed to identify rare somatic events. To date, we have examined 251 spinal cord sections derived from 10 sALS patients for the presence of expanded C9ORF72 alleles, but none was thus far detected. This nonetheless enabled us to establish RP-PCR conditions that are sensitive enough to detect expansions at a low level of mosaicism (20%).

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P08.14

Genetic and functional studies of autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS)

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Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is the second most frequent form of spastic ataxia and is caused by mutations in the SACS gene encoding sacsin. Previous subcellular localization study demonstrated that 30% of sacsin is localized to the mitochondria but its specific function is still unknown.
We have searched for mutations by direct sequencing and customized CGH array in SACS in a series of 310 patients affected by progressive spastic ataxia with age at onset < 45 years. We identified 21 mutations (5 missenses and 16 truncating) that correspond to 6% of mutated patients definitely diagnosed with ARSACS. A search for phenotype-genotype correlations is in progress.
We obtained primary cultures of patients' fibroblasts in order to perform functional analyses of sacsin. One patient carries two heterozygous nonsense mutations (p.L1180LfsX8 and p.K3747X) and the other is homozygous for a missense mutation (p.R272H). The mitochondrial network appeared quantitatively altered (with a decrease of 50% of the global mitochondrial mass) and we observed an abnormal mitochondrial shape with the presence of bubbles in both patients. We also began to study potential interactions of sacsin with partners, particularly with proteins involved in the control of mitochondrial dynamics such as DRP1 and MFN1/2.

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P08.15

De novo TITF1 gene mutation causing Benign Hereditary Chorea with Hypothyroidism and Pituitary Mass

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Benign hereditary chorea (BHC) is an autosomal dominant disorder of early onset characterised by non progressive choreic movements with normal cognitive function occasionally associated with hypothyroidism and respiratory problems. This condition is caused by mutations in TITF-1 gene (also known as NKX2.1), encoding the Thyroid Transcription Factor-1
The proband of the described family, affected with BHC, presented in infancy with delayed walking and ataxic features, then chorea and developed hypothyroidism at the age of 33. She never experienced any respiratory symptoms, and on imaging she was found to have a large cystic pituitary mass.
Genetic testing showed that the proband carries a novel TITF-1 nonsense mutation. This mutation was transmitted to her affected child, which presented in infancy with delayed walking and mild non-progressive chorea.
The genetic analysis of the whole family confirmed that the mutation arose de-novo in the proband.
We discuss the effect of this new mutation and the phenotype heterogeneity of the disease

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P08.16

Association of leukocyte telomere length and MRI correlates of brain aging: Results of the Austrian Stroke Prevention Study

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Background: Telomeres shortening is a consequence of cell division and is biological factor related to cellular aging. Shorter leukocyte telomere length (LTL) is linked to age-related diseases. The objective of the present study was to explore the relation between LTL and magnetic resonance imaging

(MRI) detected structural and morphological changes in the normal elderly individuals.
Hypothesis: We hypothesize that shorter LTL is related to loss of MRI detected total brain volume and increase in white matter lesions (WML).
Methods: Relative LTL was measured by quantitative RT PCR in 909 participants (mean age=66years, 57%female) of the Austrian Stroke Prevention Study, a community-based cohort study. All subjects underwent brain MRI with automated assessment of brain parenchymal fraction (BPF,%) by use of SIENAX and semi-automated measurement of WML volume (cm^3). The effect of LTL on MRI correlates was tested using multiple linear regressions by adjusting for age and sex (Model1) and by additionally adjusting for vascular risk factors such as hypertension, diabetes, cardiovascular disease, smoking, apoϵ4 carrier status, BMI (Model2).
Results: We observed significant association between LTL and BPF (Model1:β=0.01,*p*<.001) when adjusted for age and sex. The association remained significant when additionally adjusted for vascular risk factors (Model2:β=0.01,*p*<.001). A trend for association was observed between LTL and WML (Model1:β=0.11,*p*=0.10;Model2:β=.14,*p*=0.05).
Conclusion: Our results suggest that LTL attrition independently of vascular risk factors may be involved in the development of brain atrophy or risk of dementia. Further longitudinal studies are needed to investigate if LTL may represent a biomarker for brain aging.

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P08.17
A rearrangement in OCLN causes brain calcification and renal dysfunction

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Pediatric intracranial calcification may be caused by inherited or acquired factors. An autosomal recessive form also referred to as pseudo-TORCH syndrome is characterized by calcium accumulation in the brain, congenital microcephaly and severe developmental impairment. Mutations in the gene encoding the tight junction protein occludin (OCLN) at exon 3 or at the 5-6 intron splice site result in brain calcification and polymicrogyria with no evidence of extra-cranial phenotypes. We ascertained a consanguineous family with two cases of brain calcification coupled with significant renal dysfunction, and identified a previously unknown rearrangement of the OCLN gene. A combination of molecular techniques was used to characterize the underlying genetic cause for the disease. SNP genotyping defined a genomic region shared between affected individuals, while whole exome data revealed a deletion at exon 9 of the OCLN gene. MLPA, PCR and Sanger sequencing confirmed this deletion and further identified a rearrangement of the genetic region. Detection of the rearrangement was complicated by the presence of a replication within the genome of a section that extends from exon 5 to beyond the OCLN gene. Of the seven OCLN splice variants described by UniprotKB, all make use of exon 9 while the OCLN variants that use exons 3, 5, and 6 are tissue specific. The mutation in exon 9, as was observed in our patients, would lead to the loss of OCLN in all tissues and likely explains the observed phenotypes in our cohort that extends beyond the brain.

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P08.18
Neuroimaging algorithm of Neurodegeneration with Brain Iron Accumulation (NBIA) directs molecular genetic analyses and prompts gene identification

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Neurodegeneration with Brain Iron Accumulation (NBIA) is an emerging class of hereditary neurodegenerative diseases resulting in extrapyramidal movement disorders, intellectual deterioration and characteristic deposition of iron in the basal ganglia. The best MRI sequence to visualize iron accumulation is T2*.
While a number of NBIA causes have recently been reported, we propose a diagnostic-algorithm based on neuroimaging features to direct molecular genetic analyses. This algorithm relies on 5 very simple MRI criteria namely:
1/"Eye of the tiger" in the globi pallidi on T2
2/ Hypointensity of globi pallidi, either isolated or associated with caudate/putamen hypointensity onT2
3/ Hyperintensity of substantia nigra on T1
4/ White matter anomalies on T2
5/ Cerebellar and/or brainstem atrophy
Combining these 5 criteria in a paediatric series of 23 NBIA cases referred to our paediatric-genetic department, led to molecular diagnosis in 16/23 patients. The diagnoses comprised: Pantothenate kinase (PKAN) deficiency: 3 cases); Mitochondrial membrane protein (MPAN) deficiency: 1 case; Neuroaxonal Dystrophy (NAD): 9 cases; Fatty-Acid-Hydroxylase-associated Neurodegeneration (FAHN): 2 cases; Beta-propeller-protein associated-neurodegeneration (BPAN): 1 case.
The remaining 7 cases are still undiagnosed. Among them, 4/7 fulfilled clinical and brain MRI criteria of slowly progressive NAD. We have launched a systematic trio-exome screening of undiagnosed cases and eventually identified a novel disease gene delineating a novel physiopathological mechanism of NBIA.
In conclusion, we suggest giving consideration to brain MRI to direct molecular genetic testing in NBIA. This approach is a prerequisite for future studies aimed at identifying novel diseases genes in this increasingly recognized group of disorders.

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P08.19
MiRNA expression profiling in cortical neurons under conditions of transient focal ischemia

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Background. One of the important problems of modern medicine is to protect neurons against ischemia, traumatic brain injury and some neurodegenerative diseases. To understand the nature of these pathological processes at the molecular level it is reasonable to analyze the expression of genes and their post-transcriptional regulators, microRNAs (miRs), involved in the pathogenesis of these diseases.
Materials & Methods. Unilateral focal ischemia was performed by photochemically induced thrombosis in adult male rats. Total RNA and miRs were extracted from ischemic penumbra of rat cortex sensorimotor area and from control ipsilateral cortical region after 24 hours reperfusion by miRNeasy Miro Kit and RNeasy MinElute Cleanup Kit (Qiagen), respectively.
MiRs expression profiling of ischemic and control samples were carried out by quantitative real time RT-PCR using Custom miScript Primer Assay and miScript SYBR Green PCR Kit (Qiagen).
Results. Selection of 45 specific miRs was carried out following two criteria: cortical-specific miRs expression and target-specific regulation of genes potentially involved in ischemia using database miRanda, PicTar, TargetScan and Pubmed. Ischemic and control samples from the same rat were analyzed simultaneously on the same Custom plate. Of these 45 miRs, 3 miRs (rno-miR-30a-5p, -30a-3p, -21-5p) were upregulated and 8 miRs (rno-miR- 186-5p, -29a-3p, -223-3p, -23a-3p, -29a-5p, -497-5p, -551b-3p and rno-let-
7f-5p) were down regulated significantly.
Conclusions. We have obtained preliminary data on miRNAs expression profiling during transient focal ischemia. Further studies are needed to evaluate the possible use of miRNAs as biomarkers in stroke and related pathologies.

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P08.20

Candidate Cacna1a modifier genes in a C. elegans large scale functional RNAi screen

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Mutations in the *CACNA1A* gene that encodes the pore-forming α_1-subunit of human voltage-gated $Ca_v2.1$ (P/Q-type) Ca^{2+} channels cause several autosomal-dominant neurologic disorders, including familial hemiplegic migraine type 1 (FHM1), episodic ataxia type 2 (EA2), and spinocerebellar ataxia type 6 (SCA6).
In order to identify modifiers of uncoordination in movement disorders, we performed a large scale functional RNAi screen using the *C. elegans* strain CB55, which carries a truncating mutation in the *unc-2* gene, the worm ortholog for the human *CACNA1A*.
The screen was carried out by the feeding method in 96-well liquid culture format using the ORFeome v1.1 feeding library as previously described. We used time-lapse imaging of worms in liquid culture to assess changes in thrashing behaviour. Raw imaging data was analysed with open source Image J, and the thrashing analysis results were loaded on CellHTS2 for further exploration.
We looked for genes that when silenced either ameliorated the slow and uncoordinated phenotype of unc-2 or interacted to produce a more severe phenotype. Raw data was collected for the full library and 95% of the primary screen has been analysed by CellHTS2. During the primary screen we found 142 candidate genes improving CB55 motor function, and 148 candidate genes increasing *unc-2* impairment, through interaction with *Cacna1a*. Gene ontology revealed an overrepresentation of genes involved in development, growth, locomotion, signal transduction and vesicle mediated transport.
We are now following with more detail the genes that already scored in the first screen by expanding the panel of behavioural and neurodegeneration assays.

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P08.21

NOTCH3 unclassified variants leading to CADASIL. Importance of skin biopsy to establish causality and CADASIL diagnosis.

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CADASIL (Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), the most common familial vascular dementia, is characterized by stereotyped *NOTCH3* mutations leading to an odd number of cysteine residues in the EGF like motifs of this receptor and the presence of typical GOM (Granular Osmiophilic Material) in the basal membrane of vascular smooth muscle cells as shown by electronic microscopy (EM) study of skin biopsy.
Some patients referred for molecular diagnostic of CADASIL harbor missense variants which do not affect the number of cysteine residues and are absent from databases, raising the question of their causality. Our current hypothesis is that some of these "missense" variants might behave as splice variants leading to an odd of number of cysteine residues and therefore be true CADASIL mutations.
18 Patients showing NOTCH3 missense variants absent from EVS, 1000genomes and dbSNP137 were included. All 23 exons known to be mutated in CADASIL were sequenced and EM analysis of skin biopsy was performed in all patients.
Only one patient (P18) showed typical GOM. This patient has a G73R mutation (c. 295G>C, exon 3). cDNA analysis is on going to analyze the putative splicing effect of this mutated allele, using mRNA extracted from skin biopsy and cultured fibroblasts.
In summary, some patients with missense variants which do not affect the number of cysteine residues are true CADASIL patients showing typical GOM on EM skin biopsy. This study emphasizes the need to conduct a skin biopsy for *NOTCH3* unclassified variants.

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P08.23

West syndrome, general psychomotor retardation and dysmorphic features in a female patient due to a 2.92 Mb microdeletion leading to a haploinsufficiency of the CDKL5 gene and several additional flanking genes.

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By array CGH-analysis we identified a X-chromosomal microdeletion that occurred de novo in a now 14.5 month old girl, the second child of a Austrian couple, according our result: arr Xp22.2p22.13(15,767,378-18,684,139) x1. This microdeletion causing the loss of less than 20 genes was confirmed by Q-PCR. The first born child of this couple, a boy, is healthy. The female patient was able to sit with 8 month however still cannot walk or speak. With 7 month she showed first myoclonic seizures. Up to that age her general development was within the normal range. Since seizures became more frequent and severe, with12 month antiepileptic drugs like Keppra were applied. Several female and at least one male patient with similar but apparently not identical chromosomal breakpoints were thus far reported or communicated in databases like DECIPHER or ISCA. The apparent lack of obvious recurrent chromosomal breakpoints could suggest that for these microdeletions no common molecular mechanism might be involved. But there is a considerable overlap of the described microdeletions and because loss of the CDKL5 gene is causing a severe form of a variant of Rett syndrome, this disorder is certainly a main clinical feature of a potential contiguous gene syndrome from that genomic region. Since differences in X-inactivation may modify onset and severity of this and linked disorders, like PDHC, as reported recently, it is clearly important to determine precisely the deletion size with number of affected genes and X-inactivation (79% in our patient) to allow a better comparison and prognostic considerations.

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P08.24

Genome-wide analysis of genotype-dependent CTCF DNA binding

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The aim of the current study was to assess the effect of genetic variation in the DNA binding protein recognition sequence of CCCTC-binding factor (CTCF) and its potential involvement in Huntington's disease. CTCF is an ubiquitously expressed, highly conserved protein containing an 11 zinc finger DNA binding domain. It is essential for viability and considered to play an important role in local and long-range inter- and intra-chromosomal interactions and transcriptional regulation. CTCF is also known to act as insulator (as chromatin barrier or enhancer blocker), regulate nuclear localization, or participate in imprinting control. The DNA binding site of CTCF has a well-characterized and highly conserved binding motif. 95% of the CTCF binding sites identified by ChIPseq experiments contain the consensus motif. Single nucleotide variants within DNA binding sites are known to have a major impact on protein binding and consequently its function. Genome wide statistical analysis was performed in ChIPseq data from two fibroblast cell lines from control subjects and two from subjects with Huntington's disease. We detected 21 sites that differed significantly in CTCF binding. Twelve of these sites contained a single nucleotide polymorphism (SNP) in the binding site thereby explaining differential CTCF binding. We confirmed this SNP specific binding with an Electro Mobility Shift Assay. Examination of the SNPs in 14 more brain samples (7 for each group) showed no segregation with the neurological condition.

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P08.25

Genetic testing in hereditary neuropathies: our 10 years experience

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Objectives: Charcot-Marie-Tooth neuropathies (CMT) are a clinically and ge-

netically heterogeneous group of rare inherited neuropathies. Their prevalence is 1:2,500. Until now more than 50 CMT genes have been identified.
Aims: In this study we analysed the mutation frequencies of the most common genes (PMP22 duplication, deletion, MPZ, MFN2, Connexin 32 and EGR2 mutations) in an electrophysiologically well-characterized group of patients. In patients with gypsy origin two founder mutations (NDRG1-R148X; CCFDN-IVS6+389C>T) were also tested.
Patients and methods:
450 Caucasian (256 male and 194 female) and 15 Roma (4 male and 11 female) clinically and electrophysiologically characterized patients with neuropathy have been investigated (196 were familial cases from 85 families). The mutation analysis was performed by MLPA, real-time PCR, PCR-RFLP and bidirectional sequencing.
Results: The quantitative analysis of the PMP22 gene found 84 duplications and 65 deletions. Pathogenic mutations in the MPZ gene were detected in 11, in the EGR2 gene in 2, in the MFN2 gene in 5 and in the Connexin32 gene in 17 cases. In the Roma patients LOM type of neuropathy was present in 8, CCFDN mutation in 4 cases. In 1 family the PMP22 deletion and a pathogenic EGR2 mutation coexisted.
Conclusion: Pathogenic mutation has been identified in 41,5% of the investigated patients. In our presentation we would like to emphasize that mutation analysis of clinically and electrophysiologically well-characterized neuropathic patients reveals the genetic etiology in a relatively large percent of cases.

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P08.26
Second case of COFS Syndrome type 2 confirmed by compound heterozygous mutations in ERCC2

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Cerebrooculofacioskeletal syndrome (COFS, Peña-Shokeir syndrome type II) is a very rare progressive neurodegenerative disorder characterised by microcephaly, severe intellectual disability, congenital cataracts, facial dysmorphism, and arthrogryposis. By far, the most common subtype is COFS type 1 (COFS1), which is caused by biallelic mutations in *ERCC6*, the major gene underlying Cockayne syndrome (CSB). Most COFS1 patients originate from the Manitoba aboriginal population, in which the condition was originally described. Furthermore, a founder mutation has been identified to cause COFS1 in six individuals from a large consanguineous pedigree from northern Finland.
In contrast, until now only one patient has been described with COFS type 2 (COFS2), which is caused by biallelic mutations in *ERCC2*, the gene underlying Xeroderma pigmentosum, group D (XPD), and trichothiodystrophy (TTD). This patient was the child of nonconsanguineous Ashkenazi Jewish parents. He showed typical COFS symptoms and died at the age of 3.5 years.
Here we report on the second COFS2 case carrying compound heterozygous mutations in *ERCC2*. This 6 month old male infant was born to nonconsanguineous Kosovo Albanian parents who previously had two healthy children and one induced abortion due to prenatal diagnosed arthrogryposis. The patient presented with intrauterine growth retardation, microcephaly, developmental delay, congenital cataracts, facial dysmorphism, and congenital arthrogryposis. While complete analysis of *ERCC6* did not reveal any mutation, two heterozygous mutations in *ERCC2* could be identified: c.1703_1704delTT, p.Phe568Tyrfs*2 in exon 18 and c.1843G>A, p.Gly615Arg in exon 20. Analysis of parents indicated the compound heterozygous state of the patient.

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P08.27
Presymptomatic testing in hereditary Creutzfeldt-Jakob disease

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This study aims to describe motivations and attitudes of persons at risk for hereditary Creutzfeldt-Jakob Disease (CJD) and to compare with those at risk for Huntington's Disease (HD). Two features distinguished being at risk for CJD from HD: incomplete penetrance and the reduced time to risk discovery. In most instances, it was during the illness/death of the index case that descendants discover there at risk status whereas in HD, risk awareness might be years. Since 1992, there were 1930 requests for presymptomatic testing for late-onset neurological diseases. Most were at risk for HD (HTT, n= 1624; 85%), and CJD (PRNP, n= 20; 1%). There were 15 women and 5 men, mean age at request was 40.2 ± 9.5 years (versus 35.1 ±12.1 in HD n=1612, p=.054). The reasons to take the test was to inform descendants of their risk (n=8), " to know" (n=8) and parental choice (n=3). Only 6/20 did choose not to take the test (comparable to 33% in HD p=.78) and 13 obtained a test result (6 carriers). Parents at risk for CJD reported difficulties to inform their descendants after an unfavourable result and, as in HD, it was difficult to distinguish between being a carrier and being affected after an unfavourable test result. In conclusion, despite incomplete penetrance and the reduced time to risk discovery in CJD the outcome of presymptomatic testing for CJD is similar to HD.

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P08.28
Investigation of SORL1 variants in a Turkish cohort of dementia patients

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Background: SORL1 (Sortilin related receptor 1) is involved in intracellular trafficking and localization of Amyloid precursor protein (APP). It binds APP and regulates its sorting into endocytic or recycling pathways. Several studies showed that genetic variations in this gene increased the risk of Alzheimer's disease (AD) and down regulation of SORL1 was associated with increased amyloid beta production in cells.
Materials and Methods: We used allelic discrimination to investigate the association of rs2282649 C/T polymorphism in 116 LOAD patients and 120 controls. We also performed exome and Sanger sequencing to analyze SORL1 rare genetic variants in a cohort of 62 Alzheimer disease, 13 dementia and 16 FTD cases.
Results: Exome sequencing identified 7 non-synonymous variants in 10 patients. Five of these variants are known (N371T, R330W, E270K, A172V, T1788I) and other two are new variants. No significant differences in the genotypic distribution for SORL1 rs2282649 polymorphism in case-control, female-male, APOEe4 carrier-non carrier groups were found. As expected, the frequency of APOEe4 allele was significantly higher in the patients group (patients %39.8, controls % 19.2 p: 0.001).
Conclusions: Our results suggest that rare variants in SORL1 may be associated with Alzheimer disease while the common intronic rs2282649 C/T polymorphism is not associated with the risk of developing Alzheimer's disease in our study group. However, this study was performed in a small cohort and replication in a larger number of samples is needed.

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P08.29
Myoclonus-dystonia (DYT11) - first experience in genetic testing in Serbia

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Myoclonus-dystonia (DYT11) is an autosomal dominant disorder charac-

terized by dystonia and myoclonic jerks; the onset is most commonly during childhood and adolescence. DYT11 is caused by mutations in the gene coding for epsilon sarcoglycan (SGCE). More than 80 distinct mutations of SGCE have been described to date; most of them are localized in exons 3 to7, and in exon 9. Mutations detected in patients include nonsense, missense, splice site, insertions, partial and complete exon deletions. We have established molecular genetic testing of all coding exons, promoter and 3'UTR region of SGCE gene. The analysis comprised DNA samples of 45 Serbian patients with clinical diagnosis of myoclonus-dystonia. Detection of substitutions and small in/del mutations was performed by direct sequencing, on ABI 310 Genetic Analyzer. Also, large duplication/deletion analysis was performed using MLPA method. Three different mutations were detected in total number of 4 samples (4/45, 8.9 %). One patient harbored (nonsense) mutation in exon 6 (c.709C>; p.Arg237Ter). Small deletion in exon 7 (c.966delT; p.Val323Cysfs*11) was found in one patient. In two patients another small deletion was detected (c.835_839delACAAA; p.Thr279Alafs*16) in exon 7. The latter two patients are non consanguineous, but originate from the same geographical region. No large duplications/deletions were detected using MLPA method. These results indicate that introduction of mutation detection in SGCE gene should be considered as routine diagnostic tool in clinical practice.

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P08.30

SLC2A1 gene mutation in Serbian exercise-induced paroxysmal dyskinesia (DYT18) patient: Nonsense-mediated decay as possible pathogenesis mechanism

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Exercise induced paroxysmal dyskinesia (DYT18) is an autosomal dominant disorder caused by mutations in the *SLC2A1* gene coding for a glucose transporter type 1 (GLUT1), the main glucose transporter across the blood-brain barrier. DYT18 is just a part of phenotypic spectrum associated with GLUT1 deficiency. This condition is characterized by sudden attacks of movement disorders of various types triggered by prolonged exercise. More than 150 distinct mutations in *SLC2A1* have been described so far. In this study, we performed genomic sequencing of the entire *SLC2A1* coding region in 10 patients with clinical signs of DYT18. Novel mutation (c.516delG) was revealed in one proband. Detected mutation occured *de novo* and disrupts consensus sequence of intron 4 donor splice site. Using the splice sites analysis tool it was predicted that alternative donor splice site exists at position c.389_390 in exon 4; this splice site would create mRNA encoding truncated protein 192ak long. The cDNA sequencing of region encompassing exon 3-5 in proband showed a strong wild-type signal and a much weaker signal that corresponds to truncated allele. Real time PCR quantification by SYBR Green chemistry (ΔΔCT method) revealed that proband has 60% SLC2A1 mRNA expression compared with his unaffected father. The result indicates that described mutation exerts a major effect at the mRNA level, most likely via nonsense-mediated mRNA decay. According to our knowledge, this is the first time that nonsense mediated decay is described as pathogenesis mechanism in exercise induced paroxysmal dyskinesia.

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P08.31

Evaluating clinical validity and utility of EFHC1 and GABRA1 molecular testing in patients with juvenile myoclonic epilepsy and other idiopathic generalized epilepsies

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Juvenile myoclonic epilepsy (JME) comprises 5-10% of all epilepsies. To date, several genes have been associated with JME and other idiopathic generalized epilepsies (IGEs), although only a few presented mutations segregating with affected members in family studies. The most relevant genes are *EFHC1* and the GABA receptor α1-subunit gene (*GABRA1*). The aim of this study was to search for mutations in both genes in patients with JME and other IGEs. We performed mutation screening in 102 patients with JME and 33 with other IGEs. Missense mutations were investigated in 100 control individuals. Eight algorithms were used to estimate the impact of mutations. We identified three novel missense mutations in *EFHC1* (c.896A>G, c.887G>A, c.1766G>A) and seven previously described (c.475C> T, c.475C>G, c.545G> A, 685T> C, c.1343T> C, c.1821A> G and c.1855A> C). Only one patient with alterations did not have JME. Deleterious prediction was controversial, with most substitutions considered pathological by at least two algorithms. However, only four mutations, corresponding to the second DM10 domain and the EF-Hand motif, were not identified in control individuals. In *GABRA1*, three silent SNPs were identified. In conclusion, the absence of consensus in the algorithm predictions for *EFHC1* mutations highlights the genetic complexity of JME. In addition, most mutations were also identified in controls, suggesting that *EFHC1* is not acting as a single major gene causing JME, although it could be a predisposition factor of known significance. Therefore, our results suggest that genetic testing for *EFHC1* and *GABRA1* does not seem to be clinically relevant.

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P08.32

Mutation analysis of SLC2A1 gene in Bulgarian patients with IGE featured with absence seizures

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Mutations in the *SLC2A1* gene are the major cause for idiopathic generalized epilepsy (IGE) featured with absence seizures and an age at onset being dated from the early childhood to the early adult life. To define the mutation spectrum and related clinical characteristics in Bulgarian patients, we performed a mutation screening of the *SLC2A1* gene in 29 patients from 3 different ethnic groups (25 Bulgarians, 2 Roma gypsies and 2 Turks). All of them were displaying IGE featured by absence seizures.Sequencing data analysis of the *SLC2A1* gene revealed 3 different sequence variations - 2 of them located in the coding region (c.1078C>T, p. 360 Q>X and c.480C>A, p.160H>Q) and 1 sequence variation affecting the consensus splice-site (c.1075-14G>A).
Segregation analysis revealed that in one family the disease-causing mutation arises *de novo* and in the other two families the molecular defects came from a parent who had reported a single febrile seizure during the first year of life and latter in life.
In all three cases, the clinical picture was present with diverse forms of absence epilepsy - MAE with developmental problems, EOAE and CAE. In one family, the epileptic syndrome had begun with
febrile seizures and after that the absence and GTCS had been added to the clinical picture. This patient had been sent initially for *SCN1A* analysis.
In summary,our results broaden the mutation spectrum of the *SLC2A1* gene and enrich the clinical data of IGE with absence seizures.

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P08.33

Search of the genetic bases of a dog epilepsy: a model for the human epilepsies

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The dog, by its numerous distinct breeds considered as genetic isolates, represents a powerful model to identify genes and alleles implicated in genetic diseases. In addition, dog diseases are spontaneous, breed specific and homologous to human diseases. In dogs, epilepsy is the most widespread neurological disorder, with 5% of affected dogs, spread over 100 breeds.

The high prevalence in some breeds (>20%) supposes a genetic origin. Each affected breed is affected by a specific form of epilepsy and genetic studies in these breeds are expected to identify the responsible genes, thus constituting good candidate genes for human epilepsies. About 10% of Greater Swiss Mountain dogs are affected by a generalized epilepsy with tonic-clonic seizures, and we hypothesize that it is an autosomal recessive disease. A linkage study on a 95 dogs pedigree performed by the genotyping of 340 microsatellite markers evenly spaced on the whole dog genome, allowed the identification of a 20 Mb locus on the dog chromosome 16. The orthologous human region of this locus correspond in part to a locus previously implicated in human GEFS+ epilepsies but the genes have not been identified to date. In order to reduce this locus in dogs, we genotyped 101 dogs on the canine HD array (170 000 SNPs, Illumina). Both genetic association and genetic linkage studies are ongoing. The identification of the genetic bases of epilepsies in dog can bring novel candidate genes for human epilepsies and will allow the development of new therapies for human and dog patients.

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P08.34

Genome-wide Copy number variations in Rolandic Epilepsy

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Benign Rolandic epilepsy (RE), or benign childhood epilepsy with centro-temporal spikes (BECTS), the most common type of epilepsy in children, is a neurodevelopmental disorder with common neurocognitive impairment in speech, language, attention and executive skills. It is considered the benign end of a spectrum, including the most severe continuous spike and waves during slow-wave sleep syndrome (CSWSS) and the Landau-Kleffner (LKS) syndrome that are rare epileptic encephalopathies. An impairment of brain development with complex interplay between genetic and non-genetic factors has been suspected for RE but its pathophysiology is still elusive. We have recently reported genomic variations in patients with LKS and CSWSS, suggesting potential candidate genes.
In the present study, the involvement of rare CNVs was questioned in 50 patients with RE using aCGH (4x180K, Agilent microarrays).
We found 19 CNVs in 50 patients. Sixteen are potentially pathogenic (not reported in the database of genomic variant) and are still under investigations. Whereas highly heterogeneous CNV were found in the RE cohort, the same 16p11.2 microduplication was found in two patients. This microduplication has been associated with different neurocognitive disorders and included the PRRT2 gene, recently reported in infantile seizures. We also detected a deletion disrupting a potential major gene responsible for LKS/CSWSS, hence providing a possible biological basis for this clinical continuum. Of note, the frequent involvement of various cell adhesion molecules as detected previously in LKS/CSWSS, was not found here, suggesting that the two ends of this spectrum may be, at least partially, influenced by different genetic factors.

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P08.35

Clinical application of high resolution array Comparative Genomic Hybridization (array-CGH) in epileptic disorders

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Epileptic disorders, often co-existing with other neuropathological manifestations and/or multiple congenital anomalies/peculiar facies, compose a clinically and genetically heterogeneous entity, with a variety of unspecific and diffuse phenotypes, thus raising difficulties on genetic evaluation and diagnosis. The array Comparative Genomic Hybridization (array-CGH) technique has proved reliable and valid, allowing the detection of submicroscopic imbalances (CNVs) in the diagnostic settting. From a total of 436 unrelated patients investigated in our department, 82 presented epileptic disorders of unknown etiology accompanied by at least one additional manifestation of pathological phenotype, but with normal previous conventional karyotype and negative genetic tests (FRAX, RETT, FISH tests or metabolic screens). High resolution 4x180K and 1x244K Agilent arrays (>170.000 and >236.000 probes respectively, average resolution 8.9Kb) were used in this study. Submicroscopic genomic imbalances were detected in 55/82 patients featuring epilepsy among other phenotypic anomalies, ranging in size from 0.037 to 17Mb and involving well-known and new susceptibility chromosomal loci as; 1q44, 2q24.2-q31.1, 3p25.3, 4q34.1-q35.2, 6q21-q22.1, 7p23.2, 7q11.23, 8q24.3, 10q26.3, 12p13.31, 22q11.21, Xp22.31. Genes, implicated in syndromes which are associated with epilepsy, were identified in 36/55 cases (65.4%) as; APOD, OCLN, GABA, FLNA, LIMK1, STX1A, GTF2I, FAM36A, MBD5, C1orf199, HNRNPU, KIF26B, OGG1, IRAK2, SLC6A11, ATG7, CDKL5, NIPA1/NIPA2, IGH, CHRM5, GABRB3, SCN1A/SCN2A/SCN3A/SCN7A/SCN9A, SST, MBD5, GLRA3, RALBP1, JRK, BNIP3, KNDC1, COMT, RTN4R, GHRL, RAC1, ACTB, TUBA8, LGI3, LBX1, CLCN2, TPPP, MARCKS, HDAC2. Array-CGH offers a high yield for the identification of so far undefined or underdiagnosed epilepsy cases, elucidating plausible novel syndromes and enabling improved delineation of genotype-phenotype correlations.

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P08.36

Novel mutations in FA2H in three Arab families with a clinical spectrum of neurodegeneration and complex features of hereditary spastic paraplegia

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Fatty acid 2-hydroxylase gene (*FA2H*) is involved in the alpha-hydroxylation of the N-acyl ceramide moiety of sphingolipids fatty acids, essential components of myelin (15337768). Mutations in the *FA2H* were first identified in nine patients with recessive childhood onset spasticity, dystonia, cognitive dysfunction, and periventricular white matter disease (19068277). Mutations were also identified in a novel form of neurodegeneration with brain iron accumulation (NBIA) (20853438) as well as in a recessive complicated form of hereditary spastic paraplegia (SPG35, MIM#612319) (20104589). Together these genotype-phenotype correlations have defined a condition known as the fatty acid hydroxylase-associated neurodegeneration (FAHN). To date, few individuals with mutations in the FA2H gene have been described. Herein we report seven patients from three unrelated consanguineous Arab families with novel homozygous *FA2H* gene mutations. They were presented with progressive spastic paraparesis beginning after the age of 4 years associated with mild intellectual disabilities. Cerebellar manifestations predominated in all families including ataxia, nystagmus, intention tremors and dysarthia while infrequent limb dystonic movements were obvious with disease progression. Spastic quadriparesis and bulbar manifestations were highly evident in the oldest affecteds. Magnetic resonance imaging revealed cerebellar atrophy, high signal of white matter especially around occipital horn and low signal of basal ganglia that was also more pronounced with progression of the disease. Interestingly, nerve conduction velocity revealed sensory neuropathy of axonal nature in all affecteds; a finding recently assigned with FAHN. We suggest that FAH2 mutations result in clinical spectrum of a combined presentation of the above mentioned three disorders.

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P08.37

Phosphatidylserine increases IKBKAP levels in a humanized knock-in IKBKAP mouse model

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Familial dysautonomia (FD) is a severe neurodegenerative genetic disorder restricted to the Ashkenazi Jewish population. The most common mutation in FD patients is a T-to-C transition at position 6 of intron 20 of the *IKBKAP* gene. This mutation causes aberrant skipping of exon 20 in a tissue-specific manner, leading to reduction of the IKAP protein in the nervous system. We established a homozygous humanized mouse strain carrying human exon

20 and its two flanking introns; the 3' intron has the transition observed in the *IKBKAP* gene of FD patients. Although our FD humanized mouse does not display FD symptoms, the unique, tissue-specific splicing pattern of the *IKBKAP* in these mice allowed us to evaluate the effect of therapies on gene expression and exon 20 splicing. The FD mice were supplemented with phosphatidylserine (PS), an FDA-approved food supplement that slows cognitive degeneration in human and that increases mRNA and protein levels of *IKBKAP* in cell lines generated from FD patients. Here we demonstrated that PS treatment increases *IKBAKP* mRNA and IKAP protein levels in various tissues of FD mice without affecting exon 20 inclusion levels. We also observed that genes associated with transcription regulation and developmental processes were up-regulated in the cerebrum of PS-treated mice. Thus, PS holds promise for treatment of FD.

R. Bochner: None.

P08.38

Mutations in PRRT2 result in familial infantile convulsions with marked variability in clinical expression and SUDEP

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Benign familial infantile seizures (BFIS) is an autosomal dominant disorder characterized by afebrile seizures that begin at age 3-12 months with a favorable outcome. A recent discovery has identified heterozygous mutations in PRRT2, which encodes proline-rich transmembrane protein 2, in most families affected by BFIS. PRRT2 is also the major causative gene for familial paroxysmal kinesigenic dystonia (PKD), a rare disorder characterized by episodic attacks of choreoathetosis or dystonia. These results have corroborated the existence of familial infantile convulsions with paroxysmal choreoathetosis that shares overlapping clinical features with BFIS and PKD. In this study, we performed mutation screening of PRRT2 in our families with BFIS, ICCA or PKD phenotypes. The whole genomic region of PRRT2 was sequenced in seven Italian families, of which six with BFIS or ICCA phenotype, and one family with PKD phenotype. The previously reported mutation, p.R217Pfs*7, was found in two families with BFIS phenotype, and in one family with ICCA. In an additional BFIS family, a missense mutation, R240X, was identified. All these mutations co-segregated with the disease in these families and were not observed in 300 control subjects. In the ICCA family, two affected members displayed a more complex phenotype with episodic ataxia, mental retardation and migraine attacks. In one family that also carried the c.649dupC mutation, one affected member died at age of 13 years of SUDEP. This study confirms the major role of PPRT2 mutations in families with BFIS phenotypes and underscore the complexity of the phenotypic consequences of mutations in this gene.

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P08.39

Application of Artificial Neural Networks to link one-carbon metabolism to Alzheimer's disease risk

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Folate metabolism, also known as one-carbon metabolism, plays a fundamental role in DNA synthesis and integrity, in chromosome stability, in antioxidant defence mechanisms, as well as in epigenetic processes, and impairments of this pathway have been often linked to Alzheimer's disease (AD) risk. In addition, increasing evidence from large scale case-control studies, genome-wide association studies, and meta-analyses of the literature suggest that polymorphisms of genes involved in one-carbon metabolism influence the levels of folate, homocysteine and vitamin B12, and might be among AD risk factors.
We aimed to analyze a dataset of genetic and biochemical data (folate, homocysteine, vitamin B12, and the genotypes generated by 9 common biallelic polymorphisms of genes involved in folate metabolism) obtained from 40 AD patients and 40 matched controls to assess the predictive capacity of artificial neural networks assembled in TWIST system in distinguish consistently these two different conditions and to identify the variables expressing the maximal amount of relevant information to the condition of being affected by dementia of Alzheimer's type. Moreover, we constructed a semantic connectivity map to offer some insight regarding the complex biological connections among the studied variables and the two conditions (being AD or control).
TWIST system selected 16 variables that allowed to discriminate between AD and control subjects with over 90% accuracy. The semantic connectivity map provided important information on the complex biological connections between the studied variables highlighting the importance of folate metabolism in AD pathogenesis.

L. Migliore: None. **E. Grossi:** None. **M. Buscema:** None. **F. Coppedè:** None.

P08.40

A whole genome sequencing strategy to identify novel genes for frontotemporal lobar degeneration

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Family history has been reported in up to 50% of patients with frontotemporal lobar degeneration (FTLD), a major neurodegenerative disease of the brain. In most families the pattern of inheritance is consistent with a Mendelian segregation of a dominant mutation. Mutations in seven known genes explain the disease in approximately 40% of FTLD families. We use whole genome sequencing (WGS) to identify rare, highly penetrant genes in genetically unexplained FTLD families. The genome sequences of three affected sib pairs were obtained with an average coverage of 81-fold capturing both alleles of 96.5% of the genome, and revealing about four million variations per genome. Annotation and analysis of the genome sequences was performed using GenomeComb. Variations were filtered and prioritized using multiple strategies based on genetic and functional criteria. About 380 selected missense or splice site variations were further prioritized using mutation prediction programs, resulting in about 80 predicted harmful mutations per sib pair. The frequency of the variations was determined in neurologically healthy control individuals. About 25 variations were absent or present in less than 1% of control individuals. We tested the frequency of these rare variations in a series of 370 unrelated FTLD patients and identified around 12 variations that were present in additional patients. The genes harboring these variations are being sequenced in patients and control individuals to define their mutation profile. The identification of novel FLTD genes will provide valuable insights into the pathogenesis of FTLD and might provide insight in potential novel therapeutic targets.

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P08.41

PGRN exon 6 deletion G.101349_101355delCTGCTGT associated with Nonfluent primary progressive aphasia in a familial form of FTLD

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The Fronto-Temporal Lobar Degeneration (FTLD) is a genetically and pathologically heterogeneous degenerative disorder characterized by progressive decline in behavior or language associated with frontal and temporal lobe degeneration. The phenotypic heterogeneity consistently revealed the presence of at least two clinical manifestations such as behavioral variant FTLD (bvFTLD) and Primary Progressive Aphasia (PPA).
Several mutations either in the same gene or in different genes show a great degree of heterogeneity associated with FTLD, up to 40% of familiar cases have been described as carrying mutation/s in GRN and/or MAPT genes.

Here we describe a four-generation Southern Italian family segregating FTLD. The family consists of five siblings, three of which affected by FTLD. The three phenotypes are clinically heterogeneous with behavioral variant (bvFTLD) apathetic or disinhibited behavioral syndrome in the males and Primary Progressive Aphasia (PPA) in the female. We collected 2 of the affected individuals (one of the male and the female), and one of the non affected sibs and analyzed these for GRN and MAPT gene in search for a mutation. Specifically, we analyzed the sequence of all exons, exon-intron boundaries and 5' and 3' regulatory regions. We found g.101349_101355delCTGCTGT mutation on exon 6 of GRN, only in the affected siblings. This deletion we know causing a premature stop codon with frameshift induction and mRNA non-sense mediated decay involving GRN protein haploinsufficiency. Up to now only two sporadic cases have been described carrying these deletion, further investigation are in progress to better describe the molecular defect causing this FTLD.

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P08.42

Comparative RNA-Seq profiling from FUS mutations found in amyotrophic lateral sclerosis and essential tremor cases.

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Fused in sarcoma (FUS) is a protein involved in DNA and RNA processing and tumorigenesis. The presence of nonsense mutations affecting FUS has been observed in Amyotrophic Lateral Sclerosis (ALS) (p.Q519X) and Essential Tremor (ET) (p.Q290X) patients. While the two mutant proteins are sequestered to the cytoplasm, only the FUS(p.Q519X) still contains its RNA binding domain which could lead the FUS aggregates to act as cytoplasmic RNA trap that would prevent the normal re-entry into the nucleus of some of the RNA. Massively parallel RNA sequencing (RNA-Seq) experiments are now easily feasible and we propose to delineate the transcriptome of N2a cells expressing either FUS(WT), FUS(p.Q519X) or FUS(p.Q290X). For each of these, we would make a total, nuclear and cytoplasmic RNA preparation, converted these to cDNA and assemble them into a library compatible with a Illumina MiSeq apparatus. Cufflink will be used to align sequencing reads and evaluate the abundance of each transcript. Relative expression changes between FUS(WT), FUS(p.Q519X) and FUS(p.Q290X) will be evaluated on a transcriptome wide basis. In addition, the datasets will also be assessed for splicing defect between FUS(WT) and the two mutated FUS. Interesting variations will be confirmed by quantitative PCR and Taqman probes in the readily available ALS and ET patient's lymphoblastoid cell lines. With the implication of TDP-43, FUS, and C9ORF72 in ALS, the clues point towards disrupted RNA metabolism as a pathogenic mechanism for the disease. Thus our findings may be applicable to ALS in general and other neurological disorders associated with FUS.

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P08.43

Genomics of FXTAS (Fragile X associated Tremor Ataxia) mouse brains reveals hallmarks of altered neurodevelopmement and neurodegeneration.

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While FMR1 is silenced in Fragile X syndrome (FXS) patients carrying the full mutation, its expression is elevated (2-8 fold) in premutated individuals. These subjects may develop the Fragile X-associated Tremor/Ataxia syndrome (FXTAS), a late onset neurodegenerative disorder characterized by ataxia and parkinsonism. In addition, people carrying the premutation can be affected by a set of neurological and behavioural disorders during young age. Memory problems have been detected in these patients as well as in the mouse model for FXTAS. In those animals, by transcriptome analysis we have shown a profound deregulation of the expression levels of genes involved in learning, memory and autistic behavior, Parkinson disease and neurodegeneration. These findings suggest the presence of a synapthopathy (Zongaro et al., HMG, 2013). Furthermore, we studied gene expression profiles of whole brain obtained from both young and old FXTAS mice. We observed that in this context different neurodegenerative pathways are involved at different ages. In FXTAS mice, we also studied the expression level of several microRNAs that target FMR1 mRNA and we observed that one them, miR-221, is down-regulated at the synapse of young FXTAS mice and in the full brain of the older ones. Interestingly, many of those deregulated mRNAs at the synapse, as well as in whole brain, are target of miR-221. These findings suggest a network regulation - including miRNAs and mRNAs- for FMR1 mRNA.

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P08.44

Evaluation of NTF4 gene mutations in Brazilian patients with primary open angle glaucoma (POAG) in a case-control study

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Primary open-angle glaucoma (POAG) is a chronic neurodegenerative disease that leads to progressive damage to retinal ganglion cells (RGC) resulting in visual field loss. Although the pathophysiology of glaucoma is not well understood, members of the neurotrophin family are known to support the survival of neuronal populations, including RGCs. The goal of this study was to investigate the contribution of Neurotrophin-4 (NTF4) gene mutations in the etiology of glaucoma in a sample of Brazilian POAG patients. A case-control study involving 130 POAG patients and 90 control subjects was performed through direct sequencing of the coding region of the NTF4. Here, we report four different heterozygous mutations in the NTF4 gene, accounting for about 3% of POAG patients; no mutations were observed in the control group.
Two of the mutations were in the 3' untranslated region and the other two led to amino acid changes in the protein. The mutations D196Y and R164M imply in changes of an Aspartic acid for a Tyrosine and an Arginine for a Methionine, respectively. Both changes correspond to amino acids with different side chain polarity and charge that may affect NTF4 either the monomer or dimer structure. To confirm this hypothesis investigation about the functional consequences and phenotypic implications must be performed.
NTF4 is functionally related to POAG but the mutation frequency in Brazilian POAG patients is low, although higher than in Caucasians and Asians POAG cases. Therefore, NTF4 does not have a major contribution in the genetics of POAG in the Brazilian population.

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P08.45

Founder SPG7 mutation causing hereditary spastic paraplegia in Gypsies - clinical and genetic considerations

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Hereditary spastic paraplegias (HSP) are a group of clinically and genetically heterogeneous neurodegenerative disorders characterized by progressive spasticity and weakness of lower limbs, due to retrograde degeneration of corticospinal axons. Mutations in the SPG7 gene cause autosomal recessive HSP, characterized by both pure and complicated forms of spastic paraplegia. Recently, several heterozygous SPG7 mutations were reported in affected individuals, although the evidences for their dominant disease-causing effect are vague and contradictory. Here, we describe 6 Gypsy HSP pedigrees from Bulgaria harboring the p.L78X mutation in the SPG7 gene. The same mutation was reported in Gypsy patients with spastic paraplegia from Spain and in 2 Italian HSP pedigrees. We identified a common haplotype shared between all families, suggesting a founder effect. Importantly, the p.L78X mutation caused the HSP phenotype in both homo- and heterozygous state. Sanger sequencing excluded SPG7 compound heterozygosity in the symptomatic carriers. Genotype-phenotype correlation study of over 20 homozygous and 45 heterozygous Gypsy individuals with a homogeneous genetic background allowed characterizing the p.L78X penetrance and phenotype expressivity. Our findings broaden the clinical spectrum of SPG7 mutations, provide strong evidences favoring the dual nature of molecular defects in this gene, and allow establishing diagnostic and counseling guidelines for HSP patients from Gypsy origin.

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P08.46

Alteration of Fatty-Acid-Metabolizing Enzymes Affects Mitochondrial Form and Function in Hereditary Spastic Paraplegia

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Hereditary spastic paraplegia (HSP) is considered one of the most heterogeneous group of neurological disorders, both clinically and genetically. It comprises pure and complex forms that clinically include slowly progressive lower-limb spasticity resulting from degeneration of the corticospinal tract. At least 50 loci accounting for these diseases have been mapped to date, and mutations have been identified in 29 genes, most of which playing a role in intracellular trafficking. Here, we identified mutations in two functionally related genes (DDHD1 and CYP2U1) in individuals with autosomal recessive forms of HSP by using either the classical positional cloning or a combination of whole-genome linkage mapping and next-generation sequencing. Interestingly, three subjects with CYP2U1 mutations presented with a thin corpus callosum, white-matter abnormalities, and/or calcification of the basal ganglia. Furthermore, we recently identified a new mutation of *CYP2U1* in a Switzerland family: c.1A>C/ p.M1L. This mutation is also present in an asymptomatic sister aged of 50 years; suggesting an incomplete penetrance or a metabolic compensation in this individual. These genes code for two enzymes involved in fatty-acid metabolism, and we have demonstrated in human cells that the HSP pathophysiology includes alteration of mitochondrial architecture and bioenergetics with increased oxidative stress. Our combined results focus attention on lipid metabolism as a critical HSP pathway with a deleterious impact on mitochondrial bioenergetic function.

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P08.47

Functional study of swiss cheese, ortholog of the Hereditary Spastic Paraplegia gene

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Hereditary spastic paraplegias (HSP) are a clinically and genetically heterogeneous group of neurodegenerative diseases characterized by degeneration of corticospinal tract axons and progressive lower-limb spastic paralysis. Neuropathy target esterase (NTE) is one of the HSP genes and responsible for the development of the early-onset
autosomal-recessive HSP form. NTE is also involved in the pathogenesis of organophosphorous compound-induced delayed neuropathy (OPIDN). Both HSP and OPIDN are characterized by the distal axonopathy. The molecular mechanisms underlying the axonopathy involved in HSP and OPIDN as well as in many other neurodegenerative human disorders are poorly understood. NTE is a highly conservative gene with orthologs in many species. Mutation of NTE's Drosophila ortholog swiss cheese (sws) leads to neurodegeneration, motor impairment, and reduced life span in the insect. An understanding of sws role in axon formation, growth and deneration will help to gain insight into NTE's role in similar processes in the human nervous system. To study sws functions we use the system of the 3rd instar larval neuromuscular junctions (NMJ) of Drosophila melanogaster. In this study we show that mutations in sws (sws1, sws4, sws7615, swsolfE) alter NMJ morphology, cause abnormal distribution of synaptic proteins, disruption of axonal transport and tubulin network in NMJ of Dr. melanogaster. We propose that sws plays an important role in synaptogenesis processes as well as axon formation and functioning in Drosophila melanogaster.

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P08.48

Huntington Disease: two case reports of pregnancies continued after a positive prenatal diagnosis

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Prenatal diagnosis for Huntington Disease (HD) is uncommon. Rarer still is deciding to continue a pregnancy when the fetus has a CAG expansion in the HD range. We report two cases of continued pregnancies following a positive prenatal test. In both cases the mothers were known carriers of a CAG expansion following HD predictive testing.
Case 1: The patient was referred at 6 weeks' gestation for predictive testing Following a positive predictive test, she had amniocentesis, with the intent of ending the pregnancy if the test were positive. Testing was performed which revealed that the fetus also carried a CAG expansion. She altered her original plans, electing to continue the pregnancy. She subsequently underwent preimplanation genetic diagnosis (PGD) for her second child.
Case 2: The patient had a normal prenatal diagnosis in her first pregnancy. Subsequently, following unsuccessful PGD attempts, she had four natural conceptions which were all found to have a CAG expansion and were discontinued. When the fetus again was affected in the next pregnancy, the couple elected to continue the pregnancy.
We are not aware of any reports in the literature regarding the long term outcome for families when the presence of a gene mutation for HD is known before the birth of their child. We will discuss the follow up and general wellbeing of the current case families, including ethical and psychosocial issues raised by the circumstances. We anticipate they will help inform our practice regarding prenatal diagnosis for HD and other adult onset disorders.

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P08.49

Modulation of age at onset in Tunisian Huntington disease patients: Implication of 5 new modifier genes: DRPLA, ATXN1, TBP, DMPK and JPH3

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Our study aims to investigate the implication of CAG repeat expansion and 9 modifiers genes in age at onset (AO) of 10 Huntington's disease (HD) Tunisian patients.
The diagnosis of HD is already confirmed in our laboratory in 10 patients. Disease onset was noticed between 24 and 72 years. The number of CAG repeat varied between 40 and 49 CAG. Our patients were screened for 9 modifiers genes: JPH3, DRPLA, ATXN1, GRIN2A, GRIN2B, IT15, TCERG1, DMPK and TBP. For this we used triplet primed PCR and direct sequencing.
CAG repeat expansion varies between 40 and 49 repeats. Using SPSS 17.0 we established an inverse correlation between CAG repeat and the AO (R = -0.809, p=0.005). This CAG expansion was found to be implicated in 65.5% of the AO variance. We also established the implication of DRPLA CAG repeat at 66.3% (R square= 0.884; p=0.003) and the implication of DMPK CTG repeat at 22.02% (R square= 0.731; p=0.004) of the unexplained variance. DRPLA CAG repeat and DMPK CTG repeat were inversely correlated to the AO at respectively (R=-0.823, p=0.003) and (R=-0.641; p=0.046).
TBP, ATXN1, JPH3, GRIN2B (rs 890) and TCERG1 genes were also implicated in the AO unexplained variance at respectively 1.44%, 4.34%, 8.69%, 13.04% and 25.5%, whereas GRIN2A (rs1969060) did not show any impli-

cation in our population.
We report here the first North African study on HD. We investigated 5 new polymorphisms located at DRPLA, TBP, ATXN1, DMPK and JPH3 genes.

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P08.50
Dog as an animal model for idiopathic epilepsy: the Schipperke breed as an example

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Idiopathic epilepsy (IE) is a common neurological disease in human and dog. Relatively few risk genes have been identified for common IE to date. We have used the Schipperke dog breed as an animal model for human IE to identify disease risk genes. The seizure characteristics are similar between the two species and reduced genetic heterogeneity of purebred dogs is advantageous for genetic studies.
62 epilepsy patients were analysed through detailed owner-filled epilepsy questionnaires. 12 cases and 8 controls were clinically studied. 56 cases and 66 healthy controls were genotyped for genome-wide association (GWAS) with Illumina 170K SNP arrays. The data was analysed with the GenABEL Grammas test.
The age of onset varied between 6 months and 5.5years (mean 3years). The median seizure frequency was 5/year and the typical seizure duration was 7min (range 0.5min-30min). One-third of the owners were able to identify phenomenology preceding convulsions as a sign of focal seizure activity. The clinical examinations confirmed IE in the studied cases. In the GWAS, two loci showed tentative association on CFAs 10 (p(raw)=1.76e-05, p(gwas)=0.30) and 37 (p(raw)=5.97e-06, p(gwas)=0.12).
Based on the epilepsy questionnaires and clinical examinations, we described focal and generalised IE with variable expression in Schipperkes. The associated locus on CFA37 is also a risk locus for IE in Belgian Shepherds. This indicates that these two breeds related with each other also share common risk factors for epilepsy. We currently aim to replicate our findings in larger materials and investigate the associated loci in more detail.

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P08.51
No evidence for a role of Cystatin ß dodecamer repeat expansion in Juvenile Myoclonic Epilepsy

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Juvenile myoclonic epilepsy (JME) is a common form of idiopathic generalized epilepsy (IGE) that has a relevant genetic contribution, but so far genes related to JME families remain largely unknown. JME shares electro-features with Unverricht-Lundborg disease (EPM1) that is a form of the progressive myoclonic epilepsy characterized by myoclonus, epilepsy and progressive neurologic deterioration. EPM1 is caused by mutations in the gene that codes for cystatin ß, an inhibitor of cysteine protease. The most common mutation in EPM1 is an expansion of a dodecamer repeat located in a non coding region upstream of the transcription start site of the cystatin ß gene. Since JME and EPM1 are both myoclonic epilepsies, we investigated the role of dodecamer repeat expansion in patients with JME. Thirty-five patients (26 women; mean age: 22.4, + 6,3; mean age at onset: 15,7 + 3,5) with JME were enrolled. Twenty-four had a positive family for JME or IGE. DNA was extracted by standard methods. Dodecamer repeat expansion was amplified by expand long template PCR system and detected by electrophoresis analysis. All subjects provided written informed consent, as required by ethics committees in each epilepsy centre of all the participating investigator. The analysis of the dodecamer repeat expansion did not evidence any expanded alleles in all 35 patients with JME. Our study did not support a role for cystatin ß dodecamer repeat expansion in JME. It remains to be clarified if the so-called minor EPM1 mutations might account for a proportion of the genetic susceptibility to JME.

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P08.52
Whole exome sequencing in a cohort of 70 undetermined leukodytrophic families

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Leukodystrophies are a group of orphan genetic diseases that affect the white matter (WM) and its main component, the myelin. Diversity of the genes involved in the development and homeostasis of the WM explains the large heterogeneity of this group of disorders. During this last decade, we collected through the LeukoFrance netwok a total of 1182 leukodystrophic families. Despite advances made in gene identification of UL, 68% of our families remain without genetic marker. We selected for whole exome sequencing a cohort of 70 families with reliable clinical and neuroradiological data. We used homozygosity as an effective filter in consanguineous families (27 families). The preliminary Exome results of 11 families revealed (i) one patient mutated for a gene already involved in a leukodystrophy (POLR3) with isolated ataxia. This patient born of consanguineous union was found to be compound heterozygous, therefore genetic diagnosis have been missed by homozygosity mapping only.; (ii) for two patients mutations were found in genes known to cause other type of neurodegenerative diseases with therapeutic approaches (BOLA3 and FOLR1). The two reported patients exhibited unusual phenotypes; (iii) in 5 patients, variants have been found as possible causative mutations in 5 genes not previously reported in neurodegenerative disorders. However, the biological role of these genes and the type of mutations found highly suggested their deleterious effects. This study underlines the particular interested of Exome sequencing in leukodystrophies, in which the molecular strategy for diagnosis is up to now based on WM "MRI recognition pattern".

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P08.53
A severe form of Pelizaeus-Merzbacher disease caused by *PLP1* triplication.

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INTRODUCTION: Pelizaeus-Merzbacher disease (PMD) is a rare X-linked myelination disorder caused by genomic alterations of the *PLP1* gene. Typical forms of PMD are characterized by early nystagmus and hypotonia that later evolves into spastic tetraparesis, dystonia, and ataxia. *PLP1* gene duplications are responsible for 60% of the cases. In addition to duplication, other rearrangements, including deletions, translocations, and triplications, have been described underlying a complex genomic architecture.
CLINICAL REPORT: We report the case of a 5-month-old boy, second child of unrelated parents. Pregnancy was uneventful but nystagmus was noticed at birth and subsequent psychomotor development was severely delayed. At the age of 2 months the patient exhibited opisthotonic postures. On examination at 5 months of age, we report absent visual fixation, nystagmus, constant choreoathetoid movements, and severe hypotonia. The brainstem auditory evoked potentials (BAEP) results were seriously altered showing only wave I. His brain magnetic resonance imaging (MRI) displays a typical profound diffuse hypomyelination.
MOLECULAR ANALYSIS: *PLP1* gene dosage analysis using multiplex ligation-

dependent probe amplification (MLPA) and custom CGH array (Agilent) revealed a triplication of a 110 Kb genomic segment containing the *PLP1* gene embedded in a larger duplicated segment of 510 Kb.
CONCLUSION: To our knowledge, only 7 PMD patients with more than 2 copies of the *PLP1* gene were reported in the literature so far. Our case brings additional evidence that patients with *PLP1* triplications exhibit a more severe phenotype than patients with duplications.

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P08.54

Molecular characterization of *PLP1* duplication in a large cohort of Pelizaeus-Merzbacher disease patients

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INTRODUCTION: Pelizaeus-Merzbacher disease (PMD) is an X-linked dysmyelinating disorder characterized by nystagmus, hypotonia, spasticity, ataxia and psychomotor developmental delay. The most frequent molecular aberration responsible for about 60% of cases is a duplication of a genomic segment of variable length containing the entire *PLP1* gene. *PLP1* encodes a major protein component of central nervous system myelin and *PLP1* duplication-subsequent overexpression in oligodendrocytes results in cellular toxicity and subsequent hypomyelination. **MATERIALS AND METHODS:** This study has been performed in a large cohort of 48 duplicated patients with reliable and long term clinical data (leukofrance database). We analyzed *PLP1* gene dosage and size of the duplicated segments with high-resolution custom array-based CGH including 30,032 probes spanning a 20Mb region surrounding the *PLP1* gene. Precise breakpoint mapping has subsequently been performed using breakpoint junctions sequencing and genome walking strategy. **RESULTS:** This custom *PLP1* array-based CGH analysis in a large cohort of patient confirmed a great diversity of duplication size and breakpoints. Sequencing of the successfully amplified duplication breakpoint junction reports complex rearrangement mechanisms involving the *PLP1* gene. **CONCLUSION:** The complex local genomic architecture of the *PLP1* locus likely drives the DNA susceptibility to complex rearrangements. More investigation is required to fully understand the mechanisms involved in those genomic rearrangements and their correlation with the variability observed in the disease severity.

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P08.55

Array CGH and undetermined leukodystrophy

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Objective: Research of small-size chromosomal rearrangements in patients with undetermined leukodystrophy.
Methods: Thirty patients with undetermined hypo or demyelinating leukodystrophy were analysed by array CGH (Agilent 4x180K). Rearrangements not found in databases as polymorphism variants were confirmed by Quantitative Multiplex Fluorescent PCR (QMF-PCR). To determine their inherited or de novo status we realised familial cytogenetic investigations by QMF-PCR. Finally, in order to evaluate the deleterious status and clinical implication, expression study and X-inactivation analysis were performed to argument in favour of pathogenic or polymorphic rearrangements.
Results: Thirteen among the 30 explored patients present 1 or 2 rearrangements not described as polymorphism variants. Array CGH allowed us to identify 2 pathogenic deletions already described in leukodystrophies and taking out known causative genes: *MCT8* (Xq13.2 – 31kb) and *MBP* (18q22.3q23 – 7,88Mb). Two possible pathogenic rearrangements were identified, one deletion taking out part of *CNTNAP2* gene (Xq22.2 – 26kb) and one duplication upstream *PLP1* gene involved in Pelizaeus-Merzbacher Disease. Finally, for 4 patients, we concluded on VOUS (Variation Of Unknown Signification).
Discussion: Because of their clinical and genetic heterogeneity, array CGH, high resolutive and pangenomic technique, has its entire place in the etiologic diagnostic strategy of undetermined leukodystrophy. Indeed, this study shows that array CGH allows both to highlight rearrangements implicating genes already involved in these pathologies and to identify new candidate genes. However, because of the difficulty to conclude in some cases about the pathogenic or polymorphism status, additional analyses are still in progress (expression study, mutations screening…).

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P08.56

Protective effects of epigallocatechin-3-gallate against hydrogen peroxide-induced apoptosis in transformed lymphoblastoid cells from patients with Machado-Joseph disease

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Machado-Joseph disease (MJD) or spinocerebellar ataxia type 3 (SCA3), the autosomal dominant neurodegenerative disorder, is caused by an increased number of CAG trinuclear expansion repeats in coding regain of *SCA3/MJD1* and resulted in a disease protein with a higher polyQ domain. The study of rare, inherited mutations underlying familial forms of MJD's disease has provided insight into the molecular mechanisms of disease pathogenesis. Neurodegenerative disorders are a class of disease in which elevated levels of reactive oxygen species (ROS) and apoptosis lead to tissue damage. Epigallocatechin-3-gallate (EGCG) is a major component of green tea polyphenols which displays potential properties of neuroprotection. We investigated the protective effects of EGCG extract against hydrogen peroxide (H_2O_2), a toxin created by oxidative stress and implicated in neurodegenerative disease, in mutant ataxin-3 cells. Our results demonstrated that EGCG against H_2O_2-induced cytotoxicity in a dose-dependent manner ($p<0.05$). We examined the effect EGCG against H_2O_2-induced apoptosis through reciprocal regulating pro-apoptotic protein Bax and anti-apoptotic protein Bcl-2. Interestingly, EGCG against H_2O_2-induced apoptosis in mutant ataxin-3 cells was accompanied by the up-regulation of heat shock protein (Hsp) 70 expression. These results show that EGCG may be a potential therapeutic candidate for neurodegenerative diseases involving glutamate excitotoxicity such as MJD.

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P08.57

Autosomal Dominant Microcephaly: Identification of a Novel Locus

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Autosomal dominant microcephaly with mild to moderate mental retardation with no dysmorphism or other anomalies was diagnosed in eleven individuals of an Arab Israeli family. Craniosynostosis and environmental factors were ruled out per history as possible contributors to the disease, thus verifying the diagnosis of primary microcephaly. Brain CT scan of affected individuals showed no architectural anomalies. Nine living affected individuals were available for clinical and genetic evaluation. Association with all known microcephaly-associated loci was ruled out using polymorphic markers and genome wide linkage analysis data. Genome-wide linkage analysis revealed association of the disease to a region on chromosome 4 which was fine mapped with polymorphic markers to an area of approximately 20Mbp between markers D4S1558 and D4S2460, with a maximal LOD score of Z = 3.44, at marker D4S1534 ($\theta=0$). Whole exome sequencing has yielded several SNP mutations which are being explored in the affected family.

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P08.58

Six Novel mutations in MLC1 from 15 Iranian patients with Megalencephalic Leukoencephalopathy with Subcortical Cysts.

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Megalencephalic leukoencephalopathy with subcortical cysts (MLC) (MIM #604004) is a rare autosomal recessive neurological disorder characterized by macrocephaly, motor and cognitive decline, ataxia, spasticity and occasional seizures. Magnetic resonance imaging (MRI) shows diffusely abnormal and swollen white matter of the cerebral hemispheres, presence of subcortical cysts in the anterior temporal and frontoparietal region. Mutations in MLC1(22q13.33) and GLIALCAM have been identified in patients with MLC. Mutations in MLC1 account for 75% of mutations.

MLC was suspected in fifteen Iranian patients from thirteen families based on positive clinical findings including macrocephaly beginning in the first year, neurocognitive deterioration, seizure or loss of consciousness after minor head trauma. All except one were born to consanguineous parents. Brain MRI images were compatible with MLC and confirmed the diagnosis. Sequencing of entire coding region of MLC1 was performed for 12 patients and six novel mutations and six previously reported mutations were identified. This report shows that MLC is relatively common in Iranian population, as expected for rare diseases with high inbreeding, with surprisingly high frequency of novel mutations.

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P08.59

Cell model for Sanfilippo C syndrome using iPS cells

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Mucopolysaccharidosis III (MPS III), or Sanfilippo syndrome, includes four autosomal recessive diseases characterized by deficient heparan sulfate degradation. Clinical symptoms similar for all the types, include progressive and severe deterioration of the central nervous system during childhood. HGSNAT, the gene responsible for MPS IIIC, encodes the acetyl CoA:α-glucosaminide N-acetyltransferase, a lysosomal membrane protein. No therapies are available for Sanfilippo syndrome.

Human induced pluripotent stem cells (iPSc) offer a unique opportunity to model human neurodegenerative diseases. Several models have been obtained using that technology.

We developed a neuronal cell model for Sanfilippo C syndrome from patients' fibroblasts. Patient 1 was heterozygous for a missense (p.L445P) and a splicing (c.633+1G>A) mutation, while patient 2 was homozygous for a splicing mutation (c.372-2A>G) prevalent in Spanish patients.

Retroviral vectors encoding KLF4, OCT4, and SOX2, were used to obtain different clones of iPSc from each patient and a control healthy individual. One clone of each line was validated. Cells were positive for alkaline phosphatase, showed normal karyotypes, expressed markers of pluripotency (SSEA3, SSEA4, NANOG and TRA-1-81) and the genes encoding for the different factors were integrated in their genome. Quantitative PCR to establish the efficient repression of the exogenously introduced genes was carried out, and the ability of the cells to differentiate to the 3 germ layers was assayed in vitro and in vivo. OCT-4 and NANOG promoters were demethylated. After the validation, the cells were differentiated to neurons and astrocytes in order to study the molecular basis of the disease into these cellular types.

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P08.60

A *CIITA* gene polymorphism (rs4774*C) in conjunction with the *HLA-DRB1*15:01* allele increases susceptibility to multiple sclerosis in Brazilian females.

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We investigated the association of 83 alleles of polymorphic HLA-Class II genes with multiple sclerosis (MS) susceptibility, as well as the association of two CIITA alleles. Blood samples were taken from 52 patients (35 female) and from 126 healthy control subjects matched for ancestry, sex and age. Patients were classified according to the criteria laid out by McDonalds et al. (2001). After DNA extraction, the alleles of HLA-DRB1, DQB1 and DQA1 were identified by PCR-SSP. Sequencing of single nucleotide polymorphisms +1614G/C (rs4774*C; G500A) and -168A/G (rs3087456) on CIITA was performed by PCR, followed by capillary electrophoresis in the platform ABI PRISM® 3500 Genetic Analyzer. All data were subjected to Bonferroni´s correction. The RR for MS associated with the HLA-DRB1*15:01 allele was 2.79 (OR=3.52; p value=0.001) and the RR associated with the DQB1*06:02 allele was 2.42 (OR=2.72, p value=0.020). Mantel Haenszel-corrected p values were 0.001 and 0.020 for the OR and RR, respectively. After Bonferroni's correction, only DRB1*15:01 data within our set of polymorphic alleles were statistically significant. In addition, MS association with DRB1*15:01 seemed to be conspicuous only in female patients: 31.43% of female patients (RR=4.78; OR=3.59; p value=0.001) and 23.53% of male patients (RR=2.05; OR=1.80; p value=0.159) had the allele. The CIITA polymorphism rs4774 (+1614G/C) together with HLA-DRB1*15:01 increased the RR to 3.63 (OR = 2.65, p value = 0.005) and this finding was especially female-related (RR = 4.05; OR= 4.55; p= 0.016).These results reinforced the multifactorial and polygenic trait of the disease.

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P08.61

Exome sequencing in multiplex families reveals novel genetic variants related to multiple sclerosis

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Multiple sclerosis (MS) is a chronic inflammatory neurological disorder belonging to a group of genetically complex disorders. It is known that MS accumulates in the families and it has been proposed that burden of genetic variants contributing to disease development might be particularly high in such families. Deep sequencing in subjects from families with a large number of affected individuals may thus present a promising opportunity for delineation of key genes conferring susceptibility to MS.

With this aim, we performed whole exome re-sequencing in 12 individuals originating from distinct multiplex families and 10 control subjects from the same background population. In familial MS patients, we observed an excess of rare and damaging mutations in immunoregulatory genes associated with MS in a recent large-scale GWAS by the WTCCC2 consortium (p=0.015). In 10 of 12 patients investigated, at least one predicted pathogenic rare variant in MS-associated genes was detected. We have also detected a novel pathogenic mutation in the TYK2 gene, which was previously reported to contain mutations segregating with familial MS. Literature-based relatedness analyses revealed that genes carrying detected mutations in familial disease were 10.7 times more directly or indirectly related to MS in comparison to those found in background control population.

In the present study, we demonstrate how exome sequencing in families with MS may assist in pinpointing specific genetic variants potentially contributing to disease susceptibility and thus provide novel insight into genetic architecture and pathogenesis of MS.

A. Maver: None. **B. Peterlin:** None.

P08.62
Signal transducers and activators of transcription genes (STAT genes) and Multiple sclerosis (MS)

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Background: Multiple sclerosis (MS) is a complex inflammatory disease of the central nervous system. Both environmental and genetic factors contribute to the development of the disease. We used an integratomic approach for discovery of putative regions and genes related to MS, using a comprehensive and unique set of data sources (GWAS, linkage studies, expression studies and proteomic studies). Among 381 genomic regions and 409 genes we selected STAT3 and STAT5A for association and expression studies.
Objectives: The aim of study was to investigate the association and expression of STAT3 and STAT5A genes in patients with MS from the Central South East Europe.
Methods: A total of 1045 Caucasian patients and 986 healthy unrelated ethnically matched controls without family history of MS, were included in the study. For gene expression in blood we analyzed 50 patients and 40 controls. Diagnosis of MS was established according to McDonald's criteria. Altogether, 5 SNP were included in our study, 3 in STAT3 gene: rs7211777, rs963987 and rs1053005; and 2 in STAT5A gene: rs6503695 and rs12601982.
Results: We found a statistically significant difference in the allelic distribution of STAT5A gene (rs6503696 with P value $7{,}5 \cdot 10^{-4}$), which was supported by haplotype distributions (haplotype CA $P=3 \cdot 10^{-5}$). We haven't found any diffrences in gene expression in blood in STAT5A.
Conclusion: We provide evidence for association between genetic variation in STAT5A gene and multiple sclerosis. Further studies are required to substantiate the significance of these genetic variations.

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P08.63
High resolution genotyping analysis of Multiple sclerosis in Saudi patients.

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Multiple sclerosis (MS) has been clearly associated with human leukocyte antigen (HLA) complex that reported in several studies; nevertheless; this association is poorly examined in Saudi MS patients. This disease is spreading widely through Saudi population; apart from parental consanguinity; no clear understanding of environmental and/or genetics factors responsible. However; recent population studies reflect that the consanguinity marriages presented in 37.6% of the familial MS (FMS); 16% were first degree relatives.
This project is focus on the investigation of HLA-A, HLA-B, DRB1, and DQB1 alleles in 150 FMS and non FMS affected, 100 healthy controls were recruited. The high-resolution genotype sequencing using Roche 454 FLX+ Titanium chemistry was carried out.
MHC class I (A, B) and class II (DQ) were indicated with an odds ratio (OR) = 2.96 (95% confidence interval Cl 1.46-6.02 p=0.004) for HLA-A*01:01, and for HLA-B*41:01 the OR 3.77 (95% Cl 1.64-8.63, p=0.0025), while for DQB1*03:02 the OR 2.20 (95% Cl 1.29-3.75, p=0.0051). No differences between FMS and non FMS were observed. These findings representing the Saudi MS population and HLA complex association that may be unique when compare with other populations.
A further investigation should be carried out to confirm the above finding and attempting to understand more on the involvement of environmental/genetics of MS in Saudi and Gulf region.

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P08.64
C19ORF12 Mutations in Neurodegeneration with Brain Iron Accumulation (NBIA)

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Neurodegeneration with Brain Iron Accumulation (NBIA) comprise heterogeneous group of progressive neurodegenerative disorders that present with a progressive extrapyramidal syndrome and excessive iron deposition in the brain, particularly affecting the basal ganglia, mainly the globus pallidus. Genetic defects in PANK2 gene are the most common cause of NBIA, followed by mutations in PLA2G6, few reported NBIA families are known to carry mutations in FA2H, a gene previously associated with familial leukodystrophy and spastic paraparesis. Mutations within C19orf12 have recently been identified in patients with NBIA.This gene C19orf12, codes for a mitochondrial membrane protein and the acronym MPAN (mitochondrial membrane protein-associated neurodegeneration). In this study we report the clinical description of five patients from five families with NBIA and the subsequent molecular genetic investigation. Sequencing of PANK2, PLA2G6 and FA2H was normal; whereas the molecular analysis of C19orf12 gene revealed a novel homozygous and heterozygous C19orf12 mutations in our patients with NBIA.
Further studies are needed to explore the function of C19orf12 in NBIA, and extended genetic analysis of larger patients cohorts will provide more information about the frequency of this disease.

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P08.65
Neurodegeneration with brain iron accumulation, type 4 (NBIA4) in Russian families

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NBIA4 is autosomal recessive disease produced by *C19orf12* mutations [Hartig et al, 2011]. NBIA group also includes most common NBIA1/Hallervorden-Spatz disease (*PANK2* gene), NBIA2A/2B (*PLA2G6*), NBIA3 (*FTL1*) and adjoining SPG35 (*FA2H*), Parkinson disease 9 (*ATP13A2*), aceruloplasminemia (*aCP*). Common features are neurological signs, mainly dystonia and spasticity, and MRI picture of iron accumulation in globus pallidum. Though recently recognized, NBIA4 was found in several populations and seems not very rare. Its clinical distinctions from NBIA1 are later onset, slower course, less pronounced dementia and subtle MRI differences. We diagnosed NBIA4 in two Russian and one mixed Ukrainian/German (Patient 3) non-consanguineous families. Patient 1, 19-year-old female, had slowly progressing leg spasticity since 12 yrs, moderate dystonia in neck, hands and feet since 17-18 yrs, optic atrophy detected in 18 yrs and borderline intelligence. Her MRI was normal in 14 yrs, but in 17 yrs showed NBIA signs. Patient 2, 16-year-old male, had slowly progressing spasticity, dystonia, ataxia and mild dementia since 11-12 yrs; MRI was normal up to 16 yrs when NBIA signs appeared; optic atrophy was seen at the same age. In both patients other NBIA were excluded and homozygosity for *C19orf12* mutation c.204_214del11 was detected. Patient 3, 36-year-old female of whom we have no clinical information, was found heterozygous for c.204_214del11 (allelic mutation is in search). The mutation was most common in the first NBIA4 cohort of 20 Polish families with founder effect [Hartig et al, 2011]. Our preliminary results point to c.204_214del11 spread in other Slavic populations.

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P08.66
Altered brain development and neurodegeneration in Clp1 knockout zebrafish

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Cleavage and polyadenylation factor I subunit (*Clp1*) is an important kinase in RNA metabolism. It is involved in processes as tRNA maturation and

mRNA 3'processing. We have created an ENU induced zebrafish mutant, harbouring a p.R44X nonsense mutation in the *Clp1* gene. *Clp1* knockout fish do not survive beyond 4 days post fertilisation (dpf), have a reduced head size and show an S-curved body. At 2dpf, *Clp1* knockout fish show reduced expression of midbrain marker otx2, and increased cell death in the brain.
We show that *Clp1* is an essential gene in brain development. In humans, various RNA processing genes have been associated with neurological diseases, such as the TSEN complex - which associates with *CLP1* - in pontocerebellar hypoplasia and the *SMN1* gene in spinal muscular atrophy. Our data supports the hypothesis that RNA processing plays a crucial role in neuronal development. The exact mechanism how disturbed RNA metabolism leads to neurological diseases is still unknown.

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P08.67

A novel FTL mutation responsible for neuroferritinopathy with asymmetry of clinical features and brain anomalies

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Neuroferritinopathy is a rare autosomal dominant neurodegenerative disease caused by mutations in *FTL* gene and which belongs to the group of Neurodegeneration with Brain Iron Accumulation (NBIA). Progressive iron and ferritin deposition in basal ganglia, substantia nigra, red nuclei and dentate nuclei is responsible for abnormal movements including chorea, dystonia and parkinsonism which represent the most common features of this disorder. To date, 7 mutations in *FTL* gene have been reported, 6 *frameshifts* in exon 4 and a missense in exon 3. Here we report a patient carrying a novel *FTL* mutation with original clinical and imaging features.
First manifestations occurred by 29 years by focal dystonia limited to right foot for 10 years. At 40, dystonia extended to left upper limb, neck and was associated with dysarthria. Ferritinemia was below the normal range (49 µg/L, norm within 80-250). Brain MRI revealed hypointensities on T2* weighted images, asymmetric in basal ganglia with left predominance and symmetric in motor rolandic cortex, substantia nigra, red and dentate nuclei. The previously unknown c.468dup mutation (p.Gly157TrpfsX24) was identified and appeared to be *de novo*. The mutation leads to C-terminus modification of the protein similar to recurrent c.460dup mutation, and alters E-helix structure affecting ferritin folding, assembly and stability.
Our report confirms involvement of *frameshift* mutations located in 3' coding region of *FTL* in pathophysiology of neuroferritinopathy. This observation highlights that this disorder can manifest with asymmetric clinical features and brain anomalies. Neuroimaging and especially T2* sequences is the most sensitive investigation for NBIA diagnosis.

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P08.68

Peripheral Neuropathy and Lymphedema Associated with Mental Deficiency - Homozygosity Mapping and Gene Indentification

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Background: We present an extended consanguineous Muslim Israeli-Arab family, comprising of individuals exhibiting peripheral neuropathy, peripheral lymphedema or both. The neuropathic phenotype varies between family members in age of onset, spasticity and the existence of mental retardation. Both peripheral lymphedema and peripheral neuropathy segregate independently in affected family members as autosomal recessive traits. This study aimed to investigate the molecular basis for both disorders.
Methods: Homozygosity mapping using Illumina CytoSNP6000 and CytoSNP300K arrays was employed, followed by whole-exome sequencing in one patient.
Results: A 6K SNP array linkage analysis and whole-exome sequencing of a sibling affected with both disorders lead to the discovery of a missense mutation in the FLT4 gene (known to highly associate with lymphatic disruption). The mutation segregates as a homozygous trait within the family in line with the lymphedema phenotype, unrelated to the neuropathy phenotype. The whole-exome analysis revealed a missense mutation in the SPG7 gene (causing autosomal recessive spastic paraplegia) in the tested individual, but it did not segregate well within the family. A 300K SNP array narrowed the candidate areas to ten essentials. Areas with highest LOD scores or containing candidate genes were excluded using microsatellite markers. We are currently evaluating additional mutations identified via exome sequencing, in parallel to the linkage analysis described above.
Conclusion: While peripheral lymphedema in affected individuals was caused by a mutation in FLT4 gene, the gene causing peripheral neuropathy remains to be pinned down. Our findings reinforce the separation between both disorders within the extended family.

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P08.69

Niemann-Pick type C disease: Characterization of NPC1 mutations on mRNA and protein level

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Niemann-Pick type C disease (NPC, OMIM #257220, #607625) is a severe autosomal recessive neuro-visceral disorder characterized by progressive neurological deterioration and hepatosplenomegaly. It is associated with complex lysosomal storage of lipids, mainly of free cholesterol and glycolipids, and with mutations in one of two genes (NPC1, NPC2). We have found disease causing mutations in 38 NPC1 families and characterized molecular consequences of a subset of NPC1 mutations on mRNA and protein levels. Six genotypes (p.R1186H/S954L; p.R1186H/P1007A; p.R1186H/Y276H; p.P1007A/V950G; p. S954L/L176R; p.V664M/Arg404Glyfs*45) identified in 10 patients were inspected. We used PCR/RFLP followed by fragmentation analysis to determine the allelic expression ratio of the mutated alleles in cultured skin fibroblasts. NPC1 protein levels were assessed semi-quantitatively by Western blotting. Interestingly, the amount of immunoreactive NPC1 in p.V664M/Arg404Glyfs*45 cell line was comparable to the controls and RNA allelic transcript ratio was 50/50. In other samples RNA transcript ratios ranged from 22/78 to 30/70 and the amount of immunoreactive NPC1 protein was diminished but detectable. We have also analyzed NPC1 promoter region (up to 2 kb upstream from the start codon). We have found six common polymorphisms forming four probable haplotypes. We did not identify any rare variants, which could potentially affect regulation of NPC1 transcription.
In summary, the results show that the analyzed missense mutations allow residual protein synthesis and that the fibroblast cultures may be useful for testing of compounds stabilizing mutant NPC1 protein.
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P08.70

Recessive truncating NOTCH3 mutation in a case of severe, early-onset, progressive vascular leukoencephalopathy

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We report on a 24 years-old male patient, born to consanguineous parents, presenting with infantile-onset, progressive neurodegeneration, small vessel disease and leukoencephalopathy. Brain MRI showed marked hyperintensity on T2-sequences of cerebral white matter (WM) with enlarged ventricles and diffuse cavitations, subcortical lacunar infarcts and disseminated microbleeds. Focal hyperintensities on T2-sequences were present in the asymptomatic parents, especially in the father.
Whole Exome Sequencing (WES) identified a homozygous c.C2742A NOTCH3 mutation, inherited from heterozygous parents. No other obvious mutation emerged from WES/mtDNA sequencing. The c.C2742A mutation introduced a p.C914X stop-codon leading to a prematurely truncated protein. Testing cDNA from muscle biopsies, we observed drastically reduced NOTCH3 expression in the proband and parents vs.controls ($p<.001$), and in the proband vs.parents ($p<.05$). Alterations of smooth muscle cells (SMC)

surrounding small arteries were visible in skin biopsies of the proband and parents, showing a multilayered basal lamina. An abnormal pattern of collagen staining, characterised by unpacked collagen wall, was evident in skin vessels of proband and parents, although it was less pronounced in the mother.
Heterozygous missense NOTCH3 mutations are known to underlie CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy), the most common heritable cause of cerebral arteriopathy. Notably, no granular osmiophilic material (GOM) deposition (a hallmark of CADASIL arterioles) was detected. Molecular mechanisms by which NOTCH3 mutations predispose to ischemic stroke are poorly understood. Here, we described a novel recessive NOTCH3-related leukoencephalopathy, linking reduced NOTCH3 expression and signaling to arterial SMC degeneration and ultimately to small vessel leukoencephalopathy.

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P08.71
Identification of a novel splice site mutation of STXBP1 in Ohtahara syndrome

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Ohtahara syndrome (OS, OMIM#308350, ORPHA1934) is early infantile epileptic encephalopathy characterized by tonic spasms and a continuos burst-suppression pattern resulting in intractable seizures and severe mental retardation. Brain malformations or metabolic disorders are often associated with this syndrome but some cases remain etiologically unexplained. Mutations in *ARX*, *CDKL5*, *SLC25A22* and *STXBP1* have been identified.
We report the case of a 19-months old infant which presented with tonic, focal clonic and oculogyric seizures in the first 15 days of life. An EEG performed at the age of 45 days showed a burst-suppression pattern suggestive of Otahara syndrome. Physical examination did not reveal any specific dysmorphic features. Neuroimaging and extensive screening for inborn metabolic errors and congenital infections were uninformative. At 5 months spasms appeared. Treatment with vigabatrin and corticosteroids was effective but refractory complex partial seizures appeared. The infant has a severe psychomotor delay.
We found a novel inherited heterozygous mutation (c.1249+2T>C, G417AfsX7) in *STXBP1*, a gene that codes for syntaxin binding protein 1 involved in synaptic vesicle exocytosis. This mutation is localized in a donor splice site and eliminates exon 14 producing a truncated protein.
Loss of domain III-b and part of domain II due to this mutation could affect Rab binding and consenquently exocytosis process.

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P08.72
Evaluation of variants in Parkin, PINK1, DJ1 and SCNA genes in early-onset Parkinson's disease patients of Turkish population

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Parkinson's disease (PD) causes by the interaction of genetic and environmental factors. Recent reports showed that, variations in PARK loci genes (*Parkin, PINK1, DJ-1* and *SNCA*) cause inherited forms of typical PD in different populations. However, the knowledge about the frequency of these alterations in Turkish population is very limited. In current study, we aimed to evaluate the phenotype and frequencies of variations in these genes in Turkish early-onset PD patients.
All coding regions and exon-intron boundaries of the *Parkin, PINK1, DJ-1* and *SNCA* genes were screened by Heteroduplex analysis (HDA) followed by direct sequencing of detected variants in 27 early-onset PD patients.
Alterations were determined in 12 (44,4%) patients; 1 patient had 2 different alteration. Alterations were found in *Parkin* (n=6; IVS-35G>A and n=1; V379L) for 7 patients, in *PINK1*(IVS1-7A>G) for 5 patients and in *DJ1*(n=1; IVS3+40T>G and n=1; R98E) for 2 patients. According to Ensemble Genome Browser, IVS-35G>A in *Parkin,* IVS1-7A>G in *PINK1* and IVS3+40T>G in *DJ1* were novel alterations. There was no significant correlation between having PARK loci gene alterations and family story (P = 0,452).
Despite current data is preliminary findings, we identified three novel alterations in *Parkin, PINK1* and *DJ1*genes in PD patients of our population. Although, increasing the number of cases is required to clarify clinical significancy, we suggest that these informations may be helpful in predicting the prognosis of Turkish PD patients.

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P08.73
Analysis of *SNCA* (alpha-synuclein) mutations in Slovak Parkinson disease patients

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Parkinson disease (PD) is a chronic neurodegenerative movement disorder characterized by selective loss of nigrostriatal dopaminergic neurons and formation of Lewy bodies. Clinical manifestations include motor impairments involving tremor, bradykinesia, postural instability and rigidity. PD is generally considered to be the result of the interaction between genetic and environmental factors. Mutations responsible for the recessive early-onset PD (EOPD) were identified in *parkin* (*PARK2*), *DJ-1* (*PARK7*) and *PTEN-induced kinase 1* (*PINK1*), while mutations within *Leucine-rich repeat kinase 2* (*LRRK2*) and *α-synuclein* (*SNCA*) are associated with autosomal dominant, late onset form of PD. To detect the most common mutations (A30P, E46K, A53T) in exons 2, 3 of the *SNCA* gene, we sequenced in both direction 160 unrelated Slovakian patients with familial or sporadic PD, including early and late onset patients. However, none of these mutations was identified in our cohort. We have found three novel exonic polymorphisms (SNCA-23T>C; c.467T>C, p.V15V; c.518C>A, p.K32K), one intronic polymorphism (c.121+11C>T, IVS3+11C>T, rs35135226) in exon 2 of *SNCA* gene. But in the exon 3 we did not observe any variation after sequencing.
However, we could not completely exclude the possibility that the patients carry other mutations or rearrangements within SNCA gene. In the follow up of this study also a gene dosage studies, especially of *SNCA* and *PARK2* genes have to be considered, in order to uncover possible larger genomic deletions/duplications.
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P08.74
A rapid and simple PCR-RFLP test for the diagnosis of PARK8 form of Parkinson disease in the Algerian population

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PARK8 form of Parkinson's disease is a common disorder in Algeria (40% of familial or sporadic cases) because of the high prevalence of the c.6055G>A (p.Gly2019Ser) mutation in the LRRK2 gene. The aim of our work was to highlight the occurrence of this alteration by a simple, fast and accurate test, thus signing a diagnosis of PARK8 Parkinson's disease. Our screening strategy consisted of a targeted amplification of exon 41 of LRRK2 by a PCR-RSI approach, followed by an enzymatic digestion using restriction endonuclease AluI, with the goal of individualizing precisely and unambiguously all possible genotypic statuses: healthy homozygous and affected heterozygous/homozygous. The choice of primers allowing amplification and introducing a mismatch site downstream of the coding nucleotide c.6055 was conducted by bioinformatic analysis. Optimization of PCR conditions helped ensure the success of the amplification and the subsequent AluI digestion. Genotypes of individual controls (healthy c.6055G/G, affected c.6055G/A and affected c.6055A/A) were clearly individualized on the basis of restriction profiles obtained. The availability of this test, also avoiding the use of sequencing, provides a powerful approach for the diagnosis of the common form of Parkinson's disease PARK8, allowing to recognize the status of individuals and offering the opportunity to make a differential diagnosis among the great genetic heterogeneity that characterizes this disease. The power of our diagnostic test of PARK8 allows us to screen at first instance this form of the disease and suggests that it may be used for routine screening of the disease in the Algerian population.

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P08.75

Analysis of Parkinson's disease brain DNA reveals the novel H50Q alpha-synuclein (*SNCA*) mutation which affects copper co-ordination

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Misfolding and aggregation of alpha-synuclein (SNCA) is crucial to the pathogenesis of Parkinson's disease (PD), yet mutations in the gene are very rare, with only three missense mutations and occasional copy number variants described. The possibility of sporadic cases being due to somatic mutations leading to mosaicism, which might be undetectable in peripheral lymphocyte DNA which is usually analysed, has not been formally investigated.
We screened *SNCA* coding exons in DNA from at least one brain region from 457 cases. Sanger sequencing in five samples revealed a novel change (c.150T>G, p.H50Q) in the cerebellum and substantia nigra of one apparently sporadic, late onset, typical PD case. As no relatives or other DNA sources were available, indirect evidence of mosaicism by analysis of phase in relation to a nearby polymorphism was sought, but none was found. Subcloning of PCR products revealed 14/30 colonies with the mutation. Further samples were analysed using High Resolution Melting (HRM) analysis, which has higher sensitivity than sequencing for low level mosaicism.
Serial dilution confirmed HRM could detect 1-5% level of H50Q/ A53T. No evidence of somatic mutations was seen, although rare synonymous SNPs were detected. The conservation of H50, the absence of the mutation in controls, and bioinformatic predictions, suggested pathogenicity. As H50 participates in SNCA binding of Cu(II), recombinant wild-type and H50Q proteins were compared using electron paramagnetic resonance, and altered copper co-ordination was observed. Stably transfected neuroblastoma cell lines were established for further studies. H50Q appears to be a likely pathogenic rare germline variant.

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P08.76

Analysis of *SNCA* mRNA splicing variants in human post-mortem tissue through Real Time PCR

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Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting 1-2% of people over 60 years-old. Abnormal expression and post-translational modification of *SNCA* gene have been associated with both familial and sporadic PD. The *SNCA* gene has four mRNA transcripts: SNCA-140 (full-length isoform), SNCA-126 (lacking exon 3), SNCA-112 (lakking exon 5) and SNCA-98 (lacking exons 3 and 5). Recently, it has been proposed that a *SNCA* polymorphism in the 3'UTR (rs356165) could play a role in the risk of PD through its association with differential mRNA expression.
Our aim was to determine the level of the four α-syn isoforms through Real time PCR in human post-mortem brains from 9 PD patients and 6 controls. We obtained tissue samples from affected and non-affected brain areas, and compared the α-syn levels between rs356165 genotypes (5AA, 7AG, 3GG).
We analyzed a total of 43 samples from *sustantia nigra* (SN), cerebellum (CB) and occipital cortex (OC). We did not find significant differences in the levels of *SNCA* transcripts; patients vs. controls in the three regions. The minor transcripts (SNCA-112 and 98) were increased in the SN in PD-affected brains, without difference between the rs356165 genotypes. The level of the SNCA-140 transcript was increased in OC.
In conclusion, we found different expression levels of the *SNCA* transcripts in different brain regions, but without difference between patients and controls. However with our sample we could not support the influence of rs356165 in *SNCA* mRNA levels.

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P08.77

The study of a role of monoamine metabolizing enzyme genes in Parkinson's disease development and neuropsychological abnormalities in Parkinson's disease patients

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The purpose of this investigation was to search for genetic factors in the development of Parkinson's disease (PD) as a whole and neuropsychological impairment in Parkinson's disease patients. The analysis of association of PD with polymorphic variants of monoamine metabolism genes - TH, DAT1, COMT, MAO-B, TPH1,DRD1, DRD2, DRD3, DRD4 , 5-HTT, HTR2A, - was performed using DNA collection of PD patients (560) and controls (670). DNA samples included three ethnic groups living in Bashkortostan Republic (BR) - Russians, Tatars and Bashkirs. In all three ethnic groups PD was associated with the polymorphic variants of the DRD4 gene: allele * 7 of the polymorphic variant 48 bp in VNTR-locus (in exon 3) was revealed to be the risk marker for PD development (p = 0,02; OR = 2,0). In Tatars the genotype *H/*H (p = 0,0006; OR = 2,3) and allele *H of polymorphic locus Val108Met of the COMT gene, genotype * C/* C (p = 0,002; OR = 1,7) and allele *C (p = 0,02; OR = 1,34) of rs1800532 of the TPH1gene and allele *12 of the polymorphic locus STin2 (p = 0,04; OR = 1,3) of the gene 5-HTT were markers of the increased risk for PD development. In Bashkirs allele *C of rs6280 of DRD3 gene (p = 0,02; OR = 1,85) was the risk marker for PD. Study of neuropsychological impairments found influence of polymorphic locus Val108Met of the COMT gene on the development of dementia in PD patients.

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P08.78

Molecular genetic background of PEHO syndrome

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PEHO syndrome (Progressive encephalopathy with Edema, Hypsarrhythmia and Optic atrophy; MIM 260565) is an autosomal recessive inherited progressive infantile encephalopathy. The main features include hypotonia, infantile spasms and/or hypsarrhythmia, psychomotor retardation, absence or early loss of visual fixation, edema of the face and limbs, and typical dysmorphic features. Brain atrophy is progressive and most prominent in the cerebellum. Imaging findings suggest the presence of two subtypes of the PEHO syndrome, cortical atrophy and loss of myelin being more pronounced in type 2. In a heterogenous group of PEHO-like patients some but not all of PEHO features exist. Our aim is to characterize the molecular basis of PEHO.
Using SNP genotyping and homozygosity mapping we identified in Finnish patients with PEHO type 1 a missense mutation in a gene not previously associated with human disease. The encoded protein is strongly expressed in neural progenitor and migrating premature granular cells and its tissue expression pattern is compatible with the neuropathological findings in PEHO type 1 patients. The detected mutation affects stability and coregulatory abilities of "PEHO1". Moreover, knocking down "PEHO1" expression by RNAi in ex vivo cerebellar cultures resulted in disturbed granule cell migration consistent with previous observations of ectopic granule neurons in PEHO patient cerebellum. To identify the gene underlying PEHO type 2 we are currently analyzing exome data on two siblings and two clinically similar unrelated Finnish patients.

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P08.79

Spectrum of cerebellar and anterior horn cell degeneration caused by *EXOSC3* mutations

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Pontocerebellar hypoplasia (PCH) associated with spinal cord anterior horn cell loss has been named PCH type 1 by Barth in 1993. PCH1 is an autosomal recessive disease characterized by an early- often antenatal -onset, with arthrogryposis and limited survival. Recently mutations of the EXOSC3 gene were reported in classical as well as in mildly affected PCH1 patients.
We report 4 patients affected with cerebellar and anterior horn cell degeneration and EXOSC3 mutations. Two sibs were affected from birth and presented with hypotonia, proximal muscular deficiency, respiratory and swallowing difficulties. Electromyogram showed neurogenic changes. PCH was evident on the MRI performed at 4 months in the older child but no change was noted at day 4 in the younger. Death occurred at, respectively, 11 months and 15 days. Neuropathology showed a severe atrophy of the pons, cerebellum and anterior horn.
Patient 3 presented with a progressive disease which began at 3 months. He had severe progressive hypotonia, proximal limb weakness and amyotrophy, nystagmus, spasticity, and increased tendon reflexes. He developed respiratory failure and died at 3 years.
Patient 4 had a non progressive psychomotor retardation with PCH on MRI and he was able to sit in the first years of life. At age 10, he started to decline and presented with spastic paraplegia and proximal weakness, nystagmus and respiratory and swallowing difficulties.
The gene EXOSC3 is a major PCH1 gene but is responsible for others, milder, phenotypes of cerebellar and anterior horn cell degeneration with cerebellar atrophy without brainstem involvement.

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P08.80

Selective alteration of glutamate and GABA homeostasis in the cerebellum of SCA5 patients

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Background: Spinocerebellar ataxia type 5 is a very rare form of autosomal dominant cerebellar ataxia that is caused by mutations in SPTBN2. Mutations in SPTBN2 affect the stability of EAAT4, a glutamate transporter specifically expressed in Purkinje cells, which also negatively regulates the inhibition of GABA release. We investigated the functional consequences of SPTBN2 mutations on glutamate and GABA metabolism in 3 SCA5 patients. Methods: The levels of glutamate, glutamine and free versus total GABA were measured in patients' CSF by LC-MS/MS. In vivo 1H-NMR spectroscopy (MRS) was performed at 3T using a dedicated sequence to obtain GABA-edited spectra. The regional concentrations of N-acetyl-aspartate (NAA), GABA and glutamate-glutamine (Glx) were determined in the primary sensory-motor cortex, the striatum and the cerebellum of SCA5 patients. We also assess peripheral glutamate metabolism by measuring glutamate uptake in patients' fibroblasts. Results: The CSF levels of glutamate, glutamine and GABA in SCA5 patients were comparable to diseased controls. MRS showed normal metabolic profile in the basal ganglia and motor cortex of SCA5 patients. In the cerebellum, the levels of GABA and Glx were markedly reduced compared to controls, even when correcting for neuronal loss using NAA. The ratio of GABA/Glx was also decreased in patients' cerebellum, indicating altered GABA release. Glutamate uptake in patients' fibroblasts was normal emphasizing that glutamate metabolism is only impaired in the brain. Conclusion: We have identified a selective alteration of glutamate and GABA homeostasis in the cerebellum of SCA5 patients.

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P08.81

A new case of spinal muscular atrophy with progressive myoclonic epilepsy associated with a homozygous mutation in ASAH1

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Spinal muscular atrophy with progressive myoclonic epilepsy (SMAPME, OMIM#159950) is a rare childhood syndrome characterized by progressive myoclonic epilepsy and proximal weakness. A homozygous mutation (c.125C>T, T42M) in *ASAH-1*, a gene encoding acid ceramidase, has been recently indentified in three families with SMAPME and a homogeneous clinical picture. However, previous reports depicted a heterogeneous syndrome with respect to age at onset, associated neurological symptoms and prognosis.
Here we describe a new case of SMAPME harboring the homozygous c.125C>T mutation and compare it with those previously reported.
The patient is a 12-years-old girl born to unrelated healthy Spanish parents. Despite her early developmental milestones she walked with an unsteady gait. Progressive, predominantly proximal, muscle weakness was evident from the age of 5 years. Muscle biopsy and EMG showed evidence of a chronic denervation process. At age 7 years she began presenting frequent absences and myoclonic jerks of the upper limbs. An EEG showed frequent 3-4 Hz generalized discharges of polyspikes-slow waves. Mild cognitive decline, and distal tremor appeared later. Other diagnostic procedures ruled out common lysosomal storage disorders and mitochondrial disease. Sequencing of *ASAH-1* revealed a homozygous mutation (c.125C>T, T42M) due to paternal uniparental disomy.
Our patient shows a clinical picture that is almost identical to that described in other patients harboring this mutation. Thus SMAPME associated to the homozygous c.125C>T mutation represents an apparently clinically homogenous disorder.
Further cases are needed to confirm this clear apparently straight phenotype-genotype correlation.

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P08.82

Paradoxical somatic genetics of neurodegeneration: spotlight on post-zygotic chromosome instability in the ataxia telangiectasia brain and implications for neurodegenerative diseases

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Ataxia-telangiectasia (AT) is a chromosome instability (CIN) disease caused by ATM mutations. Probably, the most striking AT feature is a progressive neurodegeneration limited to the cerebellum. To explain this, we hypothesized that progressive neuronal death is driven by post-zygotic chromosomal (genomic) instability in the AT cerebellum. Experimental testing of this hypothesis using brain autopsy specimens showed dramatically increased level of CIN in the cerebellum. However, molecular cytogenetic analysis revealed a paradoxical feature of neurodegeneration in AT. Firstly, global aneuploidization affecting 20-50% of brain cells (20% - neurons; 80% - non-neuronal cells) in the brain unaffects basic cognitive and mental functions. Secondly, the role of CIN affecting chromosomes 7 and 14 in the degenerating cerebellum is unclear. Thirdly, pathological CIN rates have not accelerated neurodegeneration, but have been associated with increased lifespan. To explain the paradox, we hypothesized that neurodegeneration in the cerebellum can be associated with (i) activation of adult neurogenesis, (ii) migration of undifferentiated blood stem cells into the affected brain areas, and (iii) activation and proliferation of microglia (phagocyte system of the brain). Thus, the increase of numbers of glial and neuronal cells with CIN in the AT cerebellum is, probably, a result of endogenous neuroprotective processes. Finally and paradoxicaly, propagation of CIN in the brain is a sign of natural neuroprotection applicable to neurodegenerative diseases, as a whole. Supported by in part by BMBF/DLR (RUS09/006), the Grant of the President of the Russian Federation MD-4401.2013.7 and RFBR 12-04-00215-a.

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P08.83

Spastic paraplegia with thin corpus callosum (SPG11) share clinical and histological similarities with juvenile amyotrophic lateral sclerosis.

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Spastic paraplegia 11 (SPG11), the most frequent (21%) clinico-genetic entity of autosomal recessive spastic paraplegia, is essentially characterized by the degeneration of the pyramidal tract, thinning of the corpus callosum (TCC) and white matter abnormalities (WMA) at brain MRI, leading to spasticity in lower limbs, mental impairment and peripheral neuropathy in patients. The disease is predominantly associated with loss of function mutations in the *SPG11* gene, coding for Spatacsin. We report the first neuropathological analysis of a 27 years-old woman presenting with a severe progressive disability and mental deterioration, TCC, periventricular WMA, marked frontal and parietal cortical, cerebellar and medullar atrophy. Mutational analysis revealed two heterozygous stop mutations in SPG11 gene. The mutations in the Spatacsin gene cause a wide spectrum of clinical and pathological features. We show that the neuropathological profile overlaps with amyotrophic lateral sclerosis (ALS).

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P08.84

Autosomal recessive spastic quadriplegia caused by AP4M1 and AP4B1 gene mutations: expansion of the facial and cranial MRI features

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Adaptor protein complex-4 (AP4) is a component of intracellular transportation of proteins, which is thought to have a unique role in neurons. Recently, mutations affecting all four subunits of AP4 (*AP4M1*, *AP4E1*, *AP4S1*, and *AP4B1*) have been found to cause similar autosomal recessive phenotype consisting of tetraplegic cerebral palsy and mental retardation, which was named as AP4 deficiency syndrome. Using homozygosity mapping followed by whole-exome sequencing, we identified two novel homozygous mutations in *AP4M1* and a homozygous deletion in *AP4B1* in three pairs of siblings from Turkey. Spastic quadriplegia, microcephaly, severe mental retardation, limited or absent speech, and stereotypic laughter were common findings in our patients. All patients also had similar facial features consisting of bitemporal narrowing, bulbous nose with broad nasal ridge, short philtrum, and hypotonic face which were not described in patients with *AP4M1* and *AP4B1* mutations previously. The patients presented here and previously with *AP4M1*, *AP4B1*, and *AP4E1* mutations shared brain abnormalities including asymmetrical ventriculomegaly, thin splenium of the corpus callosum, and reduced white matter volume. The patients presented here also had hippocampal globoid formation and thin hippocampus. In conclusion, disorders due to mutations in AP4 complex have similar neurological, facial, and cranial imaging findings. There is no genotype-phenotype correlation. Thus, these four genes encoding AP4 subunits should be screened in patients with autosomal recessive spastic quadriplegic cerebral palsy, severe mental retardation, and stereotypic laughter, especially with the described facial and cranial MRI features.

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P08.85

Paraplegin mutations cause progressive external ophthalmoplegia with multiple mitochondrial DNA deletions in skeletal muscle

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Introduction

Spastic paraplegia 7 (SPG7) is an autosomal recessive form of Hereditary Spastic Paraplegia (HSP) caused by mutations in the SPG7 gene, which encodes paraplegin, a member of the AAA family of ATPases, located at the inner mitochondrial membrane. Paraplegin is involved in the processing of mitochondrial proteins. Respiratory chain dysfunction has been reported in muscle in SPG7 patients, but its molecular aetiology and pathogenic role remains unknown. We report a novel SPG7 mutation in two Norwegian families presenting with a phenotype consistent with mitochondrial disease, and study the disorder`s molecular pathogenesis.

Material and methods

Four patients from two Norwegian families with a phenotype of progressive external ophthalmoplegia (PEO) and spastic paraplegia were examined clinically. Muscle histology and molecular mitochondrial DNA studies were performed in one of the index patients, an additional SPG7 patient from an unrelated family and ten controls.

Results

We found a novel SPG7 missense mutation, c.2102A>C, p.H701P, which was homozygous in the first family and compound heterozygous in trans with the known pathogenic mutation c.1454_1462del in the second family. Molecular studies showed multiple mitochondrial DNA (mtDNA) deletions and deficiency of respiratory complexes I, III and IV in skeletal muscle.

Discussion

We report a novel SPG7 mutation causing a complex HSP phenotype with PEO, a common mitochondrial disease phenotype. Moreover, our findings reveal novel aspects of the molecular pathogenesis of SPG7. We show that SPG7 mutations lead to respiratory dysfunction by causing secondary mtDNA damage and therefore link paraplegin to the homeostasis of the mitochondrial genome.

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P08.86

Microrearrangements in recessive type of hereditary spastic paraplegia - SPG 11 in a group of Polish patients. Molecular findings and clinical presentation.

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There are about 27 types of autosomal recessive spastic paraplegias (AR-HSP), with the SPG11 appearing the most frequent in the European population. It is caused by mutations in the SPG11 gene, coding the spatacsin. Clinically SPG11 is characterized by early age at onset and manifests mainly as complicated HSP with mental retardation including also neuroradiological hallmark - thin corpus callosum.

The group of 116 patients, 39 familial cases with excluded autosomal dominant inheritance pattern and 77 isolated cases, were screened for SPG11 mutations by MLPA analysis.

The results of MLPA screening revealed 6 different microrearrangements in 5 families, two of them have unequivocal molecular verification of SPG11 so far. In the first family homozygotic deletion ranging from 31 to 34 exon was identified, and in the second one heterozygous variant of 31 to 34 and single 37 exon deletion were detected. Furthermore in three probands intragenic deletions in only one allele of SPG11 gene were found: ranging from 9 to 11 and single exon deletions involving exon 2 and 29. To provide molecular verification of spastic paraplegia type 11 in above mentioned three cases, direct sequencing is planned.

The phenotype of affected subjects from studied families corresponds to the complicated form of spastic paraplegia with thin corpus callosum, white matter abnormalities and polyneuropahy as well as mild to moderate cognitive impairment.

Although MLPA technique is reliable screening tool for recessive SPG11 the future analysis of SPG11 point mutations will permit more efficiently determine molecular status in our group of patients.

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P08.87

Molecular mechanism of microrearrangements in the most prevalent hereditary spastic paraplegia (HSP) - SPG4.

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Spastic paraplegia type 4 (SPG4) is the most frequent HSP genetic type and accounts for up to 40% of families with pure or complicated hereditary spastic paraplegia. Mutations in the SPG4 corresponding gene SPAST are scattered along its coding region and include all types of DNA modifications: missense, nonsense, splice site as well as microrearrangements - deletions or duplications.
To detect the deletion/duplication in the SPAST gene, the autosomal dominant spastic paraplegia MLPA (Kit P-165-B1) containing probes for all SPAST gene exons was performed for 196 patients. MLPA data were analyzed using the GeneMarker Software v.1.90 (SoftGenetics) after electrophoresis of the PCR products on ABI 3130 Genetic Analyzer with ROX500 internal size standard.
To define the breakpoints and the range of identified in MLPA analysis microrearrangements (7 deletions and 1 duplication) long-range PCR was performed. The used sets of primers were specific to the breakpoints regions of deletions and duplication. Selected LR-PCR products were directly sequenced with BigDye Terminator v.3.1.
Among 8 studied SPG4 families, 6 fusion sequences in breakpoints regions for deletions of exons: 1-9, 4-7, 5-7, 8, and 10-12 and exons 14-15 duplication were detected. Sequencing analysis revealed the microhomologic sequences flanking the microrearrangements. The presence of various, directly orientated short consensus regions, identified in 6 studied families affected with SPG4, may suggest the possible mechanism of the mutation occurrence known as non allelic homologous recombination-NAHR.
The SPAST gene architecture including distribution and frequency of repetitive elements may facilitate genomic rearrangements as a causative molecular defects of SPG4.

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P08.88

SCA36 molecular analysis in patients with spinocerebellar ataxia

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Autosomal dominant spinocerebellar ataxias (SCA) are a heterogeneous group of neurological disorders characterized by cerebellar dysfunction mostly due to Purkinje cell degeneration. Recently, a novel form of spinocerebellar ataxia (SCA36) with motor neuron involvement was described in Japanese and Galician patients. It is caused by a GGCCTG repeat expansion in intron 1 of NOP56 gene. This gene encodes a component of the ribonucleoprotein complex and plays a role in transcription and splicing. We screened a large cohort of Italian unrelated patients with familiar (n=142) or sporadic/unknown (n=254) spinocerebellar ataxia for GGCCTG repeat expansion in NOP56 intron 1. All patients were negative for the common SCA1 and SCA2 mutations. The NOP56 repeat was analysed by fluorescent triplet repeat-primed PCR analysis. NOP56 expansion was detected in 5 probands from 4 different unrelated Italian families with dominant ataxia and also in one sporadic patient and, subsequently, in his affected brother. Interestingly, all probands originated from a relatively small area in central Italy, suggesting a common ancestor and a founder effect for this mutation. In conclusion, SCA36 accounts for approximately 3% of Italian autosomal dominant SCA families negative for the common SCA mutations. Clinically, mutated patients presented with slow progressive gait ataxia with late onset (40-60 yrs) with pyramidal signs, eye movement abnormalities and, in some cases, motor neuron involvement (tongue atrophy). Neuroimaging revealed prominent cerebellar atrophy affecting the vermis, with minor involvement of cerebellar hemispheres and brainstem in later stages [Supported in part by Telethon-Italia grant GGP09301].

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Multiple genetic testing of 10 types of autosomal dominant spinocerebellar ataxias by multiplex PCR and repeat-primed PCR

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The autosomal dominant spinocerebellar ataxias (ADCAs) are clinically and genetically heterogeneous, and over 30 types of causative mutations have been reported. Although some clinical and radiological features may help in their differential diagnosis, genetic testing is required for a definitive diagnosis and adequate genetic counseling. Since genetic testing of ADCAs is widely conducted in medical genetic laboratories around the world, simple procedures are warranted. Also, the different types of ADCAs have a considerable ethnic variation in prevalence. In this study, we established simple and multiple genetic testing for the causative mutations in 10 types of ADCAs whose prevalences are higher than the other types in Japan; they include SCA1-3, SCA6-8, SCA12, SCA17, SCA31 and DRPLA. To evaluate the mutations in a concurrent manner, we designed and optimized the procedures by using multiplex PCR, repeat-primed PCR methods and DNA fragment analysis. The amplifications of 10 loci were performed with the same thermal cycle protocol. The sizes of the nucleotide repeats were determined by DNA fragment analyses, the results of which were confirmed by DNA direct sequencing. A total of 168 DNA samples obtained from patients with clinically suspected ADCAs were analyzed. Written informed consent with appropriate genetic counseling was obtained from each patient. Genetic tests were positive in 84 cases (50%). SCA3 was most common (29% of the positive cases), followed by SCA6 (26%), SCA31 (19%) and DRPLA (12%). This multiple genetic testing of ADCAs may be valuable as a routine method in medical genetic laboratories.

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P08.90

Genome-wide expression profiling and functional characterization of SCA28 lymphoblastoid cell lines reveal impairment in cell growth and activation of apoptotic pathways.

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SCA28 is an autosomal dominant ataxia associated with AFG3L2 gene mutations. We performed a whole genome expression profiling using lymphoblastoid cell lines (LCLs) from four SCA28 patients, and six unrelated healthy controls matched for sex and age. We found 66 genes whose expression was statistically different, 35 of which were up-regulated (Fold Change - FC = 2.5-10) and 31 down-regulated (FC = 0.1-0.3). The differentially expressed genes were clustered in five functional categories: (1) regulation of cell proliferation; (2) regulation of programmed cell death; (3) response to oxidative stress; (4) cell adhesion, and (5) chemical homeostasis. To validate these data, we performed functional experiments that proved an impaired SCA28 LCLs growth compared to controls ($p < 0.005$), an increased number of cells in the G0/G1 phase ($p < 0.001$), and an increased mortality of patients cells due to apoptosis ($p < 0.05$). We also showed that respiratory chain activity and reactive oxygen species levels were not altered, although lipid peroxidation in SCA28 LCLs was increased in basal conditions ($p < 0.05$). We did not detect mitochondrial DNA large deletions. An increase of TFAM, a crucial protein for mtDNA maintenance, and a decrease of DRP1, a key regulator of mitochondrial fission, suggested an alteration of the mitochondrial network remodeling system.
In conclusion, whole genome expression profiling in SCA28 LCLs allowed

the identification of several altered pathways that may be related to the disease.

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P08.91

Identification of robust biomarkers of neuronal and glial metabolic changes in spinocerebellar ataxia type 1,2,3 and 7

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Background: Spinocerebellar ataxias (SCAs) belong to the group of polyglutamine repeat disorders and lead primarily to neurodegeneration in the cerebellum and the pons. We recently demonstrated that even the most sensitive clinical scores would require large number of patients to assess any therapeutic benefit. Therefore, the identification of robust biomarkers is critical to assess disease progression for therapeutic development. Methods: 1H-NMR spectroscopy was performed at 3T to determine the neurochemical profile of 24 metabolite concentrations in the vermis and pons of a unique cohort of SCA1 (N=8), SCA2 (N=11), SCA3 (N=17) and SCA7 (N=10), as well as in healthy controls with similar median age (N=24). Results/Interpretation: Compared to controls, SCAs patients displayed a significant decrease of neuronal metabolites, N-acetylaspartate and glutamate, but increased glia-related metabolites, glutamine and myoinositol. The neuronal loss in both affected regions was associated with a significant increase in creatine and phosphocreatine suggesting compensatory energetic mechanisms. Of note, there was a strong negative correlation between the ataxia rating scores (SARA) and N-acetylaspartate levels in the pons of SCA2-3-7 patients. In the vermis, myoinositol and creatine levels were also significantly correlated with the SARA scores of SCA2 and SCA3 patients. Principal component analyses showed that the metabolic profile of SCA1-2-3 tend to differ from SCA7, which is consistent with previous observation of slower disease progression in SCA7. The correlation matrix also confirmed that the most robust biomarkers of SCAs are N-acetylaspartate, glutamate, myoinositol and total creatine, which supports preliminary data from a pilot study conducted in SCA1.

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P08.92

Identification of novel spinocerebellar ataxia disease genes using a combination of shared haplotype analysis and exome sequencing

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To date, 37 different dominantly inherited spinocerebellar ataxia types are known. Currently, genetic testing of the most frequent mutations via routine DNA diagnostic screening leaves 30% of the cases genetically undiagnosed. Here, we used a combination of shared haplotype analysis with exome sequencing to identify the disease-causing variants in 10 small Dutch families with hereditary cerebellar ataxia. We set out to pinpoint regions that have the highest probability to contain disease-causing variants by searching for shared haplotypes following genotyping affected family members on Illumina 320k cytoSNP-12-arrays. In parallel, exome sequencing was performed in 2 most distantly related affected individuals of each family. Next, we used Ingenuity software for the data-analysis and filtered subsequently for: 1. variants shared by both affected individuals, 2. novel variants, i.e. absent in dbSNP, the Exome Variant Database and GoNL, a Dutch control database containing 500 exomes, 3. variants present in genes that are located in shared haplotype regions of at least 10 Mb, 4. variants in genes that are part of an in silico generated ataxia gene-network (based on co-expression-data of known ataxia genes; unpublished data) Finally, all selected variants were validated by Sanger sequencing and cosegregation analysis.
This strategic analysis leads to relatively short lists of unique variants per family. Although the validation process is not completely finished for all families, our preliminary data strongly suggest we indeed identified novel candidate ataxia genes and that the majority of these are involved in modulating synaptic transmission.

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P08.93

Autosomal recessive spinocerebellar ataxia 7 (SCAR7) is caused by variants in *TPP1*, the gene involved in classic late-infantile neuronal ceroid lipofuscinosis 2 disease (CLN2 disease)

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Spinocerebellar ataxias are phenotypically, neuropathologically and genetically heterogeneous. We reported a unique family with a childhood onset, slowly progressive autosomal recessive spinocerebellar ataxia, referred to as SCAR7. A genome-wide linkage study mapped the causative gene on a 5.9 cM region on chromosome band 11p15. No obvious candidate gene could be assigned. Here we report the results of exome sequencing, revealing disease-causing variants in the *TPP1* gene, encoding the lysosomal enzyme tripeptidyl peptidase 1. A missense and a splice site variant in *TPP1*, cosegregating with the disease, were found in the SCAR7 family and also in another patient with a SCAR7 phenotype.
TPP1, is the causative gene for late infantile neuronal ceroid lipofuscinosis disease 2 (CLN2 disease). CLN2 disease is characterized by epilepsy, loss of vision, ataxia and a rapidly progressive course, leading to early death. SCAR7 patients showed ataxia and low activity of tripeptidyl peptidase 1, but no ophthalmologic abnormalities or epilepsy. Also, the slowly progressive evolution of the disease until old age and absence of ultra structural curvilinear profiles is different from the known CLN2 phenotypes.
Our findings expand the phenotypes related to *TPP1*-variants to SCAR7. In spite of the limited sample size and measurements a putative genotype-phenotype correlation may be drawn: loss of function variants abolishing TPP1 enzyme activity lead to CLN2 disease, while variants that diminish TPP1 enzyme activity lead to SCAR7. This finding illustrates the sometimes unexpected clinical spectrum of variants in known genes. We will encounter this phenomenon with increasing frequency using new techniques.

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P08.94

GSN, a new candidate gene in autosomal dominant spastic cerebellar ataxia

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Autosomal dominant (AD) cerebellar ataxias (CA) are severe neurodegenerative disorders, characterized by movement incoordination, variably associated with other neurological signs. As of today, 35 loci and 20 genes have been described in ADCA, but the causative gene is still unknown in half of the patients. We report a large French family with adult-onset rapidly progressive ataxia, associated with pyramidal signs, spasticity, decreased pallaesthesia and ophthalmoplegia. After exclusion of polyglutamine expansions or mutations in the commonest causative genes, susceptibility loci including

about 450 genes were identified through linkage analysis. Analysis of variants obtained from exome sequencing in two patients, filtered with data from 2 healthy controls and 6 patients with other neurodegenerative disorders, and cosegregation verification, lead to the identification of a missense mutation in the *GSN* (gelsolin) gene that was shown to appear *de novo* in the affected grandfather. The variant was not found in 380 healthy controls or in online databases. A point mutation in *GSN* is already responsible for Finnish amyloidosis. However, the mutation described here differs, and the phenotype was excluded in our family through immunohistochemistry of patient skin sample. Mutation screening in 39 patients with AD spastic ataxia revealed two new potential variants. Further screening is ongoing for another 95 patients, with a broader clinical picture. Functional studies aiming at the cytoskeleton function of fibroblasts, including immunofluorescent staining and migration tests are ongoing to validate and understand the causative nature of the mutations.

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P08.95

Dizygotic twins with ataxia telangiectasia

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We present the case of dizygotic twins (girl and boy) with ataxia telangiectasia. They both were diagnosed with strabismus, dysarthria, an ataxic gait. The girl presented dysmorphic features and muscular hypotonia, while only in the boy choreoathetotic movements and positive Romberg test was observed. The girl's electroencephalogram presented paroxysmal changes in median, central and parietal region (however, without seizures), while electromyogram showed no changes. The girl also was diagnosed with autoimmunohaemolytic anaemia, leucopenia with lymphopenia and suffered from recurrent infections. In both cases elevated level of serum AFP was observed. The concentrations of IgA and IgG were low in a girl as well as a boy. The percentage of T-cells positive with CD4 and CD19 were reduced but percentages of CD3 and NK-cell were at the normal levels in girl. The boy presented reduced percentages of T-cells positive with CD3 and CD4. However, the levels of CD8, NK-cells, B-cells, CEA, IgM, IgE (global), CRP, CPK were within the normal limits. Cytogenetic studies confirmed increased frequency of spontaneous chromosomal aberrations in both children. Additional analysis performed using PowerPlex®16 led to the observation of loss of maternal allele at locus 8q24.13. Molecular studies revealed two mutations in *ATM* gene. First mutation involves a G>A substitution of the last nucleotide of exon 43 (c.6095G>A), and it results in the deletion of exon 43. Second mutation detected in twins was deletion at position c.6754_6754delA, p.T2252PfsX5. Supported by grant NN401098240 from the Ministry of Science and Higher Education in Poland

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P08.96

Analysis of the role of GAA repeat expansion instability in Friedreich ataxia pathology in a humanised mouse model

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There is evidence that age-dependent and tissue-specific somatic instability of the GAA repeat expansion may be a determinant of the progressive pathology of Friedreich ataxia (FRDA), and is evident in FRDA patients and a YAC-based GAA repeat expansion mouse model. Interruptions in GAA repeat sequences can alleviate transcription inhibition and reduce genetic instabilities. We explored the role of instability of the GAA repeat expansion on FRDA pathology using a humanised mouse model containing an interrupted GAA repeat expansion.

An interrupted GAA repeat expansion was introduced into the first intron of the human FXN gene present on a BAC clone by recombineering. The genomic insert was used to generate humanised transgenic/KO mice.

The presence of the introduced interrupted GAA repeat expansion resulted in markedly decreased levels of human FXN transcript and frataxin protein in humanised mouse tissues. The region immediately upstream of the interrupted GAA repeat expansion region was found to be almost completely methylated. The assessment of phenotypic symptoms of FRDA by a series of behavioural, neurological, biochemical and histological tests did not reveal any significant phenotypic differences between humanised and wild type mice. Somatic instability of the interrupted GAA repeat expansion was not detected using the small pool PCR technique.

The interruption of the GAA sequence contributes to the somatic stability of the repetitive element, which in turn results in the mice lacking an obvious phenotype despite the low levels of FXN mRNA and frataxin protein and repressive epigenetic changes.

J. Sarsero: None.

P08.97

Uncommon phenotypes associated with mutations in the *WFS1* gene

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Background.*WFS1* gene mutations were mainly described in Wolfram syndrome (WS). The minimal diagnostic criteria for the autosomal recessive WS are juvenile-onset diabetes mellitus (DM) and optic atrophy (OA), frequently associated with diabetes insipidus, deafness, renal tract abnormalities and neuropsychiatric disorders. *WFS1* has also been involved in two autosomal dominant syndromes: Low Frequency Sensorineural Hearing Loss (LFSNHL) and OA associated with Sensorineural Hearing Loss (SNHL).

Patients and methods.We analysed the *WFS1* gene in a cohort of 13 families including patients presenting LFSNHL or a clinical phenotype different from typical WS with at least two symptoms including diabetes mellitus, OA, SNHL or neurological disorders. *WFS1* was sequenced in the Department of Medical Genetics, CHU of Nice (France).

Results.In this cohort, we identified *WFS1* mutations in one LFSNHL family, 3 families with autosomal dominant OA and SNHL, 5 families with late-onset WS (DM and OA occurring after 15 years of age), 1 family with recessive OA and neurological disorders and 3 sporadic cases with only one mutation in *WFS1* associated with an atypical phenotype (including isolated deafness, late-onset diabetes associated with hearing impairment, and infancy-onset DM and deafness).

Conclusion.Our data show that the *WFS1* gene is involved in a clinical spectrum wider than the commonly described WS. *WFS1* should be also tested in LFSNHL, late-onset WS and phenotypes, transmitted either in dominant or recessive fashion, presenting at least OA or SNHL associated with additional features including DM or neurological signs.

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P08.98

The mutation p.G1341D in Wilson's disease patients: how to explain clinical polymorphism?

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Background: Wilson's disease (WD) is a progressive and genetically determined disease with deterioration of copper metabolism and it's accumulation in different organs and tissues due to their functional and structural changes. The aim of the study was to evaluate the clinical forms of WD in correlation with type of ATP7B gene mutations. Material and methods: The diagnosis was based on clinical survey, biochemical and genetic tests (Germany).

Results: The missence mutation p.G1341D was the second prevalent mutation and has been revealed in 10 of 37 patients (27 %), from who 8 was heterozygous (21,6 %) and 2 homozygous (5, 4 %). The most common cha-

racteristics of this mutation was the early age of onset, mixed clinical manifestations with severe hepatic deterioration and neurological changes what lead to disability with difficulty in treatment. In a family with 2 homozygous (p.G1341D) siblings the girl had severe neurological damage and cirrhosis. In contrast, the boy didn't have neurological changes, and the liver damage was less aggressive. In the other family also with 2 heterozygous (p.G1341D) siblings the younger boy had severe clinical manifestations but his older sister had no clinical manifestations. Analysis of heterozygous and homozygous patients revealed discrepancy in neurological signs progression and efficiency of treatment being more severe in heterozygous ones. Conclusion: Discordance in clinical manifestation and age at onset in patients with similar mutations may indicate that not only genetic factors play a role in the development of the disease but also genetic or environmental modifiers.

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P08.99

Serious neurologic entity in two unrelated patients with Xq13.1 duplication

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The use of array-CGH substantially improves the diagnosis of chromosomal abnormalities that are not evident by conventional karyotype. Recently in an extended family with global developmental delay and autistic features, a novel Xq12-q13.3 duplication was identified which contained amongst others *TAF1, GJB1* genes.(Kaya et al.2012).The *GJB1* gene is associated with morphology of myelin sheath and peripheral neuropathy, while *TAF1* has been linked with torsion dystonia -3.
We present the clinical and molecular findings in two unrelated patients with Xq13.1 duplication. The first one is a 5 year old boy with truncal hypotonia, low deep tendon reflexes and proximal weakness. The other patient is a 16 year old boy with progressive difficulties in walking, tremor and learning disorder, while his mother has an identical positive family history in three deceased males. High resolution 4X180K Agilent arrays used in the study (> 236.000 probes, average resolution of 8.9 Kb) revealed a duplication on Xq13.1 (0.423 Mb) containing the *TAF1* and *GJB1* genes in both patients, while the second patient had an additional deletion on 17q21.31 (0.63 Mb) containing *KANSL1* gene. To our knowledge this is the second report of the Xq13.1 duplication extending the previously described phenotypic spectrum in that a more serious neurologic entity could be attributed to the *TAF1* and *GJB1* genes.

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P09.01

Three HLA-B*5801 positive allopurinol-induced severe cutaneous hypersensitivity reaction cases

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Allopurinol-induced severe cutaneous hypersensitivity reaction such as Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) has been reported on the genetic association with HLA-B*5801. Clinicians occasionally request HLA genotyping for evaluating the causality of drug hypersensitivity based on the evidence between HLA genotype and drug hypersensitivity. Three patients with serious cutaneous hypersensitivity reaction after allopurinol administration were tested for HLA-B genotyping. One (72-yr old woman) showed drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome and the others (63-yr old and 22-yr old men) turned out to be SJS. All patients were treated with allopurinol due to gout. The onset of the symptoms was 2-4 weeks after the drug exposure. HLA-B genotyping was performed using PCR-SBT (sequence-based typing) method. All patients have one HLA-B*5801 allele. While a patient with DRESS syndrome has HLA-B*4801/*5801, two SJS patients showed HLA-B*1302/*5801 and HLA-B*4403/*5801 genotypes, respectively. The results implied that patients with HLA-B*5801 may suffer from severe cutaneous hypersensitivity reaction with allopurinol administration. Thus PCR-SBT HLA-B genotyping could be helpful for preventing the serious problem due to allopurinol treatment although the PCR-SBT HLA-B genotyping takes long time. Therefore a simple diagnostic genotyping technology needs to be developed for using in practice.

E. Kim: None.

P09.02

A 1p36 polymorphism in the cannabinoid receptor 2 gene region is associated with CNR2 gene expression, atopic childhood asthma onset, severity, and treatment outcome with inhaled corticosteroids

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Asthma pathogenesis is currently understood through the interaction of several genes and environmental influences. It is believed that endocannabinoids act as native modulators of immune system, probably through cannabinoid receptors activation. Recent expression quantitative trait loci studies linked single nucleotide polymorphisms (SNPs) on chromosome 1p36 with cannabinoid receptor 2 (CNR2) gene expression. We analyzed rs4237 association with childhood asthma, and the effect of rs4237 on the response to inhaled corticosteroids (ICS) treatment and CNR2 gene expression. We studied a case-control cohort of 229 children with newly detected mild/moderate persistent asthma (150 atopic, 79 non-atopic, 13 undetermined atopy), and 271 controls. Blood samples were collected before treatment and 72 matching samples 4-6 weeks after treatment with ICS. According to recessive model of genetic association the frequency of CC genotype in atopic asthmatics was lower (13.2%) than in controls (21.0%, p=0.0284). Forced expiratory volume in 1 second (FEV1) was higher in atopic asthmatics with CC genotype (98%±11%) compared to those with CT or TT (90%±15%, p=0.0155). Median relative expression of CNR2 in asthmatics with TT genotype was higher (0.79±0.81) compared to those with CT or CC (0.28±0.81, p=0.0041). FEV1 increased significantly by 10.6%±11.6% of predicted value after ICS treatment in atopic asthmatics with TT genotype, compared to 6.4%±10.5% in those with CT or CC homozygotes (p=0.034). Our results suggest rs4237 is associated with asthma onset, with asthma severity, CNR2 gene expression and with inhaled corticosteroids treatment response in children with atopic asthma, and suggest the involvement of the endocannabinoid system in asthma.

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P09.03

Genetic basis of primary immunodeficiencies in Croatian patients

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Introduction: Primary immunodeficiency diseases (PID) are a heterogenic group of rare inherited conditions that occur in individuals born with malfunctioned immune system.
Objective: The objective was to confirm clinical diagnosis at molecular genetic level in patients with PID and to define carrier status by analyzing DNA samples of individuals with PID in family history.
Methods: We analyzed 24 samples of genomic DNA: 11 samples of patients with suspicion on one of the PID and 13 samples of their family members. For identification of mutations in the coding region of analyzed genes, we used the sequencing method on Applied Biosystems 3130xl Genetic analyzer and BigDye® Terminator v3.1 Cycle Sequencing Kit.
Results: In 2 patients with X-linked agammaglobulinemia, mutations in the BTK gene were found. In the first patient, mutation occurred de novo in mother's egg cells and, in the other, mutation was inherited from the mother. 3 patients with XLA suspicion didn't have mutation in the BTK gene. In 4 patients with cyclic and severe congenital neutropenia, 4 mutations occurred de novo in mother's egg cells were found. In 2 patients with suspicion on Shwachmann-Diamond syndrome mutation in SBDS gene inherited from the parents were found.
Conclusions: Understanding the genetic basis of PID is the final step in confirmation of the PID diagnosis as it provides comprehension of disease mechanisms at the molecular level and correlation between genotype and phenotype of PID.

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P09.04

EQTLs and allele specific expression of HLA haplotypes and amino acids associated to autoimmune diseases.

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HLA is the strongest associated locus in autoimmune diseases. Recent studies in celiac disease (CelD) and rheumatoid arthritis (RA) indicated the major role of DR3-DQ2 and DR4-DQ8 in both diseases, and identified additional independently associated variants both in the DR-DQ locus and in the extended HLA locus.
We hypothesized that the mechanism of downstream effect of associated variants and haplotypes is due to an impact on the expression level of HLA genes We investigated this hypothesis in a dataset of 60 unrelated CEU individuals, for whom RNAseq data, dense HLA genotyping and imputation of HLA alleles was available. We selected CelD and RA associated amino acids, HLA-haplotypes and SNPs, and assessed allele specific expression and eQTLs in the HLA locus. eQTL analysis allowed us to assess the dosage effect of HLA variants on gene expression, whereas the allele-specific analysis, performed in heterozygous individuals for each variant, indicated if one or another allele of a SNP or amino acid was preferentially expressed.
In both analyses we observed that an amino acid at position 52 of the DQB1 gene (DQB1_AA52) was significantly associated with expression of DQB1 gene both in eQTL and in allele-specific analysis. DQB1_AA52 is associated to CelD independently from the most associated DQB1 variant (AA55). We therefore confirmed that imbalanced allelic expression is the downstream effect of some HLA variants associated with autoimmune diseases. The analysis in extended number of individuals is currently in progress.

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P09.05

The IL-10 promoter genotype AA at -592 is correlated with increased susceptibility to hepatitis B but not HIV infection in Caucasian intravenous drug users.

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BACKGROUND: Interleukin (IL)-10 -592A has been associated with rapid HIV disease progression. Whether and how the IL-10 polymorphisms at the positions -592 and -1082 are associated with susceptibility to HIV and its co-infections in IDUs population is largely unknown.
METHODS: A total of 345 IDUs were recruited and of them 50% were HIV-. A control group was formed by HIV-, HCV-, HBV- 496 healthy volunteer blood donors. The IL-10 C-592A and A-1082G were determined using TaqMan allelic discrimination assay. The statistical analysis was performed using Fisher's exact test.
RESULTS: Of IDUs 88.7% were HCV+, 67.2% HBV+ and 40.6% with triple infection. IL-10 -592C allele (allelic frequencies 0.78 in IDU and 0.79 in donors) and -1082 A allele (allelic frequencies 0.57 in IDUs and 0.55 in donors) were the most common in all groups. Both polymorphisms were under Hardy-Weinberg equilibrium and the allelic frequencies did not differ between the three groups. The -592 CC/-1082 AG was the most common haplotype (29.3% in IDUs and 30.4% in donor group). No associations were found in distribution of IL-10 -592 and -1082 genotypes and HIV or HCV infections. However, persons possessing IL-10 AA had increased odds for acquiring HBV infection (OR=4.37; 95% CI = 1.01-39.63; p=0.042). After adjustment for HIV, HCV and duration of intravenous drug use, the association was not significant
CONCLUSIONS: IL-10 genotypes are not associated with HIV acquisition in Caucasian IDUs. However, IL10 -592AA genotype predisposes of being HBV positive and therefore might play an important role in HBV susceptibility.

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P09.07

Homozygous deletion of *IL-17RA* and *ADA2* in siblings with severe recurrent infections

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Chronic systemic and mucocutaneous infections can be associated with human inborn errors of immunity but the genetic background remains poorly understood. Here we report two siblings suffering since childhood from chronic severe infections mediated by *Candida albicans* and *Staphylococcus aureus,* refractory to classical treatments. DNA analysis by Array-based Comparative Genomic Hybridization (array-CGH) shows that both sibs are homozygous carriers of a 22q11.1 deletion of about 770 Kb encompassing the *IL-17RA* and *ADA2* genes. IL-17RA is one receptor for the pro-inflammatory IL-17 cytokines involved in protection against extra cellular pathogens. A homozygous nonsense mutation in *IL-17RA* has been recently reported in a patient suffering from isolated chronic mucocutaneous candidiasis. ADA2, adenosine deaminase 2, is secreted by monocytes undergoing differentiation into macrophages or dendritic cells and by dendritic cells themselves, and might be involved in immune responses. By genetic and functional analyses, we confirmed the relationship between chronic mucocutaneous infections by *C. albicans* and *Staphylococcus* species and the lack of expression of IL-17RA. Severity of the symptoms might be further exacerbated by the absence of ADA2.
This study underscores the usefulness of whole genetic analyses, allowing for precise diagnosis and genetic counselling, thereby improving the management of chronic infectious diseases.

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P09.08

Whether SNP rs12979860(C/T)of IL28B predicts virological response during peg IFN and ribavirin treatment in chronic hepatitis C patients?

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Identification of molecular markers playing role in predicting anti-HCV treatment outcome would facilitate therapy optimizing. IL28B rs12979860 polymorphism has been identified as strong predictor of Sustained Virological Response (SVR) in chronic hepatitis C (CHC) patients. The aim of this study was to examine association between IL28B rs12979860 polymorphism and VR in 35 CHC patients treated with pegIFN+RBV. Serum HCV-RNA was measured on first day and then at 4 and 12 week of treatment by quantitative RT-PCR. DNA, isolated from PBL, was used for IL28B rs12979860 genotyping by High Resolution Melting method.13 patients (37.1%) became HCV RNA negative at week 4 (RVR-Rapid Virological Response) and 10 (28.6%) at week 12 (cEVR-complete Early Virological Response). 12 patients (34.3%) did not achieve virological response until 12 weeks (PNR-Primary Non-Response). The mean baseline viral load was comparable - 6.69x10000 IU/ml vs 7.32x10000 IU/ml vs 3.51x10000 IU/ml in RVR, cEVR and PNR group, respectively. The rs12979860 CC, CT and TT genotypes found in 8 (22.9%), 23 (65.7%), 4 (11.4%) patients, respectively. Among patients with CC genotype, 75% achieved RVR and 25% achieved cEVR. Among CT genotype RVR, cEVR and PNR were observed in 30.5%, 30.5% and 39% of patients, respectively. 25% of patients with genotype TT achieved cEVR and 75% achieved PNR. The initial results confirm that IL28B rs12979860 C/T polymorphism may identify those CHC patients, who achieve RVR during early phase of treatment and thereby who are more likely to achieve SVR.
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P09.09

Effects of aging and sex difference on immune-related phenotypes.

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The complexity of the healthy human immune system and the immunological changes that occur during life have been only partially described. Furthermore several autoimmune diseases have been associated with phenotypic changes that can be interpreted as accelerated immune aging, and others show different incidence or severity in males and females. To investigate the impact of age and sex in specific immune cell types, we measured 266 immune-related traits, representing the majority of leukocyte cell populations (monocytes, T and B cells, Natural Killer cells, regulatory T cells, dendritic cells, and their subsets) as well as T cell maturation, in 1921 volunteers (aged 14-99 years) of the SardiNIA project by polychromatic flow cytometry. We then evaluated the impact of age and sex using linear regression models. We observed statistically significant ($p<0.0005$) differences for 17 traits between younger and older subjects (<= or >60 years), including the already described decrease of naïve CD8+ T cells in elderly. We also confirmed previous observations reporting stable granulocytes and monocytes, but a light increase of NK cell counts, with aging. Differences between males and females were not significant, but moderate changes were observed for dendritic cells and subtypes, as well as B cells ($p<0.005$). While this study sample already represents the largest and deepest characterized set ever described, we are currently performing immune-phenotyping in additional 2000 volunteers, including 200 healthy centenarians (aged >90 years). Our results will improve our knowledge on the immune system in health, diseases, and aging.

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P09.10

Methylation status of immune response genes promoters in cell-free DNA differ in hemodialyzed patients treated with low and high doses of erythropoietin therapy

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Anemia is a major complication of end-stage renal disease, occurring as a result of reduced erythropoietin production from the peritubular cells of kidney. Recombinat human erythropoietin therapy is successfully applied to correct the anemia but significant number of dialysis patients remain hyporesponsive to this therapy. As specific interleukins are generated during hemodialysis and monocytes are activated, the process of hemodialysis may be regarded as a stimulus activating mechanisms of inflammation. It is known that pro-inflammatory cytokines are able to suppress erythropoiesis. Therefore we decided to study the changes in methylation status of promoters of immune response genes in the cell-free DNA which is thought to fulfill regulation function in intercellular communication. We focused on hemodialyzed subjects affected with diabetic nephropathy (n=18). In our pilot study the patients were divided according to the recombinant erythropoietin therapy into two groups - with low (n=8) and with higher doses (n=18).

Cell free DNA from plasma of all patients was isolated before and after the hemodialysis procedure. The extent of promoter methylation of 24 genes involved in immune response was examined using the EpiTect Methyl qPCR Array Inflammatory Response and Autoimmunity and cluster analysis (SA-Biosciences, Qiagen). Using cluster analysis we demonstrate the complex changes in the methylation profiles of selected immune response genes and the significant differences between the profiles of patients with low and higher doses of recombinant erythropoietin.

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P09.11

Five new cases of Nijmegen breakage syndrome in Chile

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Introduction: Nijmegen Breakage Syndrome (NBS) is a rare autosomal recessive disease. It is characterized by congenital microcephaly, intrauterine growth retardation and short stature, immunodeficiency, chromosome instability and cancer proneness. The disease seems to occur worldwide, but has a much higher prevalence among Central and Eastern European Slavic populations. Over 90% of identified patients bear a common deletion. We report five new patients from Chile, South America.
Patients: Since June 2000, five patients were referred to our genetic clinic, because of Microcephaly. Their diagnosis workup established NBS.
Results: Three probands are female, referred from 10mo to 14y. Strikingly they came from one region of Chile; four had no history of consanguinity. Only one patient was referred with diagnosis of NBS. Two patients have already developed non-Hodgkin lymphoma, one dead. DNA analysis was carried out in four patients; tested individuals were homozygous for the common Slavic mutation 657del5 in the *NBS1* gene.
Discussion: NBS can be suspected in clinical grounds, although there is considerably clinical overlap with other inherited conditions. Its confirmation is useful for an appropriate surveillance and for genetic counseling. The fact that all our patients came from the same region let us to propose a founder effect. Although historical records tell us that the Chilean population was founded by the admixture of local Amerindian populations and colonizing Spaniards, it is probable that the genomic make up of current Chileans is much more complex, with multiple ethnic groups contributing at different times since the initial contact between Americans and Europeans.

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P09.12

Identification of the gene responsible for an autosomal dominant form of Common Variable Immunodeficiency

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CVID is the most common symptomatic antibody deficiency in adults, including a heterogeneous group of disorders characterized by markedly reduced immunoglobulin serum levels, poor response to vaccines and increased susceptibility to bacterial infections. The majority of CVID cases are sporadic. Approximately 20% are familial: rare autosomal recessive mutations in ICOS, BAFF-R, CD19, CD20, CD81 and CD21 coding genes have been recently reported and mutations in the TACI gene have been found in about 15% of cases.
We report a large family with autosomal dominant inheritance of CVID, diagnosed according to the ESID/PAGID criteria for CVID based on at least 2 SD below the mean for age in serum IgG and IgA, onset age >2 years, poor response to vaccines.
In this five-generation family, nine affected and eight unaffected individuals were available for the study. Based on the genomewide linkage scan, the CVID locus has been mapped to the long arm of chromosome 3 on band q27.2-q29. The CVID locus is localized in a region of 9.2 Mb spanning from marker D3S3570 to marker D3S1265, with a maximum LOD score of 3.90 at $\theta=0.00$ for marker D3S2747.
In collaboration with the Wellcome Trust Sanger Institute, we performed exome sequencing in four affected and four unaffected individuals, in order to identify shared rare variants within the linkage region. After the filtering procedure to extract heterozygous variants with a low frequency (<0.01) in the general population, we found a candidate disease-causing allele shared among the four affected subjects analyzed.

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P09.13

NLRP1 mutation in a novel autosomal recessive autoinflammatory disease with dyskeratosis and arthritis

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The inflammasomes correspond to multiprotein complexes that sense pathogens and trigger biological mechanisms in an attempt to control infection. The NLRP family (Nucleotide-binding domain leucine-rich repeat containing a PYRIN domain) plays a key role in this innate immune system by regulating inflammation via caspase recruitment. Sequence variants in genes encoding NLRPs, have been associated with autoinflammatory and autoimmune diseases. Indeed, mutations in *NLRP3* are linked to hereditary cryopyrinopathies, whereas polymorphisms in *NLRP1* are associated to autoimmune disorders such as vitiligo and rheumatoid arthritis. Recently, a non-synonymous *NLRP1* mutation in the pyrin domain (M77T) was observed in an autosomal dominant hereditary benign intraepithelial dyskeratosis.
Using an exome sequencing strategy, we have identified a non-synonymous homozygote mutation in the *NLRP1* gene in two cousins born from consanguineous parents originating from Algeria. The patients present with diffuse skin dyskeratosis (2/2), biological inflammation (2/2) and arthritis (1/2). The mutation (c.2733C>T; p.R726W) is localised near the leucine-rich repeat and is neither observed in 192 chromosomes from healthy Algerian volunteers nor web databases.
An immune phenotype in 1/2 patient revealed high numbers of granulocytes, CD64^{+} neutrophils, NK cells and immature blood B cells (CD20^{+}CD27^{-}CD38^{high}CD24^{high}).We demonstrate for the first time that *NLRP1* is involved in an autosomal recessive autoinflammatory disorder. These data, combined to the literature, highlights the pleiomorphic roles of *NLRP1* in inflammation and immunity.

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P09.14

Functional variant in the KIF5A-CYP27B1-METTL1-FAM119B locus associated with multiple sclerosis

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Multiple sclerosis is (MS) a complex inflammatory disease that is characterized by lesions in the central nervous system. Several studies have highlighted the association of the 12q13.3-12q14.1 region with MS, coeliac disease, type 1 diabetes, rheumatoid arthritis and; however, the causal variants underlying diseases are still unclear.
To identify the polymorphism determining the association with MS we performed a fine mapping of the associated region encoding 15 genes by a Tag-SNP approach (2876 MS patients and 2910 healthy Caucasian controls) and functional studies to determine the involvement of the associated MS risk variants in gene expression.
rs6581155, which tagged 18 variants within a region where 9 genes map, was sufficient to model the association. This SNP was in total linkage disequilibrium with other polymorphisms that associate with expression quantitative trait loci (eQTLs) for FAM119B, AVIL, TSFM, TSPAN31 and CYP27B1 genes . Functional annotations from ENCODE project showed that six out of these rs6581155-tagged-SNPs were located in regions with regulatory potential and only one of them, rs10877013, exhibited allele-dependent (ratio A/G=9.5-fold) and orientation-dependent (forward/reverse=2.7-fold) enhancer activity as determined by luciferase reporter assays. This enhancer is located in a region where a long-range chromatin interaction among the promoters and promoter-enhancer of several genes has been described, possibly affecting their expression simultaneously.
This study determines a functional variant which alters the enhancer activity of a regulatory element in the locus affecting the expression of several genes and explains the association of the 12q13.3-12q14.1 region with MS.

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P09.15

Expression of Mx1, OAS1, PKR (EIF2AK2) and TP53 genes during treatment of chronic HCV patients.

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Interferon stimulated genes (ISGs) play key role in antiviral responses against HCV. Aim of this prospective study was to examine association between *Mx1, OAS1, PKR* and *TP53* expression and response to pegIFN+RBV in 35 chronic hepatitis C (CHC) patients. Rapid Virological Response (RVR) and complete Early Virological Response (cEVR) was achieved by 13 (37.1%) and 10 (28.6%) patients, respectively. 12 (34.3%) did not response to therapy during 12 weeks (Primary Non-Response, PNR; less than 2 log^{10} decrease in viral titer after 12 weeks). Mean baseline viral load was comparable in RVR, cEVR and PNR group (6.7, 7.3 and 3.5x10^{4} IU/ml, respectively). Expression of *Mx1, OAS1, PKR*, but not *TP53* was low in RVR and higher in cEVR and PNR patients before therapy, but increased noticeable in cEVR and PNR and poorly in RVR patients at week 4. Between weeks 4 and 12 of therapy, expression of ISGs was stable or poorly decreased in RVR, was stable or poorly increased in cEVR and noticeable decreased in PNR group. These results indicate that exogenous IFN can stimulate transcription of *Mx1, OAS1, PKR* but not *TP53* in PBL in CHC patients. Pre-activation of endogenous interferon system (higher ISGs expression) is associated with RVR and thereby with high likelihood of achieving SVR. Treatment failure may be related to decrease in ISGs expression between week 4 and 12 of anti-HCV therapy. So, we concluded that expression of ISGs may predict the outcome of CHC.
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P09.16

Association of Perforin codon His222 missense mutations with non-immune hydrops fetalis in familial hemophagocytic lymphohistiocytosis

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Familial hemophagocytic lymphohistiocytosis (FHL) is a rare, if untreated, fatal autosomal recessive immune dysregulation disorder of early childhood. FHL may scarcely display presenting symptoms in the intrauterine life. The etiology of non-immune hydrops fetalis (NIHF), on the other hand, remains unknown in most cases and their association is of great importance to the scientists in the related fields. This is the first study demonstrating that NIHF in identical twin neonates is associated with biallelic gene defect in one of the genes causing FHL. Preterm male twins (31 wk), products of a

consanguineous family, with NIHF and hepatosplenomegaly gradually developed pancytopenia, hyperferritinemia, hyponatremia, hypoalbuminemia, and elevated ALT, AST, bilirubin, LDH levels but fever and hypertriglyceridemia of hemophagocytic lymphohistiocytosis diagnostic criteria were absent. Suspected sepsis led to antibiotic therapy. Upon detection of hemophagocytosis in bone marrow, multiorgan failure and pulmonary bleeding led to death on 15th and 18th days of life. Mutation analysis in Perforin, UNC13D, Syntaxin 11 and STXBP2 genes causing FHL revealed homozygous c665A>G substitution leading to His222Arg missense mutation in Perforin. Parents were heterozygous for this known disease causing mutation. Same mutation in heterozygous state was reported in a case with fetal acides and homozygous another missense mutation in the same codon (His222Gln) was described in a stillborn case with NIHF in the literature previously. Therefore, it is plausible to suggest that biallelic missense mutations of Perforin codon His222 may be associated with intrauterine presentation of FHL, hence with hydrops fetalis. This study was supported by TUBITAK (Grant Number:105S386-SBAG-3193).

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P09.17

Exome sequencing to identify disease causing variants in 87 families with Primary Immunodeficiency Disease

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Primary immunodeficiencies (PIDDs) constitute a heterogenous group of genetic diseases affecting the immune system. Dependent on the genetic aetiology, symptoms range from mild to severe and life-threatening. Knowledge of the molecular genetic cause and disease mechanism is important and can direct targeted and curative therapy. However, categorization of the subtypes is difficult as patients with different immunodeficiencies may have overlapping immunological and clinical phenotypes. In addition, more than 200 causal genes have been reported, and few are offered for genetic testing.

We examined the utility of exome sequencing in the diagnostic workup and research of PIDDs. As of February 2013, totally 110 patients with extensive immunological and genetic testing from 87 families have been recruited from Texas Children Hospital (Houston, USA) and from Oslo University Hospital in Norway. Based on the clinical and immunophenotypical data, family history and knowledge from similar PIDD cases, the strategies for variant evaluation have involved both candidate gene testing with known PIDD genes, trio testing, or focused on potential novel genes in regions where LOH/deletions had been identified.

Preliminary analysis has proven exome sequencing as an efficient method to detect disease-causing variants in a large set of candidate genes. Combining data from exome sequencing and array CGH with exon coverage of all known PIDD genes has proven useful, exemplified by a patient with a heterozygous deletion and frameshift mutation in the same exon of *IL7R*. Additional benefits are expected from an in-house developed high-resolution array, in average tiling all exons by 5 probes.

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P09.18

Polymorphisms and expression of PTPN22, CTLA4 and TNF-alpha genes are associated with clinical data and disease susceptibility in Rheumatoid arthritis

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Rheumatoid arthritis (RA) is a chronic, autoimmune disorder affecting about 1% of the population. Genetic factors account for 50-60% of disease susceptibility. We designed a study to test whether SNPs from selected loci and gene expression of two candidate genes are associated with the development of the disease and with clinical parameters obtained from RA patients. 4 single nucleotide polymorphisms (SNPs) and 32 bp deletion were genotyped. Expression levels of 2 candidate genes were determined. Clinical data was tested for association with genotype and gene expression.

RA patients had lower frequency of GG genotype ($p<0.0001$, OR=4.877) in rs1800896 (IL10), higher frequency of A allele and AA genotype ($p=0.008$, OR=0.529; $p=0.033$, OR=0.130, respectively) in rs2476601 (PTPN22) and higher frequency of A allele and AA genotype ($p=0.019$, OR=0.711; $p<0.0001$, OR=0.397, respectively) in rs3087243 (CTLA4).

CTLA4 gene expression was reduced in RA patients ($p<0.0001$), patients with negative family history ($p=0.027$), patients positive for rheumatoid factor (RF) ($p=0.022$) and patients with biological treatment ($p<0.0001$). CTLA4 gene expression was significantly associated with rs3087243 genotype ($p<0.0001$).

TNF gene expression was reduced in RA patients ($p<0.0001$), male patients ($p=0.049$), patients positive for RF ($p=0.011$) and patients with biological treatment ($p<0.0001$).

Our results show that genotypes in rs1800896 (IL10), rs2476601 (PTPN22) and rs3087243 (CTLA4) are associated with development of RA and that rs3087243 (CTLA4) is a cis-eQTL, regulating CTLA4 gene expression. Results also show, that TNF and CTLA4 gene expressions are affected by gender, RF presence, prescribed biological therapy and family history.

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Identification of the Cia27 quantitative trait gene

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Rheumatoid arthritis (RA) is a systemic chronic autoimmune inflammatory disease that primarily impairs joints. RA affects between 0.5-1% of the adult population in developed countries and the genetic contribution to disease is estimated to be 60%. The knowledge of the genetic factors controlling complex disorders as RA is of great importance to understand pathogenic mechanism and to improve diagnosis and therapy. Human genome heterogeneity and gene-environment interactions are great challenges for the identification of RA susceptibility genes in human studies. In contrast, mouse models allow to work in homogeneous genetic background and under controlled environment. Our group has used the collagen-induced arthritis (CIA) mouse model to identify a new potential genetic factor involved in arthritis. In this study, we fine mapped the *Cia27* QTL, which is associated with antibody production in a F2 cross between the arthritis-susceptible DBA/1J strain and the arthritis-resistant FVB/N. By haplotype block analysis, 6 candidate genes were pointed out. Next, based on exon sequencing and differential gene expression analysis, *Thrap2* was pinpointed as the main candidate. This gene was confirmed to be involved in CIA by using a congenic strain approach. Knockdown assays in mouse primary B cells culture resulted in decrease of specific cell death, suggesting a role of this gene in B cell survival.

In summary, we have identified a putative gene involved in disease development, presumably by acting on B cells, in a murine arthritis model. Furthermore, we have generated a tissue-specific knock-out mouse.

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P09.20

Phenotypic analysis of Peptidylarginine deiminase type 4 knock-out mice

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Rheumatoid arthritis (RA) is well-known as an autoimmune disease and is a chronic inflammatory disorder characterized by the destruction of multiple joints. Many genome wide association studies were performed and multiple RA-susceptibility loci and autoimmune-susceptibility loci have been identified. These studies suggested that multiple genes and its functions were related with disease causing and development.
Previouly, peptidylariginine deiminase type 4 **(**PADI4) was identified as a susceptibility gene for RA in a Japanese population by case-control association study (Ref 1). PADI4 is a member of the PADI gene family and converts arginine residue (peptidylarginine) to citrulline residue (peptidylcitrulline). PADI4 is highly expressed in bone marrow, macrophages, neutrophils and monocyte. Peptidylcitrulline is an interesting molecule in RA, because it is an antigen of ACPA and only PADs (translated protein from PADI genes) can provide peptidylcitrullines, via modification of protein substrates. To evaluate the importance of PADI4 gene in the progression of RA, we generated Padi4 -/- DBA1J mice by speed congenic method. We used Padi4-/- mice to show that PAD4 is affected to development and progression of collagen induced arthritis (CIA), well known as an RA model animal. Padi4-/- DBA1-Jand WT mice were immunized with bovine type II collagen (CII) for CIA. Clinical disease score was significantly reduced. In Padi4-/- mice sera, the concentrations of serum anti-CII IgM, IgG, and TNF-a also decreased significantly rather than in WT mice. Resulting from these studies, we suggested that Padi4 enhanced collagen-initiated inflammatory responses.
1) Suzuki, A. et al Nat. Genet.34, 395-402 (2003)

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P09.21

Is a functional variant of ANXA11 R230C associated with impaired apoptosis?

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Sarcoidosis is a granulomatous disorder of an unknown aetiology, where the granuloma formation has been associated with impaired apoptosis of activated inflammatory cells. There is no information concerning the influence of *ANXA11* R230C, a functional annexin A11 variant associated with protection/disease modification of sarcoidosis, on the sensitivity of peripheral blood mononuclear cells (PBMC) to apoptosis yet.
We therefore compared the sensitivity to apoptosis of PBMC obtained from 92 sarcoid patients and 14 healthy controls. Tributyltin was used as apoptosis stimulus; annexin V positive cells were detected by flow cytometry. Additionally, sarcoid patients were subgrouped according to ANXA11 R230C (rs1049550) genotype (TT, n=13; CC, n=45) and clinical phenotype (CXR-stages, organ involvement).
When compared to healthy controls, lower number of annexin V positive cells was detected in stimulated PBMC from sarcoid patients (mean 82.0%; range 43.9–97.4%) than in cells from controls (88.9%; 71.3–96.5%; p=0.01). After subdivision according to genotypes, the number of annexin V positive cells differ between patients carrying TT genotype (71.0%; 43.9–94.2%) and those with CC genotype (82.6%; 53.4–97.4%; p=0.01). There was no difference in apoptosis between patients with particular CXR-stages or organ involvement, respectively.
In conclusion, PBMC obtained from sarcoidosis patients showed more apoptosis resistant phenotype than cells from control subjects. PBMC obtained from sarcoidosis patients with TT *ANXA11* genotype showed more apoptosis resistant phenotype than cells from CT and CC patients. Further studies in a larger patient cohort are under progress.
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P09.22

MTNR1B polymorphism rs10830962 in healthy women and patients with Systemic lupus erythematosus

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Background: The pineal hormone melatonin is an important modulator of the immune system in animals and humans. However, the role of the melatonin receptors for the development of autoimmune diseases in humans remains obscure. The present study aimed to investigate whether the MTNR1B rs10830962 gene polymorphism could influence on the clinical characteristics of systemic lupus erythematosus (SLE) in women.
Methods: The MTNR1B rs10830962C/G gene polymorphism was determined by RFLP analysis in 95 healthy women and 101 patients with SLE. The differences between genotype distribution in patients and controls and the interrelations between the C/G alleles and clinical features (ACR criteria) of the disease were studied.
Results: No differences in the allele distribution between patients with SLE and healthy controls were found (p=0.295). CC-homozygous patients had significantly more lupus ACR criteria in comparison to heterozygous GC and GG-homozygous patients (5.79±1.47 vs. 5.07±1.09, p=0.017) and a tendency for development of the disease at younger age (31.53±12.63 vs. 35.91±11.93, p=0.090). 76.5% of CC-patients had hematological disturbances in comparison to 58.2% of patients with other genotypes (p=0.082), probably because of the increased prevalence of leucopenia (38.2% vs. 17.9%, p=0.031). No significant interrelations between CC genotype and other ACR criteria were found.
Conclusions: Our preliminary results in SLE patients showed that MTNR1B rs10830962C/G polymorphism is not crucial for the development of SLE but it could modulate some clinical characteristics of lupus patients and especially the development of some haematological disturbances.
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P09.23

Genome-wide association study in a French familial T1D cohort using the Illumina Immunochip beadarray

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Our objective was to characterize susceptibility alleles in a French cohort of T1D families, using a recently developed genotyping microarray, the „immunochip". Resulting from a collaborative effort, this array includes ~200,000 SNPs including some 200 loci of interest to immune phenotypes. We performed a family-based association study in 236 trios. We replicated the well-known association of HLA class II alleles DR3 and DR4 ($p < 5x10^{-8}$). The most associated SNP in this region, rs9273363 ($p = 2.21x10^{-18}$, OR = 9.36), is in linkage disequilibrium with both DR3 and DR4. Outside the MHC, we replicated the association in the *BACH2* gene, with two new SNPs showing stronger effects than previously described ($p = 7.5x10^{-6}$, OR = 3.42). Suggestive evidence of association ($P<10^{-4}$) was found in four new regions (1p21, 3q25, 6q21 and 21q22). In contrast, other previously reported loci, notably including *INS*, *PTPN22*, and *IL2RA*, did not reach this threshold. We are currently validating these findings in 796 additional trios. Altogether, this work constitutes the first whole genome association study of a French T1D cohort.

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P09.24

Defective autophagy in TNFR associated periodic synfrom (TRAPS) accounts for mutant TNFR1 accumulation and enhanced inflammation

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Down-regulation of autophagy has been observed to enhance the inflammatory response, thus representing a possibly common pathogenic event underlying a number of autoinflammatory syndromes. So far, Tumor necrosis factor receptor associated syndrome (TRAPS) associated *TNFRSF1A* mutations have been reported to induce cytoplasmic retention of the receptor, defective TNFalpha-induced apoptosis and production of reactive oxygen species.
To search a link between *TNFRSF1A* mutations and inflammation in TRAPS, by means of both in vitro and ex vivo systems, represented by HEK293T cells transfected with expression constructs for WT and mutant TNFR proteins and by monocytes, derived by TRAPS patients, respectively, we have investigated the cellular response to mutant TNFR1 proteins.
We have found that autophagy is the main mechanism involved in mutant TNFR1 elimination and that it is impaired in the presence of TNFR1 misfolded proteins, thus likely accounting for their accumulation, for associated induction of NF-kappaB activity and excessive IL-1beta secretion.
We also show that autophagy inhibition due to TNFR1 mutant proteins can be reverted by geldanamycin, found to rescue membrane localization of mutant TNFR proteins, to reduce their accumulation, and to counteract the enhanced inflammation by decreasing IL-1beta secretion.
Overall, these observations provide a rationale to the apparent paradox that so far the most effective therapy in TRAPS is represented by inhibition of the cascade signaling induced by IL-1beta rather than by the use of drugs counteracting the TNFR-mediated pathway; as a consequence, we propose autophagy as a novel therapeutic target for TRAPS and other inflammatory diseases.

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P09.25

SNPs at the 3q21 -region are associated with type 1 diabetes and the appearance of beta-cell autoimmunity in Finland

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IDDM9-region on chromosome 3q21 has previously shown evidence for linkage to (LOD=3.4) and association with (TDT p=0.0002) type 1 diabetes (T1D) in Finnish families. As an extension to previous findings, we are analyzing genes at 3q21 region for association with T1D using tag-SNPs in an independent set of Finnish trio families. Currently two genes have been analyzed. Furthermore, two markers were examined by survival analysis for genotypic association with the appearance of beta-cell autoantibodies (AABs) and T1D and also for progression from autoimmunity appearance to clinical disease in a follow-up cohort of children at increased HLA associated risk for T1D.
Datasets included 977 Finnish T1D trio families and a follow-up cohort of 520 children with AABs (with or without T1D) and 992 AAB negative children. PLINK v1.07 and UNPHASED v3.0.13 were applied for TDT and haplotype-TDT and SPSS 19.0 for AAB appearance and progression analyses (Cox-regression).
Markers rs12485336 and rs777236 were significantly associated with T1D (p=0.034, p=0.009) and appearance of beta-cell autoimmunity (p=0.015, p=0.001) but not with progression to clinical disease from AAB positivity. In TDT, only haplotype analysis revealed significant association with T1D (rs9860012-rs777216, p=0.0014 and rs6796916-rs1345187, p=0.0022). Haplotypes showed independent association with T1D (rs6796916-rs1345187 conditioned by rs9860012-rs777216, p=0.018). Both AAB-associated markers were also associated with T1D in families due to LD with T1D-associated haplotypes.
Evidence from four independent data sets strongly suggests that 3q21-region is harboring T1D locus/loci. More comprehensive association studies are required to identify the functional polymorphisms affecting beta-cell autoimmunity and T1D susceptibility at this region.

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P10.01

Asian-specific mitochondrial genom polymorphism (9 bp deletion) in the Hungarian population

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Objectives: Several polymorphisms in the mitochondrial genome have population-genetically and anthropologically interest. The 9 bp deletion is anthropological marker for people of East-Asian origin.
Methods: The mitochondrial A8344G mutation was investigated by PCR-RFLP, performed on DNA samples isolated from blood and postmitotic muscle biopsy specimens. The mitochondrial COII/$tRNS^{Lys}$ and hypervariable regions were sequenced bidirectionally.
Results: From 890 patients we found 13 cases with 9 bp deletion (CCCCCTCTA) in the mitochondrial hipervariable non-coding region. Among them in 11 cases the 9 bp deletion was present with homoplasmic C8270T substitution. Their coexistence determines the M haplogroup. In one family (3 patients) beside these alterations we found a new heteroplasmic A8332G mutation in the $tRNA^{Lys}$ gene, wich was absent in 150 normal controls.
Conclusion: M haplogroup is in European populations very rare. It is mainly present in Asia, America and Australia, because of the human migration directions, these populations migrated eastwards. The frequency of 9 bp deletion in the Hungarian population is 1,5%. This polymorphism can be explained by the Westward migration of Hungarians from Siberia (in the matriarchal lineage). The deletion induce instability of this mitochondrial DNA-region like enough, and provoke the conformation other pathogen mutations.

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P10.02

ABCC5 as a candidate gene for Type 2 diabetes

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Type 2 diabetes (T2D) is a common, multi-factorial disorder caused by environmental and genetic risk factors. We have conducted a candidate gene study of a linkage disequilibria (LD) genomic region including *PARL* and *ABCC5* for European and African American samples, in relation to the phenotypes of healthy fasting plasma insulin and glucose levels, visceral fat accumulation and T2D disease. Previous functional studies and animal models of T2D have implicated *PARL*, but human association studies have yielded contradictory results.
We found no evidence of phenotypic association with *PARL* using commercial and fine map genomic and expression data. By contrast, we observed strong evidence of phenotypic association with *ABCC5* for European and African American samples. The genomic location estimate for the *ABCC5* functional variant associated with T2D (p=1E-06) and expression data (p=1E-11) was identical for all samples (185,136Kb), suggesting the identified genomic variant is an eQTL for *ABCC5*. We will also present preliminary hepatocyte cell culture results testing if *ABCC5* influences glucose uptake.
Previous observations of human genomic association with *PARL* may be due to LD confounding with *ABCC5*. The *ABCC5* variant is associated with expression, fasting insulin and glucose levels and T2D, while *ABCC5* expression is itself an intermediate phenotype for T2D. Given the *ABCC5* risk variant is cosmopolitan, common and observed in populations of disparate ancestry, this suggests the polymorphism is likely to be old and that the transporter gene plays an aetiological role in the onset of T2D.

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P10.03

Severe Form of Niemann-pick Type B Disease in The Iranian Population

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The non-neuronopatic type of acid sphingomyelinase (ASM) deficiency also designated as Niemann-Pick disease type B [NPD-B] is regarded as mild, late-onset and non-neurological form of acid sphingomyelinase (ASM) deficiency that presents with visceromegaly, and interstitial lung disease.
Here we report data on ten Iranian patients (5males, 5 females, under 18 years of age) with confirmed diagnosis of NPD-B. Longitudinal data were available for all patients in this study.
Most patients presented with thrombocytopenia, while anemia and leucopenia were less common. HDL cholesterol was reduced in most patients. Viseromegaly and Pulmonary disease were extremely severe in 50% of the cases.
A unique feature of this population was the observance of facial coarseness and severe growth retardation, which were present in more than 50% of cases. There was no evidence of skeletal abnormalities. In these cases motor development was also significantly delayed, perhaps secondary to cachexia and severe organomegaly. However in all cases, mental developments assessed by language and communication skills were progressive. Consanguinity of parents were documented in all the cases and various homozygot missense mutation in exon 6 of SMPD1 gene showed a high prevalence.
This is the first report of severe form of NPD-B with high morbidity and mortality seen among the Iranian population. In these cases pulmonary disease and viseromegaly are the most debilitating features. As enzyme replacement therapy (ERT) with recombinant sphingomyelinase is being developed special attention on this group of patients is warranted.

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P10.04

A *de novo* novel *POLG* mutation associated with Alpers syndrome

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A 16-month old boy was admitted to pediatric neurology with a status epilepticus and subsequent epilepsia partialis continua, elevated liver enzymes and increased blood and CSF lactate. EEG showed epileptic discharges on both occipital regions, brain MRI showed edema of the right hippocampus and muscle biopsy revealed decreased enzyme activities of respiratory chain complexes II+III and IV. The symptoms correspond with Alpers syndrome, a recessive disease in children characterized by progressive degeneration of the central nervous system due to mutations in the gene *POLG* coding for the mitochondrial DNA polymerase gamma. We sequenced DNA of the proband for mutations in *POLG* and identified 2 heterozygous mutations: a single base mutation in exon 7 leading to the known recessive mutation p.Ala467Thr and a frameshift insertion of 1-bp in exon 17 p.Ala880Glyfs*11, leading to protein truncation deleting the polymerase domain and activity. Analysis of the parents' DNA indicated that the mother carried p.Ala467Thr while the father did not carry any mutation in *POLG*. Non-paternity could be excluded by genetic fingerprinting. Both father and son carried the common 4-bp insertion polymorphism in intron 17 which was absent in the mother. Therefore, we predicted that p.Ala880Glyfs*11 might have arisen *de novo* on the father's allele. We were able to proof the *de novo* appearance by using a primer with its 3' end matching the insertion sequence and amplifying only the mutant allele. In conclusion, the patient is a compound heterozygote carrier of 2 *POLG* loss-of-function mutations, p.Ala467Thr and p.Ala880Glyfs*11, leading to recessive Alpers syndrome.

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P10.05

POLG-related Alpers-Huttenlocher syndrome in four Polish children.

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A wide spectrum of POLG-related diseases, including the most severe Alpers-Huttenlocher syndrome (AHS) is caused by dysfunction of the polymerase γ, the only mammalian enzyme able to replicate and repair mtDNA. Mutations in *POLG* account for ~25% of all mitochondrial defects. The diagnostic triad of AHS includes: drug-resistant epilepsy, hepatic impairment triggered by sodium valproate and mitochondrial DNA depletion. Here we describe four Polish children born in a good condition,

with infantile development delay due to muscle hypotonia, who died with severe liver failure at the age of 10-34 months. Intractable seizures followed by hemiplegia, and failure to thrive dominated at disease onset in all cases. Hepatic injury features were associated with valproic acid administration. Abnormalities characteristic for liver damage (hypertransaminasemia, coagulopathy, significant INR increase, decrease in fibrinogen concentration, hyperbilirubinemia, hipoalbuminemia, hipocholesterolemia), lactic acidemia, and OXPHOS dysfunction were found in most of the patients.

Severe mtDNA depletion confirmed in authopsied liver specimens ranged from <1% to 18% of control values in all cases.

Molecular analysis showed the presence of c.2243G>C(p.W748S) *POLG* mutation associated with c.3428A>G(p.E1143G) variant in all children. One homozygote and three compound heterozygotes with c.2419C>T(p.R807C), c.3630C>G(p.Y1210X), and c.2605C>T(p.R869X) were identified. This study shows similarity of genotypes identified in Polish and Nordic patients, with dominant p.W748S mutation. *POLG* mutation searching and mtDNA depletion assessment should be included in differential diagnosis of intractable epilepsy associated with hepatopathy. The study was supported by NCN grants: 2857/B/P01/2010/39, 01627/B/NZ2/2012/05, VENTURES 2011-8/3 FPS, MSHE0751/B/P01/2009/37, CMHI S126/12.

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P10.06

Mutations in mitochondrial aminoacyl tRNA synthetases identified by exome-sequencing

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Mitochondrial disorders include a group of heterogeneous clinical syndromes, frequently presenting as encephalo- and/or cardiomyopathy, with a broad range of associated causative genes. In the last years, several mutations in genes associated with defects of mitochondrial protein synthesis, affecting either the mitochondrial DNA (mtDNA) or nuclear genes, have been reported: an increasing group consists of mutations in mitochondrial aminoacyl tRNA synthetases (aARSs) genes, that are associated with diverse clinical presentations, usually with an early-onset and transmitted as autosomal recessive traits. A strict genotype-phenotype correlation has been reported for most of these genes, albeit it is not clear the reason for specific and different cellular or tissue damages, being all aARS2 ubiquitous enzymes working in the same pathway.
Whole-exome analysis in patients with clinical presentations suggestive of mitochondrial disorder, and OXPHOS deficiency, allowed the identification of mutations in: 1) *VARS2,* in one subject with microcephalia and epilepsy; ii) *AARS2,* in one subject presenting cerebellar atrophy; iii) *TARS2,* in two siblings presenting with axial hypotonia and severe psicomotor delay. All

identified variants are not present in SNPs databases, are predicted to be deleterious by bioinformatic analysis, and segregate within the families; moreover the pathogenic role of *VARS2* and *AARS2* variants was proven using yeast models.
This study reports two new mitochondrial aminoacyl-tRNA synthetase (VARS2 and TARS2) as cause of mitochondrial diseases, and presents a new phenotype associated with *AARS2* mutations; moreover, we confirm the value of exome sequencing for the identification of disease genes in mitochondrial disorders.

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P10.07
Autosomal dominant vitamin B12 deficiency: a new form of hereditary juvenile megaloblastic anemia

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Familial selective vitamin B12 (cobalamin, Cbl) malabsorption (Imerslund-Gra¨sbeck syndrome, IGS, OMIM 261100) is a group of autosomal recessive disorders characterized by selective malabsorption of Cbl from the terminal ileum in the presence of normal histology. Mutations in the amnionless (*AMN*), cubilin (*CUBN*) and Gastric Intrinsic Factor (*GIF)* genes are known to be causes of IGS.
Here we report, a family affected by vitamin B12 malabsorption for whom molecular analysis failed to identify any mutation for *AMN, CUBN* and *GIF.* Clinically, these patients resembled those with typical IGS; cobalamin absorption tests had been inconclusive regarding
the nature of the defect. The transmission of the disease from the mother to her children supports an autosomal mode of inheritance.
Therefore, we propose that this family shows a new form of hereditary juvenile megaloblastic anemia due to vitamin B12 (cobalamin) deficiency.

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P10.08
Biotin-responsive basal ganglia disease revisited: Clinical, radiologic, and genetic findings

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Background Biotin responsive basal ganglia disease (BBGD) also called Thiamine transporter-2 deficiency, is a treatable autosomal recessive neurometabolic condition resulting from mutations in the SLC19A3 gene**.**
Objective To investigate the clinical, genetic, and neuroradiologic data of BBGD and clarify the disease spectrum.
Methods We investigated all Patients with a genetically proven diagnosis of BBGD between 2009 and 2012. All patients underwent a detailed medical history and clinical examination, extensive laboratory investigations including genetic tests, and brain MRI. Finally, we conducted a systematic review of the literature.
Rresults We enrolled 15 with BBGD, and analyzed the data on 14 patients from 4 previous reports. The BBGD occurred predominantly in preschool/school-aged patients in the Saudi population, but it was also observed in other countries. The typical clinical picture consisted of recurrent subacute encephalopathy leading to coma, seizures, and extrapyramidal manifestations. The brain MRI typically showed symmetric and bilateral lesions in the caudate nucleus and putamen, infra- and supratentorial brain cortex, and in the brainstem. Early treatment with a combination of biotin and thiamine resulted in clinical and neuroradiologic improvement. Death and neurologic sequelae were observed in those who were not treated or were treated late.
Conclusions BBGD is an underdiagnosed pan-ethnic treatable condition. Clinicians caring for patients with unexplained encephalopathy and neuroimaging showing vasogenic edema in the bilateral putamen and caudate nuclei, and brainstem should consider this disorder early because a therapeutic trial with biotin and thiamine can be lifesaving. Our study also showed that thiamine is main treatment of this disease.

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P10.09
P30L and V281L mutations distribution in Macedonian and Serbian patients with nonclassic 21-hydroxylase deficiency

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Background: The mild nonclassic form of steroid 21-hydroxylase deficiency is one of the most common autosomal recessive disorders, occurring in almost 1% of Caucasians and 3% of Ashkenazi Jews. Although rarely recognized in infants, it may cause premature adrenarche and pubarche, virilization in young women and variable symptoms in young men. The missense P30L and V281L mutations in CYP21A2 gene for 21-hydroxylase are commonly associated with nonclassical congenital adrenal hyperplasia (NCAH) that produce enzymes retaining 20-60% of normal activity.
Method: We have performed direct molecular P30L and V281L detection in 20 Macedonian and 13 Serbian NCAH patients diagnosed according to standard clinical criteria at the Department of Endocrinology and Genetics, University Children's Clinic, Skopje, Republic of Macedonia and Institute for Mother and Child Health, Belgrade, Serbia, using PCR/ACRS method.
Results: The P30L mutation was detected in 40% (8/20) Macedonian NCAH patients on the 27.5% (11/40) alleles and 46.2% (6/13) Serbian patients on the 34.6% (9/26) alleles. Three homozygotes for P30L in each population and 5 heterozygotes in Macedonian and 3 heterozygotes in the Serbian patients were detected. The V281L mutation was observed only in 3/20 (15%) Macedonian NCAH patients on 3/40 (7.5%) of the alleles.
Conclusion: The P30L distribution in Macedonian and Serbian NCAH patients was higher compared with other European populations. On the other hand, lower V281L distribution in Macedonian patients than other European populations was observed while it was undetected in the Serbian NCAH patients.
Key words: Nonclassical congenital adrenal hyperplasia (NCAH), CYP21A2 gene, P30L mutation, V281L mutation.

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P10.10
Structural Elucidation of the Calcium-Sensing Receptor Extracellular Domain: 3D mapping of mutations observed in hyper- and hypocalcemic patients

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The Calcium-Sensing-Receptor (CASR) is a model for G-Coupled-Protein-Receptors (GPCR) more generally. The dimeric CASR protein plays a central role in regulating extracellular Ca2+ concentrations. Gain-of-function mutations of the CASR gene have been identified in patients with sporadic or familial-autosomal-dominant-hypocalcemia (ADH). Inactivating mutations of the CASR gene cause familial-hypocalciuric-hypercalcemia (FHH). More than 63% of these CASR mutations are located in the extracellular-domain (ECD). Despite more than a decade of effort, crystals of neither whole CASR nor CASR-ECD have been obtained. We developed by homology modeling and molecular dynamic (MD) simulations a complete 3D model of CASR-ECD, based on the rat mGluR1 structure. This new model allowed five regions in CASR-ECD to be precisely defined: the Membrane-Anchor-Domain (MAD), the Internal-Domain (ID), the Crown-Domain (CD), the Extended-Dimerization-Domain (EDD) and Calcium-Binding-Sites (CBS). The known amino acid substitutions associated with FHH and ADH were mapped onto this 3D model. The CD was the only domain in which all known mutations were activating. We further studied this region by functional and structural analyses of a new mutation. We showed that this mutation is an activating mutation and that it results in the loss of a disulphide bond, such that there is no covalent bonding in the CD. This new model thus provided evidence of the importance of the CD in CASR regulation activity. More generally, it will help to clarify the role of each region of this protein by allowing the effects of known and new mutations to be correlated to their 3D location.

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P10.11

Nitrosylation regulates Carnosinase activity substrate-specific

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Objectives: Diabetic nephropathy (DN) is the final stage in 40% of long term diabetic patients. A common variant in carnosinase (CNDP1, "Mannheim-Allele") is associated with lower activity and a reduced probability of developing a DN. Carnosinase catalyzes the degradation of histidine-containing dipeptides such as carnosine and anserine, compounds with cytoprotective functions. Several factors were identified regulating carnosinase activity. Since nitric oxide concentrations are altered under diabetic conditions, we investigated the role of both cysteine residues in the carnosinase as possible targets for nitrosylation and its effect on carnosinase activity.
Methods: We cloned three different CNDP1 variants: The wild type enzyme as well as two variants where each cysteine (Mut 1^{C102S} and Mut 2^{C229S}) residue has been substituted by a serine, respectively. Recombinant FLAG-tagged proteins were purified from CHO supernatant. The high-purified proteins have been studied for altered enzyme activity after nitrosylation with a NO donor (e.g. 3-Morpholinosydnonimine).
Results: Carnosine degradation was about 20-fold higher for the wildtype compared to anserine degradation. Enzyme activity was completely diminished for Mut 1 (Mut 1^{C102S}), whereas Mut 2 (Mut 2^{C229S}) showed activities in the same range as measured for the wildtype. Nitrosylation reduced enzyme activity of wild-type and Mut 2 carnosinase in a concentration-dependent manner. Contrary to these findings, nitrosylation had no effect on anserine degradation.
Conclusion: Nitrosylation regulates carnosinase activity in a substrate-specific manner. These findings suggest an important role of NO in the regulation of carnosinase activity. Therefore, modulation of NO-production can be of important therapeutic value.

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P10.12

Cerebellar ataxia and atrophy due to mutations in CABC1/ADCK3, a diverse clinical presentation in two sisters and potentially treatable.

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Abstract
We describe two sisters with cerebellar ataxia and atrophy. The younger sister demonstrates early onset rapidly progressive cerebellar ataxia accompanied by motor and non-motor cerebellar features, as well as cognitive decline and psychiatric problems. Mitochondrial respiratory chain enzyme analysis in muscle showed a decrease in complex I+III. Progressive cerebellar atrophy was demonstrated on serial brain MRI imagings. CoQ supplementation was started at the age of 5 years with significant improvement in motor and cognitive abilitiesand partial lessening of the cerebellar signs. Discontinuation of the treatment with CoQ resulted in worsening of the ataxia, cognitive decline and severe depression associated with significant progression in the cerebellar atrophy. The older sister, who is 32 years old, has non progressive dysarthria and clumsiness from the age of 10 years and cerebellar atrophy shown on MRI. Both were found to be compound heterozygote for 2 mutations in the CABC1/ADCK3 gene, one of the mutations was not previously described.
Patients with primary CoQ deficiency due to *CABC1/ADCK3* mutations show wide spectrum of clinical presentations even in the same family.
The remarkable clinical response of some of these patients to CoQ supplementation highlights the importance of treatment trials with CoQ10 in patients with cerebellar ataxia and atrophy even before analyzing the *CABC1/ADCK3* gene. Hence it might improve the outcome for these patients

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P10.13

The association of common SNPs with HDL cholesterol levels in Latvian population

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Low level of high-density lipoprotein cholesterol (HDL-C) is one of the main risk factors for cardiovascular disease (CAD) and the heritability of HDL-C level is estimated at approximately 50%. Earlier association studies of HDL-C have found numerous genes and their respective proteins influencing lipid phenotype. The precise genetic profile determining heritability of HDL-C however are far from complete and there is substantial room for further characterization of genetic profiles influencing blood lipid levels.
Here we report an association study of 139 SNPs from more than 30 loci with HDL-C level. We genotyped 1273 individuals selected from Genome Database of Latvian Population.
58 SNPs from 13 loci were nominally associated ($p<0.05$) with HDL-C levels. 10 SNPs from CETP gene and two from MLXIPL retained significant association with low HDL-C levels (rs1800775, rs3764261, rs173539, rs9939224, rs711752, rs708272, rs7203984, rs7205804, rs11076175, rs9929488, rs17145738 and rs2286276) after Bonferoni correction. We have also prepared case-control haplotype association analysis of extreme ends of HDL-C level distribution and identified haplotypes from CETP with distinct effects on determination of HDL-C levels. Moreover we performed case-control allele dosage test of extreme tails of DHL-C level for 20 nominally associated SNPs and found that more than 25 risk alleles significantly decreases HDL-C level.
Our conclusion: CETP gene is the strongest genetic factor influencing HDL-C levels in the Latvian population.

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P10.14

Functional studies of novel mutations in the CYP21A2 gene of Norwegian patients with 21-hydroxylase deficiency

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Background: Congenital adrenal hyperplasia (CAH) with 21- hydroxylase deficiency is caused by mutations in *CYP21A2*. Recently we reported 4 novel mutations in the Norwegian population of patients with CAH.

Objective: To characterise the novel *CYP21A2* mutations by *in silico* analyses, develop an *in vitro* assay for determination of the enzyme activity of each mutations, and compare the results with the patient phenotypes.

Methods: In silico analysis was performed by Clustal Omega multiple sequence alignment tool, Polyphen, and Pymol. The QuickChange II Site Directed Mutagenesis Kit (Stratagene) was used to generate point mutations. TNT® Quick Coupled Transcription/Translation system (Promega) was used for protein expression. 21-hydroxylase activity was assayed by LCMS-MS (conversion of 17-hydroxyprogesterone to 11-deoxycortisol).

Results: The mutation p.L388R revealed no activity, while the p.E140K showed an activity of less than 10% compared to the wild type, which were in accordance with the *in silico* analysis. The mutation p.P45L gave slightly impaired activity by the *in vitro* assay, but was expected to interfere with the protein activity by Polphen. The mutation p.V211M showed an activity of 30-60% compared to the wild type, but were predicated as benign by Polyphen.

Conclusion: The results from the *in vitro* study of the novel mutations were in accordance with the severity of the phenotype of the patients, but only partly in agreement with the *in silico* results.

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P10.15

Single heterozygous mutations in ABCC8 are a common cause of diazoxide-unresponsive diffuse form of congenital hyperinisulinism

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Background *ABCC8* encodes the SUR1 subunit of the β-cell ATP-sensitive potassium channel whose loss of function is responsible for congenital hyperinsulinism (CHI). Diffuse forms of diazoxide-unresponsive CHI are classically associated with the presence of two mutations with a recessive inheritance. However several dominant missense mutations were recently reported.
Methods Clinical and molecular characterization of 27 probands with a diagnosis of diazoxide-unresponsive diffuse CHI and carrying a unique *ABCC8* mutation were performed. - Nine missense *ABCC8* mutations were subjected to in vitro studies testing 1) processing efficiency of mutant channels by Western blots and surface immunofluorescence staining and 2) channel function using inside-out patch-clamp recording.
Results Twenty-three distinct *ABCC8* heterozygous mutations were identified in these 27 patients: 15 missense mutations (62%), 4 truncating mutations (17%), 3 splice mutations (13%) and 1 in frame indel mutation. None of the missense mutations altered the processing of the SUR1 protein. All mutants but two had dramatically reduced response to MgADP or to diazoxide (<10% of WT current).
Conclusion This analysis confirms that mutated channels with preserved trafficking to the membrane are associated with a dominant inheritance. It also raises the question of the truncating mutations identified as sole event and generally associated with defective trafficking. Consequently, diffuse forms of CHI may be associated with either recessive or dominant inheritance. These findings are of major importance in terms of differential diagnosis with focal forms associated with a paternally-inherited mutation and in terms of genetic counselling.

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P10.16

Connexin50 mutation L7Q attenuates hypertension in spontaneously hypertensive rat SHR/OLAIPCV

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The aim of this study was to determine the metabolic, hemodynamic and transcriptomic outcomes of recently identified connexin50 gene (Gja8) mutation L7Q that arose spontaneously in the spontaneously hypertensive inbred rat strain SHR/OlaIpcv, creating thus a coisogenic rat strain SHR-Dca.
Adult, standard chow-fed male rats of SHR/OlaIpcv and SHR-Dca strains were used (n = 8/strain/procedure). We assessed metabolic and morphometric profiles of the two strains, measured arterial blood pressures continuously by radiotelemetry (36 days). We used Affymetrix GeneChip® Rat Exon 1.0ST Array to assess the heart and renal transcriptome. Immunohistochemistry was performed using polyclonal rabbit anti-Connexin50 (H-65) antibody .
The distribution of triglycerides and cholesterol across major lipoprotein fractions was similar in both strains except for significantly lower high-density lipoprotein cholesterol concentrations in SHR-Dca. There were no differences in morphometry, glucose tolerance, adiponectin and leptin levels. We found significant 10-15mmHg decrease of both systolic and diastolic blood pressures in SHR-Dca compared to SHR (repeated measures ANOVA $p<0.01$ and $p<0.05$, respectively). By immunohistochemistry we localized Cx50 in heart, kidney, aorta, liver and lungs, mostly in endothelium. There were 15 transcripts common to the heart and kidney sets including S100A9, Bcl6 and Clec4d downregulated and Cyr61, Frs2 and Dusp1 upregulated in both heart and kidney of SHR-Dca compared to SHR.
We show that Cx50 mutation L7Q attenuates hypertension in SHR-Dca strain and significantly changes the renal and cardiac expression of sets of genes involved in blood pressure regulation and related pathways.
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P10.17

COQ6 mutations in patients with nephrotic syndrom, sensorineural deafness and optical atrophy

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Primary Q10 coenzyme (COQ10) deficiency is a rare autosomal recessive mitochondrial disease accessible to a substitutive treatment. Clinical presentation is heterogeneous depending upon the gene involved. Mutations in COQ2, COQ6 and PDSS2 genes are responsible for steroid-resistant nephrotic syndrome associated to various extra-renal features. COQ6 gene mutations have been reported in 13 individuals belonging to 5 unrelated families who presented steroid-resistant nephrotic syndrome and sensorineural deafness.
We report on two brothers of Turkish decent with COQ10 deficiency with renal involvement, sensorineural deafness and optical atrophy. Patient 1 (the older brother) was diagnosed at the age of 5 years with end-stage kidney disease and bilateral sensorineural deafness. He received a kidney transplant at the age of 6. Patient 2 (the younger brother) developed nephrotic range proteinuria and bilateral sensorineural deafness at the age of 5 years. Kidney histology revealed focal segmental glomerulosclerosis not otherwise specified. ACE inhibitors and diuretics led to a sustained remission. At the age of 17 years, Patient 1 presented sudden and severe bilateral blindness with flat electroretinogram secondary to optical atrophy that did not improve with methylprednisolone pulses. Direct Sanger sequencing of the COQ6 gene revealed a previously published homozygous missense mutation (pA353D) in the two brothers. Subsequently, both patients were treated with oral COQ10 (Idebenone) which allowed a progressive improvement in visual acuity in Patient 1.
We report herein the first patient with COQ6 mutations and ocular involvement in association with renal and auditive defects. COQ10 substitution might significantly improve the disease course in the patients.

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P10.18

Genomic anatomy of chronic progressive external ophthalmoplegia patient (CPEO) related multiple deletions on mitochondrial genome

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Mitochondrial genome (mtgen) deletion syndromes comprise three major phenotypes, progressive external ophthalmoplegia (PEO), Kearns-Sayre syndrome (KSS), and Pearson syndrome. PEO is conventionally defined as progressive limitation of eye movements with normal pupils and ptosis of the eyelids. 1.1 kb to 10 kb of multiple deletions were previously reported from the mtgen analyses of skeletal muscle, however systematic consideration and molecular mechanism of the multiple deletions are unknown.
Here we report detail mitochondrial genome analysis using a patient (31 y/o male) who suffering from PEO, muscle weakness, hearing loss, leukoencephalopathy and hypogonadism. After receiving his agreement through a genetic counselling, we performed extraction of the genomic DNA from muscle tissue, peripheral blood cells and oral mucosa, long range PCR covering the whole mtgen region, sub-cloning of the PCR products, nucleotide sequencing of the sub-clones, and identification of the deletion points by comparing with normal mtgen sequences structures.
At least four kinds of deletions, ranging from 7627 bp to 8976 bp, were detected in the muscule tissue, but these deletions were not observed in peripheral blood cells and oral mucosa. Of them all deletion points involved in whole mtgen region were identified, and none of them had identical short nucleotide sequences near the deletion points that have not been so far reported. These findings suggest that the onset of the CPEO in caused by accumulation of *de novo* deletions and it may lead to solution why he has the unique symptoms.

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P10.19

Molecular analysis of mitochondrial DNA in children with vomiting cyclic syndrome

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Cyclic vomiting syndrome (CVS) is a disabling condition affecting mostly children characterized by recurrent attacks of unremitting nausea and vomiting, often associated with lethargy and separated by asymptomatic intervals. Neuromuscular disease manifestations are present in approximately 25% of cases. In CVS children with a family history of migraine, migraine was identified on the maternal side only in 63% versus the paternal side only in 16% . Therefore, it has been suggested that at least some of the predisposing genetic factor is located on the maternally-inherited mitochondrial DNA (mtDNA). In order to further study the relationship between mtDNA sequences and CVS, we screened the entire mtDNA D-loop in 37 Italian children (23 males and 14 females) (age 11.9±4.8 years) with CVS, in their first-degree relatives and in 103 control subjects. The subjects included in our study had mtDNA haplogroup H.
Over 1200 bp of mtDNA, including the D-loop region, were amplified by nested PCR using primers L15990-H617. Four overlapped nested PCRs were performed using primers L15990-H16434, L16431-H162, L039-H407, and L361-H617. PCR products were analyzed by automatic sequencing. Excluding insertions in the ultravariable 302-315 region, 22 different mtDNA variants were identified in two or more CVS subjects. Most prominently, one of the SNPs, 16519C>T, was found to be highly associated with CVS vs. controls ($p<0.001$). Another, 73A>G SNP was found to be highly associated with CVS in subjects with 16519T vs. in controls ($p>0.01$) with 16519T. Our findings provide further evidence that there is a component of mitochondrial dysfunction in CVS.

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P10.20

DHTKD1 mutations cause 2-aminoadipic and 2-oxoadipic aciduria and suggest a therapeutic strategy for glutaric aciduria type 1

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Glutaric aciduria type 1, caused by mutations in GCDH, results in a severe neurological phenotype for which the only available treatment is a lysine reduced diet. However, the identification and characterisation of novel metabolic disease genes together with the elucidation of their physiological function can provide guidance for therapeutic interventions.
Recently, by exome sequencing of individuals with 2-aminoadipic and 2-oxoadipic aciduria we identified mutations in DHTKD1. This gene encodes for dehydrogenase E1 and transketolase domain-containing protein1. The accumulation metabolites argued that this enzyme acts upstream of GCDH in the L-lysine-degradation pathway. Elevated levels of 2-oxoadipate in individual-derived fibroblasts could be restored upon lentiviral complementation using wild-type DHTKD1 mRNA. Moreover, experiemts with deuterium-labeled 2-oxoadipate showed accumulation in DHTKD1-deficient cells, indicating that DHTKD1 mediates the last unresolved step in the L-lysine-degradation pathway.
Since patients with DHTKD1 mutations showed only a mild phenotype with developmental delay, this opened the posibility to treat glutaric aciduria type1 patients with DHTKD1 inhibitors. An existing GCDH- mouse is known to develop a severe phenotype upon lysine-rich diet. To clarify whether inhibition of DHTKD1 activity can rescue the severe phenotype of GCDH deficiency we are creating a DHTKD1-/GCDH- double KO mouse by using a TALENs (transcription activator-like effector nucleases) approach. These nucleases bind to the desired DNA sequence and disrupt gene function by small insertions or deletions with high specificity, which has been verified in an in vitro system. This approach mimics the condition of a Glutaric Aciduria Type 1 patient, who receives an inhibitor for DHTKD1 activity.

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P10.21

Exploration of the impact of low-frequency and rare coding variation in the genetic architecture of type 2 diabetes susceptibility

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Custom genotyping arrays, like the Illumina HumanExome Beadchip, provide a cost-effective alternative to sequencing to explore the role of low-frequency (LF; 1-5% MAF) and rare (<1% MAF) coding variants in type 2 diabetes (T2D) susceptibility.We studied 12,778 cases and 24,474 controls of European ancestry from the UK, Finland and Sweden, genotyped on this array to uncover novel T2D loci and examine whether LF and rare coding alleles could explain established common variant GWAS associations. We combined summary statistics (single-variant and gene-based) of up to 109,227 high-quality autosomal variants across studies by meta-analysis. We identified two LF nonsynonymous (NS) variants achieving study-wide significance ($P<2.5 \times 10^{-7}$) in genes not mapping to established T2D loci: *KRTAP4-3* (P152S; $P=1.7 \times 10^{-17}$; 0.3% MAF) and *DOCK6* (R430H; $P=6.2 \times 10^{-8}$; 0.4% MAF). One additional NS variant in *PSORS1C1* approached significance (E34K; $P=6.4 \times 10^{-7}$, 9% MAF). We found no evidence of LF or rare coding variants with large effects (Odds ratio>1.5) in established T2D risk loci that could explain common SNP GWAS associations. Gene-based analysis (SKAT-o) of rare NS variants in four genes *C16orf91*, *IL22RA2*, *FAM63A*, and *GSG2* met the gene-based significance threshold ($P< 5 \times 10^{-6}$), but each is driven by association in a single study and further confirmation is required.Our results indicate that the genetic landscape of T2D susceptibility is not dominated by LF and rare coding variants of large effect. In an effort to provide power to identify variants of weaker effect, we are expanding the sample size to >80,000 subjects.

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P10.22

Humans have an active endogenous D-lactate dehydrogenase

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Lactate exists as two stereo-isomers, the L and the D form. In human cells L-lactate is produced from pyruvate during the anaerobic metabolism of carbohydrates, mammals are not believed to produce and metabolize D-lactate. Increased levels of bacterial produced D-lactic acid are only occasionally seen in patients that underwent gastrointestinal surgery.
Here we present a patient with mental retardation and a normal intestine length. He was found to have chronic increased D-lactic acid levels in urine, which did not decrease after repeated antibiotic treatment, indicating an inborn metabolic defect in D-lactate metabolism.
Because his parents were consanguineous, we performed homozygosity mapping and identified several large stretches of homozygosity. The third largest homozygous stretch contained a gene with homology to D-lactate dehydrogenases from lower organisms. Sanger sequencing subsequently identified a homozygous missense variant Thr463Met in human lactate dehydrogenase D (LDHD), the variant is predicted to have a damaging (Sift) and deleterious (Polyphen) effect on protein function. Currently we are performing functional studies to assess the effect of the mutation on protein function.
We conclude that contrary to common textbook knowledge the human body does require endogenous D-lactate dehydrogenase activity for proper D-lactate clearance.

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P10.23

Clinical Exome Sequencing Leads to the Diagnosis of Mitochondrial Complex I Deficiency in a family with global developmental delays, ataxia, and cerebellar and pons hypoplasia

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Exome sequencing was performed on a 14 year-old female with familial ataxia, global developmental delays, and cerebellar and pons hypoplasia. The family history was remarkable for a 3 year-old sister with a similar phenotype. Nearly a decade of molecular, cytogenetic, and biochemical testing was uninformative. Exome sequencing revealed compound heterozygous alterations of the NUBPL gene (c.311T>C; p.L104P & c.815-27T>C). The c.311T>C missense alteration is located at a highly conserved amino acid. The c.815-27T>C alteration is located at a highly conserved nucleotide and previous in vitro analyses demonstrated splicing defects. The affected sister manifested both alterations; each parent carried one alteration. Alterations within the NUBPL gene occur in an autosomal recessive fashion in association with mitochondrial complex I deficiency syndrome (CI deficiency) (MIM_252010). The NUBPL gene was first discovered in association with disease in 2010 and has only been reported in two other families, both of which displayed remarkable clinical overlap with the family herein. Exome sequencing is an especially powerful tool to aid in the diagnosis of CI deficiency given the extreme clinical and genetic heterogeneity making establishing a clinical diagnosis exceedingly difficult. Further, the underlying mutation has not been discovered in about half of patients with CI deficiency, thought to be due to yet undiscovered associated genes. Diagnostic exome sequencing led to the successful identification of the NUBPL alterations and, after years of unsuccessful analyses, led to a molecular diagnosis for the family.

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P10.24

Pro-oxidant state and effect of anti-oxidants in Fanconi anemia and Down syndrome patients

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Background: Fanconi anemia and Down syndrome are two genetic disorders caused by chromosomal aberrations. Oxidative stress is an important pathological factor in both disorders.
Objective: Evaluation of the effect of antioxidant treatment on the level of oxidative stress and DNA damage in Fanconi anemia and Down syndrome patients.
Patients and methods: Seventeen Egyptian patients diagnosed to have Fanconi anemia, and Fifteen Egyptian patients diagnosed to have Down syndrome were recruited from the outpatient clinic of Clinical Genetics Department, National Research Centre. Fifteen healthy children were used as controls. Oxidative stress parameters including total antioxidant capacity (TAC), Superoxide dismutase (SOD) enzyme activity and Malondialdehyde (MDA) biomarkers were estimated. DNA damage was determined using the alkaline comet assay. Diepoxybutane (DEB) test was used for Fanconi anemia patients. Re-estimation of these parameters was done after six months of antioxidant intake.
Results: In Fanconi anemia patients after antioxidants intake, there was an improvement of DEB ($p< 0.01$), and DNA damage levels, ($p< 0.01$). Improvement in oxidative stress parameters occurred with significant lowering of MDA levels ($p< 0.01$) and significant elevation in TAC ($p< 0.01$) and SOD levels ($p< 0.01$). DNA damage levels in Down syndrome patients improved after antioxidants administration ($p< 0.01$). Also, improvement in oxidative stress parameters occurred after antioxidant administration with lowering of MDA ($p< 0.01$) and SOD levels ($p< 0.05$) and elevation in TAC levels ($p< 0.01$).
Conclusion: Improvement in oxidative stress parameters and DNA damage after giving antioxidants occurred in each of Fanconi anemia and Down syndrome patients.

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P10.25

Haplotype-based regulation of frataxin by microRNAs in Friedreich Ataxia

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MicroRNAs (miRNAs) are small non-coding RNAs that can modulate gene expression by interfering with translation or stability of messenger RNAs (mRNAs). The differential expression of miRNAs in patients with Alzheimer disease or various ataxia suggest a crucial role of miRNAs in neurodegeneration. We hypothesized a differential regulation of frataxin by miRNAs that could affect protein levels in patients with Friedreich ataxia (FRDA). To this end, we searched for miRNA binding sites in the frataxin gene using various computational tools, and identified a subset of target sites. By sequencing the 3'-UTR of frataxin in our cohort of patients with FRDA (n = 57), we were able to identify single nucleotide polymorphisms (SNPs) defining several haplotypes with one reaching 89% of homozygosity in patients versus 24% in controls. This result was confirmed in another cohort of FRDA Reunionese patients, with 94% patients who were found homozygous for this haplotype. Finally, by evaluating the potential regulator of those 3'-UTR haplotypes, we established differential regulation, and specifically demonstrated the involvement of miR-124 in the down-regulation of the FRDA haplotype. Our results suggest for the first time that post-transcriptional regulation of frataxin occurs through the 3′-UTR and involves miRNA targeting. We propose that the involvement of miRNAs in a FRDA-specific regulation of frataxin may provide a rationale to increase residual levels of frataxin through miRNA-inhibitory molecules.

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P10.26

Mutation spectrum in Indian patients with mucopolysaccharidosis IVA

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Mucopolysaccharidosis IVA is an autosomal recessive inborn error of metabolism caused by the mutations in the N-acetylgalactosamine-6-sulfatase (GALNS) gene and manifests with a spondyloepiphyseal dysplasia. Bidirectional sequencing of all the coding region was performed in 60 patients from 58 families. In silico analysis was carried out for novel missense mutations to peredict the effect of mutations on protein function. We identified 37 different mutations, 26 of which had not been previously described. The 26 novel mutations consisted of 22 missense mutations (p.MlI, p.N32T, p.L36R, p.P52L, p.P77S, p.C79R, p.S82P, p.L86P, p.H142P, p.G188S, p.Y191D, p.N204T, p.F216S, p.W230C, p.R251Q, p.A291S, p.T313M, p.G317R, p.H329P, p.R386S, p.E450G, p.C501S), one nonsense mutation (p.Q414X), 3 intronic variations (c.120+1G>C, c.1003-3C>G and c.1139+1G>A) that affect the splice sites and 11 previously reported mutations. The missense mutations p.S287L and p.A291S account for 9.1 % (10/111) and 7.3 % (8/111) of the cases respectively and were the common mutations in Indian patients. This is the first report of screening for mutations in GALNS gene in the Indian population and the largest number of patients studied in the literature till date. The identification of these mutations has helped 5 families for prenatal diagnosis.

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P10.27

Increased level of alpha-synuclein oligomers in blood plasma in Gaucher disease

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At present alpha-synuclein is regarded as the key pathological molecular in pathogenesis of Parkinson's disease (PD). Oligomeric alpha-synuclein, the form of the protein most likely causing neuronal death, has been found in brain tissues of PD patients. Gaucher disease (GD) is the most common lysosomal storage disorders (LSD). Mutations in the glucocerebrosidase (GBA) gene result in lysosomal dysfunction and glucosylceramide accumulation. The carriers of GBA mutations are at an increased risk for PD. Dysfunctions in alpha-synuclein cellular degradation may link PD and LSD.
The aim of our work was to estimate the level of alpha-synuclein oligomers in blood plasma in GD patients.
We generated blood plasma of 22 GD patients (mean age 14, range1-42, 9 males) and 24 individuals of control group (mean age 20, range 1-41, 13 males). Diagnosis of GD is based on assay of glucocerebrosidase activity and genetics genotyping of mutations in the glucocerebrosidase (GBA) gene. We present a quantitative measurement of blood plasma alpha-synuclein oligomers in healthy control subjects and GD patients using enzyme-linked immunosorbent assay ELISA (Human Synuclein OLIGO kit aj Roboscreen, Germany).
The level of oligomeric alpha-synuclein was significantly elevated in blood plasma of GD patients (median 4.75 ng/ml, range 0.80-36.3 ng/ml) compared to controls (median 1.22 ng/ml, range 0.00-40.10 ng/ml), $p < 0.02$.
This is the first report of elevated level of alpha-synuclein oligomers in blood plasma in GD patients. Our results allow to suggest that GBA mutations promote alpha-synuclein aggregation in GD, which might explain the pathological mechanisms underlying GBA-associated PD.

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P10.28

GCK mutations are not common cause for MODY2 and Gestational Diabetes in Turkey

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Maturity-onset diabete of the young (MODY) is an early-onset non autoimmune form of diabetes with an autosomal-dominant mode of inheritance. MODY has at least 10 subtypes associated with distinct genes. MODY2 associated with glucokinase (GCK) gene mutations is the second most common type of MODY. It accounts for 20-30% of all MODY cases. MODY2 is characterized by a glucose sensing defect and mild chronic hyperglycemia. In addition GCK gene mutations have been asssociated with increased risk of gestational diabetes. Studies have demonstrated that heterozygous GCK mutations are associated with mild fasting hyperglycemia and gestational diabetes while homozygous and compound heterozygous mutations are associated with mutations cause more severe clinical phenotypes like Permanant Neonatal Diabetes Mellitus. The purpose of the study is to determine the genetic profile of MODY2 and Gestational diabetes in the Turkish patient population by analyzing GCK gene. In order to identify GCK gene mutations, GCK gene exonic sequences, exon-intron boundaries and the promoter sequence was analyzed by direct DNA sequencing in 35 MODY suspected and 15 gestational diabetes patients. Studies have revealed presence of one novel mutation (c.765delC) and two previously known mutations (c.3G>A and c.686delC) in the MODY suspected patients. On the other hand, no pathogenic alteration in the GCK gene was detected in the gestational diabetes patients. This study demonstrated that GCK gene mutation is not a common cause of MODY and gestational diabetes in Turkey.

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P10.29

Genome-wide association study of 10,947 type 2 diabetes cases and controls from five ancestry groups provides novel insights into the genetic architecture of the disease

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We undertook genome-wide association studies (GWAS) of type 2 diabetes (T2D) in five ancestry groups: African American (AA), East Asian (EA), European (EU), Hispanic American (HA) and South Asian (SA). We performed trans-ethnic meta-analysis (MANTRA) of GWAS, imputed up to the 1000 Genomes Project Phase I reference panel (~32.3 million variants), in 5,464 T2D cases and 5,483 controls. We identified three novel T2D susceptibility loci at genome-wide significance, defined by $\log_{10}$ Bayes' factor (BF)>6 (equivalent to $p<5\times10^{-8}$ under a fixed-effects model), mapping near *PDGFRL* ($\log_{10}$BF=6.43), *CLDN10* ($\log_{10}$BF=6.37) and *LEP* ($\log_{10}$BF=6.36). The lead variant near *PDGFRL* is common (MAF≥5%) in all ancestry groups and has homogeneous allelic effects on T2D susceptibility across ethnicities (OR = 1.20 [1.13-1.28]). The lead variant near *LEP* is also common in all ancestry groups, but the allelic effect is heterogeneous between ethnicities ($\log_{10}$BF=5.07), and is specific to EA populations (MAF=10.5%, OR=1.85 [1.50-2.27]). At *CLDN10*, the lead variant is monomorphic or rare (MAF<1%) in EA, EU, HA and SA populations, so that the effect on T2D appears specific to AA populations (MAF=8.7%, OR=1.98 [1.58-2.49]). We identified no low-frequency variants (1%≤MAF<5%) at novel or established loci at genome-wide significance. The common lead SNPs are specific to one ancestry group, or are polymorphic across ethnicities with predominantly homogenous allelic effects. Our results highlight the benefits of trans-ethnic meta-analysis for discovery and characterisation of complex trait loci, and emphasize the opportunity to extend insights into the genetic architecture of human disease. across diverse populations.

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P10.30

Diabetes associated endothelial dysfunction begins in utero

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The 'Developmental Origin of Health and Disease (DOHaD)' paradigm proposes that environmental factors in pregnancy act *in utero* to program the risk for adverse diseases, i.e. gestational diabetes mellitus (GDM) that confers an increased risk for vascular and endothelial dysfunction.
The feto-placental compartment is also affected by the maternal diabetic environment. We hypothesized that primary human arterial endothelial cells isolated from term placentas after healthy pregnancies (normal AEC) and pregnancies complicated with GDM (diabetic AEC) would differ in their intrinsic biological program. We focused on two key endothelial processes i.e., proliferation and angiogenesis.
Viable and dead cells were counted to determine proliferation of normal and diabetic AEC. *In vitro* angiogenesis (2-D network formation) was studied in media containing 2% normal or diabetic cord blood serum (CBS). In addition, the DNA methylation profile was determined by 450k methylation arrays and global gene expression profile by Human GeneChip 1.0ST arrays. Biological interpretation of the candidate genes was assessed using IPA.
Diabetic AEC had reduced proliferation (ANOVA $p<0.003$) after 96h culture than normal AEC. In the presence of normal CBS total tube length was increased in diabetic vs. normal AEC (ANOVA $p<0.001$). Diabetic CBS did not influence network formation potential. Thus the difference in proliferation and tube formation is the result of an intrinsic program of the cells. In fact principal component analysis revealed differences in the global methylation and expression pattern between normal and diabetic AEC. IPA identified that differentially methylated and expressed genes clustered to cell growth and proliferation related processes.

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P10.31

Glycogen storage disease type III in Tunisian patients

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Purpose: Glycogen storage disease type III (GSD III) is a rare autosomal recessive disorder caused by a deficiency of the glycogen debranching enzyme, amylo-1, 6-glucosidase (AGL). GSD III is characterized by the storage of structurally abnormal glycogen, termed limit dextrin, in both skeletal and cardiac muscle and/or liver, with great variability in resultant organ dysfunction. Here, we present the molecular and enzymatic analyses of 22 Tunisian GSD III patients.
Methods: To examine the heterogeneity Glycogen Storage Disease Type III, we genotyped the AGL gene and sequenced all its exons in 22 Tunisian patients with a clinically and biochemically confirmed diagnosis.
Results: Molecular analysis revealed three novel mutations; a nonsense (Tyr1148X) and two deletions (3033_3036del AATT and 3216_3217del GA), and five known mutations; three nonsense (R864X, W1327X and W255X), a missense (R524H) and an acceptor splice-site mutation (IVS32-12A/G). Each mutation is associated to a specific haplotype.
Discussion: This is the first report of screening for AGL gene mutations in the Tunisian population. We concluded that Glycogen Storage Disease Type III is a highly heterogeneous disorder in our population, requiring full gene sequencing to identify commun or specific mutations.

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P10.32
Genomic, transcriptomic, and lipodomic profiling demonstrates the benefits of extreme phenotype approach and highlights the role of inflammation in individuals low HDL-cholesterol

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Low HDL-C is a major risk factor for CVD. To elucidate novel pathways behind low HDL-C, we have employed 3 different omics:genomics, transcriptomics,and lipidomics.
We performed GWAS on 450 Finns with extreme HDL-C-phenotypes(90th percentiles). We obtained subcutaneous fat biopsies for transcriptome-analysis, and isolated plasma HDL particles for MS-lipidome analysis.
We first conducted network analysis for low-HDL-C-associated loci observing that SNPs within inflammatory pathways(e.g.antigen-presentation) were enriched among low-HDL-C-associated genes (p=10^-5). Also,these inflammatory pathways were over-expressed in SC-fat of low-HDL-subjects(p=10^-10). We then calculated genetic risk scores based on low-HDL-C-associating SNPs from these pathways,observing that high risk score resulted in both decreased HDL-C-levels and increased expression of these inflammatory pathways. Moreover, individual genes of these pathways (e.g. HLA-DRB1[p=10^-7],TAP2[p=10^-27]) exhibited cis-eQTLs, their expression inversely correlating with HDL-C.
Consistent with this,the inflammatory and potentially less vasoprotective nature of the HDL-particle of low-HDL-C-subjects was further highlighted by the elevation of proinflammatory ceramides and reduction of anti-oxidative plasmalogens in the lipidomic analysis, which was also GRS/genotype-dependent.
Interestingly,in a replication meta-analysis of 5 Finnish population-cohorts(n=11,211) we demonstrate that the genes found are indeed 'low-HDL-genes';the SNP-effects get stronger and more significant using more extreme low/high-HDL-C-criteria (e.g.HLA-DRB1; OR for low HDL-C<25%/high HDL-C>75%=1.11,OR(10%/90%)=1.38,OR(5%/95%)=1.77), but not observed for HDL-C as quantitative trait.
Here we demonstrate the following novel phenomena in cardiovascular genetics: extreme phenotype-approach may detect additional novel rare or common variants not detected in population-GWAS; and the major effect of HDL-C associated variants on cardiovascular phenotypes may in fact result from their association with impaired lipoprotein quality rather than quantity.

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P10.33
A fourth type of Autosomal Dominant Hypercholesterolemia characterized by an alteration of the LDL endosomal transport

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Autosomal Dominant Hypercholesterolemia (ADH) is characterized by high LDL levels, high risk of premature cardiovascular disease and is due to LDLR, APOB or PCSK9 mutations. Through the analysis of a large ADH family which excluded these three genes, we localized a fourth gene (HCHOLA4) at 16q22 (Marques-Pinheiro, Eur J Hum Genet 2010). Functional candidate genes in the locus were sequenced but no causal mutation was detected.
In parallel to the genetic approach, we searched for specific intermediate metabolic traits that could point out a metabolic pathway. Several analyses were performed for two members of the HCHOLA4 family. In vivo kinetic studies of apo B-100-containing lipoproteins showed a decreased LDL catabolism. At the cellular level in primary fibroblasts, the repression of the gene expression in response to a LDL overload was delayed in time for two sterol-dependant genes (HMGCoAR and LDLR), the LDL binding to their receptor is increased, indicating a likely increased receptor recycling. Immunohistochemistry analyses showed alterations of LDL endosomal transport, and larger early endosomes (EE) than in control cells. Labeled LDL-Dil, or labeled Shiga toxine B subunit, do not concentrate around the nucleus as seen in control cells, pointing out a delayed traffic between EE and Golgi. And, this was also observed for the intracellular distribution of the small GTPase Rab6a known to regulate the transport of vesicles into the Golgi compartment. Altogether, these observations indicate impaired endosomal sorting machinery. These results provide more insight into the alterations associated with mutations within the still unidentified HCHOLA4 gene.

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P10.34
LDLR and APOB gene analysis in the Slovene population of the children and adolescents with hypercholesterolemia

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Introduction: Hypercholesterolemia (HCH) is a major risk factor for atherosclerosis and its premature cardiovascular complications. HCH can be multifactorial or less frequently monogenic. Monogenic form leads to Autosomal Dominant Hypercholesterolemia (ADH) and is most frequently associated with mutations in the LDLR gene, while much rarer with mutations in the PCSK9 or APOB gene.
Objective: To analyze the LDLR and APOB gene in the Slovene population of the children and adolescents with the probable or definite HCH according to the clinical criteria.
Methods: We have used various molecular genetic methods (PCR, dHPLC, HRM and sequencing). We analyzed all 18 exons of the LDLR gene and most common missense mutation p.Arg3527Gln in APOB gene in 98 patients.
Results: 44 patients have had 21 known and 3 novel (p.Asp100Glu, c.1587-1G>C and c.1706-1G>C) causative mutations in the LDLR gene. 13 patients have had mutation p.Arg3527Gln in APOB gene.
Conclusions: In addition to the known pathogenic LDLR and APOB mutations, 3 novel LDLR gene mutations were identified. Remaining 41 patients without identified causative mutation will be subsequently analysed for PCSK9 mutations. The genetic characteristics of the Slovene HCH population were comparable as in neighbouring and/or related populations.

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P10.35

After LDLR, APOB and PCSK9, APOE is another major gene of autosomal dominant hypercholesterolemia

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Autosomal Dominant Hypercholesterolemia (ADH) is characterized by high LDL levels, high risk of premature cardiovascular disease and is due to LDLR, APOB or PCSK9 mutations. We found further genetic heterogeneity of ADH through a genomewide scan in a large French family with a new disease locus at 19q13.31-13.32. Whole exome and candidate genes sequencing showed an unique APOE mutation: p.Leu167del. This finding was unexpected since APOE mutants are essentially associated with familial combined hyperlipidemia (FCHL). APOE p.Leu167del was not detected in over 440 control chromosomes and two other APOE mutations (p.Arg235Trp, p.Arg269Gly) were found in three unrelated ADH probands.
In silico structural prediction of the mutant protein p.Leu167del showed an alpha-helix disruption within the receptor-binding domain that could affect apoE affinity for its receptors. In vivo apo B-100 kinetics from one APOE p.Leu167del carrier, showed: an increased LDL pool, which was the consequence of both an increased LDL production and a decreased LDL catabolism. These findings are similar to those from patients with a LDLR mutation. Together, these observations indicate a decreased catabolism of LDL-bearing the mutant apoE, explaining ADH, and underscoring the hypothesis that some APOE mutations may be responsible of ADH and should be looked for.
The APOE p.Leu167del was also reported in FCHL. Conversely, hypertriglyceridemia is sometimes observed with ADH, mainly because of the many genetic/environmental factors contributing to triglyceride elevation. Thus, mutations in LDLR or APOE may amplify the effects of these factors, and thus, could be associated with FCHL or ADH.

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P10.36

A new homozygous frame shift mutation of the leptin receptor-(LEPR)-gene identified by SNP array analysis is causing an early onset of a severe form of generalized obesity that is also associated with intellectual disability and general psychomotor retardation.

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Extreme obesity in children of less than a year of age is rare and in nearly all cases a genetic basis can be established. In the majority of these patients a mutation of the leptin gene can be found. Involvement of further genes like the melanocortin-4 receptor gene, the leptin receptor gene, and the Proopiomelanocortin gene (POMC) has recently been shown, however, additional genes might be of importance as well. In association studies and in particular in affected patients born to consanguineous parents SNP-array analysis can be helpful to identify candidate gene loci which could be of relevance. Homozygous or compound heterozygous mutations of the leptin receptor (LEPR) gene are infrequent but represent a very important cause of this disorder. Since sequencing of a number of these large genes is challenging and the probability that another new gene might be responsible the mentioned strategy was chosen. Here we describe a patient with a very severe obesity, and the use of SNP-array analysis to identify a homozygous frameshift mutation, c.461dupA[p.N154Kfs*3] in exon 5 of the LEPR-gene, leading to a loss of function of the membrane and leptin binding domains. Since this also the first report describing a severe form of intellectual disability associated with LEPR-gene mutations, this aspect is discussed as well.

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P10.37

Anorexia associated with a *de novo* 7q32 duplication spanning the *LEP* gene: characterization of the duplication using Whole Genome Sequencing, DNA combing and qRT-PCR of the duplicated genes

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Next generation sequencing technologies had revolutionized genomic research. While whole genome sequencing has been used several times to identify successfully physical breakpoints of apparently balanced *de novo* chromosome translocations, here it allowed us to characterize a *de novo* 7q32 duplication in an index-case with unexplained precocious anorexia and ponderal growth retardation. The whole-genome sequencing revealed that the duplication was a direct tandem duplication. The exact identified breakpoint suggested a gene fusion and was validated by Sanger sequencing. In addition the DNA combing approach was used to confirm the structure of the duplication.
The 5' part of the gene-fusion is unknown in current human gene databases. This potential pseudogene is paralogous to the *TLK2* gene. The 3' part of the gene-fusion includes the whole coding region of the *LEP* gene, coding for the well-known adipokine protein involved in the regulation of appetite. Several fusion transcripts by alternative splicing were detected by RT-PCR on patient's fibroblasts and lymphocytes.
In parallel, real-time quantitative RT-PCR revealed that the 7 genes included in the duplication were over-expressed in patient's fibroblasts compared with controls. Despite of the gene *LEP* over-expression in the patient's fibroblasts and lymphocytes, his leptinemia was normal.
We will present the exact nature of the gene fusion and the preliminary molecular characteristics of any fusion protein possibilities. Several hypotheses trying to explain the phenotype from the *de novo* duplication will be discussed, however these studies provide insight into the current ways to characterize a duplication.

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P10.38

Wolman Disease: the importance of early diagnosis. A review of 7 cases with complete lysosomal acid lipase deficiency.

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Background. Wolman disease (WD) is a recessively inherited disease (<60 cases since 1961) resulting from complete lysosomal acid lipase (LAL) deficiency (OMIM#278000). Although normal at birth following normal pregnancy, Wolman infants die by age 6-mo of multiorgan failure caused by generalized lysosomal neutral lipid storage. Despite secure biochemical and genetic diagnosis and potential for curative therapy, WD remains largely underdiagnosed.
Aim. Identify early clinical, imaging and biological signs from 7 WD cases.
Results. Parental consanguinity was present in 5 cases. All had <0.08N leucocyte LAL activity, resulting either from severe homozygous or compound heterozygous gene mutations (c.193C>T, c.419G>C, c.429-1G>C, c.482del, c.538G>A, c.676-2A>G, c.1024G>T, c.1055_1057del). In all cases, between 1 & 3 wks of life, alerting signs were persistent regurgitation/vomiting and abdominal pain, fast followed by hepato-splenomegaly, gut distension and

growth failure (<-1SD) by age 4 wks; low grade fever was unusual (2/7 cases). By age 8wks, bilateral adrenal calcification and mesenteric adenomegaly were constant on ultrasound or MRI imaging. Fasting TG and TC were moderate-high; liver cytolysis with anicteric cholestasis, very low HDLC (<0.5N), and inflammation (CRP>10N, Ferritin >5N) associated with foamy leucocytes were constant. Serum and stool bile acids and sterol precusors were high. Untreated cases died by age 16wks of malnutrition, liver and multi-organ failure.
Conclusion. Wolman disease a lethal condition of infancy may be underdiagnosed because early signs are unspecific after a 4wk lag phase. Abdominal imaging, serum liver, lipids and inflammation profiling combined with enzyme activity and genotyping may early identify infants amenable to curative therapy.

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P10.39
Expanding the mutation spectrum in beta-mannosidosis: Identification of two novel beta-mannosidosis associated sequence variants

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Beta-mannosidosis (OMIM #248510) is a rare lysosomal storage disorder caused by deficient activity of the enzyme beta-mannosidase (MANBA, E.C. 3.2.1.25). The disorder has been reported in goats, cattle and man. It is a severe autosomal recessive neurological disease in animals, but the human disorder is generally milder, and the clinical spectrum is heterogeneous. So far, only 21 cases and 15 disease associated sequence variants have been reported, indicating substantial genetic heterogeneity in beta-mannosidosis. By sequence analysis of genomic DNA from an African patient with demyelinating peripheral neuropathy, but without apparent mental retardation (Graber *et al.* 1994 *Ann Neurol* 35:116-119), heterozygosity for a 2-bp deletion in exon 17 in the *MANBA* gene was the only obvious disease associated variant detected. However, RNA analysis revealed a heterozygous frameshifting deletion of exon 15 from the transcript. A mutation located outside the splice site sequences in exon 15, and originally believed to be silent, was shown by use of hybrid splicing reporter minigenes to cause aberrant splicing. Bioinformatics analyses predicted that the mutation weakens a potential binding site for splicing stimulatory protein SRSF1, and our preliminary results from RNA affinity binding studies corroborates this. Studies are in progress to evaluate this further. The present study provides a new and interesting example on how translationally silent substitutions can be deleterious by disrupting the finely tuned balance between splicing regulatory elements in constitutive exons.

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P10.40
Megdel syndrome in two Turkish siblings: a novel SERAC1 mutation

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MEGDEL syndrome was described as a distinct entity with association of 3-methylglutaconic aciduria with impaired oxidative phosphorylation, deafness, encephalopathy, Leigh-like lesions on brain imaging, progressive spasticity and dystonia. It is an autosomal recessive disorder and, recently, it has been reported that SERAC1 (Serine Active Site Containing 1) gene mutations cause this syndrome.
Here we present two siblings with 3-methylglutaconic acid and 3-methylglutaric aciduria, microcephaly, growth retardation, dysmorphic features, severe sensorineural deafness, progressive spasticity, dystonia, seizures, basal ganglia involvement and degeneration consistent with leigh-like syndrome.
Other types of methylglutaconic acidurias were excluded by enzymatic analysis and clinical findings. Since the clinical pictures of our affected siblings are very comptible with reported patients with MEGDEL syndrome, SERAC1 gene was selected as a candidate gene for mutation screening. Sequencing analysis of SERAC1 gene in patients showed novel homozygous c.799_800delC (p.Pro267Leu fs*10) mutation in exon 9.
It is the first report of MEGDEL syndrome due to SERAC1 gene mutation after the report of Wortmann et.al. (2012) that describes a novel mutation ,its molecular effects and clinical correlation in the literature.
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P10.41
Reduction of mutated mitochondrial DNA in MERRF cells by Peptide Nucleic Acids modified twice

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The intracellular number of mutated mitochondrial DNA (mtDNA) can increase during life resulting in cellular dysfunctions, e.g. in patients with the mitochondriopathy MERRF. We developed a method using peptide nucleic acids (PNA) modified twice to inhibit the replication of mutated mtDNA specifically. For this purpose, PNAs targeting the coding strand were modified C-terminal with a cell penetrating peptide and N-terminal with a mitochondrial targeting peptide. Transfection of a MERRF cybrid cell line with these PNAs results in a highly specific and efficient inhibition of replication of mutated mtDNA. The inhibition of replication strongly depends on the length of the PNAs, their concentration and the duration of treatment. The dramatic reduction of mutated mtDNA after 4 PNA treatments persists for at least 60 additional days in the MERRF cell line. We suggest that the used PNA modifications and the choice of the targeted strand are crucial for the successful inhibition of mtDNA replication by PNAs.

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P10.42
Genomic determinants of metabolic syndrome features in the rat recombinant inbred strain panel PXO

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Background: Metabolic syndrome is a prevalent disease characterized by concurrent manifestation of 3 symptoms (elevated waist circumference, triglycerides, reduced HDL cholesterol, hypertension, elevated fasting glucose). We preformed genome-wide association and linkage study of major metabolic syndrome components in recombinant inbred strain panel PXO.
Methods: Adult male rats of 14 PXO strains and two progenitor strains SHR-Lx a BXH2 (n=183) were subjected to one-week of high-sucrose diet feeding. We established morphometric and metabolic profile of the whole PXO panel including glucose tolerance and triacylglycerol (TG) and cholesterol (C) concentrations in 20 lipoprotein fractions were determined. The association and linkage analyses utilizing > 20,000 SNPs were performed using MapManager, the significance validated by 2000 permutations per trait.
Results: In most of the phenotypes we identified substantial gradient among the strains (e.g. area under the glycemic curve from 237±25 to 606±35 mmol/l/180 min). Using interval mapping, we have identified 14 loci showing suggestive or significant linkage to studied traits. Except for linkage signals of LDL-TG on chromosomes 3 and 12, PXO strains carrying the SHR allele displayed significantly higher values of the lipid linked traits, e.g. LDL-C (21.2±0.4 vs. 12.5±0.4 mg/dl in PXO strains with SHR allele vs. BXH2 allele in D3Rat50-D3Got19 block). C concentrations in large, medium and very small LDL particles were significantly associated to a single gene (Lrp1b).
Conclusion: Using genome-wide linkage and association we have identified new genetic determinants of glucose tolerance, TG and C distribution into lipoprotein fractions.
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P10.43

Molecular diagnosis of mitochondrial diseases in Egyptian pediatric patients : A two year study

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Mitochondrial diseases (MCDs) are an important cause of morbidity and mortality in both adults and children. MCDs are caused by mutations in both nuclear and mitochondrial encoded genes. This report is the first one to describe the screening and diagnostic molecular service for MCDs routinely started at the Inherited Metabolic Disease Unit laboratory at Cairo University Children's hospital from January 2011.

DNA was extracted from whole blood samples of 62 Egyptian Pediatric patients using standard protocol. Eight patients were clinically and radiologically suspected to have Myoclonus epilepsy and ragged-red fibers (MERRF), 17 patients with mitochondrial encephalomyopathy , lactic acidosis and stroke-like episodes (MELAS), 31 patients with and Leigh's syndrome/Neuropathy Ataxia Retinitis Pigmentosa syndromes (LS/NARP), 3 with Leber's hereditary optic neuropathy (LHON), 2 patients with Mitochondrial Neurogastrointestinal Encephalopathy (MNGIE) and one with chronic progressive external ophthalmoplegia (CPEO).

Common mtDNA point mutations were screened using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism and confirmed by direct sequencing. Sequencing analysis of the nuclear Thymidine hosphorylase (TP) gene was done in MNGIE patients.

The A3243G mutation was detected in one patient with MELAS and the T14484C was detected in two patients with LHON. The c.3371A>C and c.4183G>A in TP gene were detected in MNGIE patients.

As Egypt is a country with high rate of consanguineous marriage, the molecular pathogenesis of MCDs is suspected to be of nuclear genetic origin. Mitoexome and whole exome sequencing represents an appealing approach for detection of disease causing variants.

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P10.44

Interest of exome sequencing in prenatal diagnosis of mitochondrial disorders.

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Mitochondrial dysfunction is a major cause of metabolic disorders and accounts for a large variety of clinical symptoms in both childhood and adulthood. Genetic diagnosis is complex and challenging because of the involvement of mitochondrial or nuclear DNA and of primary or secondary nature of respiratory chain (RC) dysfunction. While mutations in nuclear genes underlie the vast majority of RC deficiencies, in most patients, genes and molecular mechanisms responsible remain unknown. Here, we report three consanguineous families with two affected siblings born from healthy parents suggesting an autosomal recessive transmission. Affected children presented with severe symptoms from the mitochondrial disease spectrum, beginning before 2 months of age, and associated to a RC deficiency. No mutation was found in mtDNA nor in known nuclear genes possibly involved regarding phenotypes and affected RC complexes. By using Whole Exome Sequencing (WES), we identify causative genes in all cases with mutations in *SERAC1*, *COQ2* and *SIAT9* encoding the GM3 synthase (GM3S). In the last family, we showed that GM3S deficiency was responsible for a secondary RC defect with a decrease of mitochondrial membrane potential leading to apoptosis in patients' fibroblasts. Moreover, our findings allow direct benefit for parents since prenatal diagnosis was performed in the 3 families and mutation analysis showed the non affected status of the fetus. In conclusion, our work highlights the importance of WES in prenatal diagnosis of mitochondrial disorders.

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P10.45

Prevalence of rare mitochondrial DNA mutations in mitochondrial disorders

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Background. Mitochondrial DNA (mtDNA) diseases are rare disorders whose prevalence is estimated around 1/5000. Patients are usually tested only for deletions and for common mutations of mtDNA which account for 5-40% of cases depending on the studies. However, the prevalence of rare mtDNA mutations is totally unknown.

Methods. We analysed the whole mtDNA in a cohort of 743 patients, suspected of manifesting a mitochondrial disease, after excluding of deletions and common mutations. Both heteroplasmic and homoplasmic variants were identified using two complementary strategies (Surveyor and MitoChip). Multiple correspondence analyses followed by hierarchical ascendant cluster process were used to explore relationships between clinical spectrum, age at onset and localisation of mutations.

Results. A total of 7.4% of deleterious mutations and 22.4% of novel putative mutations were identified. Pathogenic heteroplasmic mutations were more frequent than homoplasmic mutations (4.6% *versus* 2.8%). Patients carrying deleterious mutations showed symptoms before 16 years of age in 67% of cases. Early-onset disease (<1 year) was significantly associated with mutations in protein-coding genes (mainly in complex I) while late-onset disorder (>16 years) was associated with mutations in tRNA genes. *MTND5* and *MTND6* genes were identified as "hotspots" of mutations, with Leigh syndrome accounting for the large majority of associated phenotypes.

Conclusions. Rare mitochondrial DNA mutations probably account for more than 7.4% of patients with respiratory chain deficiency. This study shows that a comprehensive analysis of mtDNA is essential, and should include young children, for an accurate diagnosis that becomes now accessible with the development of Next-Generation Sequencing technology.

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P10.46

The effect of citrulline and arginine supplementation on lactic acidemia in MELAS syndrome

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Background: Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome is a common mitochondrial disorder in which nitric oxide (NO) deficiency can play major roles in the pathogenesis of several complications including stroke-like episodes, myopathy, and lactic acidosis. Arginine and citrulline act as NO precursors and their administration can restore NO production in MELAS. Lactic acidemia in MELAS results from an inability of dysfunctional mitochondria to generate sufficient ATP, leading to shunting of pyruvate to lactate. Hypoperfusion may result in lactic acidosis. Therefore, NO deficiency in MELAS can result in decreased blood perfusion and therefore aggravates lactic acidosis.

Methods: We measured plasma lactate concentrations in 10 adults with MELAS before and after 48-hour supplementation of oral L-arginine at a dose of 10 g/m^2 body surface area/day. The study was subsequently repeated before and after L-citrulline.
Results: The average plasma lactate concentration was lower after arginine (3.16→2.99 mmol/L) and citrulline (3.17→2.94 mmol/L) supplementations. This reduction was statistically significant after citrulline ($p<0.05$), but not after arginine.
Conclusions: The reduction in lactate after arginine and citrulline supplementations add more evidence to their potential therapeutic utility in MELAS. Previous study showed that both arginine and citrulline supplementations increase NO production in MELAS with citrulline resulting in higher increment. In this study the lactate reduction was more significant after citrulline supplementation which can be due to the superiority of citrulline in increasing NO leading to a better perfusion and lower lactate levels. These results also suggest that citrulline can have a better therapeutic effect.

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P10.47
The RedMIT mouse

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Mitochondrial diseases are common and symptoms range from isolated presbyacusis, to loss of vision, to catastrophic neurodegeneration. Treatments are ineffective but mitophagy, mitochondrial recycling which is important in mitochondrial quality control, has been suggested in studies to have a role in neurodegeneration. We have generated a transgenic mouse strain (RedMIT mouse) expressing the dsRed-monomer targeted to mitochondria to follow mitochondrial turnover.
We confirmed that dsRed is targeted to mitochondria in tissues section and in MEFs from this mouse. We used Imagestream (Amnis) to investigate the co-localisation of mitochondria and the autophagic marker LC3; an increase in co-localisation with a chloroquine treatment validated the system.
We identified two populations of mice with either low or high proportion of blood cells expressing dsRed. Interestingly the low expressing population shows the most LC3 labelling. Similarly MEFs with more LC3 have less dsRed. These observations suggest that dsRed expressing mitochondria could be removed by mitophagy and thus that dsRed might impair the mitochondrial functions.
Oxygen consumption measurements of RedMIT tissues and MEFs showed a slightly affected function compared to wild-type. A lower mitochondrial potential in RedMIT MEFs than wild-type MEFs is also found.
Finally we monitored the blood level of dsRed in mice and showed a drop with time. This latter result recapitulates the age-dependent fall in level of 3243 mutant mtDNA in mitochondrial disease patients.
In conclusion we have a mouse model with dsRed targeted to mitochondria that mildly impairs mitochondrial functions. This RedMIT mouse may usefully recapitulate features of heteroplasmic mtDNA disease.

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P10.48
Identification of the first nonsense mutation in the *PDX1* gene in a family with diabetes mellitus

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Maturity onset Diabetes of the Young type 4 (MODY4) is a monogenic form of diabetes mellitus caused by mutations in the *PDX1* homeobox gene. *PDX1* encodes a pancreatic beta-cell specific transcription factor essential for the embryonic development of the pancreas.
The index patient presented with a gestational diabetes at the age of 28 years (1-hour-OGTT 192mg/dl). No insulin therapy was necessary. The pregnancy ended at a spontaneous full term delivery (birth weight 3530g). Three months after the pregnancy the patient showed impaired glucose tolerance (1-hour-OGTT 148 mg/dl) but no other symptoms of diabetes. Her habitus is slender (BMI 19). Currently, she shows normal blood sugar levels (fasting glucose 98 mg/dl) and slightly elevated C-peptide level (1,42 nmol/l).
Direct sequencing of the *PDX1* gene revealed the heterozygous mutation c.204C>G resulting in a preliminary stop codon (p.Tyr68*). Sequencing and MLPA analysis of *HNF1A*, *GCK*, *HNF4A*, and *HNF1B* revealed no pathogenic mutations.
We analysed the maternal family of the index patient and detected the mutation in her mother (suffering from non-insulin dependent diabetes mellitus) and aunt (showing elevated blood sugar levels but no diabetes). In her grandmother with type 2 diabetes requiring insulin therapy the mutation was not detected. The diseased grandfather, known to have diabetes, is thus an obligate carrier of the mutation.
Until the year 2013, altogether 13 mutations in the *PDX1* gene have been described. Here we describe the first nonsense mutation in the *PDX1* gene. In a heterozygous state, this mutation is associated with a mild form of MODY.

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P10.49
HNF1A gene analysis in the MODY suspected Turkish patients

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Maturity-onset diabetes of the young (MODY) is an early-onset non autoimmune form of diabetes with an autosomal-dominant mode of inheritance. MODY has at least 10 subtypes associated with distinct genes. MODY3 associated with hepatocyte nuclear factor- 1 alpha (HNF1A) gene mutations is the most common type of MODY. It accounts for 12-65% of all MODY cases in Europe. MODY3 is dignosed during adolescence or early adulthood. MODY3 requires pharmocological treatment and late-onset microvascular complications are frequently observed in the patients. More than 300 different mutations were identified in the MODY3 patients. Functional studies revealed that they cause insulin deficiency by the loss of function or by the dominant negative effect. The purpose of the study is to determine the genetic profile of MODY3 in the Turkish patient population by analyzing HNF1A gene. To identify HNF1A gene mutations HNF1A gene exonic sequences, exon-intron boundaries, the promoter sequence was analyzed by direct DNA sequencing in 35 MODY suspected patients. One novel (p.Ser345Tyr) and two previously known (p.Thy10Met, p.Arg263Cys) mutations were identified. In addition 6 of the patients were heterozygous for p.Ile27Leu polymorphism that was associated with insulin resistance. Besides,a common haplotype (p.459Leu, p.487Asn, and IVS7+7G>A polymorphisms) was identified in 38 percent of the patients. Case control association study revealed that it was not assssociated with MODY3.In conclusion, this study revealed that the frequency of MODY3 in the Turkish MODY suspected patients is 8.5 percent. Future studies will reveal whether there are other genes responsible for the MODY phenotype in the Turkish population.

O. Yalcin Capan: None. **M. Sargin:** None. **E. Berber:** None.

P10.50
Genetic heterogeneity of patients with Familial Young-Onset Diabetes: data from the Slovak nation-wide survey in years 2003-2012

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INTRODUCTION: Maturity Onset Diabetes of the Young (MODY) is a heterogeneous group of monogenic diabetes with early onset, familiar appearance, and autosomal dominant inheritance. Familial Young-Onset Diabetes (FYOD) represents a part of MODY caused predominantly by mutations in the genes for transcription factors.
THE AIM of this study was to identify the etiology of the Slovakian families with the clinical suspicion on the Familial Young-Onset Diabetes by the DNA analysis of the known MODY genes.
METHODS: 399 patients from 184 families were recruited from outpatient clinics throughout Slovakia. Relevant genes responsible for FYOD (HNF1A, HNF1B, HNF4A, NEUROD1, KCNJ11, insulin, ABCC8) were sequenced and MLPA analyzed.
RESULTS: 66 patients from 28 families had a mutation in one of the target genes: 24 probands and their 37 family relatives had a mutation in HNF1A gene; one family (2 pts) had a mutation in the HNF4A gene, one proband had a mutation in insulin gene, one proband had a HNF1B whole gene deletion

and one proband had a novel mutation in ABCC8 gene. No KCNJ11 and NEUROD1, gene mutation carriers were found. In 44 families no mutations in the analyzed genes were found (MODY-X).
CONCLUSIONS: Among 399 from 184 families with clinical suspicion on FYOD the monogenic diabetes was confirmed in 66 patients from 28 families. 20 different mutations (from which 8 are novel) were found in 4 out of 6 analyzed genes. These results indicate a high heterogeneity of etiology of the Familial Young-Onset Diabetes in Slovakia.
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P10.51
Mutational spectrum of glucokinase (GCK) gene and prevalence of monogenic diabetes type GCK-MODY in Slovakia

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Heterozygous inactivating glucokinase (GCK) mutations cause a subtype of maturity-onset diabetes of the young (GCK-MODY) characterised by familial mild stable fasting hyperglycaemia. Over 600 GCK mutations have been reported, nevertheless none of them prevails between GCK-carriers. The aim of the study was 1) to determine the GCK mutation spectrum and 2) to disclose the prevalence of GCK-carriers among the Slovakian population.
Patients and methods: 369 unrelated probands with clinical suspicion on MODY were referred for genetic testing to our laboratory between 2004 - 2012 from >100 clinical diabetologists and endocrinologists across Slovakia. Following the proband's clinical phenotype consistent with GCK-MODY, direct sequencing and MLPA analyses of pancreatic GCK gene were carried out.
Results: DNA analyses identified 31 different GCK mutations in 55 probands and 90 relatives located in promoter, coding and intronic regions. Missense mutations occurred most frequently - 77% (24/31), followed by deletions 10% (3/31), splice-site substitution 6% (2/31), promoter substitution 3% (1/31) and intronic variant 3% (1/31); no exon deletion was detected. Different exon mutations were scattered along the entire GCK length, no hotspot region was found. The most frequent GCK mutation in Slovakia (promoter -71G>C) occurred in 27% (15/55) of unrelated families. In Slovakia with population of 5,4 millions the minimum prevalence of GCK-MODY was estimated to 27 cases/million inhabitants.
Conclusion: We have determined the GCK mutation profile with the Slovakian prevailing mutation (-71G>C). The minimum prevalence of GCK-MODY in Slovakia is 27 cases/million inhabitants, which is higher than our previously published data.
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P10.52
A comparative transcriptome analysis identifies FGF23-regulated genes in HEK293 cells stably expressing KLOTHO

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Phosphate is the most abundant anion in the human body and is crucial for various biological functions like cellular activity and bone mineralization. A key regulator of phosphate homeostasis is fibroblast growth factor 23 (FGF23). It is mainly secreted from osteocytes, circulates in the blood and binds to receptor heterodimers composed of FGF receptor 1 (FGFR1) and KLOTHO in the kidney. As elevated FGF23 levels are the main cause of hypophosphatemia in monogenic disorders of phosphate homeostasis (XLH (MIM 307800), ADHR (MIM 193100), ARHR1 (MIM 241520) and ARHR2 (MIM613312)), further studies on the regulation of phosphate metabolism are necessary to identify possible therapeutic targets. FGF23 activates KLOTHO/FGFR1 to inhibit renal phosphate reabsorption and to suppress 1,25-dihydroxyvitamin D3 synthesis. Little is known about FGF23/KLOTHO/FGFR1 signalling and downstream targets of FGF23 contributing to its phosphaturic action. For this purpose, we established an in vitro cell system of FGF23-inducible HEK293 cells that stably express KLOTHO (HEK293-KL). To find early expressed FGF23-induced transcripts, we performed whole transcriptome analysis. We used the technology of RNA-Seq, which is a massively parallel sequencing approach to allow genome-wide analysis of gene expression profiles at a far higher resolution than is available with microarray-based methods. Genome-wide transcriptional changes in HEK293-KL cells specifically caused by FGF23 were defined by comparing the transcriptome of FGF23-induced HEK293-KL cells with the transcriptomes of not induced HEK293-KL cells and mock-treated HEK293 cells. We tried to identify new FGF23-responsive genes that might belong to a network of factors involved in the regulation of phosphate homeostasis.

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P10.53
Clinical and Genetic Spectrum of Mucopolysaccharidosis Type VI (Maroteaux-Lamy Syndrome) at Eastern Province of Saudi Arabia

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Mucopolysaccharidodis (MPS) type VI or Maroteaux-Lamy Syndrome (MIM#253200) is an autosomal recessive lysosomal storage disease caused by deficiency of the enzyme N-acetylgalatcosamin-4-sulfatase or Arylsuulfate B. This will lead to a progressive intracellular accumulation of the glyacsosaminoglycans, dermatan sulfate resulting into a wide range of multi-systemic dysfunction. It is a clinically heterogeneous disorder ranging from a mild to moderate and severe phenotype. So far, more than 130 different mutations in the ARSB gene have been identified. However, most are unique to individual families making population screening is difficult. Here we report 16 Saudi Arab affected patients from 6 unrelated families from the Eastern Province of Saudi Arabia presented with a severe phenotype. All of the tested patients were found to be homozygous the nonsense mutation c.753C>G (p.Y251X) at ARSB gene. This is a good reflection of the high consanguinity rate among this population. We propose that this mutation could be utilized for screening individuals at risk within this region of the country.

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P10.54
Prenatal diagnosis of Mucopolysaccharidosis by two-dimentional electrophoresis of amniotic fluid glycosaminoglycans: An Egyptian experience.

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Abstract: Objective: Prenatal diagnosis of Mucopolysaccharidosis(MPS) in pregnancies at risk by means of two-dimensional electrophoresis for analysis of glycosaminoglycans(GAGs) in amniotic fluid. Subjects and methods: This study included 53 pregnant females having previously affected child or more with one of the MPS types. Those cases came from allover Egypt during the period from 2000 till 2012. Consanguineous marriages was present in 39 cases (73.6%) and gestational age at time of amniocentesis ranged between 15-19 weeks. All the pregnant females were subjected to history taking, pedigree construction, clinical examination, amniocentesis guided by ultrasound scan. Two-dimensional electrophoretic separation of GAGs was done in amniotic fluid of all cases.
Results: 18 cases (34%) out of the 53 pregnant females were affected. Eight cases showed electrophoretic pattern of MPS type-I with dermatan and heparin sulfate spots, 4 cases MPS type-II with small dermatan and heparan spots, one case showed pattern of MPS type-III with heparan and heparan sulfate spots and 5 cases showed pattern of MPS type-VI with big dermatan spots.
Conclusion: Prenatal diagnosis of MPS for women with previously affected child or more using two-dimensional electrophoretic separation of GAGS in amniotic fluid is a sensitive, rapid and reliable method for diagnosis. It helps those families to have healthy infants and prevent having another affected child in the family.

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P10.55

Association of mitochondrial respiratory chain polymorphisms with obesity and type 2 Diabetes in the spanish population

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OBJECTIVE: To study the association between genes codifying for mitochondrial respiratory chain (MRC) subunits and BMI and obesity as well as the impact on the risk to develop type 2 diabetes in the general population from Spain.

RESEARCH DESIGN AND METHODS: Three thousand seven hundred and thirty-one subjects (age range 21 to 89) from three different population-based studies of Spain, were studied. Forty-eight single nucleotide polymorphisms (SNPs) of chromosomal genes which codify MRC proteins were selected and processed by the SNPlex method.

RESULTS: Significant associations were observed between polymorphisms rs4600063 (SDHC gene), rs11205591 (NDUFS5 gene), rs10891319 (SDHD gene) and BMI (p-value= 0.04, 0.0011 and 0.0004, respectively) and obesity risk (OR=0.72, p-value=0.0072; OR=0.72, p=0.039; OR=1.25, 0.0038, respectively). In addition, polymorphisms rs11205591 and rs10891319, showed a significant epistatic interaction for BMI levels and obesity risk. Finally, the GG genotype of rs11205591 polymorphism significantly reduced the risk of being diabetic after including age and sex as covariables (OR= 0.32, 0.17-0.62; p-value =0.0001), and BMI (OR=0.37, 0.19-0.72; p-value =0.0008).

CONCLUSIONS: Polymorphisms of genes codifying MRC can be involved in BMI variation and can be related to the risk for being obese in the Spanish general population. Furthermore, the rs11205591 (NDUFS5 gene) polymorphism might contribute to the risk to develop type 2 diabetes.

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P10.56

Dysmorphic features in Niemann-Pick type C disease: Blurring the frontiers between dysmorphology and biochemical genetics

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Background: Niemann-Pick type C disease (NP-C) is a rare lysosomal disorder whose presentation in early infancy is usually nonspecific and may be overlooked, specially because at this age, the classical neurological features of the disease - as the vertical supranuclear gaze palsy - are absent. Facing this challenge, it is important to develop tools and to recognize clinical clues that can lead to an early diagnosis. Here we report, for the first time, a cohort of NPC patients whose facial features point out to a similar "facial gestalt" that could help both the non-specialist clinician and the clinical geneticist to a early recognition of the disease or at least to raise a clinical suspicion. Discussion: In an apparent opposition to other lysosomal storge disorders (LSDs), NPC was generally considered a non-dysmorphic inborn error of metabolism, nevertheless there are some facial features that can make possible to recognize a facial phenotype in infants with NPC. This fact is not surprising since dysmorphic manifestations can be expected as a result of disorders which affect the synthesis, traffic or early steps in the degradation of the large molecules. Conclusion: Identification of some dysmorphic features can add one more 'layer' in the investigation of NPC in early infancy, although that there is still a need to further development of screening tools in a effort for a better and earlier recognition and management of the disease.

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P10.57

Mutation Screening in NPC1 and NPC2 Genes in 37 Turkish Patients Suspected with Niemann-Pick disease Type C: Six Novel Mutations

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Niemann-Pick disease Type C (NPC) is a rare autosomal recessive lysosomal lipid storage disorder with probable minimal incidence of 1/120,000 live births. Clinical presentation of NPC is extremely heterogeneous, featuring a range of systemic and neurological signs that are not specific for the disease progression and prognosis. As a result, the diagnosis of NPC can be a prolonged and complicated. NPC is caused by mutations in the NPC1 (in 95% of cases) or in the NPC2 (in around 5% of cases) genes. To date more than 300 disease-causing mutations have been reported.

In this study, mutation screening of 25 exons in NPC1 gene and 5 exons of NPC2 was performed in 37 unrelated Turkish patients suspected with NPC. Twelve different mutations in NPC1 and NPC2 gene were detected in 13 of 37 patients. In regard to novel mutations in NPC1 gene, c.2229_2230delTG, p.Trp949Gly, p.Asn906Tyr were detected as homozygously and c.3246-2delAG¬¬_¬3246-3247delTG mutation was detected with p.Pro1007Ala mutation in compound heterozygously. In addition, two novel mutations p.Ala181Thr and p.Thr375Pro were detected in patients as heterozygously in NPC1 gene.

NP-C diagnosis in these individuals were confirmed by this molecular genetic analysis study. The mutation detection rate which resulted in confirmed molecular genetic diagnosis, is found 25% in this selected group of patients. This study would be helpful for to raise the awareness and improve the early diagnosis and therapy of the disease in Turkish patients.

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P10.58

Characterization of the human ornithine carbamoyltransferase promoter

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Full transcriptional activity of human ornithine carbamoyltransferase (OTC, EC 2.1.3.3; MIM#300461) promoter depends on an upstream enhancer. The promoter-enhancer interaction contributes to tissue specific expression of OTC in the liver. The aim of our study is a detailed characterization of basic OTC regulatory elements in human.

Using the luciferase reporter assay with constructs containing a series of truncated promoter variants, we specified the 173 bp core promoter region carrying three positive and a negative *cis*-acting element. The *Cis*1+ positive regulatory element reaching 80% of the promoter activity was found in a short sequence of 35 base-pairs located close to the transcription start site. Another regulatory elements *Cis*2+, *Cis*3- and *Cis*4+ were localized within the positions 128-179 bp, 180-219 bp and 220-269 bp upstream the initiation codon.

We set up an innovated DNase I footprinting method based on the capillary electrophoresis of fluorescently labeled fragments after DNase I cleavage. The comparative analysis of electrophoreograms revealed protected regions corresponding to *Cis*1+ and *Cis*2+ elements.

Introducing the electromobility shift assay we demonstrated DNA-protein interaction in all investigated *cis*-acting elements. Two of them, *Cis*1+ and *Cis*2+, contained short sequences recognized by HNF-4, a transcription factor playing an essential role in the *OTC* gene regulation in rodents. A binding site for COUP-TF, an antagonist to HNF-4 in rat was found in the *Cis*3- region. The nature of the *Cis*4+ element remains unknown; however it contains a conserved TATA-like domain and could serve as a binding site to the TBP transcription factor.

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P10.59

Fatal Outcome of Iron-Sulfur Cluster Scaffold *NFU1* Gene mutations in a Neonatal Patient.

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Mitochondrial diseases are a heterogeneous group of disorders characterized by a variety of clinical symptoms and a defect of the OXPHOS system, but only part of the genes involved in these pathways have been identified. The NFU1 protein is implicated in the iron-sulfur (Fe-S) cluster biogenesis. The latter are conserved protein co-factors with an essential role in a wide variety of enzymatic processes, including the e-transfer of complexes I to III of the respiratory chain. Recently, mutations in the *NFU1* and *BOLA3* genes were identified in patients with a multiple mitochondrial dysfunction syndrome.

Here we report on a patient with lactic acidosis, tubulopathy, failure to thrive and cardiac hypertrophy who died at 3 months of age. He is compound heterozygous for the common c.622G>T (p.Gly208Cys) missense mutation and a novel c. 62G>C (p.Arg21Pro) mutation changing a completely conserved arginine residue into a proline. Both parents are heterozygous carriers. His metabolic profile was largely characterised by an enzymatic complex II defect in lymphocytes, fibroblasts, muscle and liver tissue. Preliminary observations by BN-PAGE analysis concord with the conclusions of Ferrer-Cortès et al. 2012. They also observed a profound complex II deficiency as the main molecular signature of a pNFU1 malfunction and patients surviving the first months of life as compared to patients with an *NFU1* null allele. Today, only a small number of patients with Fe-S cluster protein defects, most of them compound heterozygous for the p.Gly208Cys mutation in the NFU1 gene, have been identified.

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P10.60

Association between dyslipidaemia and mutations in the genes *PCSK9, LDLR*

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Introduction: Coronary heart disease (CHD) is the main cause of death in developed countries. One of the main causes of CHD is atherosclerosis, in which pathogenesis dyslipidemia is a crucial factor. There are several genes involved in cholesterol metabolism, e.g. *PCSK9* and *LDLR*. Mutations and polymorphisms in these genes can be associated with hyper- or hypocholesterolemia, and can cause Familial hypercholesterolemia.
Aim: To determine the association of the polymorphism E670G in the gene *PCSK9* with dyslipidemia and to test patients with dyslipidemia for seven mutations in the gene *LDLR*.
Material and methods: The study population included 38 patients with dyslipidemia and 66 healthy individuals served as a control population.
The E670G polymorphism was detected by RFLP analysis, but the mutations: D374Y, P664L, L458P, R329X, E207X, D200G, E80K in the gene *LDLR* were detected by ELUCIGENE FH20 (*Tepnel Molecular Diagnostics*).
Results: Polymorphism E670G - the G allele's frequency in affected individuals was 0.3947, in control population - 0.1339 ($p=3.9x10^{-5}$, OR 4.217, CI95% 2.069-8.587). After linear regression analysis there was not found significant association ($p>0.05$) with any of biochemical markers. Mutation E80K in the gene LDLR was found in two individuals with dyslipidemia.
Conclusions
1) Polymorphism E670G in the gene PCSK9 is one of the genetic risk factors in development of dyslipidemia, but it is not associated with specific biochemical markers;
2) Mutation E80K in the *LDLR* gene suggests that two patients have Familial hypercholesterolemia, but to make precise diagnosis, the mutations should be confirmed by other molecular method.

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P10.61

Transcription factor gene *MNX1* is a novel cause of permanent neonatal diabetes in a consanguineous family

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Background: Permanent neonatal diabetes mellitus (PNDM) is a rare monogenic form of non-autoimmune diabetes. Genetic defects have been identified in ~60% of cases, with mutations in *ABCC8*, *KCNJ11* and *INS* being the most frequent causes of PNDM. Recognition of genetic subtypes strongly impacts on both patients care and family counselling.
Objective: To identify the genetic aetiology of PNDM in a diabetic child born from consanguineous parents of Egyptian origin.
Methods: DNA samples from both the proband and non-diabetic parents were analysed for homozygosity mapping using the Illumina 660K-SNP chip. We then focused on the runs of homozygosity (ROHs) over 2.5 Mb, which were detected only in the patient. Sanger sequencing of candidate genes (*MNX1* and *GATA6*) present in those ROHs was subsequently performed, as well as expression analyses on human embryonic and adult pancreatic islet samples.
Results: We identified a putatively causal homozygous mutation in the transcription factor gene *MNX1* (c.816C>A/p.Phe272Leu) in the PNDM patient, who was clinically diagnosed as a typical case of PNDM without any pancreas developmental defects or other clinical features. The probably deleterious mutation is located within MNX1 homeodomain helix 2 that is highly conserved between species. In human embryonic pancreatic islet samples, we showed that *MNX1* expression was significantly enriched in pancreatic epithelium compared to mesenchyme, suggesting a role of MNX1 in human pancreatic beta-cell development.
Conclusion: We report a new putative cause of PNDM from a consanguineous family. Replication in other cohorts would be helpful to draw the clinical spectrum of *MNX1*-mutated PNDM patients.

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P10.62

LC-MS/MS Analysis of Peroxisomal Biogenesis Disorder (PBD) Patients' Plasma Bile Acids (BA) Implicate Cytosolic BA-CoA:amino acid N-acyltranferase (BAAT) Activity in BA Reconjugation and Homoeostasis

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Bile acids (BA) are required for lipid and vitamin absorption, and cholesterol elimination among other functions. BA synthesis originates in the liver, and requires thirteen enzymatic steps, including cholesterol nucleus modification and side-chain oxidation. Glycine- and taurine-conjugation via BAAT is the last step before BA excretion into bile. Intestinal bacteria deconjugate BAs, but 95% of the BA pool is reabsorbed within enterohepatic circulation. BAAT is mostly peroxisomal, implying that deconjugated BAs must circulate to peroxisomes for reactivation and reconjugation via a currently unknown mechanism. As investigations with PBD patients are indispensible for the elucidation of the peroxisome's role in BA synthesis, we used an LC-MS/MS method to measure 18 conjugated and unconjugated plasma BAs (all primary, secondary, glycine-, and taurine-conjugates) alongside BA precursors, dihydroxycholestanoate (DHCA) and tetrahydroxycholestanoate (THCA), in PBD patients (n = 52) and controls (n = 279). Whereas PBD plasma contained elevated BA precursors, DHCA and THCA (median: 379x and 19x > controls, respectively), indicating BA synthesis impairment, taurine-conjugated primary BAs (taurocholate and taurochenodeoxycholate) were also markedly elevated (median: 136x and 26x > controls, respectively). In addition, the percentage of glycine-conjugated BAs in PBD specimens was half that of controls (42% vs. 89%, respectively), suggesting preferential taurine-reconjugation of BA pools. In summary, the analysis of BA levels in PBDs demonstrates the existence of BAAT activity without functional peroxisomes. Moreover, the reduction of the proportion of glycine-conjugated BAs suggests a cytosolic localization of BAAT activity in PBD hepatocytes and questions the functional purpose of peroxisomal BAAT in BA reconjugation.

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P10.63

Biallelic mutations in the mitochondrial caseinolytic peptidase, CLPP, cause Perrault syndrome, an inherited disorder of deafness and ovarian failure

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Perrault syndrome is a genetically and clinically heterogeneous autosomal recessive condition characterized by sensorineural hearing loss and premature ovarian failure. By a combination of linkage, homozygosity mapping and exome sequencing in three families, we identified mutations in *CLPP* as the cause of this phenotype. In each family, affected individuals were homozygous for a different pathogenic *CLPP* allele: the missense mutations c.433A>C (p.Thr145Pro), c.440G>C (p.Cys147Ser), and an experimentally demonstrated splice donor site mutation c.270+4A>G.
CLPP encodes a highly conserved endopeptidase, a component of a mitochondrial ATP-dependent proteolytic complex that forms an element of the evolutionarily ancient mitochondrial unfolded-protein response (UPR^{mt}) stress signaling pathway. Protein modelling studies indicate the pathogenic nature of the mutations. Our findings define biallelic variants in *CLPP* that cause a strikingly variable phenotype of profound pre-lingual sensorineural hearing loss and ovarian failure/ovarian dysgenesis. This expands the understanding of the contribution of mitochondrial proteins to health and disease and allows accurate risk assessment and counselling in families with Perrault syndrome.

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P10.64

The spectrum of mutations PAH gene in patients with phenylketonuria in Kazakhstan

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In 2007, in the Republic of Kazakhstan has been introduced the State program of genetic screening of newborns for phenylketonuria (PKU). With the introduction this program, 54 patients with PKU were identified. The national register of patients with PKU in Kazakhstan includes information about 106 patients. 57 children with PKU were studied by molecular genetic method. The average frequency of PKU in Kazakhstan is equal of 1:18593. The aim of our study was detection in genome of PKU patients known mutations in PAH gene.
Molecular genetic analysis of mutations in PAH gene (R408W, R261Q, R252W, IVS10-11, IVS12 +1, R158Q, P281L, IVS14 +5) carried out using PCR technique.
The most widespread R408W mutation was detected in 20 patients (frequency was 0.538), 4 patient were homozygous and 16 heterozygotes. R261Q mutation was found in 5 patients with a frequency 0.096 in heterozygous state. 5 patients had P281L mutation with a frequency of 0.096. IVS12+1 was detected in getorozigous state in 2 patients (frequency 0.038). IVS10-11 mutation identified only 1 patient with a frequency 0.019. Other patient with PKU had unknown mutation, which requires a whole genome sequencing RAH gene. 2 patients had a whole genome sequencing RAH gene. It was found Arg243Glu (c.728g.a.) and R243Q/R243Q in 7 exon of PAH gene.
Application of molecular genetic techniques provided opportunities for diagnosis of hereditary diseases including ethnicity. Molecular genetic study of patients allows PKU prenatal diagnosis and detect heterozygous carriers. To date, this is the most adequate prevention of PKU in Kazakhstan.

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P10.65

Characterization of PMM2 mutations: identification of target mutants for specific pharmacological chaperone therapy

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Congenital Disorders of Glycosylation (CDG) are a group of multisystemic, inherited, metabolic diseases resulting from defects in the protein N-glycosylation pathway. Phosphomanomutase 2 (PMM2) is the affected protein in CDG-PMM2 disorder, the most common CDG, for which there is currently not an effective treatment. PMM2 is a cytosolic enzyme which converts mannose 6-phophate to mannose 1-phosphate. The screening of target mutations for specific therapies was performed through the functional analysis of nine PMM2 mutations. All the studied mutations presented lower amounts of PMM2 protein detected by western blot from crude bacterial extracts and purified proteins. The analysis of the oligomerization pattern showed an oligomeric profile similar to the wild-type protein with abundance of dimers for several mutations others showed increased levels of aggregates while one mutation, p.Pro113Leu, did not present any oligomeric state. Protein stability, studied by differential scanning fluorimetry, showed that all mutants are more unstable than wild-type shifting the Tm from 2 to 7 °C. Finally, the specific activity of mutants from pure protein and bacterial crude extracts showed a reduction from 50 to 100% compared to the wild-type protein. Based on this results and the localization of residues in the PMM2 crystal structure, we have classified the mutations as catalytic, destabilizing and mutations affecting the dimerization. The destabilizing mutations pV44A, p.D65Y, p.R162W, p.C241S, p.F207S and p.T237M are promising candidates for testing the 15 pharmacological chaperones previously selected by high-throughput screening from 100 000 library´s compounds using the wild-type protein.

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P10.66

Interest of QMPSF analysis in the detection of large intragenic POLG1 rearrangements through the study of a large French cohort

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POLG1 is the most commonly gene involved in mitochondrial disorders with mitochondrial DNA instability and causes a wide range of diseases with recessive or dominant transmission. More than 170 mutations have been reported. Most of them are missense mutations but nonsense, splice-site mutations and small deletions and insertions have also been identified. However, to date, only one large-scale rearrangement has been described in

a child with Alpers syndrome. Herein, we report a large cohort of 160 patients with clinical, molecular and/or biochemical presentation suggestive of POLG deficiency. Using sequencing, we identified POLG1 variants in 25 patients including 5 novel pathogenic mutations. Two patients with novel mutations had unusual clinical presentation: one patient exhibited an isolated ataxic neuropathy and one child presented with endocrine signs. We completed sequencing step by QMPSF analysis in 40 patients with either only one POLG1 heterozygous variant or a family history suggesting a dominant transmission. We identified a large intragenic deletion encompassing part of intron 21 and exon 22 of POLG1 in a child with refractory epilepsia partialis continua. In conclusion, we describe the first large French cohort of patients with POLG1 mutations, expanding the wide clinical and molecular spectrum observed in patients with POLG1 mutations. We also show that large deletions are probably less rare events than previously thought and the great interest of using QMPSF in patients with a single heterozygous POLG1 mutation, particularly in severe infantile phenotypes.

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P10.67

Genetic variants of organic cation transporters (Octs) in patients with polycystic ovary syndrome (PCOS) and their association with glucose metabolism

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Metformin, a well-known oral insulin-sensitizing drug, is commonly used in the treatment of polycystic ovary syndrome (PCOS). It decreases also hepatic lipogenesis and activates hepatic SHBG production. There is a great variability in the clinical response to metformin, mainly due to genetic variances influencing the pharmacokinetics/dynamics of metformin or its action. Metformin is a substrate of organic cation transporters (Octs), especially Oct1 (liver) and Oct2 (kidney).
We investigated the pattern of metformin response mediating genotypes in PCOS patients to identify potential "bad responders" and "non responders" and determined their relation to glucose metabolism prior to metformin treatment.
We genotyped R61C, G465R, S14F, S189L, G220V, P160L and M408V (Oct1), A270S (Oct2) and rs11212617 (ATM) variants in 676 PCOS and 90 control women and performed oral glucose tolerance tests in 422 patients and 88 controls prior to metformin treatment.
In PCOS women, we found 30% with non-functioning alleles and 50% with decreased metformin response alleles. Oct variants were associated with elevated C-peptide levels at baseline (p=0.006) and following oral glucose load (AUC C-peptide p=0.005). A high percentage of our PCOS patients might be "bad responders" or "non-responders" due to their Oct genotypes. Oct genotypes are able to modulate the pharmacokinetic and pharmacodynamic profile of metformin, thus contributing to differences in metformin efficacy. In addition, Oct genotypes might have effects on glucose metabolism which are independent from metformin.

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P10.68

The effect of Mediterranean diet and exercise on insulin resistance in the presence of PPARG2 rs1801282 polymorphism in a central Romanian population

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As the environment has a role in modifying the relationship between genetic variants and clinical measures of disease, consideration of gene-environment interactions (GxE) is mandatory. Based on the Nutritional Phenotype Database initiated to facilitate GxE GWAS or single-gene studies, we proposed to follow the role of lifestyle and rs1801282 on insulin resistance in a non-diabetic population from central Romania.
In 210 subjects (median age 52) living in an urban environment, insulin resistance has been estimated by QUICKI (Quantitative Insulin Sensitivity Check Index, IRI=1/QUICKI), while genotyping was done by PCR-RFLP using BstUI. Lifestyle has been evaluated by the PhenX Toolkit and DataShaper questionnaires.
In case of an increased calorie intake, low fiber ingestion and sedentary lifestyle, IRI was found to be significantly higher (3.1 vs 2.71, p=0.003). Differences have been maintained and reproduced in the presence of the unmodifiable inherited factor (PP vs PA+AA), in subjects following (2.76 vs 2.68, p=0.02) or not (3.06 vs 2.93, p=0.03) a healthy lifestyle.
In conclusion, diet and physical activity may have beneficial effects even in the presence of risk alleles contributing to insulin resistance. Given the complex nature of GxE, minor effect of rs1801282 complicated by population differences and contradictory data reported in the literature, extension of analysis on a high number of subjects is necessary. Efforts, though, are motivated by insight that could directly facilitate a more active intervention through lifestyle choices to reduce cardiometabolic risk.

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P10.69

Unusual presentation of propionic acidemia : dilated cardiomyopathy of adult onset and premature ovarian failure

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Propionic acidemia (PA) is characterized by neonatal or infancy-onset metabolic decompensation. The vast majority of patients develop long-term complications including progressive encephalopathy, cardiomyopathy and seizures in spite of adapted clinical management. We report an unusual late-onset PA diagnosis in an adult female patient.
The patient was referred to our department of genetics at the age of 28 years in the context of premature ovarian failure (POF). At the age of 17 years, she had presented acute heart failure secondary to a dilated cardiomyopathy which had required cardiac transplantation. Clinical examination, serum creatine phosphokinase concentration and karyotype were normal. No episode of metabolic acidosis nor neurocognitive impairement has been reported. Nevertheless, her personal history prompted us to perform metabolic investigations, which revealed an abnormal urine organic acid profile with elevated methylcitric acid. Acylcarnitine analysis showed an important increase of propionyl-carnitine. Enzymatic analysis showed no detectable PCC activity on cultured fibroblasts. Sequence analysis revealed two mutations within the PCCB gene: c.1218_1231del14ins12 and c.1514T>C. Both parents were shown to be heterozygous for one PCCB mutation. This observation is the second description of POF in a patient with PA, suggesting that POF is a complication of the disease. Dilated cardiomyopathy is commonly described as a long term complication in PA, but to our knowledge, only three other cases of PA revealed by DCM have been reported. This observation illustrates that a systematic metabolic evaluation should be performed in patients with dilated cardiomyopathy.

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P10.70

Transient expression of ATIC and ADSL restore affected purinosome assembly in cultured skin fibroblasts from patients with ADSL deficiency

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De novo purine synthesis (DNPS) is one of the basic processes in eukaryotic cells. Purinosome is multienzyme complex composed by enzymes of DNPS that cells transiently assemble in their cytoplasm upon depletion or increased demand of purines. Dysfunction of DNPS is represented by two genetic diseases: AICA-ribosiduria and adenylosuccinate lyase deficiency (dADSL). We hypothesized that some of the mutations may affect formation or stability of the purinosome.
We investigated purinosome formation in cultured skin fibroblasts from one AICA-ribosiduria patient and nine dADSL patients representing three groups of the disease - neonatal form, type I form and type II form. Skin fibroblasts cultured in purine depleted medium were immuno-labeled with

various combinations of DNPS proteins. We observed correlation between purinosome formation and the disease severity : no creation of purinosome in neonatal and type I forms and reduced formation in type II form of dADSL and AICA-ribosiduria.
Then we investigated the possibility of the purinosome formation recovery in AICA-ribosiduria skin fibroblasts transfected with wtATIC; and dADSL skin fibroblasts transfected with wtADSL. We observed renovation of purinosome formation in transfected dADSL fibroblasts comparable to control fibroblasts in the purine depleted medium. In contrast, no purinosome formation was observed in transfected AICA-ribosiduria fibroblasts.
These results indicate that genetic defects of DNPS affect not only activity and structure of the individual enzymes, but according to disease severity, also functionality of the DNPS pathway by purinosome complex destabilization.
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P10.71

Infantile Refsum disease: a clinical diagnosis for a complex biochemical algorithm

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Background
Peroxissomal biogenesis disorders (PBD) include Infantile Refsum disease (IRD), a rare autosomal recessive disorder, belonging to the mild end of the Zellweger spectrum disorders (ZSDs). Mutations in *PEX* genes, coding for peroxins, result in general impairment of peroxisomal functions. We report a fifth Portuguese patient with IRD, establishing a comparison with the remaining Portuguese patients.
Case report
A 3 years-old girl born from consanguineous parents was referred to the Medical Genetics consultation for retinitis pigmentosa, bilateral sensorineural hearing loss, developmental delay and dysmorphic cranio-facial features. Hypotonia was observed at birth. She had had neonatal jaundice and increased hepatic enzymes. Nystagmus, detected at 3 months, prompted ophthalmologic evaluation that later documented retinal dystrophy. Pyramidal syndrome was evident at 16 months. Plasma levels of very long chain fatty acids were slightly increased, which in combination with increased phytanic acid and pristanic acid levels supported the hypothesis of a peroxisomal disorder, either a ZSD or a D-bifunctional protein deficiency. The deficient activity of dihydroxyacetone-phosphase acyltransferase in fibroblasts is in accordance with diagnosis of ZSD.
Discussion
When comparing to the other Portuguese patients with IRD, biochemical abnormalities in the present patient were found to be milder. Hearing and visual impairment should prompt a comprehensive investigation of PBD, as the onset and severity of manifestations is variable and numerous clinical differential diagnoses exist. Since the ZSDs are a continuum of at least 3 phenotypes (Zellweger syndrome, neonatal adrenoleukodystrophy, IRD), to name a specific profile of ZSDs is useful when discussing prognosis and counselling families.

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P10.72

Common g.1541G>A SCO2 gene mutation carriership among Polish patients with negative molecular testing for spinal muscular atrophy.

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Introduction
Spinal muscular atrophy (SMA) is one of the most frequent among rare diseases in Poland (1:7000-10000 births). Direct molecular testing for *SMN/NAIP* deletions is a routine diagnostic approach. About 80-100 such tests are performed per year.
Muscle histology and EMG records in *SCO2*-related encephalomyopathy (with g.1541G>A as a common mutation) are similar to these observed in SMA, which may suggest necessity of *SMN/NAIP* testing.
The aim of this study was to determine frequency of g.1541G>A mutation in a cohort of 468 archive DNA samples negative for *SMN/NAIP* deletions.
Material and Methods
We examined samples of DNA isolated from peripheral blood leukocytes. Genotyping with Taqman's probe (based on Real Time equipment) was used for specific mutation identification. Presence of the mutation in selected samples was confirmed by direct sequencing.
Results
The study revealed nine g.1541G>A positive samples: three g.1541G>A carriers , in addition to six finally established non-SMA diagnoses (5 cases of *SCO2*-related encephalomyopathy and one SMARD). The carrier frequency in the studied group was finally established as 1:156 (3/468).
Conclusion
It remains to be clarified if the number of g.1541G>A carriers identified in this study corresponds to the true population frequency or may be associated with a neurogenic muscle pathology.
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P10.73

The effects of the (TAAAA)n VNTR on serum SHBG and lipid levels in men with coronary heart disease

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HDL-C levels are inversely associated with the CHD risk. Serum SHBG concentration is partially under genetic control and positively associated with the HDL-C levels. In our study, the possible relation between SHBG (TAAAA) n VNTR and low HDL-C were investigated in 75 men with CHD and in 50 healthy men by analyzing this polymorphism and also measuring SHBG levels. In CHD patients who had SHBG>35nmol/l, the possibility of having a HDL-C levels >0.90mmol/l was observed significant (p=0.004;OR:6.319). The frequency of six repeats allele was higher in controls (p=0.002;OR:0,293). Six repeats allele in patients and short repeats (≤8) in controls were found to be associated with higher SHBG ($p<0.05$ and $p<0.01$, respectively). In patients with eight repeats allele, HDL-C/LDL-C was observed lower than in non-carriers ($p<0.05$). Triglycerides were lower in controls with six repeats allele and short repeats and higher in controls with nine repeats allele ($p<0.05$). HDL-C/LDL-C was lower in controls with nine repeats allele. Our findings indicate six repeats allele has a positive effect on SHBG levels in both patients and controls. Consequently, this is a preliminary study to establish the link among (TAAAA)n VNTR, SHBG and HDL-C levels in CHD pathogenesis in a Turkish population. It was demonstrated 6 repeat allele may protect against CHD risk. Additional studies with larger sample sizes are needed to define the influence of SHBG (TAAAA)n VNTR on clinical outcomes. Finally, we suggest a positive correlation between SHBG and HDL-C levels in CHD patients, and this association might be affected by SHBG gene variations.

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P10.74

Study of an asymptomatic mother heterozygous for X-linked creatine transporter defect

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Female carriers of SLC6A8 mutations may have learning disabilities or be asymptomatic. Therefore, X-linked creatine transporter defect is likely to be under diagnosed in females and almost all of female carriers reported in the literature are related to an affected boy.

An increased creatine/creatinine ratio in urine or a relatively low cerebral creatine signal in 1H-magnetic resonance spectroscopy (MRS) compared to other metabolic peaks (choline-containing compounds, myo-inositol, N-acetylasparate) is used to detect the creatine transporter defect in females.

We report two female carriers of SLC6A8 mutations (c1006-1008 delAAC), aunt and mother of an affected boy: the aunt has mild cognitive impairment, the mother is asymptomatic.

Interestingly, the aunt has normal urinary creatine/creatinine ratio but low cerebral creatine signal in MRS whereas her sister has increased urinary creatine/creatinine ratio but normal cerebral creatine signal.

The cognitive evaluation of the asymptomatic mother shows IQ scores in the range of low average intellectual functioning with inter-scale heterogeneity. However, it represents a 35-40 point deficit when compared to her two healthy brothers' IQ scores.

In the same way as it is necessary to take into account the urinary creatinine or cerebral metabolic peaks to interpret respectively the urinary creatine and the cerebral creatine peak of individuals, we suggest considering cognitive levels of family members to assess effects of SLC6A8 mutations on IQ female carriers in further research.

Screening based on urinary creatine/creatinine ratio and MRS could be proposed to females with learning difficulties after global cognitive evaluation.

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P10.75

Growing up with cholesterol biosynthesis deficiencies

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In the last two decades, several genetic defects affecting different steps of the cholesterol biosynthesis pathway were described and nine inherited errors of the distal part of cholesterol biosynthesis have been recognized. Affected patients present complex malformation syndromes involving different organs and systems.

We report on the biochemical data, genotype and evolution, since young life till adulthood, of three patients with enzymatic defects at three distinct, steps of cholesterol biosynthesis pathway, namely: Smith-Lemli-Opitz syndrome (SLO); X-linked dominant chondrodysplasia punctata type 2 (CDPX2) and congenital hemidysplasia with ichthyosiform erythroderma and limb defects (CHILD) syndrome in order to contribute for a better knowledge of the natural history of these genetic disorders. The differences on clinical manifestations depended on several factors: the step in cholesterol synthesis that is blocked, the type of mutation and its impact in the structure and function of the enzyme, and the amount of exogenous cholesterol that the patient received either via placenta during embryonic development or later by oral intake.

None of our patients showed life threatening episodes and their phenotypes actually improved over time probably due to the fact that the western adult diet became progressively enriched in cholesterol (due to the increased amount of animal products ingested), which may partially compensate for the deficiency after birth. In order to diagnose such disorders it is important to look for malformations, minor dismorphic features and chronic skin changes in patients with low or borderline cholesterol levels at any age.

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P10.76

Discovery of new potentially associated loci from pathway analysis of known influencing genes for Type 2 Diabetes

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Genome-wide association studies have contributed to the description of 71 loci that influence susceptibility to T2D; however, our understanding of the underlying pathophysiological processes is still limited.

We explored the mechanistic basis of T2D associations assuming their functional dependence and, therefore, connectivity via non-interrupted gene networks.

We considered 84 T2D-associated genetic variants within 71 known loci (Jan 2013) and explored their gene network connections using the LocusSpider tool, enabling interrogation of functional gene-gene relations from KEGG, Reactome and PPI pathways. LocusSpider prioritised genes having: (i) maximal connectivity within the 71 input loci; and (ii) located most closely to the associated variants.

The LocusSpider gene network model (p_{model}=0.01) connected variants from 62 T2D-associated loci (41 genes were <50kb distant from the SNP) and suggested 44 additional genes required to connect genes from the input loci. The most represented pathways included signal transduction (WNT/EGFR-signaling), transcription, cell cycle and insulin secretion regulation. At 22 known loci, pathway analysis suggested a gene different from the previously assigned locus label, underscoring a need for further functional characterisation. We observed at least nominal associations (p-value<0.05, FDR q-value<0.20) with T2D in DIAGRAMv3 meta-analysis (Morris et al. 2012) within 25 loci (+/-50kb regions) surrounding the 44 suggested genes, with strongest signals at rs9270986 (*HLA-DQA1, p*=2.7x10^{-7}), rs7169735 (*p*=4.7x10^{-5}) at *RASGRF1* as *ARAP1-CAMK2B* inter-link, and rs603939 (*p*=5.7x10^{-4}) at *PRKACB* connecting *ADCY5* and *KCNJ11*.

Our findings suggest that novel pathway analysis methods may underscore important biological mechanisms of T2D pathogenesis and highlight biologically relevant associations for subsequent follow-up.

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P10.77

Congenital Disorders of Glycosilation: Antisense therapy in TMEM165 deficiency

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Deficiency of glycosyltranferases, glycosidases or nucleotide-sugar transporters involved in protein glycosylation leads to Congenital Disorders of Glycosylation (CDG), a rare group of diseases characterized by multisystemic phenotype. Recently, others proteins implicated in Golgi trafficking and Golgi pH homeostasis have been identified as CDG causing genes. In this work, we describe the functional analysis of the intronic change c.792+182G>A identified in TMEM165 causing new CDG type II. The protein function is unknown, but points to an important role in Golgi glycosylation and Golgi morphology maintenance. Using a functional in vitro splicing assay based on minigenes and transcriptional profile studies in patient-derived fibroblasts, we have validated its pathogenic effect. Once functionally analyzed the deep intronic change we have applied a mutation specific therapy. We have designed a specific antisense oligonucleotide targeted to rescue the abnormal splicing process. After transfection we have detected normal transcript mRNA which is efficiently translated into a well localized protein. We have observed a protein recovery (3-fold) detected by double inmunofluoresce using TMEM165 and the Golgi-specific marker GM130 antibodies. The recovery was dose and sequence specific with no effect on patient-derived fibroblasts harboring missense changes. The results show the efficiency of the antisense therapy targeted to intronic changes, which are detectable by combined DNA and RNA studies. Up to now, five TMEM165-CDG patients have been described and three of them are homozygous for the intronic change c.792+182G>A which makes antisense morpholino therapy an excellent candidate treatment for this new CDG type II.

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P10.78

Disruption of *NUBPL* due to balanced translocation [t(3;14)(q26.33;q14)] increases severity of a family-specific PGK1 mutation

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An intriguing group of familiar translocations are those which not always segregate with the "associated" disorder. Here we report the genetic alterations underlying a clinical phenotype characterized by haemolytic anemia and neuro-myopathy, seemingly associated with the familial translocation [t(3;14)(3q26.33;q14)]. Two affected probands and two unaffected relatives have been identified as carriers. The 3q26.33 breakpoint was mapped about 40 kb from the *TTC14* 5' end, at position 180.28 Mb and the 14q14 breakpoint within IVS 6 of *NUBPL*. The latter has been implicated in the aetiology of mitochondrial complex I deficiency (OMIM 252010). The most important additional possible candidate gene identified in this region is *DNAJC19* causing an autosomal recessive disorder (OMIN 610198) that partially overlaps the reported phenotype. The recognition that a deceased relative most likely suffered from a similar disorder suggested the possibility of an X-linked disorder. Exclusion of additional genomic alterations within the breakpoint regions or elsewhere in the genome, familial X-chromosome segregation analysis and whole exome sequencing identified a novel missense mutation, c.358G>A, p.Glu120Lys, in exon 4 of phosphoglycerate kinase 1 (*PGK1*). Segregation analysis confirmed the association of this mutation with the disease phenotype. Re-evaluation of clinical data indicates that myopathy is considerably more severe in PGK1 deficient patients carriers of the translocation. The confirmation of this observation is currently underway. In conclusion, we have identified a novel *PGK1* mutation whose clinical phenotype is exacerbated by co-inheritance of the disrupted *NUBPL* and/or by alterations affecting the genes in the breakpoint regions.

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P10.79

Novel mutations in HPD causing tyrosinemia type III in Northern Israel

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Impaired tyrosine catabolism result in elevated plasma tyrosine concentrations. Malfunction enzymes along the tyrosine catabolic pathway results in hereditary tyrosinemia. Tyrosinemia type III is caused by deficiency in 4-HPPD. To date, only 14 cases have been described, showing a wide clinical spectrum. Reported patients have either presented with mental retardation and neurological symptoms while others, detected by neonatal screening, have been asymptomatic.
We report 4 new patients, 3 from a highly consanguineous Druze family, presenting elevated blood tyrosine levels with normal development and intelligence. Clinical evaluation revealed novel eye and skin phenotype not previously described in tyrosinemia type III. The fourth patient, from a Muslim origin consanguineous family, was diagnosed with hypertyrosinemia in the neonatal metabolic screen. Her psychomotoric development is normal for her age. Sequencing revealed two novel splicing mutations in HPD. Healthy control screening from two Druze villages detected 3:100 carriers in one village and none in the second.
We suspect tyrosinemia type III to be under-diagnosed and more common than is currently known. With neonatal metabolic screen becoming more widespread, more patients are likely to be diagnosed. Molecular analysis is the gold standard for diagnosing tyrosinemia type II and III. In light of the minute number of patients with tyrosinemia type III worldwide, clinical characterization of new patients is highly significant, determining the natural history of the disease, and describing the treatment and its contribution to normal growth and development of the patients.

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P10.80

A homozygous *UQCRC2* mutation cause a neonatal onset metabolic decompensation due to complex III deficiency.

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The mitochondrial respiratory chain generates energy as adenosine triphosphate (ATP) by means of the electron-transport chain and the oxidative-phosphorylation system. The mitochondrial respiratory chain, located in the inner mitochondrial membrane, is composed of five complexes: I, II, III, IV, and V. Among them, mitochondrial complex III (CIII) comprises 11 subunits encoded by one mitochondrial and 10 nuclear genes. Until now, mutations in four genes have been known to cause autosomal recessive CIII deficiencies: *UQCRB*, *UQCRQ*, *BCS1L* and *TTC19*. *UQCRB* and *UQCRQ* encode components of CIII itself, while *BCS1L* and *TTC19* produce mitochondrial assembly factors. Here, we report three patients from a consanguineous Mexican family presenting with neonatal onset of hypoglycemia, lactic acidosis, ketosis, and hyperammonemia. By whole exome sequencing combined with linkage analysis, we successfully found a homozygous missense mutation in *UQCRC2* that encodes mitochondrial ubiquinol-cytochrome c reductase core protein II. In its native state, the CIII monomer is quickly converted into a catalytically active homodimer that is incorporated into a supercomplex, and this supercomplex functions as a single enzyme. Based on structural modeling, the mutation (p.Arg183Trp) was predicted to destabilize the hydrophobic core at the subunit interface of the core protein II homodimer. In vitro studies using fibroblasts from the index patient clearly indicated CIII deficiency, as well as impaired assembly of the supercomplex consisting of complexes I, III, and IV. This is the first described human disease caused by *UQCRC2* abnormality.

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P10.82

Association and molecular analysis of 3' UTR polymorphisms of the WFS1 gene

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Wolframin is a major protein of the endoplasmic reticulum, loss of function mutations of the WFS1 gene result in the monogenic Wolfram-syndrome, characterized by optic atrophy, diabetes insipidus, diabetes mellitus and deafness, whereas polymorphisms of the gene are putative risk factors of diabetes. Our aim was the association and molecular analysis of two SNPs (rs1046322 and rs9457) in the 3' UTR of the WFS1 gene, which are supposed to alter the miRNA binding according to in silico data.
Association analysis was carried out by case-control study. 617 patients and 1147 healthy controls particiapted. Genotype analysis was carried out using PCR based methods, functional analysis was done by luciferase reporter system.
Our results showed that rs9457 "C" allele was significantly more frequent among patients with type 2 diabetes, whereas the rs1046322 variant showed a significant association with the type 1 form of the disease. Luciferase reporter experiments suggested that the rs1046322 and the rs9457 SNPs altered the binding of miRNA-668 and miRNA-185, respectively.
Our results suggest that the rs9457 and rs1046322 polymorphisms are the genetic components of diabetes mellitus. Earlier studies showed an association between WFS1 rs1046320 and diabetes, however no biological function of the SNP could be observed. We suggest that this result is due to the strong linkage disequilibrium between rs9457 and rs1046320, thus the latter polymorphism can be a genetic marker of rs9457.
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P10.83

X-linked adrenoleukodystrophy: Two manifesting heterozygotes in a cohort of Czech and Slovak patients

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The most common peroxisomal disorder, X-linked adrenoleukodystrophy (X-ALD; MIM# 300100), is a recessive neurodegenerative disease. The major phenotypic forms are
childhood cerebral (ccALD) and adrenomyeloneuropathy (AMN). The diagnosis of X-ALD was established in 25 Czech and Slovak families. All patients had elevated concentration of C26:0, elevated ratios of C26:0/C22:0 and C24:0/C22:0 and a mutation in *ABCD1* gene.
In the cohort there are two symptomatic females. The first presented with AMN symptoms from the age of 27 years. She is a carrier of previously published mutation c.887A>G (p.Tyr296Cys). Sequencing of cDNA showed a strong prevalence of the peak corresponding to the mutated allele.
The second female patient was a two-year-old girl with microcephaly, psychomotor delay, hearing and vision disorder, seizures, and leukodystrophy. Pathological VLCFA profile and a previously found a pathogenic mutation c.1553G>A (p.Arg518Gln) in a heterozygote state was identified. Sequencing of cDNA showed 70 - 80% prevalence of the mutated allele. However, the sequencing of the patient's asymptomatic mother revealed very similar results (including biochemical findings). HUMARA analysis confirmed the unfavorable X-inactivation skewing in the mother blood sample.
These data show that molecular diagnostics in heterozygous females is not straightforward. It may reflect at least three possible explanations: 1) The X-inactivation status does not correspond to the phenotype in X-ALD heterozygotes in all cases. 2) The genotype-phenotype correlation is poor. 3) Clinical picture of heterozygotes may result from a combination of X-ALD and other factors, like severe perinatal risks.
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P10.84

Arrested leukodystrophy in a young man with adrenomyeloneuropathy (AMN)

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Background X-linked adrenoleukodystrophy (X-ALD) is a peroxisomal disorder which presents with progressive leukodystrophy in boys or as progressive spasticity and neuropathy in men (AMN).
Case Report A 30 year old male presented to the Neurometabolic Clinic with worsening stiff gait and paresthesias, suggestive of AMN, and a recent diagnosis of X-ALD. He was diagnosed with Addison's Disease at age 4. He was a gifted student until high school when he had a change in personality, decreased attention and executive functioning. This was progressive during high school and then stabilized. IQ testing at age 16 years revealed a verbal IQ of 115 and performance IQ of 92.
At the time of his evaluation, neuropsychological testing revealed a verbal IQ of 115 and an improved performance IQ of 110. MRI of the spine revealed generalized atrophy of the spinal cord and the EMG revealed sensorimotor neuropathy.
The MRI scan revealed abnormal signal in the white matter of the parieto-occipital, temporal and cerebellar white matter bilaterally which has remained stable. There was no enhancement or restricted diffusion to suggest active demyelination. MR spectroscopy was normal.
Conclusion This case demonstates a rare arrested form of leukodystrophy in a young man with AMN.

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P10.85

Genetic heterogeneity in Mabry syndrome

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Mabry syndrome (hyperphosphatasia with developmental disability) was first described in 1970 (OMIM#239300). At first considered rare, improved syndrome identification has led to recruitment of more than twenty families world-wide. Salient features of the disorder include:
characteristic facial dysmorphology (hypertelorism, a broad nasal bridge and a tented mouth); variable shortening of middle and distal phalanges; and neural abnormalities on biopsy (plaques disrupting Schwann cells). Like many infantile metabolic storage disorders, seizures associated with Mabry syndrome have an onset in the first year of life. Persistently elevated serum alkaline phosphatase (ALP) levels are now known to be associated with abnormalities of the phosphatidylinositol glycan (GPI) anchor due to mutations in the PIGV, PIGO and PGAP2 genes. Lysosomal storage material detected in some patients with Mabry syndrome. It has been putatively identified as glycolipid in nature - possibly resulting from changes in lipid raft functions that result from GPI anchor disruption. Not all patients with a clinical diagnosis of hyperphosphatasia with neurologic deficit result from known GPI disruptions. We present data on patients that suggest there are further subtypes of the disorder.

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P10.86

Newborn screening for X-linked adrenoleukodystrophy

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Objective: X-ALD is the most common peroxisomal disorder (incidence 1:21,000 males). It is a progressive and fatal disorder that affects nervous system, adrenal cortex and testis. Adrenal insufficiency is totally prevented by presymptomatic therapy, while the cerebral phenotype, affecting 40% of all male patients, can be prevented by hematopoietic cell transplantation. However, this intervention has a narrow window of opportunity to be effective. Lysophosphatidylcholine species (lyso-PC) on blood spots have been shown to be abnormally elevated in newborns affected with X-ALD and peroxisomal biogenesis disorders (PBD), allowing identification of presymptomatic patients.
Methods and Results: Using a flow injection analysis MS/MS (FIA-MS/MS) method, we have measured lyso-PC species (C26:0 to C20:0) on dried blood spots from 16 male patients with X-ALD (12 adults and 4 newborns), 12 XALD adult heterozygotes, and 6 patients with PBD (4 adults and 2 newborns). Data were compared with 340 controls (NBS dried blood spots).
Results are shown in the table
Conclusions: The data of the present study strongly indicate that our method is analytically suitable as a screening tool for X-ALD. Patients affected with peroxisomal biogenesis disorders and 70 - 85 % of XALD female carriers will be detected by this assay. With support from NICHD and the NBSTRN, a pilot study on 100,000 leftover NBS sample is ongoing to test the clinical validity of this approach.

Concentration of C26 Lyso-PC species in peroxisomal cases and controls

	C26 (ug/ml) 1 percentile	C26 (ug/ml) 99 percentile
XALD (16)	0.28	0.75
XALD H (12)	0.15	0.51
PBD (6)	0.25	1.06
controls (340)	0.07	0.28

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P11.001

Intellectual disability and cancer susceptibility in a family with inherited 14q32.13q32.2 deletion

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Located in 14q32.13, *DICER1* codes for an RNase III endoribonuclease essential in the processing of microRNAs. These microRNAs are functional non-coding RNAs that regulate gene expression at a post-transcriptional level by interfering with translation and degradation of target messenger RNAs. Their deregulations have been implicated in several human diseases and cancers. Germline mutations in *DICER1* are associated with a low susceptibility risk to tumor development. Inactivating mutations have been identified in patients and families with various benign and malignant tumors, especially pleuropulmonary blastoma, cystic nephroma and ovarian Sertoli-Leydig tumors. Germline *DICER1* deletion has not been reported so far. We report a 7-year-old girl with an inherited *DICER1* deletion. She was referred for mild intellectual deficiency, a medical history of cystic nephroma and dysmorphic features. Her father, two paternal uncles and the paternal grandmother had mild to moderate intellectual disabilities. The deceased grandmother had presented uterin and ovarian tumors. Array-CGH analysis identified a 5041kb deletion in 14q32.13-q32.2 in the patient, the father, the two uncles and the paternal grandmother. The deletion contained 50 coding genes and led to *DICER 1* haploinsufficiency which was responsible for cancer susceptibility. Moreover, transmission of the deletion was related to intellectual disability in this family. The 14q32.13q32.2 locus has been previously associated with autosomal recessive mental retardation, suggesting the presence in the region of genes implicated in cerebral development and cognitive functions.

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P11.002

MiRNA related polymorphisms as susceptibility markers of childhood Acute Lymphoblastic Leukemia

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Acute Lymphoblastic Leukemia (ALL) is the most common malignancy in children. ALL is developed in early life, and consequently, a strong genetic influence could be expected. In fact, previous Genome-Wide Association Studies (GWAS) have provided evidence of this fact. Nevertheless, most of these studies are focused in coding regions.

MicroRNAs (miRNA) are non-coding RNAs that act as negative regulators of the expression of other genes. Up to 171 deregulated miRNAs have been reported in pediatric ALL, showing their importance in the disease.

Recent studies have shown that SNPs in miRNAs-related genes might be associated with risk of cancer. These polymorphisms could affect miRNA function. Nevertheless, there are no studies in ALL risk.

The aim of this study was to evaluate the role of SNPs in miRNAs-related genes in ALL susceptibility and to determine their functional implication.

We analyzed 213 childhood B-ALL patients in complete remission and 387 healthy controls. We studied 118 SNPs in pre-miRNAs and genes of miRNA biogenesis pathway. The implication of these SNPs in miRNA function was evaluated by qRT-PCR.

We found 11 polymorphisms significantly associated ($p<0.05$) with ALL risk. Of them, 8 SNPs were in processing machinery genes and 3 in pre-miRNA genes. The functional effect of each SNP was evaluated.

Our results suggest that SNPs in miRNAs and miRNA biogenesis pathway may affect childhood ALL susceptibility.

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P11.003

Cytogenetic abnormalities and monosomal karyotypes in a large series of adult Greek patients with acute myeloid leukemia

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Cytogenetic abnormalities constitute one of the most important prognostic factors in Acute Myeloid Leukaemia (AML). We performed a conventional cytogenetic study in AML patients in order to define the chromosomal abnormalities and their frequencies in *de novo* and secondary AML (*s*-AML). Our study includes 619 patients of whom 503 had *de novo* AML and 116 *s*-AML. Chromosome studies were performed on unstimulated bone marrow cells. The sex ratio (males/females) was 1.2/1. The median age of *de novo* AML and *s*-AML was 59.46 and 68.9 years respectively. The most common FAB subtype was M4 in 23.5% of patients followed by M2 in 22.6%, M3 in 19.7% and M5 in 15.3%. A successful karyotypic result was achieved in 98.2% of patients. Among them, normal karyotypes were found in 183 (30.1%) patients; 165 with *de novo* AML (33.5%) and 18 with *s*-AML (15.6%). Complex karyotypes had 32.6% of *de novo* and 34.5% of *s*-AML patients, while monosomal karyotypes were observed in 19.9% and 27.8% respectively. The most common chromosome aberrations in *de novo* AML patients were +8 (25.5%), -7/del(7q) (15.7%), -5/del(5q) (11.2%), abnormalities of 11q23 (6.5%), inv(16) (5.9%), t(15;17) (5.9%), t(8;21) (5.7%) and +21 (3.3%). In *s*-AML the most common abnormalities were +8 (27.4%), -7/del(7q) (28.7%), -5/del(5q) (28.7%), t(9;22) (6.1%), +21 (6.1%), -Y (4.3%) and abnormalities of 11q23 (3.5%). In conclusion the most common abnormality in AML was +8 followed by -7/del(7q). Secondary AML occurred in older adults and showed a higher frequency of abnormal karyotypes and monosomal karyotypes than *de novo* AML.

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P11.004

Association of $A^{313}G$ glutathione S-transferase P1 germline polymorphism with the susceptibility of *de novo* acute myeloid leukemia

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Genetic approaches for understanding the etiology of Acute Myeloid Leukemia (AML) comprise polymorphic variants in xenobiotic metabolizer loci. Glutathione S-transferases (GSTs) are phase II detoxification enzymes, involved in the metabolism of carcinogens and anticancer drugs. *GSTP1* gene is subjected to a single nucleotide polymorphism ($A^{313}G$) that decreases the catalytic activity, and consequently, the detoxification capacity. Individuals homozygous for the mutant allele (G/G) have a lower conjugating activity than individuals homozygous for the wild type allele (A/A), while heterozygotes (A/G) display intermediate activity. This study investigates the possible implication of *GSTP1* polymorphism in AML susceptibility and AML-specific chromosomal abnormalities. PCR-RFLP assay was applied to detect the *GSTP1* polymorphism in 184 primary AML patients and 370 unrelated healthy donors. Abnormal karyotypes were found in 62.6% of patients and among them, 32.1% and 20.5% had complex and monosomal karyotypes, respectively. A significantly higher incidence of the mutant *GSTP1* genotypes (A/G and G/G) was observed in AML patients as compared to the controls ($p<0.0001$). No association was found between the *GSTP1* polymorphism and gender or age. Stratification of patients according to karyotype revealed an increased frequency of G/G homozygotes with normal karyotypes as compared to the abnormal karyotypes (19.5% vs 8.9% respectively, $p=0.056$). Furthermore, a higher frequency of heterozygotes A/G was observed in patients with -Y, -7/del(7q) and +22. Our results suggest that the *GSTP1* $A^{313}G$ polymorphism may be a predisposing factor for the development of AML. Moreover, homozygosity of the *GSTP1* polymorphism seems to be associated with development of AML with normal karyotype.

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P11.005

Immunophenotypic and clinical features of acute myeloid leukemia patients with normal cytogenetics and mutation of FLT3 gene

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The goal of the research was to determine distinctive features of de novo acute myeloid leukemia (AML) with FLT3-ITD mutation. Objects: to review the variability of morphological and cytogenetic characteristics of AML patients with FLT3-ITD; to identify homogeneity of the group of AML patients with normal karyotype (NK) with FLT3-ITD based on haematological data and immunophenotype of blast cells. The investigation group included 101 AML patients. FLT3-ITD mutation were detected by PCR method in 21 cases (20,8%). Meanwhile 13 patients (61.9%) from this group had NK. The group of patients with expression of several antigens more than 20% (diagnostically significant number) were analyzed. There were no significant difference in number of patients with high expression of CD34 in control group and group with FLT3-ITD: 83,3% against 90,0%; *p=0,635.* At once, the number of patients with high expression of HLA-DR and CD7 were significantly higher in group with NK and FLT3-ITD: 6,2% against 50,0%; *p=0,007* and 55,6% against 100,0%; *p=0,014*, respectively. Correlation analysis showed FLT3-ITD to be determined significantly often in patients older 51 years in comparison with young*; r=0,400; p=0,034.* Received data allowed to make a conclusion that AML with NK and FLT3-ITD represent the group, homogeneous on a number of haematological data and biological characteristics of leukemic cells. Thereby such markers as leukocytosis in combination with aberrant expression of HLA-DR and CD7 on blast cells allowed to prognosticate the probability of detection FLT3-ITD mutation in AML patients with NK.

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P11.006

Detection by array-based comparative genomic hybridization in a case of adenoid cystic carcinoma of an unbalanced translocation t(6;9)(q23;p22.3~23) which generates a MYB-NFIB fusion oncogene.

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Background: adenoid cystic carcinoma (ACC) has been shown as a histologically distinctive neoplasm for nearly 150 years. It is among the most common carcinomas of the salivary glands. A recurrent t(6;9)(q23; p22.3~23) translocation was reported in ACC. Recently, it has been shown that the ACC-specific t(6;9)(q23;p22.3~23) translocation results in a MYB-NFIB fusion oncogene which is the hallmark of ACC. Array comparative genomic hybridization (aCGH) is a suitable method for studying oncogenomic imbalances.
Results: a 44k aCGH analysis was performed on 17 frozen ACC. In one tumor, genomic imbalances involved only 6q and 9p. A 35.24 Mb deleted region was detected on 6(q23q27) and a gain segment of 13.87 Mb was observed on (9)(pterp23) <0.20-14.08 Mb>. Genomic mapping indicated that breakpoints lied within MYB/6q22.3~23 sequence and NFIB/9p23 gene. The unbalanced form of t(6;9)(q23;22.3~23) translocation: der(6)t(6;9) was hence suspected. The MYB-NFIB chimeric transcript was detected by RT-PCR. The latter fusion gene link MYB exon 9b to NFIB exon 11.
Discussion and conclusion: Analysis of 17 frozen ACC by aCGH has allowed the suspicion -of an unbalanced t(6;9)(q23;p23p24) translocation in one case. This tumor displays the der(6)t(6;9) as the sole cytogenomic imbalances, indicating that it is a primary rearrangement in this carcinoma. The der(6) is the critical derivative chromosomal of the t(6;9) translocation because the MYB-NFIB transcript was detected by RT-PCR. In cancer, the aCGH data should be analysed carefully and the chimeric fusion gene should be looked for because this type of abnormality may change the prognosis and the treatment.

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P11.007

Exome sequencing identifies potential new candidate genes for colorectal adenomatous polyposis

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Background: In up to 50% of families with colorectal adenomatous polyposis no germline mutation in the currently known genes APC causing familial adenomatous polyposis (FAP) or MUTYH causing MUTYH-associated polyposis (MAP) can be identified. Missense mutations in polymerase genes have recently been found as rare cause of multiple colorectal adenomas/carcinomas. Methods: To uncover new causative genes, the exomes of ten unrelated APC and MUTYH mutation negative polyposis patients were sequenced (Illumina HiSeq platform). For data analysis and variant filtering the GATK software and in-house tools were applied. Results: Altogether, 66 genes affected by biallelic truncating variants (recessive model) in at least one patient and 63 genes affected by truncating heterozygous variants (dominant model) in at least two patients were found. After detailed manual investigations of the variants and data mining according to functions and pathways seven genes of high interest remained (three for the recessive, four for the dominant approach), some of which are involved in cell adhesion, proliferation, or recombination repair. Moreover, we found a missense variant in the polymerase gene POLD2 in one patient, which is predicted to be damaging by in-silico analysis. In another patient, an APC nonsense mutation in mosaic state below the detection threshold of Sanger sequencing (10% of reads) was recognised. Conclusions: Using exome sequencing we identified new potentially causative genes for adenomatous polyposis. The clinical relevance of the genes is presently clarified in a validation sample of 200 polyposis patients. (Supported by German Cancer Aid and BONFOR programme of the University of Bonn)

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P11.008

Mosaic pattern of familial Beckwith Wiedemann syndrome revealed by isolated benign adrenocortical tumor: a case report

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We report a familial BWS presented with a benign hypersecretant adrenocortical tumor (ACT) which proved to be isolated in a 2 months old baby girl. Family history revealed a paternal aunt who developed at 60 years a benign ACT. Our patient was managed surgically and tumoral tissue so as blood sample and skin tissue underwent molecular analysis using MS-MLPA (Methylation-sensitive multiplex ligation probe analysis) in order to detect epigenetic alterations associated with BWS.
Genetic investigations noticed in blood sample a methylation mosaic pattern at imprinted domain 2 (IC2) encompassing KCNQ1, KCNQ1OT1, and CDKN1C genes. This mosaicism was associated in tumoral tissue to a complete loss of methylation at IC2, however skin tissue analysis showed a normal methylation profile.
This rare epigenetic constellation may explain the mild phenotype observed in both patient and her aunt. However appropriate genetic counseling to the affected patient and her family members should be provided and clinicians should be aware that patients may develop isolated benign ACT in the context of hereditary syndromes.

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P11.009

Exome sequencing identifies drivers of progression of Transient Myeloproliferative Disorder to Acute Megakaryoblastic Leukemia in children with Down Syndrome

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Megakaryoblastic leukemia in children is almost exclusively associated with Down Syndrome (DS) - trisomy 21 and acquired mutations in GATA1. Up to 10% of DS neonates develop Transient Myeloproliferative Disorder (TMD), which is self-regressing in the majority of cases, but in 20-30% it progresses to malignant acute megakaryoblastic leukemia (AMKL). Currently there is no evidence of a genetic component underlying the progression of the disease. In this study we have performed exome-sequencing of 7 megakaryoblastic leukemias in DS-children to test the hypothesis that somatic genetic variants contribute to the progression of TMD to AMKL.
In four out of seven studied AMKL/TMD cases we have observed somatic mutations, additional to GATA1, occurring in genes with established function in different types of leukemia (EZH2, APC, FLT3 and JAK1). In addition, the AMKL genomes were characterized by genomic instability which was not observed in the TMDs. In one patient we have detected two putative driver mutations, a frameshift indel in EZH2, and a hemizygous loss of APC in AMKL which were absent at the TMD stage of disease in this patient. Our findings favor a three-hit hypothesis, where transition of TMD to AMKL requires a 3rd genetic event additional to T21 and GATA1 mutation. These additional driver mutations were observed in genes of WNT, JAK-STAT and MAPK/PI3K pathways, aberrant activation of which may enhance the effect of GATA1 mutation resulting in overexpression of MYC therefore facilitating transition towards malignancy.

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P11.010

Array CGH analysis of acute myeloid leukemia patients with normal and complex aberrant karyotype.

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Acute myelogenous leukemia (AML) is the most common type of leukemia affecting adults, and is responsible for a large number of cancer-related deaths. Lymphocytes of approximately 55% of AML patients show various chromosomal aberrations, including structural and numerical alterations. Such changes may not only correlate with morphological and clinical data, but also serve as prognostic factors.
In our study we analyzed bone marrow samples of 60 AML patients with normal (18 patients) or complex (42 patients) karyotype by conventional cytogenetics. We then assessed the genomic changes by the array CGH (aCGH) method. Genomic losses were found more frequently than gains. The most frequent losses affected 5q (25%), 7q (20%) and 3p (18%), and the most frequent genomic gains included 11q (20%) and 21q (17%).
In patients with initially normal karyotypes it was possible to determine single or multiple aberrations by means of aCGH. In this group losses were also more frequent than gains. Changes detected using aCGH were verified by FISH analysis in all cases.
In the second part of this study we analyzed the prognostic effects and interactions of the nucleophosmin (NPM1) gene mutation and fms-like tyrosine kinase-3 internal tandem duplication (FLT3-ITD) in patients with normal-karyotype (AML-NK).
Identification of all possible rearrangements is valuable for better understanding of the mechanisms of leukemogenesis, as well as for more precise stratification of patients into appropriate risk groups.

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P11.012

Mutation of the ARID1A gene in Endometrial Carcinoma in Spanish patients

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Endometrial carcinoma (EC) is the most common gynaecological tumor in developed countries. There are several common mutated genes depending on the type. PTEN, PI3K, K-RAS and B-RAF genes are frequently altered in endometrioid carcinomas and P53, E-Cadherin, p16 and PPP2R1A genes in non endometrioid.
ARID1A is a recently identified tumor suppressor which participates in chromatin remodeling, specifically, in forming switch/sucrose non-fermentable chromatin remodeling complexes. It has been reported that ARID1A gene is mutated in ovarian clear cell, endometrioid carcinomas and in uterine endometrioid carcinomas. Most ARID1A mutations are nonsense, frameshift or in-frame, leading to loss of expression of the protein which encodes.
The aim of our study has been the analysis of the ARID1A gene in order to determine the incidence of mutations in the different types and grades of EC.
We extracted DNA from a set of 48 fresh tumor from patients diagnosed with EC (33 endometrioid carcinoma: 17 grade I, 13 grade II and 3 grade III; 15 non endometrioid: 7 mixed carcinoma; 6 serous carcinoma; 1 carcinosarcoma; 1 clear cell carcinoma. We studied the ARID1A gene by PCR, CSGE, cloning and automatic sequencing.
The results are showed in the annexed table. We found 21 ARID1A pathogenic mutations including some deletions, insertions and base substitutions.
Our results confirm that ARID1A gene mutations are a frequent event in EC.
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Tumor type	Patients mutated (%)
Endometrioid carcinoma	57.57
-I grade	27.27
-II grade	27.27
-III grade	3.03
Non endometrioid carcinoma	40

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Molecular profile of low- and high-grade astrocytomas: a multivariate approach

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Malignant astrocytomas are the most common and aggressive primary brain tumors in adults with a dismal prognosis. In an attempt to better understand the biology of astrocytomas, we have performed a molecular analysis of TP53, IDH1, EGFR, PI3KCA, PTEN and BRAF genes in a set of 105 astrocytomas (24 low-grade astrocytomas and 71 high-grade astrocytomas).
Nineteen low-grade (79.2%) and 71 high-grade astrocytomas (87.7%) had at least one alteration in these genes, and more than 50% of cases showed two o more alterations. Mutations in TP53, IDH1 and BRAF genes were more frequent in low-grade astrocytomas while alterations in EGFR, PTEN and PI3KCA genes were more represented in high-grade astrocytomas (Table 1).

Table1. Alterations in low- and high-grade astrocytomas

	TP53 mutation	EGFR amplification	EGFRvIII expression	PI3KCA mutation	LOH 10q23	PTEN mutation	BRAF mutation	IDH1 mutation
Low-grade Astrocitomas	13(56.5%)	0(0.0%)	1(4.3 %)		4(33.3%)		2(8.7%)	15(65.2%)
High-grade Astrocytomas	17(22.7%)	26(35.1%)	23(33.3%)	5(6.7%)	49(75.4%)	19(25.3%)	1(1.3%)	3(4.0%)
Total	30(30.6%)	26(35.1%)	24(26.1%)	5(6.7%)	53(68.8%)	19(25.3%)	3(3.1%)	18(18.4%)

43.3% of tumors with TP53 mutation carried IDH1 mutation; 26.7% of cases showed both EGFR amplification and PTEN loss; 20.3% of tumors displayed PTEN deletion and mutation, and 14.3% of tumors carried TP53 mutation and PTEN mutation/deletion. However, mutations in PTEN and PI3KCA genes were mutually exclusive.

Multivariate analysis by logistic biplot and hierarchical clustering classified high-grade tumors in proneural subtype distinguished by TP53 and IDH1 mutations and EGFRvIII expression; classical subtype defined by EGFR amplification and PTEN deletion along with EGFRvIII expression and PTEN mutation; and mesenquimal subtype characterized by PTEN deletion with TP53 and PTEN mutations. Furthermore, proneural subtype patients demonstrated improved survival over the proliferative or mesenquimal groups.

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P11.014

The malignant transformations in Ewing's sarcoma cells are correlated with beta arrestin1 level

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Ewing's sarcoma is a malignant tumor which can have mesodermal and ectodermal origins. It appears most frequently in children and adolescents and in most of the cases is the result of a translocation between chromosomes 11 and 22 which affect Ewing's sarcoma and Friend leukemia integration 1 transcription factor genes. Beta arrestin1 and insulin-like growth factor receptor has an important role in the activation of a signaling cascade in MAPK pathway which is involved in the initiation and transformation of the malignant phenotype.
The aim of this study was to experience proper cell culture techniques in order to mimic pathological characteristics of Ewing sarcoma and provide adequate experimental model for drug screening. It is known the fact that cell culture lines represent an actual experimental study model of epigenetic and genetic mechanisms involved into the initiation of pathological processes. Many of these new research activities are based on genetic reprogramming of the patient derived cell lines. The experiment envisaged to evaluate the effect of beta-arrestin1 concentratons in two types of ES cell line, genetically modified by B-arr1 shRNA. Quantifying cell proliferation and estimation of cellular viability were used for the correlation between Beta arrestin level and its tumorigenicity. Results indicate that malignant transformations are connected with beta-arrestin1 level and the malignant phenotype is modified when the intracellular levels of beta-arrestin1 decrease. The protein beta-arrestin 1 play an important role in IGF1R signaling and also in the initialization process of tumorigenicity in ES cells.

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P11.015

Unexpected diagnosis of Fanconi anemia with biallelic FANCD1/BRCA2 mutations associated with early onset colorectal tumors in adulthood: potential role of pre-mRNA splicing in phenotypic variability.

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Fanconi anemia (FA) is characterized by progressive bone marrow failure, congenital anomalies, and predisposition to malignancy. A small proportion of FA is due to biallelic FANCD1/BRCA2 mutations which is associated with earlier onset and increased incidence of leukemia and solid tumors. Here we describe the clinical and molecular features of a family in which the diagnosis of FA was unexpectedly made from a female proband with early onset breast cancer. Remarkably, she and her sister, also diagnosed with FA, had uneventful childhoods and no morphological anomalies. Both patients presented colorectal adenocarcinomas (microsatellite stable) with either gastric lymphoma or breast cancer around the age of 30. Another sibling died at the age of 5 of acute myeloid leukemia. Biallelic FANCD1/BRCA2 mutations were identified: one frameshift mutation (c.1845_1846delCT, p.Asn615LysfsX6) and one missense mutation (c.7802A>G, p.Tyr2601Cys) with a frame-shifting splicing anomaly leading to both a truncated protein and a residual point-mutant protein. This protein, predicted to be deleterious, is presumably partially active and might explain the clinical spectrum with delayed FA diagnosis. Spontaneous and mitomycin-induced chromosomal instability was pathognomonic of FA and only the molecular investigations permitted the diagnosis. The lack of other symptoms in the two older siblings and the occurrence of colon cancers raise the hypothesis that variations in spliceosomal proteins might participate in subtle defects in the splicing machinery and thus lead to constitutional and tissue-specific variability of the phenotype. An interaction between BRCA2 and the MMR pathway could also be questioned in presence of early colorectal adenocarcinomas.

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P11.016

Genetic variants involved in specialized DNA replication and their relation with bilateral breast cancer

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One of the hallmarks of cancer is the occurrence of high levels of chromosomal rearrangements as a result of inaccurate repair of double-strand breaks (DSB). Germline mutations in *BRCA* and *RAD51* genes, involved in DSB repair, are strongly associated with bilateral breast cancer. Pol θ, a "DNA repair" polymerase specialized in the replication of damaged DNA may also be involved in DSB repair and could act in this pathway with *BRCA* and *RAD51.* It is noteworthy that *POLQ* is highly expressed in breast tumors and this expression is able to predict patient outcome. Here we analyzed *POLQ* variants in hereditary (HBC) and sporadic (SBC) breast cancer. We recruited 229 breast cancer patients (94 SBC and 135 HBC) and 206 controls. In this case-control study seven SNPs (rs61757736, rs55748151, rs41545723, rs1381057, rs587553, rs13065220, rs3806614) were analysed using Taqman Real Time PCR. The rs581553 SNP located in a promoter region of POLQ was strongly associated with HBC (g.121265913C>T; HBC TT=18, Control TT=8; OR=2.30, CI95%= 1.47-3.55; p<0.0001). Interestingly, all of the homozygous for this polymorphism fulfilled criterions for HBOC (Hereditary Breast and Ovarian Cancer) syndrome, where 16 of them developed bilateral breast cancer and one had familial history of bilateral breast cancer. The age at diagnosis (> or < 50 years) did not show statistic differences when compared patients with or without this polymorphism. Considering the evidences that *POLQ* may be involved in DBS repair our study suggest that this polymorphism may contribute to the etiology of bilateral breast cancer in our population.

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P11.017

DNA repair gene polymorphisms involved in bladder cancer development

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To assess c.839G>A and c.1196A>G XRCC1, g.45854919T>G XPD, c.-4A>G XPA gene polymorphisms contribution in the development and severity of bladder cancer we carried out a study 306 DNA bladder cancer patients samples and 271 healthy individuals DNA samples. It was shown c.839 G>A XRCC1 associated with bladder cancer development in several models, but significantly in log-additive (p<0.0001, OR=2.40, AIC=765.9). Second polymorphism c.1196G>A XRCC1 associated with disease in recessive (p=0.03, OR=1.61, AIC=784) and overdominant (p=0.02, OR=0.66, AIC=782.7) models. No association with bladder cancer was observed for c.-4A>G XPA and

g.45854919T>G XPD. In order to estimate the contribution of studied polymorphisms in the development of invasive and non-invasive bladder cancer sample of patients with these forms were compared separately with control cohort. Interestingly, c.839 G>A XRCC1 contributes to the development of both non-invasive and invasive bladder cancer (p = 0.0017, OR= 2.06, CI (1.31-3.26) and p<0.0001, OR= 2.70, CI (1.78-4.10), respectively). Thus we can assume XRCC1 polymorphisms make a definite contribution to the development and severity of bladder cancer. However, the results should be confirmed in independent studies.

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P11.018

Expression of B7-H4 gene polimorphisms in transitional cell carcinoma of the bladder

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B7-H4 is one of the most recently identified members of B7 superfamily of costimulatory molecules serving as an inhibitory modulator of T-cell response. The expression of B7-H4 has been observed in various types of human cancer tissues and its soluble form has been detected in blood samples.

In this study B7-H4 gene rs10754339, rs10801935 ve rs3738414 SNPs were studied by PCR-RFLP method in paraffin embedded tumor specimens from 62 transitional cell bladder cancer (TCC) patients and in the control group including 30 patients without bladder cancer. Both groups were in the similar age interval (patient group 63.34+12.5 vs control group 60.8+11.1; p>0.05). Male gender was more frequent among cancer patients (55 male vs 7 female) and the same gender distribution was detected in non-bladder cancer patients (28 male vs 2 female) (p>0.05).

According to WHO 2004 bladder cancer classification system 19 non-muscle invasive low-grade carcinomas, 20 non-muscle invasive high-grade carcinomas and 23 high-grade invasive carcinomas were included. We detected rs3738414 and rs10754339 polimorphisms were more frequent in cancer patients when compared with control group (p<0.05). Only rs3738414 polimorphism showed statistically significant difference in frequency between pathologic diagnostic groups (p<0.04).

In both rs10754339 and rs3738414 polymorphisms, AA and GG genotype distributions were found to have higher frequencies in the cancer group when compared with control group (p<0.05). None of the genotype distributions showed statistically significant difference from the control group in rs10801935 polimorphism.

We conclude that B7-H4 has the potential to be a useful prognostic marker for patients with TCC.

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Chemotherapeutic drug sensitivity determination through expression profiling of bladder tumors

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Background: Cancer of the bladder is a disabling disease with multifocality, frequent recurrence and growing incidence in the economically developed countries. The majority of tumors at presentation are low grade, non muscle-invasive, papillary tumors. The aim of our study is investigation the expression levels of the genes responsible for sensitivity and resistance to known chemotherapeutic agents.

Materials & Methods: Tumor sample of 40 patients with bladder neoplasia in different stages: precancerous, pTa, pT1 and pT2 were analyzed. 168 genes from two panels for Cancer drug resistance and metabolism (84 genes) and Cancer drug targets (84 genes) were investigated.

Reaults: Our results reveal a 3-5 times up-regulation of five genes for drug response related to xenobiotic metabolization, degradation of aromatic compounds, polycyclic aromatic hydrocarbons and the anti-cancer drug taxol. A more than 10 fold up-regulation of PPARG, CYP3A5 and ABCC3 genes related to metabolism of cyclosporine and steroid hormones was observed in a tumor stage dependent manner (pTa, pT1 and pT2).

The results from Cancer drug targets panel show up-regulation of TOP2A and genes from MRP subfamily (ABCC1 and ABCC5), involved in resistance to thiopurine anticancer drugs in the muscle invasive versus non-invasive bladder tumors.

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P11.020

Prediction of recurrence in bladder cancer by real-time quantitative PCR analysis: cDNA microarray results

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The aim of the study was to define specific genetic profile in Ta and T1 urinary bladder carcinoma patients with and without recurrence by gene expression microarrays. Eleven patients with the time to recurrence shorter than one year (patients with recurrence) and 11 patients with time to recurrence longer than 4 years (patients without recurrence) were enrolled.

Data from microarrays were subjected to a panel of statistical analyses to identify bladder cancer recurrence-associated gene signatures. Initial screening revealed a putative set 47 genes differing in gene expression in both groups. After the validation, 33 genes manifested significant differences between both groups. The significant expression was observed in the group of patients without recurrence by 30 genes of which the highest differences were detected by ***ANXA1***, ***ARHGEF4, FLJ32252, GNE, NINJ1, PRICKLE1, PSAT1, RNASE1, SPTAN1, SYNGR1, TNFSF15, TSPAN1,*** and ***WDR34***. These genes code for signal transduction, vascular remodeling and vascular endothelial growth inhibition mainly. In the group with recurrence, 3 genes had significant differences, the highest differences were identified by two genes ***(PLOD2*** and ***WDR72)***. We have selected and validated 15 genes that are differentially expressed in superficial bladder cancer. We hope that this cohort of genes will serve as a promising pool of candidate biomarkers for early stage bladder cancer. Our results indicate that it may be possible to identify patients with a low and high risk of disease recurrence at an early stage using a molecular profile. . Research was supported by MSM 0021620808

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P11.021

Detection of p53 gene mutations and ATPase6, Cytb, ND1 and D310 mtDNA mutations in bladder carcinomas

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Introduction: Bladder carcinoma is the most common malignancy of the urinary tract. Analysis of p53 gene and mtDNA mutations might be useful to monitor the prognosis of bladder carcinoma. Therefore the aim of this study is to investigate the relation between mtDNA and p53 gene mutations in bladder carcinomas.

Methods: 30 patients and 27 controls were recruited to the study. Bladder cancer tissues were obtained by radical cystectomy or transurethral resection. Genomic DNA was extracted from peripheral blood. ATPase6, Cytb, ND1, and D310 regions of mtDNA as well as the exon 5,6,7 and 8 regions of p53 gene were amplified by PCR and sequenced directly. Results were evaluated statistically.

Results: In patient group, 33 mtDNA mutations and 3 p53 gene mutations were found in which 3 of them are novel and 15 of them cause amino acid substitutions. A15607G mutations in Cytb gene and 12570 A-insertion in p53 gene were found statistically significant in patient group whereas C12570A was found statistically significant in control group. Additionally, 8 different 100% correlations between mtDNA mutations and 3 different positive correlations between p53 gene and mtDNA mutations were detected.

Conclusion: High incidences of A15607G mutation and 12570 A-insertion may be important markers for the detection of bladder tumors. Despite that,

C12570A mutations may have a protective effect against the disease. Additionally, based on correlation values, it has been suggested that there is a relation between p53 gene and mtDNA gene mutations and this relationship could play an important role in bladder carcinogenesis.

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P11.022

Bortezomib Induces Apoptosis by Interacting with JAK/STAT Pathway in K-562 Leukemic Cells

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In the current study, we aimed to identify the cytotoxic and apoptotic effects of Bortezomib (BOR) on human K-562 chronic myelogenous leukemia cells and to evaluate the potential roles of JAK/STAT pathway members STAT3, STAT5, JAK2 and IL-6 on BOR induced cell death of leukemic cells.
Cell viability was assessed via Trypan blue dye exclusion test and cytotoxicity of the BOR treated cells was conducted by XTT [2,3-Bis (2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide inner salt] assay. The relative mRNA expression levels of STAT3, -5A -5B, JAK2 and interleukin- 6 were analyzed by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR). On the other hand, their protein expression levels were detected by western-blot method.
The obtained results indicated that, BOR treatment reduced cell viability and induced leukemic cell apoptosis in a dose and time-dependent manner as compared to untreated control cells. While mRNA expression levels of STAT5A, STAT5B and STAT3 were significantly
reduced following BOR treatment when compared to untreated controls; it had no effect upon JAK2 and IL-6 mRNA expressions. As for protein levels, STAT expressions were downregulated after BOR treatment especially at 72th and 96th hours.
Our results pointed out that, BOR treatment had a significant potential of being an anticancer agent for chronic myelogenous leukemia therapy; and this effect could be due to the expressional downregulations of JAK/STAT pathway members.

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P11.023

Splicing functional assays of a RCA1 minigene with exons 15-19

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Deleterious mutations in BRCA1 and BRCA2 increased up to 20-fold the risk of developing breast cancer. Previously published data of our group showed that a third part of pathogenic mutations affected pre-mRNA processing so that splicing could be one of the most relevant etiopathogenic mechanism in hereditary breast/ovarian cancer. We constructed a hybrid minigene with BRCA1 exons 15 to 19 in the new splicing vector pSAD (Spanish patent: P201231427). Bioinformatics studies of DNA variants in exons 16 and 17 of BRCA1 were performed with NNSplice and Human Splicing Finder, to select them according to the following criteria: disruption of canonical splice sites, broken branch points or creation of de novo silencers. Functional assays performed either in lymphocyte RNA from patients or in hybrid minigenes in MCF7/HeLa cells. We investigated the impact on splicing of pre-selected variants listed in the international databases and to correlate these results with those obtained in lymphocyte RNA of patients from Castilla y Leon (Spain).
All the RT-PCR products were sequenced to characterize all the splicing outcomes. Splicing functional assays allow a better molecular characterization of DNA variants of unknown clinical significance. The ultimate goal of this research is to improve the quality of life of patients and their families that will benefit from new preventive measures, surveillance and prophylaxis.
Acedo et al. 2012. Breast Cancer Res 14: R87
Sanz et al. 2010 Clin Cancer Res 16: 1957-1967

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P11.024

Intronic sequence variants in BRCA genes among breast and ovarian Romanian patients

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Molecular diagnosis in cancer predisposition is mainly targeting *BRCA1* and *BRCA2* genes, involved in hereditary breast and ovarian cancer syndrome (HBOC). Carriers of deleterious mutations are at significantly higher risk of developing cancer than general population. Screening for BRCA mutations is now standard practice in Europe, and permits medical follow-up and adapted genetic counselling.
Currently, most laboratories performing diagnostic analysis of the *BRCA* genes use direct Sanger sequencing of exons and intron-exon boundaries. Thousands of BRCA sequence variations have already been reported, but not all variants can be considered pathological. Deleterious mutations and common non-pathogenic SNPs are usually detected, but almost a half of the observed variations are of uncertain clinical significance. *In-silico* analysis is therefore essential for understanding possible effects on protein function and pathogenicity.
While completely sequencing *BRCA1* and *BRCA2* genes in routine molecular diagnosis, we found a total of 14 intronic variants, equally distributed to *BRCA1* (7 variants) and *BRCA2* (7 variants). Half of these variants were novel, not known in BIC, UMD or NCBI databases. By *in-silico* analysis, one variant showed to be pathogenic, by completely destroying a splicing site, while two other variants have an unclassified status (type 3 in the UMD 5-levels pathogenicity classification). Eight variants proved to be benign SNPs, and were used to define novel BRCA1 haplotypes.
This proves that a better investigation of intronic regions can bring useful information, either from pathogenic variants responsible for the predisposition to the disease, or form rare benign SNPs defining haplotypes important for population studies.

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P11.025

Breast and prostate cancers in men in the French National *BRCA1/BRCA2* carriers cohort (GENEPSO)

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Breast cancer in men is extremely rare, less than 1% of all breast cancers. The presence of germline mutations in the *BRCA2* gene increases the risk of developing breast cancer and prostate cancer during man's lifetime.
Objectives: To describe breast and other cancers in men with deleterious mutation of the *BRCA1/BRCA2* genes and to investigate correlations between type of mutation, age and cancer phenotypes.
Materials and Methods: The study population included males participants in the French National cohort study GENEPSO of *BRCA1/2* carriers recruited at 33 cancer genetic clinics. This cohort study was initiated in 2000 to estimate breast and other cancer risk and to assess risk-modifying factors in *BRCA1/BRCA2* mutation carriers.
Results: To date, the GENEPSO cohort study has included 376 men, 60% of them carrying a *BRCA1* mutation. 24 breast cancers were observed. Mean age at breast cancer diagnosis was 62.4 years (range: 45-77). All the 3 *BRCA1* mutation positive male breast cancer (MBC) were invasive ductal carcinoma, RE+, RP+ and grade 3 for 2 men. 52% of *BRCA2*-related MBCs (n=21) were grade 2 or 3, 71% were RE+, 66% RP+ and 3 of them (14%) presented an invasive lobular carcinoma. Prostate cancers ranked second in this cohort: 5 *BRCA1* and 10 *BRCA2* mutation carriers. Median age at prostate cancer diagnosis was 64 years (range: 53-77).
Conclusion: In men, breast and prostate cancers are mainly associated with *BRCA2* mutation and breast cancer phenotypes seem to be different according to sex and type of gene.

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P11.026

Next-generation sequencing and analysis of the BRCA1 and BRCA2 genes in 95 Bulgarian women

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Background: Breast cancer is the most common type of cancer in women. Most of its hereditary forms are caused by mutations in the *BRCA1* and *BRCA2* genes, the main function of which is DNA repair of double-stranded breaks. Genetic testing in women with familial history is recommended to determine whether they have a hereditary predisposition to this type of cancer.
Methods: After giving their informed consent, blood samples from 25 women diagnosed with cancer and 70 controls were collected, from which genomic DNA was isolated. Sequence-targeted *BRCA1/2* libraries were then prepared using the TruSeq Custom Amplicon technique, which were then sequenced on an Illumina MiSeq system.
Results: A wide range of mutations were found, of which 55 were in *BRCA1* and 69 in *BRCA2.* In total, 5 (4%) were categorised as deleterious, including a donor splice-site mutation in intron 2 of *BRCA2,* 11 (9%) supposed deleterious, e.g. an in-frame trinucleotide deletion in exon 17 of *BRCA1* within the BRCT functional domain and non-synonymous mutations within other functional domains of *BRCA1* and *BRCA2,* 28 (23%) variants with unknown significance, 21 (17%) favoured polymorphisms and 59 (48%) benign polymorphisms.
Conclusion: This is the first genetic study, in which the *BRCA1* and *BRCA2* genes of women from the Bulgarian population were sequenced paving the way for finding local founder mutations as well as polymorphisms typical for this region.

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P11.027

Women with sporadic breast cancer between age 36 and 41 should be offered BRCA1/BRCA2 testing

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BACKGROUND: *BRCA1* and *BRCA2* are the two best-known breast cancer predisposition genes. Gene analysis is warranted in women with both a personal and family history of the disease, or with sporadic breast cancer at a very young age (≤35). There is however no consensus regarding testing in women with sporadic breast cancer between ages 36 and 41, as the probability of finding a mutation might be too low to justify a long and expensive procedure.
METHODS: We offered *BRCA* testing to all women with sporadic breast cancer ages 36-41, without other personal or familial history, and treated between 2006 and 2012 in our hospital. We searched for point mutations (Sanger sequencing) and large rearrangements (MLPA).
RESULTS: Out of 99 tested women, 11 (11%) carried a mutation, 7 in *BRCA1* and 4 in *BRCA2*. Four and four carriers had triple-negative (*BRCA1* only) and bilateral disease, respectively.
DISCUSSION: There is a consensus within the Cancer Genetics community that genetic testing should be performed when the probability of finding a mutation exceeds 10%. According to this large single-center study, women with sporadic breast cancer between ages 36-41 fit in this category, and should therefore be offered *BRCA1* and *BRCA2* analysis, especially if their tumour is triple-negative or bilateral. The identification of a mutation in a substantial proportion of these women would lead to a personal management, resulting in decreased morbidity and mortality.

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P11.028

Haplotype analysis and ancient origin of the BRCA1 c.4035delA Baltic founder mutation

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Uncertainty exists about the origin of BRCA1 c.4035delA mutation which is prevalent in Baltic countries, with the highest frequency being in Lithuania (53% of all BRCA1 mutations), although formal founder mutation analysis by haplotype has not yet been undertaken. In this study we genotyped 78 unrelated BRCA1 c.4035delA mutation carriers families from Lithuania, Latvia, Poland and Russia. The results from the haplotype analyses were used to estimate the age of the mutation. Using maximum likelihood methods we estimated that the mutation arose approximately 1550 years (62 generations of 25 years) ago (ca. 5th century) somewhere in the present territory of Lithuania, in the area inhabited by ancient Baltic tribes at that time. Our results show that this mutation gradually entered the gene pool in the neighbouring countries.

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P11.029

Design of a multiplex PCR panel for semiconductor sequencing of the *BRCA1* and *BRCA2* genes on the Ion Torrent PGM

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The introduction of benchtop massive parallel sequencing machines enables the development of methodologies that are faster and more cost-effective than conventional sequencing. In this study we describe a collaborative effort for the validation of a strategy for the screening of the breast cancer susceptibility genes *BRCA1* and *BRCA2*, making use of the most recent advances on Ion AmpliSeq multiplex PCR technology combined with Ion PGM System.
Strict criteria were set to the design of the amplification primers, including 100% coverage of all coding exons and exon-intron boundaries and the absence of recurrent SNPs in the last five nucleotides of primer. Twenty-five Dutch and twenty-five Portuguese mutations were selected for this analysis. The first 20 cases were used to test and optimize the methodology and were selected based on the presence of mutations in or within close proximity to homopolymeric regions. The additional 30 cases were used for an independent evaluation of the new methodology. These samples included common mutations in Portugal and the Netherlands. In all these cases all SNPs and mutations that were previously identified by capillary electrophoresis sequencing were detected with our multiplex-based approach. The mutations identified included missense, nonsense and indel mutations. The number false positive results were consistently less than five per sample and reproducible. This work demonstrates the potential for mutational screening of *BRCA1* and *BRCA2* using the AmpliSeq technology combined with the Ion Torrent PGM.

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P11.030

Lack of referral for genetic counseling and testing in BRCA1/2 and Lynch syndromes: A nationwide study from the French National Cancer Institute based on 240·134 consultations and 134·652 genetic tests

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Background: Based on nationwide data from the French national cancer institute (FNCI), we analyzed the evolution of cancer genetics consultations and testing over time, and the uptake of targeted tests in relatives of families carrying BRCA1/2 or MMR gene mutations.
Methods: All French cancer genetics centers completed annually a survey collecting standardized parameters for the FNCI from 2003 to 2011. We report on the analysis of a total of 240·134 consultations and 134·652 genetic tests that identified 32·494 mutation carriers.
Results: From 2003 to 2011, 141·639 (59 %) and 55·698 (23·2 %) patients were referred for a breast or a colorectal cancer predisposition syndrome, respectively. During this period, we found a dramatic and steady increase in genetic tests performed for the *BRCA1/2* genes (from 2095 to 7393 tests/year, P<0·0001) but not for the MMR genes (from 1144 to 1635/year, P=NS). The percentage of deleterious mutations identified in the probands tested was 13·8 % and 20·9 % in BRCA1/2 and Lynch syndromes, respectively. In families with a *BRCA1/2* or a MMR identified mutation, there was an average number of 3·03 relatives performing target tested.
Conclusion: This nationwide study shows a lack of referral and genetic testing in Lynch syndrome as compared to BRCA1/2 syndrome. Only a third of relatives of a proband carrying a BRCA1/2 or MMR gene mutations performed a targeted test. Enhanced information about benefit of genetic testing is crucial in Lynch syndrome and for relatives of a proband carrying an identified BRCA1/2 or MMR genes mutation.

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P11.031
BRCA1 missense mutation p.Met18Lys detected in 11 Czech high-risk hereditary breast cancer families: segregation analysis and clinical data

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Identifying women who carry pathogenic mutation in BRCA1 is important for cancer prevention and individualised treatment. Many of tested women are discovered to have variant of unknown clinical significance. One of them, which is nearly exclusively detected in Czech population, is BRCA1 missense variant c.53T>A, p.Met18Lys located 6 aminocids upstream of the highly conserved C3HC4 - RING zinc finger domain. The p.Met18 is highly conserved residue where p.Met18Lys substitution represents replacement of non-polar hydrophobic methionine to a polar charged lysine with highest value of Align-GVGD prediction: C65; likely to induce distortion of helical bundle. Functional tests of p.Met18Lys exhibits loss of ubiquitin ligase activity (Morris 2006) and significantly resulted in a reduction of BRCA1/BARD1 complex formation (Sarkar 2008).
Here we present available segregation data and clinical data of 11 Czech high-risk hereditary breast/ovarian cancer families. In six families segregation with cancer diseases were apparent and in 4 families only insufficient data were available to make any conclusion. Out of these 10 families: 12 women with breast cancer (diagnosed from age of 26 to 64) and 4 women with ovarian cancer (diagnosed from age of 41 to 65) were carriers of BRCA1 p. Met18Lys; whereas only 2 individuals diagnosed with cancers were not carriers (glans penis cancer diagnosed at 53 and breast cancer diagnosed at 61).
Two mutations were segregating in the last family where 2 affected woman were double heterozygote for BRCA1 p. Met18Lys and BRCA2 p.Arg3052Trp (IARC class 5).
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P11.032
BRCA1 Splicing

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BRCA1 is integral for maintaining genome integrity and correct cellular function. It has been shown to play a pivotal role in DNA damage repair, cell cycle control, and gene expression regulation. Importantly, BRCA1 function has been shown to be altered through alternative splicing of BRCA1 pre-mRNA, leading to the expression of BRCA1 splicing isoforms which exhibit diminished function. Two key BRCA1 splicing isoforms have been identified in both breast cancer tissue and cell lines: BRCA1 Δ11 and 11Q. The BRCA1 Δ11 splicing isoform, generated through skipping of exon 11, is associated with dramatic loss of BRCA1 function, particularly in DNA repair. The 11Q isoform is produced through use of an internal 5' splice site in exon 11, generating an mRNA containing a partial section of exon 11. This isoform has a relatively unconfirmed function, exhibiting a contextual effect of promotion or inhibition of cancer cell growth. Developing an understanding of the mechanism involved in the expression of these splicing isoforms in breast cancer, which also utilizes patient identified point mutations, helps us to understand how to regulate their expression for therapeutic benefit. Antisense oligonucleotide's (ASO's) can be used to alter the pattern of splicing, leading to either stimulation or suppression of particular splicing isoforms. ASO's can be applied to alter the splicing pattern of BRCA1 exon 11, and in turn alter the function of the wild-type protein. Endogenously altering BRCA1 splicing isoform expression in this way allows us to explore novel avenues for the development of new breast cancer therapeutics.

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P11.033
Identification pathogenic regulatory and non-coding variants in the 5' region of *BRCA1* and *BRCA2* genes

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BACKGROUND : *BRCA1* and *BRCA2* are major breast/ovarian cancer susceptibility genes, deleterious mutation increase in risk of breast/ovarian cancer. More than 60% of families with a significant cancer history do not have any coding deleterious mutation identified. The hypothesis is there are some variants in the non-coding variants which can have an impact in the risk of breast/ovarian cancer.
METHODS : 384 breast/ovarian cancer probands, *BRCA1/2* negative with the routine screening process, have been explored in four non-coding regions with qPCR-HRM approach and Sanger sequencing: *BRCA2* exon 1, *BRCA1* exon 1a, *BRCA1* exon 1b, *BRCA1* intron 2.
RESULTS : In *BRCA2* exon 1, 4 families with variants were identified (c.-277_-272dup in two families, c.-273G>T, c.-213T>G). All variants were related to very conserved interspecies regions, typical family histories (ovarian cancer, male breast cancer and several breast cancer under 60 year-old) and with several transcription factor sites. In *BRCA1* exon 1, no medium size deletion was identified in a 1.5kb fragment. Only one variant could have an impact (c.-20+279 G>A) close to the Alu sequence. In *BRCA1* intron 2, one variant was detected (c.81-3985A>T) in a family with breast cancers under 40 years-old and which have a potential interaction with the promoter.
DISCUSSION/CONCLUSION: As expected, those variants are very seldom in those regions (1.5% -6/384). Further analyses are needed to confirm any implication in the cancer predisposition (RNA allelic imbalance and luciferase assays). Currently, in the ENIGMA consortium, a database has been created to initiate collaborative work in their classification.

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P11.034
Confirmation of cancer family history: Impact on models predicting *BRCA1/BRCA2* germ-line mutations

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Background: *BRCA1* and *BRCA2* mutations are responsible for 5 to 10% of all breast and ovarian cancer. Risk assessment tools based on personal and family history are used to evaluate the probability of identifying *BRCA1/BRCA2* germ-line mutations. To provide accurate counselling, gathering a detailed family history is important. The aim of this study is to evaluate the importance of confirming all familial cancer cases before using risk assessment tools.
Methods: Clinical and histopathological characteristics of cancer affecting index cases, 1st and 2nd degree-relatives and cousins, have been extracted from medical files for 204 families with *BRCA1/BRCA2* screening performed between 1998-2009. BRCAPRO, BOADICEA and Manchester Scoring System have been retrospectively applied with 3 distinct criteria: 1) using information collected during the 1st consultation; 2) integrating histopathological characteristics for index cases; 3) integrating clinicopathological features for all available cancer diagnoses after the confirmation process. Receiver operating characteristics (ROC) curves were generated to evaluate the performance of each risk assessment tool within these 3 situations.
Results: Histopathological characteristics were validated for all (n=189) affected index cases. Overall confirmation rates among affected relatives were 39% (144/372) for breast cancer and 51% (19/37) for ovarian cancer. Only 7% (27/396) diagnoses changed after the confirmation process. All risk assessment tools showed a statistically significant better performance ($p \leq 0.05$) when all cancer features were taken into account after the confirmation process.
Conclusion: Confirmation of cancer diagnosis among affected index cases and close relatives is associated with a significantly better evaluation of the likelihood of *BRCA1/BRCA2* mutations.

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P11.035

Fifteen years of *BRCA1/BRCA2* screening in a Swiss diagnostic laboratory: Issues with unclassified variants (UVs)

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Introduction: *BRCA1* and *BRCA2* testing has moved from research to clinical setting some years ago. Analytical performance improvement of screening methods has added complexity in interpreting genetic results due to an increased detection of unclassified variants (UVs). We extensively reviewed all *BRCA1/BRCA2* UVs identified between 1996-2009 in a diagnostic laboratory in Geneva, Switzerland.
Methods: Various techniques [SSCP, HA, PTT (1996-1999); DHPLC prescreening, Sanger sequencing confirmation (1999-2007); HRM prescreening, Sanger sequencing confirmation (2007-2009); MLPA (since 2005)] were successively used to identify *BRCA1/BRCA2* germ-line modifications. Significance of all UVs was re-evaluated as follows: 1) referring to gene-specific databases (BIC, LOVD, UMD); 2) using *in silico* tools (Align-GVGD, SIFT, PolyPhen); 3) reviewing literature. BRCAPRO scores, as well as family history characteristics, were collected and compared to *BRCA1/BRCA2* testing results.
Results: 1'163 complete *BRCA1/BRCA2* analyses were performed: 218 (18.7%) index cases were identified as carriers of pathogenic mutations (*BRCA1*: n=134; *BRCA2*: n=84) and 127 (10.9%) as carriers of UVs. Among 114 distinct UVs (*BRCA1*: n=45; *BRCA2*: n=69), 77 were missense variants, 33 intronic variants and 4 in-frame deletions. Before 2009, 3 (2.6%) UVs were reclassified as pathogenic. Distribution of BRCAPRO scores and family history were not statistically different between families with UVs or non informative testing results. Higher BRCAPRO scores ($p<0.0001$) and some particular familial phenotypes, such as ovarian cancer or multiple early-onset breast cancer cases, were significantly associated with identification of *BRCA1/BRCA2* pathogenic mutations.
Conclusion: In our cohort, most *BRCA1/BRCA2* UVs were associated with similar features as non informative testing results.

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P11.036

Spectrum of mutations in predisposing genes to hereditary breast and ovarian cancer in Czech Republic

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BRCA1 and *BRCA2* are major predisposition genes to hereditary breast/ovarian cancer (BOC) but germ-line mutations in these genes account for only 24.1% of inherited BOC cases in Czech Republic. Mutations in additional susceptibility genes, i.e. *CHEK2*, *ATM*, *TP53*, *NBN1*, and *PALB2* were studied in families at high risk of BOC negatively tested for *BRCA1/2* mutations.
Coding sequences of both *BRCA1* and *BRCA2* were analyzed by protein truncation test (PTT) and direct sequencing; patients negative for point mutations and small deletions/insertions were screened for large genomic deletions and rearrangements (LGRs) at *BRCA1/2* by MLPA, long range PCR and genomic sequencing. In patients negative for *BRCA1/2* mutations, the coding region of *TP53* was sequenced using cDNA, two most frequent Czech *CHEK2* alterations (c.1100delC and a 5395 bp deletion comprising exons 8 and 9) were detected by MLPA, and the coding region of *PALB2* was sequenced via cDNA; all mutations were confirmed by direct DNA sequencing.
The four most frequent mutations in *BRCA1* (c.300t>G, c1806C>T, c3819_3823del5, and c 5385dupC) accounted for 69.1% and LGRs for 11.4% of all *BRCA1* mutations. In *BRCA2*, the eight most frequent mutations accounted for 43% and no LGRs were found. Mutations in *PALB2* were present in 2% and two most frequent *CHEK2* mutations tested in 1.2% of our patients. Mutations in *TP53*, *ATM*, and *NBN1* genes were rare.
Mutation testing of other predisposing to BCO genes with moderate risk would help in clinical genetic consulting in Czech families at high risk of BCO negative for *BRCA1/2* alterations.

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P11.037

Evaluation of *ERBB2*, *C-MYC* and *CCND1* gene status and its association with tissue aluminum concentration in breast cancer

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Introduction: It has long been hypothesized whether body tissue uptake of aluminum may have biological or clinical implications in breast cancer. *In vitro* and a few *in vivo* studies have shown that aluminum may trigger genomic instability by interfering directly on the assembly of DNA strands. **Objective:** to examine the relationship of aluminum concentration in peripheral and central areas of breast tumors with the instability of key genes in breast cancer, *ERBB2*, *C-MYC* and *CCND1*, and aneuploidy of chromosomes harboring these genes. **Subjects and Methods:** For this study, 118 samples of breast cancer tissue were obtained. Evaluation of tissue aluminum content was carried out using Graphite Furnace Atomic Absorption Spectrometry. A tissue microarray slide containing the samples was used in FISH assays to assess *ERBB2*, *C-MYC* and *CCND1* and the respective chromosomes 17, 8 and 11 centromere status. Clinicopathological data were obtained from patient records. **Results:** Levels of aluminum >2.0mg/kg were found in 20.3% and 22.1% of the central and peripheral areas, respectively. The amplification and/or aneuploid status for the *ERBB2/CEP17*, *C-MYC/CEP8* and *CCND1/CEP11* were detected in 24%, 36.7% and 29.3% of the tumors, respectively. We found that aluminum concentration was not related to any of these altered gene statuses. **Conclusions:** Recent lines of evidence, including our findings, suggest that aluminum concentration may not directly affect genomic stability in breast tissues. Tissue microenvironment modifications due to the presence of aluminum compounds seems more appealing as possible targets to current and future studies on the implications of aluminum in breast carcinogenesis.

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P11.038

Mutation spectrum and prevalence of BRCA1 and BRCA2 genes in patients with familial and early-onset breast/ovarian cancer from Tunisia : Novel and high proportion of recurrent germline mutations

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Background: Hereditary factors are the most significant risk causing breast cancer and many of these are depending on mutations in BRCA1 or BRCA2 genes. Estimate of pathogenic mutations prevalence of BRCA1/2 genes varies within different populations. The contribution of BRCA1 and BRCA2 mutations to hereditary breast cancer in the Tunisian population has not been accurately estimated.
Methods: We studied 48 unrelated Tunisian high-risk cases selected for their family history of breast /ovarian cancer . Coding regions of BRCA1, BRCA2 and exon-intron boundaries were screened using direct sequencing.
Results: We identified five different pathogenic mutations in twelve families, three in BRCA1 and tow in BRCA2 with a prevalence of 25%. We also identified 20 distinct polymorphisms and unclassified variants. Among BRCA1 mutation carriers, four unrelated families shared the c.211dupA deleterious mutation which seems to be Tunisian specific mutation, since it was described for the first time in Tunisia and was not reported elsewhere. Morover analysis of twelve microsatellite markers in the BRCA1 locus has shown a common haplotype associated with this mutation in all carriers, suggesting a new Tunisian founder mutation.
Conclusion: This study indicates that our population has a spectrum of prevalent BRCA mutations which account for a high proportion of hereditary breast/ovarian cancer, and demonstrated a predominance of BRCA1 germline recurrent mutations, accounting for more than 66% of all identified alterations and appears as founding mutations. Identification of a recurrent mutation could reduce the cost of mutation analysis, for genetic counseling and cancer risk assessment.

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P11.039

The SWEA study - an extended analysis of hereditary breast cancer in Sweden

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The SWEA study (The SWE-BRCA Extended Analysis) is a national collaborative study involving all cancer genetics clinics in Sweden. Within the SWEA study, index cases from breast cancer families are screened for mutations in 17 known breast cancer susceptibility genes: BRCA1, BRCA2, TP53, PTEN, STK11, CDH1, CDKN2A, CHEK2, PALB2, BRIP1, ATM, RAD50, RAD51C, RAD51D, BARD1, NBN and MRE11A. In addition, we screen the protein coding exons of 47 candidate susceptibility genes, selected based on the functional networks in which the known 17 genes exert their effect. Putative risk alleles will be followed up with segregation analysis. All patients who undergo clinical BRCA1/2 screening in Sweden are offered participation in SWEA. The study opened for inclusion in April 2012. In the first nine months over 400 index individuals were screened, and we estimate that over 1200 prospective families will be included within the first two years of the study. We will also perform pooled mutation screening in 5000 healthy controls, 5000 consecutive breast cancer patients and 4000 familial high-risk cases. Preliminary results will be presented.

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P11.040

Detection of copy number variations on oncogenes and tumor suppressor genes by multiplex ligation probe amplification analysis

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Copy number variations (CNVs) on chromosomes 8p, 11q and 17q are among the most common aberrations in breast cancer (BC). Several oncogenes and tumor suppressor genes involved in development and progression of the tumor are located on these chromosomes. Some of them are proposed to predict and guide therapy, but their prognostic value is still a subject of intense study and controversy. The aim of the study was to examine the CNVs in 21 BC related genes and to couple the data with pathological features of tumor that are currently used for prognosis and treatment selection. We analyzed 71 female invasive breast cancers with multiplex ligation-dependent probe amplification kit P078 (MRC Holland). We identified PRMD14 amplification in 42.2% of the patients, MTDH in 35.2%, CCND1 in 31%, MYC in 28.1%, ERBB2 and CDC6 in 22.5%, FGFR and MED1 in 21.2%, IKBKB, TOP2A and AURKA in 19.7%, BIRC5 in 16.9%, CDH1 in 15.5%, ADAM9 in 14.9%. Co-amplification of ERBB2/TOP2A, ERBB2/CCND1, ERBB2/MYC, MYC/CCND1 and MYC/TOP2A genes were observed in 15.5%, 8.5%, 5.6%, 12.7% and 7% of the tumors. Significant association was observed between HER2 positive tumors and high level amplification of 17q genes: MED1, ERBB2 and TOP2A (p=1.63x10-6, 3.52x10-4 and 0.001); between IKBKB gain and increased p53 expression (p=0.003); and CDH1 loss and lobular type of tumor (p=0.036).
In conclusion, MLPA is fast, simple and accurate method for detection of CNVs and can contribute to better understanding of the role of oncogenes and tumor suppressor genes in development and progression of BC.

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P11.041

Qualitative analysis of the CTC levels in Bulgarian patients with metastatic breast cancer

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Background: The vast majority of cancer-related death is due to the metastatic spread of the primary tumour. Circulating tumour cells (CTC) detection has been considered as a reliable biomarker for overall survival and assessment of the therapeutic response in patients with metastatic breast cancer (MBC).
Materials and methods: We have analyzed the CTC levels in 18 MBC patients at three specific points: before therapy, after three and after six cycles of chemotherapy, by commercial AdnaGen diagnostic tests based on CTC isolation from 5 ml blood samples with immunomagnetic separation and RT-PCR of three BC-associated expression markers GA733-2, Her-2 and Muc-1. Samples from 12 healthy women were used as negative controls. The PCR products were analysed by 4% agarose gel electrophoresis and Agilent Bioanalyzer 2100.
Results: CTC were detected in 44.4% (8/18) of MBC patients before therapy. All of the initially negative CTC patients remained negative in the course of treatment. All CTC positive patients lost their expression markers after three cycles of chemotherapy, but one of them became positive after the sixth course. Two patients died and could not be followed. The frequencies of the studied expression markers were 44.4% (8/18) for GA733-2, 33.3% (6/18) for Muc-1 and 11.1 % (2/18) for Her2. Interestingly two of the patients with Her2 negative primary tumor demonstrated positive Her2 expression in CTC.
Conclusions: Our results demonstrated that CTC levels determined by RT-PCR can be used as reliable surrogate biomarker of the clinical outcome and treatment response in Bulgarian patients with MBC.

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P11.042

Breast cancer risk prediction using the novel germline signatures in epigenome regulatory pathways.

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Epigenetic regulatory pathways are intensely studied for their involvement in breast tumorigenesis, however little is currently known about the genetic variation in epigenome components contributing to the risk and/or prognosis of breast cancer. In this study we have tested how novel germline genetic signatures identified in epigenetic regulatory genes (ERGs) may potentially contribute to clinical prediction of breast cancer risk. We have genotyped 711 SNPs tagging 87 ERGs in 1985 breast cancer cases and 1609 controls. The samples were of white European origin with a fraction of Ashkenazi Jewish (AJ) ancestry (n=1642). Associations with risk were assessed using logistic regression, adjusted by age and AJ status. The strongest associations were observed for RUNX1 (rs7280097, OR=0.83, CI 95%: 0.71-0.94, p=0.006) and PRDM16 (rs12135987, OR=1.22, CI 95%: 1.06-1.42, p=0.007). The predictive ability of SNP signatures was tested by ROC curves using logistic regression models, and the area under the curve (AUC) was used to assess their utility in the classification of breast cancer risk. A signature of 20 SNPs tagging 13 ERGs (20-SNP-ERG) was significantly associated with breast cancer risk. The inclusion of predictor variables (age, AJ status) and 20 associated SNPs in the ROC analysis yielded a best fitting model involving 10 SNPs tagging 8 ERGs with AUC of 0.723, compared to 0.660 with predictor variables alone (p=0.003). These results suggest the promising clinical potential of 20-SNP-ERG signature in identification of high-risk individuals in the population, and point to possible biological implication of germline patterns in epigenetic enzymes in breast tumorigenesis.

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P11.043

Prognostic and predictive significance of HOXB13:IL17BR expression ratio in breast cancer

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Breast cancer progression is directly related to estrogen hormone (ER) status of patients. Although most of ER (+) cases benefit from adjuvant endocrine treatment with tamoxifen, almost 40% of these patients are not respond to this therapy. Recent studies have shown that *HOXB13* and *IL17BR* have both significant prognostic and predictive utility. The aim of this study was to investigate the significance of *HOXB13*: *IL17BR* ratio in recurrence/distant metastasis potential of ER (+) primary Turkish breast cancer patients.
HOXB13 and *IL17BR* gene expression levels were determined by quantitative RT-PCR analysis from formalin-fixed paraffin-embedded tissues of 40 tumor and 40 normal primary breast cancer patients receiving adjuvant tamoxifen. ΔCt values were used to build a *HOXB13: IL17BR* index. All statistical analyses were performed using MedCalc 12.4.0 Statistical software.
The expression level of *HOXB13* was 12,2 fold higher and *IL17BR* was 0,0006 fold lower in tumors. Although there was no significant correlation, *HOXB13* and *IL17BR* demonstrated opposing patterns of expression (r=-0.03465, *P*=0.8341). According to two sample T test, the ratio of *HOXB13*:*IL17BR* significantly different between metastatic and non-metastatic tumors of patients ($P < 0.0001$). Also, ROC Curve analysis validated to this significancy (AUC: 0.725, *P*=0.017). In addition, hormonotherapy was associated with longer disease-free (P =0,0166) and median (P =0,0101) survival in the entire cohort.
Even further studies needed, a higher *HOXB13*:*IL17BR* expression may be a candidate biomarker of recurrence /distant metastasis in breast cancer in the setting of adjuvant tamoxifen therapy.

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P11.044

The Importance of MIR-126 in Breast Cancer

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There are many effective mechanisms in cancer development. Breast cancer is the most seen cancer type in women on the World. The effect of microRNAs in cancer and its mechanisms haven't been fully elucidated. Therefore, micro-RNAs are important because of the possibility of as a biomarker of cancer.
In this study, we have investigated and the effect of miR-126, which is thought to play a role in vessel development and the cell movements control like metastasis and invasion in breast cancer cases. Furthermore, in this study, we have also researched the gene expression of miR-126*, the complementary of miR-126, and the Epidermal Growth Factor Like-7 (Egfl-7) mRNA expression, which includes miR-126/126* in breast cancer patients. The decrease of miR-126 gene expression caused an increase in gene expression of Egfl-7, which was inhibited by miR-126.
Firstly we collected peripheral blood samples of breast cancer patients and isolated mononuclear cells. Then, we isolated RNA from these mononuclear cells and determined gene expression levels by using Real Time PCR method.
In our study, we observed a decrease in miR-126 gene expression and an increase in Egfl-7 gene expression when compared to that of healthy people. The decrease of miR-126 gene expression caused an increase in Egfl-7 gene expression, which is then inhibited by miR-126.
Our results supported that miR-126 can be a tumor suppressor in breast cancer.

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P11.045

Analysis of miR-195 Expression and Role in Breast Cancer

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Because of the utility of currently available tumor markers is limited by disappointing sensitivities and specificities, the discovery of novel classes of molecular markers in cancer has provided exciting, potentially viable biomarkers that may have utility in early cancer detection. The potential of microRNAs (miRNAs) as novel tumor markers has been the focus of recent research because of their tissue specificity, stability, and association with clinicopathological parameters. The objective of this study was to investigate expression and potential role in prognosis of miR-195 in breast cancer.
miR-195 expression levels were determined by quantitative RT-PCR analysis from fresh frozen tissues of 72 tumor and 72 normal primary breast cancer patients not receiving neoadjuvant chemotheraphy. The Statistical analysis were performed to clarify the correlation with miR-195 expression level and clinical features of breast tumor tissues.
The expression level of miR-195 was 0,283 fold lower in tumors than normal tissues. Although miR-195 expression levels were not significantly different between tumor and normal tissues of patients according to Independent Sample T test (*P*=0.217), Binary Logistic Regression Analysis demonstrated that there was a significant correlation between miR-195 expression levels and tumor grade (P = 0.023). Also, miR-195 expression levels were associated with tumor size and tumor type (*P*=0.020 and *P*=0.018, respectively).
A low miR-195 expression was related with increased tumor size and grade. So our data imply that miR-195 play role in breast cancer malignancy and may be the potential therapeutic target.

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Segregation Analysis Offers a Mechanism for Variant Reclassification in a Small Subset of Cases but is Especially Powerful in Classifying Deleterious Mutations

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Accurate classification of variants in regards to their clinical significance is a critical challenge associated with gene sequencing tests. Thus, it is important to evaluate the effectiveness of various strategies for variant reclassification. This study compares the use of segregation analysis against other methodologies for the reclassification of variants in Hereditary Breast and Ovarian Cancer (*BRCA1/2*). Reclassification methods independent of additional family testing and powered by Myriad's extensive *BRCA1/2* variant database resulted in 61% of reclassifications, mostly downgrades. Determining if a novel variant segregates with cancer in a family is the classic genetic tool used to assess a variant's effect on hereditary cancer risk. Myriad Genetics' test reports of variant of unknown significance (VUS) and favor polymorphism (FP) are accompanied by an offer of research testing to additional family members. These data are then used to reclassify variants. In this study, we recorded family history submissions from 17% of the VUS/FP results. Research testing was offered to an average of 2.3 relatives per family history submission, with a 24.1% response rate. While we made a significant effort to collect segregation data, this effort resulted in only 3.5% of all variant reclassifications during a 13-month time period. However, of the 10 variant upgrades to deleterious or suspected deleterious, two (20%) were based on co-segregation data. These results illustrate the power of segregation analysis in identifying deleterious variants and suggest that the tailoring of family analysis to specific families may be the most productive use of laboratory and community resources.

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P11.047

A Clinical History Weighting Algorithm Accurately Classifies BRCA1 and BRCA2 Variants

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Genetic testing, including full gene sequencing and large rearrangement analysis for germline BRCA1 and BRCA2 mutations, can identify individuals with Hereditary Breast and Ovarian Cancer syndrome. Current genetic analysis identifies BRCA1 and BRCA2 deleterious mutations as well as variants of unknown clinical significance. Reclassification of uncertain variants to more clinically interpretable categories is critical for patient management. We have developed a statistical algorithm that aids in the assignment of clinical classifications to uncertain variants. This algorithm is based on the premise that disease-associated mutations will be observed more often in individuals at high risk for carrying a mutation, as determined by personal and family history. Statistical analysis weights the family histories of each proband carrying the variant of interest and compares these histories to those of control probands carrying variants known to be benign or deleterious. Data from over 400,000 probands were utilized for algorithm development. This technique was validated by and used to analyze over 6000 BRCA1 and BRCA2 variants. The algorithm successfully classified well-documented variants such as BRCA1 c.181T>G (Deleterious), BRCA1 c.1065G>A (Polymorphism), and BRCA2 c.2808_2811del (Deleterious). The BRCA1 c.5096G>A (Suspected Deleterious with reduced penetrance) and BRCA2 c.7878G>C (Suspected Deleterious with reduced penetrance) mutations were classified as „Not Callable" by the algorithm, consistent with their previous hypomorphic interpretations. This 'history weighting' algorithm allows for the accurate reclassification of BRCA1 and BRCA2 uncertain variants and improved clinical management of at-risk patients. With modifications, this algorithm is expected to be applicable to other autosomal dominant cancer-associated and non-cancer-associated genes.

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P11.048

Germline mutation in the *RAD51B* gene confers predisposition to breast cancer

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Background. Most currently known breast cancer predisposition genes play a role in DNA repair by homologous recombination. Recent studies conducted on *RAD51* paralogs, involved in the same DNA repair pathway, have identified rare germline mutations conferring breast and/or ovarian cancer predisposition in the *RAD51C, RAD51D* and *XRCC2* genes. The present study analysed the five *RAD51* paralogs to estimate their contribution to breast and ovarian cancer predisposition.

Patients and Methods. The study was conducted on 142 unrelated patients with breast and/or ovarian cancer either with early onset or with a breast/ovarian cancer family history. Patients were referred to a French family cancer clinic and had been previously tested negative for a *BRCA1/2* mutation. Coding sequences of the five genes were analysed by EMMA (Enhanced Mismatch Mutation analysis). Detected variants were characterized by Sanger

sequencing analysis.
Results. Three splicing mutations and two likely causal missense variants were identified: *RAD51B* c.452+3A>G, *RAD51C* c.706-2A>G, *RAD51C* c.1026+5_1026+7del, *RAD51B* c.475C>T/p.Arg159Cys and *XRCC3* c.448C>T/p.Arg150Cys. No *RAD51D* and *XRCC2* gene mutations were detected.
Conclusions. This study identified *RAD51B* as a new breast cancer predisposition gene and is the first report of *XRCC3* mutation analysis in breast and ovarian cancer. It confirms that *RAD51* paralog mutations confer breast and ovarian cancer predisposition and are rare events. In view of the low frequency of *RAD51* paralog mutations, international collaboration of family cancer clinics will be required to more accurately estimate their penetrance and establish clinical guidelines in carrier individuals.

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P11.049
Germline copy number variations in BRCA1/2-negative Finnish breast and ovarian cancer families

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BACKGROUND Breast cancer (BrCa) is the most common cancer among women in western countries, including Finland. Inherited factors are known to account for 5-10% of all BrCa cases. Several BrCa susceptibility genes have been recognized but the predisposing factors remain mostly unidentified in majority of the hereditary breast and ovarian cancer (HBOC) families. In recent years, copy number variations (CNVs) have been shown to have an important role in predisposition to several complex diseases. Our aim was to identify germline CNVs contributing to HBOC predisposition in the Finnish population.
METHODS A genome-wide SNP array was performed for 84 HBOC individuals, who have been tested negative for *BRCA1/2*-founder mutations and pre-screened for most common BrCa genes, and 36 healthy controls. Gene-affected CNVs were analysed by Gene Ontology term enrichment, pathway analyses, and database searches. Six potential CNVs were validated and genotyped in 209 additional healthy controls by qPCR.
RESULTS Intronic deletion at *EPHA3* receptor tyrosine kinase was enriched in HBOC individuals (8 of 81, 9.9%) compared with controls (7 of 185, 3.8%) (OR=2.7; *P*=0.05). EPHA3 was identified in several enriched molecular functions including receptor activity. A rare novel deletion affecting intronic region of tumor suppressor gene, *CSMD1*, was observed in 1 of 81 (1.2%) HBOC individuals but it was absent in healthy controls implicating a disease susceptibility.
CONCLUSION The study reveals new information about the germline CNVs that likely contribute to HBOC susceptibility in Finland.

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P11.050
DNA repair gene (XRCC2, XRCC4 and RAD51) polymorphisms and breast cancer in Saudi females

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Double strand breaks in DNA are repaired by different mechanisms, including homologous recombination and non-homologous end joining (NHEJ). We investigated seven SNPs in XRCC2 and RAD51 (homologous repair) and XRCC4 (NHEJ) in 100 Saudi breast cancer patients and 100 controls, to study the association between rs3218536 (G>A) in XRCC2; rs1801320 (G>C), rs1801321 (G>T) and rs2619681 (C>T) in RAD51; and rs2075685 (G>T), rs10474081 (C>G) and rs1120476 (A>G) in *XRCC4* and occurrence of breast cancer in Saudis. Genotyping was performed using genomic DNA obtained from peripheral blood, by TaqMan genotyping assay, PCR-RLFP and DNA sequencing. We observed a highly significant association between breast cancer occurrence and RAD51 rs1801321 (G>T) where the G allele (OR=7.93; p<0.0001) homozygocity (GG) was observed only in the patients and the T allele was significantly protective (OR=0.13; p<0.0001). For RAD51, rs2619681 (C>T) also showed a significant association, where the C allele increased breast cancer risk (OR=2.05; p<0.01) and the T allele was significantly protective (OR=0.49; p<0.01). rs1801320 in RAD51 and the SNPs in XRCC4 did not show any association with breast cancer. rs3218536 (G>A) in XRCC2 showed a marginal association where the OR for the mutant allele was 1.82 and for the wild type allele was 0.54. We will present the results of association between the genotypes and age of diagnosis, family history, disease severity and recurrence risk of cancer in Saudis.

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P11.051
Two novel *RAD51C* mutations in families with breast and ovarian cancer

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Since its discovery by Meindl et al. in 2010, *RAD51C* is defined as a breast and ovarian cancer (BC, OC) susceptibility gene besides the two highly penetrant BC genes *BRCA1/2*. Heterozygous mutations in *RAD51C* are predominantly found in families with both BC and OC. While *BRCA1/2* mutations are found in 21% of the families that fulfill the inclusion critieria of the German consortium of hereditary breast and ovarian cancer (GC-HBOC), *RAD51C* mutations are only found in 1 - 1.5 % of theses families Seven distinct pathogenic *RAD51C* mutations have been described so far in 14 families by the GC-HBOC.
We here describe two novel *RAD51C* mutations segregating in one BC and one BC/OC family respectively. One large genomic deletion including exons 5 to 9 was discovered in a BC family by MLPA technique during screening of 168 non-BRCA BC and 151 BC/OC families. The genomic break point was determined by quantitative real-time PCR followed by Sanger-sequencing. The second mutation, an exonic splice site mutation affecting the last nucleotide of exon 2, *c.*404G>C, was found during clinical screening of *RAD51C* by Sanger-sequencing. RT-PCR revealed the activation of a cryptic splice site in intron 2 with subsequent inclusion of 27 nucleotides from intron 2, leading to a truncated protein *p.*Met136X. The finding of two novel deleterious *RAD51C* mutations supports previous findings that *RAD51C* mutations are prevalent in high risk BC/OC as well as BC only families in Germany and may be included in routine clinical diagnostic testing in selected high risk families.

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P11.052
Identification of breast cancer susceptibility loci in genes unregulated in breast cancer gene-expression arrays

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Breast cancer is a complex, multifactorial, polygenic disease. Some of the familial risk for breast cancer can be explained by rare mutations in high-penetrance genes such as BRCA1 and BRCA2. The failure of linkage studies to identify further susceptibility genes suggests that most familial clustering of cancer is due to a combination of multiple lower penetrance alleles. In an attempt to gain a further insight into the inherited basis of breast cancer we performed a meta-analysis of publically available gene expression profiling data for breast cancer and used this information for prioritizing candidate genes to be investigated. A total of 243 single nucleotide polymorphisms (SNPs) located within or in close proximity to the top 50 meta-analysis genes were selected and subsequently genotyped in the MASTOS study cohort (which consists of 1109 Cypriot breast cancer patients and 1177 healthy controls) using the Sequenom Mass-ARRAY platform. Of the 243 SNPs studied, eighteen were found to be statistically significantly associated with breast cancer risk. Of these eighteen SNPs, two are located in the HMMR gene, two in the NTRK2 gene and the rest in the FLJ10357, KIT, TGFBR3, TXNIP, ZBTB16, MME, GSN, CKS2, EGR1, PTRF, NEK2, SYNM, ANXA1 and MTHF02 genes. Our study demonstrates that this approach is useful for disease risk loci identification in genes which are upregulated in breast tissues and are thus potentially important in the carcinogenesis pathway. However, large-

scale replication in multi-ethnic cohorts is warranted in order to replicate our results and confirm the associations observed in our population.

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P11.053

Analysis of the BRCA1 promoter methylation in triple-negative sporadic breast cancer

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Background: Breast cancer is the most frequent neoplasia in women. Approximately 5% of them are hereditary, caused by inherited mutations in breast cancer susceptibility genes: BRCA1 and BRCA2. The 95% remaining are sporadic. Epigenetic regulation, especially promoter methylation, is an important mechanism for down-regulating tumour suppressor genes in human cancer cells. Methylation of the BRCA1 promoter has been reported in sporadic breast cancer with links to reduced mRNA and protein expression in tumours and cancer cell lines.
The aim of our study was to estimate the percentage of methylation in the promoter region of BRCA1 oncosupressor gene, in patients from Salamanca (Spain), diagnosed with sporadic breast cancer and to compare these results with their clinical data.
Patients and methods: Genomic DNA was extracted from paraffin blocks from triple-negative breast cancer samples of 98 women. After bisulphite conversion, DNA methylation was measured by quantitative analysis of methylated alleles (QAMA), to estimate the extent of methylation of 2 CpG sites in the promoter region of BRCA1 gene. Association between methylation and clinico-pathological features was evaluated using statistical tests with SPSS.
Results and conclusion: Preliminary analysis showed a big percentage of cases with BRCA1 methylation with an inverse relationship between C-KIT gene expression and BRCA1 methylation (p=0.017).
Our results show that BRCA1 promoter is an important mechanism of BRCA1 gene silencing in sporadic triple-negative breast cancer.

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P11.054

Breast cancer, IL6 gene polymorphism and infection with Torque teno virus: a pilot association study

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Inflammation leads to dysplasia and was associated with cancer development.
The -174C allele of interleukin 6 -174G>C gene polymorphism is assumed to confer breast cancer risk. Torque teno virus (TTV) was proven to increase production of IL6.
The aim of this study was to assess if the combination of IL6-174G>C genotypes and TTV infection represents a risk factor for breast cancer.
Blood samples were collected from 200 subjects (100 breast cancer patients and 100 healthy controls). Subsequent to total DNA extraction, genotyping of IL6 polymorphism and presence of TTV were assessed with PCR based methods.
IL6-174G>C genotype distribution respected the Hardy-Weinberg equilibrium (p>0.05). The IL6-174CC genotype was more frequent in cancer patients (p=0.7). In our study, the presence of the C allele was not associated with breast cancer (p=1.7).
The average prevalence of TTV was 77%, with a significantly higher value in cancer patients (p=0.001; OR=0.3). The presence of C allele was not associated with TTV infection (p=0.3) when both cohorts were analyzed. However, cancer patients carriers of C allele were prone to TTV infection (p=0.01).
TTV was more prevalent in cancer patients. Our results disagreed with other studies that reported IL6-174C allele as a risk factor for breast cancer. Nevertheless, the combination of IL6-174C allele and infection with TTV showed a modest association with breast cancer in our cohort.

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P11.055

Genetic heterogeneity of amplification of ERBB2 status in breast invasive carcinoma with 2+ HER2 immunostaining:a challenge for genetic scoring.

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The genetic heterogeneity of *ERBB2* gene amplification (GA) in breast cancer has previously been described, but its clinical significance remains unknown. We studied the genetic categories of a series of consecutive 4491 invasive breast carcinomas 2+ IHC, with a focus on cases with *ERBB2* GA detected in minor clone(s). We compared the *ERBB2* status in primary tumors and positive axillary lymph nodes (ALN) to test the hypothesis that HER2 amplified cells are more aggressive and metastase quicker than non amplified cells. Heterogeneous amplification was defined as the presence of 5-50% of amplified cells (ratio>2,2) within the tumors otherwise classified as not amplified or borderline.
The genetic heterogeneity of IHC 2+ tumors is a common event. FISH can classify these tumors into 5 genetic categories (monosomy, disomy, polysomy, ERBB2 gain and GA). 48 cases (10% of 2+ cases) declared as negative cases contained one or several *ERBB2* amplified clones. All types of clones (amplified and not amplified) have a metastatic capacity. We continue our work in collaboration to:
1) Find out a proportion of such patients in HER2 IHC 2+ group in other hospitals
2) Study a status of the gene in relapsed cases
Institut Gustave Roussy Guidelines for *ERBB2* FISH heterogeneous and difficult cases will be proposed in order to help to analyse the breast cancers and reach accurate conclusions.

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P11.056

Genetic polymorphisms and breast cancer risk

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Breast cancer risk in BRCA1/2 mutations carriers appears to be modified by different genetic or environmental factors. GWAS (genome-wide association studies) identified genome regions that contain SNPs that appear to be associated with breast cancer risk in high penetrance genes mutation carriers. Specific variations in this regions act as risk modifiers, however their mechanisms of action remain unknown.
The aim of the study was to determine if genetic variants could be associated with different phenotypes of cancer, segregated in function the presence/absence of BRCA mutations and whether risk modification by these genetic variants is associated to immunohistochemical characteristics.
Genomic DNA was extracted from peripheral blood by standard techniques. We selected 8 non-synonymous SNPs that are involved in risks modification. Studies were performed using TaqMan probes for the analysis of polymorphisms: rs2981582 (FGFR2), rs13281615 (8q24 region), rs2910164 (miR146), rs799917 (BRCA1), rs1042522 (TP53), rs16941 (BRCA1), rs16942 (BRCA1) and rs2279744 (MDM2).
Allelic distribution were studied in three group of patients: Familiar breast cancer patients with BRCA mutation, breast cancer patients without BRCA mutation and less than 3 relatives affected, and breast cancer patients without BRCA mutation and 3 or more relatives affected. Statistical analysis was performed using SPSS.
Preliminary analysis showed a strong association between rs2981582 and BRCA mutation carriers, the association of rs2910164 with breast cancer incidence according to age, and association of rs799917 with breast cancer incidence according to presence/absence of hormonal receptors. These results support the hypothesis that SNPs in high penetrance genes mutation carriers could act as risk modifiers.

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P11.057

Determining a role for germline mutations in genes associated with an intermediate risk for breast cancer in the Belgian population

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In our patient population *BRCA1&2* germline mutations are identified in less than 15% of patients referred for genetic testing. In this study we evaluated the mutation prevalence of *PALB2* and *ATM* mutations in *BRCA1/2* mutation negative families referred for genetic testing.
We screened the complete UTR, coding and splice site regions of these genes with high resolution melting curve analysis followed by Sanger sequencing of the aberrant melting curves. *In silico* predictions of variants with an unclear clinical significance was performed with the Alamut software.
We identified 10 mutations predicted to lead to a premature stop codon or to affect splice sites: 4 *PALB2* mutations in 357 unrelated patients (1.1%) and 6 *ATM* mutations in 190 unrelated patients (3.2%). In case more family members were available, we confirmed segregation of the mutation with the disease. In addition, we detected 11 missense variants (4 in *PALB2* and 7 in *ATM*), which may also affect protein function, based on *in silico* prediction programs. Deleterious *PALB2* and *ATM* mutations were exclusively identified in familial and not in sporadic cases with early onset or bilateral breast cancer. Interestingly, the average age at diagnosis for the *PALB2* (62y; range 48-71) mutation carriers was higher compared to the average age at diagnosis of the *ATM* carriers (44y, range: 33-66) and the non carriers (39y, range: 25-78).
Cancer prevention guidelines have not been established for *PALB2* or *ATM* heterozygous carriers. Currently screening schedules are based on family history of breast cancer.

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P11.058

BRCA2-c.2808_2811del is located in a mutational hotspot and has multiple origins in Spanish population

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BRCA2-c.2808_2811delACAA is one of the most reported germline mutations in non-Ashkenazi breast/ovarian cancer patients. We aimed to investigate its genetic origin in Spanish carrier families.
Mutation carriers of 51 families were genotyped with seven microsatellites and four intragenic *BRCA2* SNPs. Mutation age was calculated with the DMLE software. DNA secondary structure was predicted with Quikfold. Random mutagenesis experiments were performed over a 923-bp fragment of *BRCA2*.
Three independent founder haplotypes associated with c.2808_2811delACAA were clearly distinguished that accounted for 23 families (West Castilla y León/WCL), 20 families (East Castilla y León/ECL) and 6 families (South of Spain). Mutation age was estimated in a range of 45-68 and 45-71 generations for WCL and ECL haplotypes, respectively. The most prevalent variants of this DNA region, c.2808_2811delACAA and c.2803G>A, were located in a double-hairpin loop structure (c.2794-c.2825) that was proposed as a candidate mutational hotspot. To check this hypothesis, random mutagenesis was performed and a total of 86 DNA variants were characterized. Interestingly, three mutations reported in the mutation databases (c.2680G>A, c.2944delA and c.2957dup) were replicated in this experiment and 20 affected the same position with different nucleotide changes. Moreover, five variants were placed in the same hairpin-loop of c.2808_2811delACAA, and one of them (c.2808A>G) affected the same position.
Our results support that at least three different mutational events occurred to generate this mutation. A relevant fraction of DNA variants, including *BRCA1*-c.68_69delAG and *BRCA2*-c.5946delT and c.8537delAG, are preferentially concentrated in predicted hairpin-loops suggesting that these structures may represent mutational hotspots.

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P11.059

A high rate of mutations is identified in a single exon of the *CHEK2* gene in Greek breast/ovarian patients

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CHEK2 gene encodes for a cell cycle checkpoint kinase that is activated by ATM in response to DNA damage and interacts with and phosphorylates BRCA1 and influences its role in DNA repair pathway by homologous recombination.
Certain mutations in CHEK2 are associated with increased risk of breast cancer in women, while 80% of CHEK2-associated breast cancers are ER and PR positive.
The aim of this study is to elucidate CHEK2 breast cancer susceptibility in Greek breast/ovarian patients.
We analyzed, 466 women with breast/ovarian cancer diagnosed with synchronous or metachronous cancer, or having at least one family relative diagnosed with breast and/or ovarian cancer and 223 women diagnosed with early onset breast/ovarian cancer (<45 years). All patients have been previously tested negative for BRCA1 founder and recurrent mutations.
Missense and loss-of function mutations were detected in 17/466 (3.6%, mean age 42,2 years) patients previously reported with family history and in 10/223 (4.85%, mean age 34,7 years) of early onset breast cancer patients. Remarkably, all the identified variants were located on exon 3 of the gene.
The present study demonstrates the existence of a large proportion of CHEK2 mutations within breast/ovarian cancer patients in Greece, all of which identified in exon 3. Women with early-onset breast/ovarian cancer should be considered for CHEK2 mutation testing, after a negative BRCA1 and BRCA2 result.
This research has been co-financed by theESF and Greek national funds through the Operational Program „Education and Lifelong Learning" of the NSRF - Research Funding Program of the GSRT: THALIS.

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P11.060

Identification of tumor microRNAS in plasma samples from hereditary breast cancer patients

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There is increasing evidence that microRNAs (miRNAs) may be good biomarkers for diagnosis, prognosis or monitoring of various diseases. It has been observed that miRNAs are present in detectable levels in a variety of body fluids, and it has been shown that serum/plasma miRNAs are stable and resistant to degradation. We analyzed the expression of miRNAs in plasma samples from patients with hereditary breast cancer compared to the expression in blood plasma of healthy women to establish their role as disease markers with diagnostic utility.
Based on miRNA expression profile data of hereditary tumors previously set by microarray analysis, 19 miRNAs were selected because they were globally expressed at high levels in tumors, to study their presence in plasma of breast cancer patients. Expression in tumors was previously validated by quantitative PCR.
RNA from plasma samples was extracted (miRNeasy kit, Quiagen) in 20 women with hereditary cancer (BRCA1, BRCA2 and BRCAX) and 20 female controls. The expression level of the 19 tumoral miRNAs, 3 control serum miRNAs, 2 interplate calibrators, were analyzed by quantitative PCR (qPCR), using a customized designed Pick&Mix specific panel (Exiqon), and the detection system of miRNAs from Exiqon (LNA miRCURY miRNAs Universal RT PCR).
We were able to identify significant increased expression of 4 tumor specific miRNAs in plasma samples of women with breast cancer, compared to the control samples ($p<0.05$).

The detection of tumor-specific miRNAs in plasma support them as promising noninvasive biomarker with potential utility in the diagnosis or monitoring of these tumors.

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P11.061
The common missense polymorphism rs2229742 in NRIP1 is associated with non-BRCA hereditary breast cancer risk

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Non *BRCA1* or *BRCA2* related hereditary breast cancer (HBC) represents 80-85% of all HBC families. Increasing evidence highlights the contribution of low-risk variants such as common polymorphisms to a polygenic HBC model. Many of the genes involved are related to either DNA repair or hormone metabolism. Several studies have suggested that a common polymorphism in the *NRIP1* gene, Arg448Gly (c.1342C>G; rs2229742) might be associated with pathologies involving estrogen signaling. In this case/control study, we investigated the contribution of this variant in 159 non-*BRCA* HBC cases and 416 unaffected controls. The frequency of the G allele (Gly448) was significantly higher in cases compared to controls (16.0% *vs* 9.7%; p = 0.0024). The risk of breast cancer increased in carriers of at least one copy of this allele (p < 0.001; 95% CI, 1.33-3.05) with an OR of 1.79 (95% CI, 1.23-2.61). Functional tests confirmed that the Gly448 variant reduced the intrinsic transrepression activity of the central NRIP1 repressive domain. Altogether, our data indicate that *NRIP1* Arg448Gly might influence the risk of HBC in the absence of *BRCA* mutations.

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P11.062
Comprehensive and rapid approach detects the most frequent BRCA1 and BRCA2 mutations in Slovak HBOC population

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Detection of germline mutations in BRCA1/2 genes represents, according to many facts (e.g. lack of mutational hot spots, several types of mutations, genomic rearrangements, size and number of exons) serious methodical problem. Complex analysis of both genes by direct sequencing is also time-consuming and financially demanding and even after all this effort, approximately 70% of families remains negative for the presence of any causal mutation.
Here we describe a rapid and comprehensive approach for the detection of most frequent mutations in BRCA1 and BRCA2 genes in Slovak HBOC population. Currently, we are able to detect 77% of all BRCA1/2 mutations detected in our population, namely 13 mutations in BRCA1 (c.68_69delAG, c.181T>G, c.1938_1947del10, c.1953_1956del4, c.2068delA, c.2921T>A, c.3016_3019del4, c.3018_3021del4, c.3700_3704del5, c.3770_3771del2, c.4065_4068del4, c.4243delG, c.5266dupC) and 4 different mutations in BRCA2 gene (c.3076A>T, c.5645C>A, c.9098dupA, c.9403delC).
Altogether, methodology comprises only 3 multiplex PCR reactions, 2 of which serve as PCR templates for SNaPshot PCR and third is a multiplex fluorescently-labeled PCR. SNaPshot PCR analysis detects selected point mutations (substitutions as well as 1-2 bp deletions/insertions) and the fluorescently-labeled PCR detects frame-shift mutations in three large regions of exon 11 of BRCA1 gene.
This approach allows us to pre-screen all the samples, assign preliminary results and finally, in the case of mutation carriers, apply a preventive management to specific patients as soon as possible. This method represents very time and financially efficient approach with relevant sensitivity (77% of all HBOC positive families), which can be offered to a broad spectrum of women.

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P11.063
Pharmacogenomic assessment of outcomes of cisplatin-based chemotherapy in ovarian cancer patients

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Background: Platinum drugs are among the most active and widely used chemotherapeutic agents. However, their effective clinical use is impeded by significant variations in both the response rate and the rate of adverse reactions and requires the identification of genetic markers that can be used to screen patients before treatment. Here, we have evaluated the associations between polymorphisms in approximately 100 genes, involved mainly in drug metabolism, DNA repair, cell cycle and apoptosis, and outcomes in ovarian cancer patients receiving cisplatin-cyclophosphamide chemotherapy. **Methods:** DNA was isolated from blood samples of 104 patients by proteinase K treatment. 228 single nucleotide polymorphisms were genotyped using the arrayed primer extension technology. Correlations between the polymorphisms and survival were assessed using the Kaplan-Meier product limit method and the log-rank test and $\chi 2$ test. To evaluate the associations between the genotypes and treatment toxicity, patients were classified as having good or poor tolerance. **Results:** SNPs in *GSTP1*, *TPMT*, *NQO1*, *COMT* and *GRPR* genes were significantly associated with progression free survival. Polymorphisms in *NAT2*, *GSTA4*, *GSTM3* and *CCNH* genes were associated with anemia. SNPs in *ERCC5*, *RAD52*, *MUTYH* and *LIG3* genes correlated with the occurrence of severe neutropenia. Thrombocytopenia, emesis, nephrotoxicity and neurotoxicity were associated with SNPs in *MSH3*, *MSH6*, *EPHX1* and *CYP1A1* genes, respectively. SNP in *ADH1C* gene correlated with complete tumor response. **Conclusions:** The obtained results suggest that polymorphisms in different genes involved in drug metabolism can be important in distinguishing subgroups of cancer patients who would have benefit from cisplatin-based treatment.

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P11.064
RAD51D germline mutations in Greek ovarian cancer patients

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Ovarian carcinoma is the most common cause of cancer death from gynecologic tumors. One in four ovarian cancer cases is now known to be attributed to germline mutations in at least 16 genes. Germline mutations in the BRCA1 and BRCA2 genes contribute to approximately 18% of hereditary ovarian cancers, 1% is associated with deleterious mutations in MMR genes, while 5% is associated with other genes of the Fanconi Anemia- BRCA DNA repair pathway.
Recent studies have shown that 0.8-0.9% of BRCA1 and BRCA2-negative ovarian cancer cases are positive for loss-of-function mutations in RAD51D. The aim of this study was to identify the prevalence of RAD51D mutations in Greek ovarian cancer patients, both in familial and sporadic cases that were previously found negative for the most common BRCA1 mutations. Subsequent analysis of the full BRCA1 gene in familial cases did not identify any mutations. We thus sequenced the full coding sequence and intron-exon boundaries of RAD51D in 235 sporadic and 60 familial cases. We identified one deleterious mutation and two probably damaging unclassified variants. The present data provide the first evidence for the existence of RAD51D mutations in hereditary ovarian cancers in Greece. Women with ovarian cancer should be considered for RAD51D gene testing after a BRCA1-BRCA2 negative test result, irrespective of ovarian cancer family history, or age at diagnosis.This research has been co-financed by the European Union (ESF) and Greek national funds through the Operational Program „Education and Lifelong Learning" of the NSRF - Research Funding Program of the GSRT: ARISTEIA.

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P11.065
DICER1 mutations in non-epithelial ovarian tumours

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Non-epithelial ovarian (NEO) tumours make up 10% of all primary ovarian cancers. They include germ cell tumours (GCTs), sex cord-stromal tumours (SCSTs), and other rare tumours. GCTs include dysgerminomas, embryonal carcinomas, yolk sac tumours, teratomas, and mixed GCTs. SCSTs represent 3-5% of ovarian malignancies and include granulosa cell tumours and Sertoli-Leydig cell tumours (SLCTs). SLCTs and juvenile granulosa cell tumours, like GCTs, usually occur in younger women.

DICER1 is involved in cleavage of dsRNA to miRNA. Somatic mutations in the *DICER1* gene have been found in various types of NEO tumours, specifically around the RNase IIIb domain metal-binding sites. These mutations reduce the number of miRNAs from 5p strands, leaving those from 3p strands to target mRNAs. Given that recent reports and unpublished data (Foulkes lab) suggest that deleterious somatic mutations outside of RNase III are uncommon, we focused on the RNase III domain. We have sequenced the RNase III regions of *DICER1* in 184 cases of NEO tumours, and 32 samples of lymphocyte DNA from affected patients (Tables below). Our study confirms that somatic mutations in *DICER1* are frequent in Sertoli-Leydig cell tumours (SLCTs) and could represent a therapeutic target. They are uncommon in other types of NEO tumours.

Table 1. Mutations found and Number of Samples Analyzed

Tumour Type	Mutations found/ Total Tumour Samples	Mutations found/ Total Lymphocyte DNA Samples
GCT		
Dysgerminoma (5 cases of Gonadoblastoma)	1/55	0/4
Mixed GCT	1/18	0/9
Yolk Sac Tumour	2/40	0/3
Embryonal Carcinoma	0/2	-
Immature Teratoma	0/29	0/5
Mature Teratoma	0/21	0/10
SCST		
Juvenile Granulosa Cell Tumour	0/2	-
SLCT	7/14	0/1
Unclassified SCST	0/3	-
Total	**11/184**	**0/32**

Table 2. Mutations found in *DICER1* RNase IIIb region

Tumour Type	Codon Change	Predicted Effect
Gonadoblastoma/Dysgerminoma	c.5429 A>G	Exon 25 Skip
Yolk Sac Tumour	c.5428 G>T	Cryptic Splice site
Yolk Sac Tumour	c.5438 A>G	Exon 25 Skip
Yolk Sac Tumour/Dysgerminoma	c.5438 A>G	Exon 25 Skip
SLCT	c.5429 A>T	p.D1810V
SLCT	c.5439 G>C	p.E1813D
SLCT	c.5438 A>G	Exon 25 Skip
SLCT	c.5437 G>A	p.E1813K
SLCT	c.5437 G>A	p.E1813K
SLCT	c.5437 G>C	p.E1813Q
SLCT	c.5437 G>C	p.E1813Q

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P11.066

C1orf24 is negatively regulated by miR-106b-5p in thyroid tumorigenesis process.

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We have previously shown that the *C1orf24* geneis highly expressed in follicular thyroid carcinomas while is not expressed in benign lesions. However, little is known about the molecular mechanism involved in *C1orf24* expression. It is widely demonstrated that microRNAs (miRs) are potent regulators of gene expression. The miRs expression varies in according of tissue, development stage, and tumor types. In this study, employing three algorithms (miRBase targets, TargetScan and microRNA.org) we identified miR-17-5p and miR-106b as potential candidates to regulate the expression of *C1orf24*. We initially investigated the expression of miR-17-5p and miR-106b in 64 thyroid lesions by qPCR. Although both miRs showed lower expression in thyroid carcinomas, compared to benign lesions, the difference was considered statistically significant only for miR-106b ($p<0,01$). Then, miR106b was transiently transfected into the follicular thyroid carcinoma cell line (WRO). Ectopic expression of miR106b inhibited *C1orf24* mRNA and protein in WRO cells, when compared to negative control. To show that miR-106b directly interacts with *C1orf24* mRNA at 3' UTR, we then transfected PCCL3 cells with both *C1orf24* 3'-UTR mRNA wild type and mutated. We observed that miR-106b directly interacts with *C1orf24*. Our findings indicate that miR-106 acts as tumor suppressor gene and plays a role in thyroid carcinogenesis, at least in part by negatively regulating *C1orf24* expression. However, further analysis is needed to fully demonstrate the role of miR-106b in regulating *C1orf24* expression and, therefore, its role on thyroid carcinogenesis.

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P11.067

A European OncoNetwork Consortium study to develop Ion AmpliSeq™ Colon and Lung Cancer Panel to detect most frequent somatic CRC or NSCLC mutations on FFPE samples.

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Several somatic mutations have been associated with therapeutic benefit in CRC or NSCLC. Thus, there is a need for simultaneous screening of multiple targetable gene mutations using small amounts of DNA. The OncoNetwork Consortium made up of 8 European Cancer Translational Research Labs developed an approach to identify hotspot mutations in 22 CRC or NSCLC related genes using IonAmpliSeq™ technology. The analyses were performed using the IonAmpliSeq™ workflow, with 10 ng of DNA amplified in a single multiplex PCR. Sequencing was carried out with the PGM™ Sequencer on Ion316 chip (8 samples per chip) and results were analyzed using the IonReporter™ Software. In phase 1, all labs tested the same 5 control FFPE samples. In phase 2, each lab sent 10 characterized FFPE samples for blind analysis by another lab. In phase 3, each lab tested 15 of their own FFPE samples. A total of 140 unique FFPE samples were screened. Overall, the percentage of on target reads was greater than 85 % with an average per base accuracy >98%. The 6 labs that completed all phases confirmed detection of all expected variants in both controls and blind samples, corresponding to a 100% true positive rate. For example, a sample of phase 3 with low allele frequency had a mean coverage of 6119, allowing detection of KRAS-G12D variant at 2%. The Ion AmpliSeq™ Colon and Lung Cancer Panel allows fast, highly sensitive and reproducible detection of frequent mutations from only 10ng of DNA from FFPE CRC or NSCLC samples.

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P11.068

Prognostic significance of IDH1 mutations and 1p/19q co-deletion in patients with gliomas

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Background:

Somatic mutations in IDH1, which encodes cytoplasmic isocitrate dehydrogenase 1, were reported to occur at high frequency in glial tumours. In astrocytomas these aberrations are commonly found together with loss of 1p and/or 19q. Chromosomal co-deletion of the short arm of chromosome 1(1p) and the long arm of chromosome 19(19q) are found most frequent in oligodendrogliomas. Both events, separately or combined, are associated with favourable prognosis and improved overall survival not only in oligodendrogliomas, but also in astrocytomas even though they are rarer in astrocytic tumours.

Methods:

We screened 65 gliomas (astrocytomas and oligodendrogliomas) by direct

sequencing of exon 4 of IDH1 gene and MLPA analysis of large genomic deletions within chromosome 1p and 19q performed using SALSA MLPA kit P088.
Results:
IDH1 abberations were found in 25 (38%) glial tumours of WHO grade II, III and IV. In 24 patients mutations were at position 395 causing amino acid substitution R132H and in one -amino acid substitution R132L. Mutations in IDH1 were associated with better overall survival (median survival 22.9 months vs. 9.1 in non-mutated cases; p=0.006). MLPA analysis showed loss of 1p and/or 19q in 28% of cases but no significant association with the overall survival of patients. However, the combination of 1p/19q co-deletion and IDH1 mutations were associated with increased overall survival (p=0.002).
Conclusion:
Our results indicate that IDH1 mutations combined with chromosomal co-deletions of 1p and/or 19q identify gliomas with better survival and may be used as specific prognostic biomarkers in patients with gliomas.

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P11.069

A Functional Variant, rs967591G>A, in the 19q13.3 and Survival of Early-Stage Lung Cancer

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To define the causative functional SNP of the association, this study was conducted to investigate the associations between single nucleotide polymorphisms (SNPs) in 19q13.3 and survival of early-stage non-small cell lung cancer (NSCLC) patients.
A two-stage study design was used to evaluate five SNPs in relation to survival outcomes in 328 patients and then to validate the results in an independent patient population (n= 483). Luciferase assay and real-time PCR was performed to examine functional relevance of a potentially functional SNP. Of the five SNPs, three SNPs (rs105165C>T, rs967591G>A and rs735482A>C) were significantly associated with survival outcomes in a stage 1 study. The rs967591A allele had significantly higher promoter activity of *CD3EAP* compared with the rs967591G allele (*P* = 0.002), but the SNP did not have an effect on the promoter activity of *PPP1R13L* in promoter assay. The rs967591G>A was associated with the level of *CD3EAP* mRNA expression in lung tissues (*P* = 0.01). The rs967591G>A exhibited consistent associations in a stage 2 study. In combined analysis, the rs967591 AA genotype exhibited a worse overall survival (adjusted hazard ratio = 1.69, 95% confidence interval = 1.29-2.20, *P* = 0.0001). The rs967591G>A affects *CD3EAP* expression and thus
influences survival in early-stage NSCLC. The analysis of the rs967591G>A polymorphism can help identify patients at high risk of a poor disease outcome.

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P11.070

Large rearrangements involving intron 2 of *CDH1* and breast cancer susceptibility

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Background: Germline mutations in the *CDH1* gene are associated with hereditary diffuse gastric cancer. Large rearrangements in *CDH1* are now being reported as well. Because *CDH1* mutations can be associated with breast cancer, rearrangements could also impact susceptibility for this disease.
Methods: A zoom-in CGH array that covered exonic, intronic and flanking regions of the *CDH1* gene was used to screen a cohort of 148 *BRCA1/2* negative probands. *CDH1* intron 2, including several regulatory regions, was specifically targeted. Real-time RT-PCR has been used to assess *CDH1* expression in related lymphoblastoid cell line and also in breast tumors.
Results: Two large rearrangements of *CDH1* exon 3 causing a premature stop were detected. In both families, screened also for *BRCA1/2*, lobular breast and gastric cancers were reported. Two large rearrangements within intron 2, a deletion and a duplication, were also reported in probands with breast cancer and without any familial gastric cancer. Recurrent duplication copy number variations are found in this region. The deletion encompasses an expressed sequence tag and resulted in *CDH1* RNA downregulation. Two novel transcripts starting at the deleted region were characterized.
Conclusion: No germline rearrangements in the *CDH1* coding region were detected in families without gastric cancer and with a broad range of breast cancer susceptibility. This study confirms the diversity of large rearrangements in *CDH1*. The intron 2 deletion highlights a putative role for intron 2 in *CDH1* regulation. The four herein described *CDH1* rearrangements involving intron 2 could modulate the risk of breast cancer.

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P11.071

Genome-wide association study identifies multiple loci for cervical cancer

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Cervical cancer is caused by infection with human papillomavirus (HPV). Our understanding of the genetic basis of cervical cancer is still limited. We performed the first genome-wide association study (GWAS) by analyzing 731,422 single nucleotide polymorphisms (SNPs) in 1,075 cervical cancer cases and 4,014 controls, followed by replication in 1,140 cases and 1,058 controls. Three independent loci in the major histocompatibility complex (MHC) region at 6p21.3 were identified to be associated with cervical cancer; the first is adjacent to the MHC class I polypeptide-related sequence A gene (MICA) (rs2516448, odds ratio [OR]=1.42, Pcombined=1.6×10-18), the second is between HLA-DRB1 and HLA-DQA1 (rs9272143, OR=0.67, P-combined=9.3×10-24) and the third at HLA-DPB2 (rs3117027, OR=1.25, P-combined=4.9×10-8). Using imputed alleles at classic human leukocyte antigen (HLA) loci we confirmed previously reported associations of B*0702 and DRB1*1501-DQB1*0602 with susceptibility (OR=1.42, P=7.9×10-8; OR=1.39, P=3.8×10-7, respectively) and DRB1*1301-DQA1*0103-DQB1*0603 with protection (OR=0.47, P=8.8×10-10). However, none of them can account for the effects of the three novel loci. The nature of the new loci and their effect on the susceptibility will be discussed. The results demonstrate the presence of additional loci in the MHC region with a stronger effect on the disease susceptibility than those previously identified.

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P11.072

Mitochondrial (mt)DNA D-loop region SNPs in human papillomavirus-related cervical neoplasia

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Cervical cancer is the second most common malignant neoplasm in women and high risk human papillomavirus (hr-HPV) is known to be its etiological agent. On the other hand, alterations in the non-coding displacement (D) loop mtDNA are present in many cancers, suggesting that the extent of mtDNA mutations might be useful in the prognosis of cancer outcome.
In order to evaluate the role of T239C, A263G, C150T, T16189C and C16223T mtDNA polymorphisms in cervical lesions, 60 cervical specimens were investigated. Isolated DNAs from cervical cells were tested for HPV presence. TaqMan SNP Genotyping Assays were used for SNP detection.
In patients with normal cytology and ASCUS (Atypical Squamous Cells of Undetermined Significance), hrHPV was absent. 72.2% (13/18) LGSIL (Low-Grade Intraepithelial Lesion) and 93.3% (14/15) HGSIL (High-Grade Intraepithelial Lesion) patients presented infection with hrHPV in single or

co-infection represented mainly by HPV16,18,31 and 33. We detected a significantly greater incidence of mtDNA polymorphisms T239C, A263G and C150T and a lower incidence of T16189C, C16223T in cervical lesions. C150T and T239C polymorphisms were corelated with hrHPV-positive LGSIL and HGSIL lesions (P<0.05). HPV-positive individuals were more likely to carry the C150T, A263G and T16189C polymorphisms than HPV-negative controls (P<0.003). A positive corelation between HPV-positive HGSIL patients and A263G SNP was found. In all cancer subjects, an increased risk of HPV infection was associated with the selected polymorphisms.
These findings suggest that mitochondrial SNPs in the D-loop region may represent a cofactor in HPV-induced neoplasia but their carcinogenesis mechanism remains to be solved.

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P11.073

Molecular cytogenetic analyses of *hTERC* (3q26) and *MYCC* (8q24) genes amplifications in 30 patients with cervical carcinomas: correlations with clinical outcome

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Cervical cancer remains one of the most common malignancies among women worldwide in both incidence and mortality. Although infection of high-risk human papilloma virus (HPV) is recognized as an essential initiating event in cervical tumorigenesis, this alone is not sufficient for the progression to invasive cancer. Some chromosomal aberrations have been associated with the progression of CIN to carcinoma, especially the amplification of *hTERC* (3q26) and *MYCC* (8q24) genes.
In this study, tumour samples of patients with cervical spino- or adenocarcinoma (stage IA-IVA) were studied using fluorescence in situ hybridization with the Cervical FISH Probe Kit and Agilent 244K or 180 K microarray. The results of molecular cytogenetic analyses were compared with clinical findings.
HPV infection was proven in 97 % (29/30) of patients, *hTERC* copy number changes were found in 26 from 30 patients (87 %). *hTERC* alteration were detected in 3/4 patients with IA, in 17/19 patients with IB, in all 5 patients with IIIB and in 1/2 patients with IVA TNM stage. Copy number changes of *MYCC* gene were found in 18/30 patients (60 %); in 1/4 patient with IA, 14/19 patients with IB, 2/5 patients with IIIB, and in 1/2 patient with IVA TNM stage.
No single amplification of *MYCC* was detected. Twelve patients with carcinoma were positive for lymfangioinvasion. All patients were in high risk group and had amplification of *hTERC* gene. In 13 cases, array CGH profiling revealed additional genomic alterations associated with the progression of cervical carcinoma.
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P11.074

Evaluation of circulating DNA as an alternative marker in multimodal care for patients with advanced colorectal cancer

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Background: A presence of circulating cell-free DNA (cfDNA) has long been observed in patients with advanced colorectal cancer. It is expected that cfDNA may represent a useful biomarker, especially for monitoring course of therapy.
Aim: The main purpose of this work was to evaluate a feasibility of using cfDNA in process of monitoring patients with progressive colorectal cancer, namely the follow-up after the surgery and in between chemotherapy cycles.
Methods: A total of 21 patients in advanced stages (III and IV) were included in the study. Peripheral blood samples were collected at various stages of the disease management i.e. before and after the surgery and in some patients also in several time intervals during chemotherapy regimens. A mutation-based approach was applied for cfDNA detection in which a panel of genes was tested for presence of a mutation in peripheral blood-derived plasma samples. Mutations were tested by denaturing capillary electrophoresis (DCE) exhibiting detection sensitivity limit of 1% mutated alleles.
Results: Cell-free DNA was detected in 16 patients (76%). In most cases the cfDNA occurrence did not correlate with elevated levels of CEA or CA19-9. No correlation was found when comparing presence of the cfDNA mutation in primary tumor and/or metastatic tissue.
Conclusions: Presence of cfDNA in peripheral blood may be used for observation of disease development as an alternative to standard tumor markers. Apparently the kinetics of cfDNA release is defined by characteristics of both, primary as well as metastatic lesion.
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P11.076

Immune infiltration in choroidal melanomas

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Introduction: Choroidal melanomas are the most common primary, intraocular malignant tumors in adults. In these tumors, the presence of CD8+ T lymphocytes is associated with bad prognosis. In contrast, in most other solid tumors, the CD8+ T cell infiltrate is related to good prognosis.
Materials and Methods: The primary untreated choroidal melanomas were analyzed for gene expression using Affymetrix U133 plus 2.0 array (n=15) and by immunohistochemistry (n=89).
Results: Gene expression profile analysis led to the identification of a gene signature consisting of 39 upregulated genes in a subset of choroidal melanomas. These genes were associated with: antigen processing and presentation, cell adhesion molecules, interferon-gamma and chemokine signaling pathways. On immunohistochemistry, signature positive tumors displayed a dense intra-tumoral infiltrate of HLA-DRA+CD163+ macrophages and CD3+CD8+ T-cells. It was mild to moderate in tumors lakking the signature. On this basis, additional tumors were analyzed by immunohistochemistry. In total, 19 melanomas had high immune infiltrate and 70 low. Kaplan-Meier plots demonstrated tumors with high immune infiltrate had shorter disease-free survival (log-rank P < 0.001).
Conclusions: We identified in choroidal melanomas an interferon-gamma induced gene signature associated with infiltration of macrophages and CD8+ T lymphocytes. The presence of tumor-infiltrating immune cells correlated with higher risk of occurrence of metastases.

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P11.077

Instability of chromosome 5 in myelodysplastic syndromes (MDS).

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Aberrations of chromosome 5 are common findings in bone marrow cells of MDS patients. The most frequent is del(5q) occurring in 10-15% of them. Extent of deletion differ among patients, however region 5q31 is commonly deleted. Monosomy 5 is described in 3-8% cases. We performed genome wide analysis in MDS patients with monosomy 5 detected by G-banding with aim to determine real existence of total monosomy 5 in MDS.
Bone marrow cells of 67 patients with suspected monosomy 5 were analyzed by I-FISH (Abbott), mFISH/mBAND (MetaSystems) and array CGH (BlueGnome). All 67 patients presented a complex karyotype with confirmed del(5)(q31). In all cases the deleted 5 entered into unbalanced rearrangements with various partners (most frequently chromosomes 3, 7, 12 and 17). Chromosomes 8, 22 and Y were never included in these translocations. We confirmed our hypothesis that pure monosomy 5 does not exist in MDS

as separate cytogenetic entity, as declared in the literature and WHO classification. In all patients with suspected monosomy 5 we showed that at least part of chromosome 5 is retained elsewhere in the genome. Minimal common preserved region was assessed at 5p11.1-p14.2 (22.31Mb). Complex karyotypes involving chromosome 5 identified at diagnosis gave extremely poor prognosis (median OS 3 months).
We assume that del(5q) is arising in myeloid precursor cells as the primary event and subsequently leads to chromosome instability and higher susceptibility to the breakage and rearrangements. This process is resulting in increased genomic damage and fast disease progression.
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P11.078

Characterization of a new rearrangement and identification of a novel fusion transcript, FNIP1/ETV6 in an uncommon chronic eosinophilic leukemia (CEL) with an atypical t(5;12).

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Chronic eosinophilic leukemia (CEL) is a clonal proliferation of eosinophil precursors with recurrent chromosomal rearrangements, such as del 4q- or t(5;12)(q33;p13), leading to *FIP1L1/PDGFRA* and *ETV6/PDGFRB* fusion genes respectively, enhanced tyrosine kinase activity, uncontrolled cellular proliferation.
We describe here a patient first referred to us in 1984 with a high eosinophil count (20.000/µL). A diagnosis of CEL was made and eosinophilia resumed incompletely with interferon-α. Later on, a t(5;12) was recognised, but subsequent TKI treatment remained ineffective. These atypical features (protracted course, resistance to TKI) prompted us to analyse breakpoints. BAC-FISH positional cloning showed that the breakpoints occurred at *ETV6* on chromosome 12 (p13) and at *FNIP1* on chromosome 5 (q23). A fusion transcript, ETV6/FNIP1 was identified on the der(5) whereas no fusion transcript was identified on the der(12) in which an interstitial deletion encompassing *ACSL6*, was found.
The underlying mechanism of leukemogenesis in this patient still remains elusive. However, *FNIP1* could be involved as this gene has been shown to interact with mTOR signaling pathway in Birt-Hogg-Dubé syndrome, predisposing to kidney tumors. On the other hand, we found that the IL-3 gene located near the breakpoint on chromosome 5 and ending up on the der(12) was overexpressed. The encoding eosinopoiesis cytokines IL-5 and GM-CSF genes, located in the vicinity of the translocation could also be involved. In sum, we describe a new rearrangement in CEL. The deregulation of key genes of eosinopoiesis, located near the rearrangement or implication of *ETV6/FNIP1* fusion transcript remains to be determined.

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Automated cartridge based method for BCR-ABL detection in chronic myeloid leukemia patients achieved major and complete molecular response on tyrosine kinase inhibitors therapy

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Introduction. Automated cartridge-based GeneXpert Dx System for detection of *BCR-ABL* level was recently developed. There is lack of findings concerning the results' concordance obtained by routine and automated methods. **Purpose.** To compare the results of *BCR-ABL* gene expression analysis obtained by GeneXpert and manual standardized RT-PCR in 45 CML patients on TKI therapy, most of them were in major molecular response (MMR, n=12) or complete molecular response ($CMR^{4.0}$, n=33). "Xpert BCR-ABL Monitor" assay was developed according to WHO reference standard and allows to present results in %IS. **Results.** Four patients demonstrated *BCR-ABL* >0.1%IS by both methods. MMR (0.01-0.1%IS) was detected in 8 patients, $CMR^{4.0-5.0}$(0.0001-0.01%IS) was revealed in one patient and negative results were obtained in 12 patients by both methods. Concordance rate was 56% (25 of 45 patients). In 15 cases (33%) GeneXpert results were more sensitive than manual ones: two cases of MMR by GeneXpert were CMR by conventional RT-PCR and 13 cases of CMR by GeneXpert were negative by manual. In 5 cases (11%) GeneXpert demonstrated less sensitive results than obtained by manual method: 2 cases of CMR by GeneXpert were MMR by manual method and 3 negative results by GeneXpert were one MMR and two CMR by manual. Probably it was closely related to samples' quality. **In conclusion**, the results have demonstrated appropriate concordance rate of GeneXpert Dx System and conventional RT-PCR for *BCR-ABL* transcript measurement. GeneXpert allows detecting fusion gene transcript in higher percentage of cases.

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P11.080

The role of VHL gene inactivation in sporadic clear cell renal cell carcinoma development.

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Sporadic clear cell renal cell carcinoma (ccRCC) is the most common type of adult kidney cancer. It's often associated with aberrations on chromosomes 3p. Aberrations on chromosome 3p are associated with inactivation of tumor suppressor gene von-Hippel Lindau (VHL), which activates the hypoxia-inducible factors HIF1α and HIF2α. The goal of the study was to investigate inactivation of VHL gene by mutations, loss of heterozygosity (LOH) and methylation of its promoter in cancer tissues of ccRCC patients. We studied 93 paired DNA samples of tumor tissues and normal renal parenchyma in ccRCCpatients from Bashkortostan Republic of Russia. DNA extraction was performed by phenol-chloroform extraction. Analysis of mutations in the coding part of the VHL gene was performed by direct sequencing. Mutations in VHL gene were found in tumor tissues with the frequency of 22,6% (21/93). We found out 10 somatic mutations which hadn't been described in literature previously. Analysis of LOH was performed using D3S1038 and D3S1317 microsatellite loci. LOH at D3S1038 was detected in 25,3%, at D3S1317 - 28% (27/83 and 14/50, respectively) of informative cases. Besides, we analyzed VHL gene promoter methylation. In 5 cases hypermethylation of VHL gene promoter was identified (3,8%). VHL inactivation through promoter hypermethylation and through sequence alterations in tumor DNA didn't differ by histopathologic characteristics or occupational exposure. Thus, somatic inactivation of VHL gene is the main molecular event in most sporadic RCC and a number of drugs have been developed in the treatment of RCC that target HIF-responsive gene products and block angiogenesis.

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P11.081

Assessment of microarray analysis as a prognostic clinical tool in chronic lymphocytic leukemia.

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Chronic lymphocytic leukemia (CLL) is a B-cell malignancy with a highly variable clinical course. In addition to the mutational status of the immunoglobulin heavy chain, recurrent genomic abnormalities are among the strongest prognostic markers. For risk classifications and clinical decision-making in CLL a standard panel of commercially available FISH probes is most often used to identify deletions at 11q22, 13q14 and 17p13, duplications of 6q, and trisomy 12 in the clinical setting. In order to assess the usefulness of CGH+SNP array analysis as an alternative to conventional karyotyping and iFISH in routine clinical use, 100 CLL patients were analyzed both by iFISH and CGH+SNP arrays. In addition, all cases in which no abnormalities were identified were subjected to G-band analysis. The iFISH panel included del(13)(q14)(DLEU1), del(11)(q22)(ATM), del(17)(p13)(TP53)

and trisomy 12. For array analysis a 180K custom-designed cancer focused array was used from OGT. The array was designed for the detection of single exon abnormalities in 17 selected genes (average coverage 1probe/100bp) as well as the simultaneous identification of CNLOH by adding 36000 SNP probes (12000 different SNPs across the genome). In addition, the array covers 1500 cancer-associated genes with an average resolution of 2kb, whilst retaining an average backbone resolution of 50kb. From this study we concluded that CGH+SNP array analysis is a robust, high resolution and sensitive screening method for routine clinical use in CLL with a higher diagnostic yield than iFISH. It also reliably detected genomic abnormalities present in only 10% of the cells.

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P11.082

Exome sequencing during disease evolution in CML patients displaying an isochromosome 17q

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The acquisition of a Philadelphia chromosome in a hematopoietic stem cell, resulting in a constitutively active tyrosine kinase, BCR-ABL1 is a hallmark of chronic myeloid leukemia (CML). The P210 BCR-ABL1 fusion protein is critical and most likely sufficient for establishing the initial chronic phase (CP), but secondary genetic changes are likely to be important for disease progression in to the acute phase, referred to as blast crisis (BC). Here we describe the clinical features and mutation patterns in three CML patients exhibiting an isochromosome for the the long arm of chromosome 17, i(17)(q10), which is one the most common structural cytogenetic change at the disease progression of CML. Exome sequencing was performed at different stages of the disease to identify genomic changes critical for the disease progression. Exome enrichment was performed using the Truseq exome enrichment kit (Illumina) and paired end 100 bp sequencing was performed on a HiScanSQ (Illumina). Mutations in genes involved in DNA repair, cell cycle control and epigenetic regulation such as *TP53, ASXL1* and *EZH2* were detected, but no recurrent mutations were identified. Interestingly, the *TP53* mutation preceded the occurrence of an i(17q) in one patient, suggesting that that the loss of 17p-material as a consequence of i(17q)-formation, partly served to unmask the effect of the *TP53* mutation. However, no *TP53* mutations were detected in the other two patients, suggesting that i(17q)-formation leads to disease progression by causing dosage imbalances of several genes encoded by chromosome 17 rather than via specific gene mutations on this chromosome.

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P11.083

Clonal evolution in chronic myeloid leukemia revealed by whole-exome sequencing

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CML is one of the best examples of a disease that can be targeted by molecular therapy however, the success is restricted to the chronic phase (CP) of the disease. If not cured at this stage, CML transforms into a blast crisis (BC). To investigate the genetic changes associated with CML progression we performed whole-exome sequencing (WES) of an individual patient at three different time points: CP, complete hematological remission (CHR) and BC.
We validated mutations in genes known to be involved in CML (*ASXL1* and *TP53*) as well as in genes that have not been described so far in the disease (*UBE2G2*, *ZEB2* and *IKZF3*). *TP53* mutation (p.E286K) was found in the three phases of CML progression. However, *ASXL1* (p.G679*), *UBE2G2* (p.D35V), *ZEB2* (p.L420R) and *IKZF3* (p.E272K) were present only in the CP and BC. The evaluation of the number of mutated reads allowed us to study clonality and clonal evolution : 93% of the selected SNSs that were present in the CP were also seen in BC. In fact, the percentages of reads of the mutant alleles were the same, both at CP and at BC.
WES allowed us to identify a large number of mutated genes, even at the chonic phase of CML, that harbour clear prognostic and predictive significance (*TP53*, *IKZF3*, absence of *ABL1* mutations). The study of the mutation profile through the disease progression indicated that, at least in this patient, the number and the type of mutations were rather similar at CP and BC.

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P11.084

Copy number variation analysis in 222 patients with colorectal polyposis reveals potential new causative candidate genes

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Background: In up to 50% of patients with colorectal adenomatous polyposis, a tumour predisposition syndrome, no germline mutation in known genes (APC, MUTYH) can be identified. Loss-of-function copy number variants (CNVs) contribute significantly to the mutation spectrum of hereditary tumor syndromes and thus those CNVs might also be the underlying cause in yet unidentified genes responsible for adenomatous polyposis.
Methods: Genomic DNA from 222 unrelated mutation negative polyposis patients was genotyped (HumanOmni1-Quad BeadArray, Illumina). Putative CNVs were identified by QuantiSNP v.2.2, filtered according to various criteria by use of the Cartagenia Bench™ software, in-silico-analysis, comparison with data from 531 healthy in-house controls, and validated by qPCR. The expression of affected genes was examined by cDNA analysis of normal colonic mucosa. Candidate genes were prioritized by function, pathways, and segregation analysis.
Results: 33 unique deletions and 64 unique duplications, encompassing 151 protein coding genes, were found in 28 (13%) and 50 patients (23%), respectively. Each except one CNV occurred only once in the cohort. After expression analysis and data mining, the number of candidates could be further reduced to 23 deletions and 57 partial duplications, including protein kinases, transcription factors, and potential tumor suppressors. To identify pathogenic point mutations, 24 candidates are currently sequenced in the whole patient group.
Conclusions: By applying stringent filter criteria, we identified a group of rare and potentially causative loss-of-function CNVs. The clinical relevance of the most promising candidates will be verified by a target sequence approach. The study was supported by the German Cancer Aid.

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P11.085

Expression of GA-733 gene family in colorectal cancer.

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Aim of study: Colorectal cancer is the third more frequent malignancy among the newly diagnosed cancer cases in the United States and the third cause of death from malignancy. It has been and being an object of study in regards to the mechanisms that promote oncogenesis as well as the implicated genes. Genomic and protein analyses in colon cancer have proved the significant participation of GA733 protein family. However, so far a small number of studies have been reported in human-tissue material.
Experimental Design: Cancerous and noncancerous tissues were obtained from 40 patients with colorectal cancer and RNA was extracted from each sample (cancerous - non cancerous tissue samples). In order to quantitate GA733-1 and -2 mRNA transcripts, we developed and evaluated real-time fluorescence PCR assays. Correlation of GA733-2 mRNA levels in noncancerous versus cancerous tissue with clinicopathologic parameters was performed in adenocarcinoma colon samples.
Results: mRNA expression of GA733-1 gene was almost undetectable in both noncancerous and cancerous tissues. This can be explained since no

metastasis was diagnosed before or after surgery. In noncancerous tissues the median ratio GA733-2/h-PBGD mRNA levels was 273.31 (range, 65.24-14862.41), while in cancerous tissues of the above patients, the median ratio GA733-2/h-PBGD was 115.64 (range, 11.58-1189.14) (p<0.05).
Conclusion: Our results reveal a strong correlation between reduced GA733-2 mRNA levels and lymph node perforation (p=0.0343) in contrast to GA733-1 gene expression which was almost undetectable in our samples. Therefore, GA733-2 mRNA measurement could be a potential predictive marker for metastasis and poor cancer prognosis.

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P11.086
The allelic expression of the beta-catenin is polymorphic in the general population and allelic imbalance of the beta catenin constitutes a risk factor for colorectal cancer

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In order to identify new genetic risk variations associated with colorectal cancer (CRC) risk, we conducted a prospective case-control study in 716 CRC patients without detectable germline mutations and highly selected on criteria suggestive of an increased genetic risk for CRC: (i) CRC before 61 years with a first degree relative presenting with CRC; or (ii) CRC before 51 years of age; or (iii) multiple primitive CRC, the first one diagnosed before 61 years. We recruited 550 controls that were CRC-free and had no familial history of CRC in their first-degree relatives. Analysis of the allelic expression of beta catenin and TCF4 revealed that, in controls, the ratio of allelic expression was dispersed for beta catenin, as compared to TCF4 (0.76 +/- 0.26 versus 0.91 +/- 0.07; interquartile range: 0.22 versus 0.09; p < 0.0001). Using cut-off values corresponding to the 5th and 95th percentiles, we observed, among the 355 informative patients and 274 informative controls, a beta catenin allelic imbalance in 73 patients (20.6%) and in 37 controls (13.5%) (p = 0.0208; OR = 1.66, CI [1.08; 2.55]). This difference was even more significant after taking into account age at blood sampling and sex (p = 0.0062; OR 2.07, CI [1.25-3,43]). This study reveals that allelic expression of the beta-catenin, the main regulator of the APC pathway, is polymorphic in the general population and that allelic imbalance of the beta catenin constitutes a risk factor for CRC.

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P11.087
Association between microsatellite instability, BRAFV600E mutation and MLH1 promoter hypermethylation in sporadic and familial colorectal cancers

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In our ongoing study investigating the genetic and epigenetic mechanisms of colorectal cancer (CRC) development among patients from the Republic of Macedonia, we have evaluated the microsatellite instability (MSI), BRAF-V600E mutation and methylation status of the MLH1-promoter in a total of 389 patients with histopathologically confirmed CRC. Fluorescent multiplex PCR followed by capillary gell electrophoresis; bidirectional DNA sequencing and quantitative methylation-specific PCR after bisulfite DNA-conversion were employed for the above analyses respectively. MSI was detected in 11.2% of all CRCs, acting as a mechanism that enables development of cancers with early phenotypic presentation (55 years on average) in the proximal colon (p<0.001) and lower tumor stage at diagnosis (p=0.046). Nearly one third of all MSI cancers (29%) were isolated from patients with a clinical diagnosis of Lynch Syndrome. MLH1 promoter hypermethylation was responsible for MSI in 54% of cancers with this type of genetic instability, suggesting sporadic character of the disease. The BRAFV600E mutation was significantly associated with MSI (p=0.03), being detected in 12% of MSI cases, and 2% of MSS cancers. All of the evaluated "BRAF+" cancers were MLH1 hypermethylated, and only half of the MLH1-hypermathylated cancers bear the BRAFV600E mutation, supporting the previous hypothesis that the gene- promoter-hypermethylated phenotype creates a permissive environment for the BRAFV600E mutant clones. A family with MSI cancers without defects in the mismatch repair genes, but bearing the BRAFV600E mutation and hypermethylated MLH1 promoter was identified, suggesting a role of the later two mechanisms in development of the currently unexplained familial CRC cases.

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P11.088
Identification of cancer relevant variants by comparison with data from 200 healthy Danes using CLC Genomics Workbench

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It is well known that people with a different native background respond differently to drugs and have different prepositions to develop rare diseases. Furthermore, the knowledge about common genetic variants in a population can be helpful to identify disease related variants in patients.
Projects like the HapMap and 1000 Genomes project have been proven to be excellent repositories to explore common as well as rare variants. However, not all different ethnic groups are part of these projects, yet. Also, if a lot of publicly available population data exists, the accurate identification of common and rare variants from this data is a difficult and often time-consuming step, which requires a lot of specialized knowledge.
In this presentation we will show how fast and easy common and rare variants can be identified using CLC Genomics Workbench and how they can be used to distinguish between common and disease related variants in a patient with a certain genetic disease. As examples we will use publicly available data from 200 healthy Danes and one cancer sample from a patient of Danish ethnicity.

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P11.089
Stool DNA methylation markers suitable for colorectal cancer screening

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Background: Colorectal cancer is the third most commonly diagnosed malignancy in the world. Research over the last decade has shown that population screening for CRC can reduce mortality by 15 - 33%. Until recently the method most often used to screen for early CRC has been based on the findings of faecal occult blood. The problem with this test is, however, that the sensitivity is only 50-60% when used as a single examination. Tumour specific DNA methylation in stool is a new method for detecting colorectal cancer and precancerous lesions at an early stage.
Materials and method: In order to define suitable markers for a DNA methylation based screening assay we have conducted analysis of normal colonic mucosa and adenoma tissue samples. First we used a DNA methylation

array platform based on immunoprecipitation and then we performed an Illumina Infinium 450K Methylation assay analysis. We have conducted various filtering analysis and performed statistical and bioinformatic analyses to define aberrant methylated candidate CpG sites suitable for a stool based screening test.
Results: We have identified these 21 candidate genes; ADAMTS5, DLX5, DMRTA2, DPP10, FAM19A4, GRIA4, HAND2, HPSE2, ITGA4, KCNA1, LRFN5, MEOX2, MIR129-2, PAX6, PRMT8, SLC6A15, TBX5, VCAN, ZIC1, ZNF568 and ZNF804B, p-value of <0.001, with more than one differential methylated CpG site by the Infinium 450K array.
Conclusions: Further work is necessary in order to develop „high-throughput" methods for each of the potential markers and hopefully it will result in a more sensitive screening tool for early diagnosis of colorectal cancer.

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P11.090
Folate metabolism and gene promoter methylation in colorectal cancer

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Colorectal cancer (CRC) occurs as consequence of a complex interplay between genetic and epigenetic alterations. Epigenetic modifications represent the dynamic link between the environment and the genome. DNA methylation is potentially reversible and therefore is a good target for research and therapy. One-carbon metabolism is necessary for DNA synthesis, repair and methylation and several studies reported its pivotal role in carcinogenesis. Folates may influence CRC risk either through dietary intake as well as by intrinsic factors. Our study is aimed to evaluate the methylation levels of APC, CDKN2A, hMLH1 and MGMT genes in CRC and healthy adjacent tissue specimens. The correlation among the methylation status of these genes and the clinical-pathological features of the patients and the association among MTHFR, TYMS, DNMT3B, RFC1, MTRR, MTR folate metabolism gene polymorphisms and the methylation levels of CRC key genes were assessed. Allele and genotype frequencies between CRC and healthy control subjects for the gene polymorphisms were evaluated. Some interesting correlations have been observed, such as an interaction between MTRR A66G polymorphism and CRC risk; a higher gene promoter methylation in CRC tissue with respect to the healthy adjacent tissue; a statistically significant positive correlation between age and hMLH1 and MGMT promoter methylation and between gender and hMLH1 promoter methylation. Moreover low folate levels were associated with an increase of hMLH1 and APC methylation while TYMS 1494 6bp ins/del polymorphism correlated with CDKN2A promoter methylation. All these data corroborate the critical role of folate metabolism in CRC.

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P11.091
The VEGF/VEGFR pathway in advancing stage colorectal cancer

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Angiogenesis as the process of new capillary formation plays an important role in colorectal cancer (CRC) development. The VEGF/VEGFR pathway is one of the most important regulators of angiogenesis. Vascular endothelial growth factor (VEGF) binds to its receptors (VEGFR1/2) and leads to cell proliferation and new vascular formation. VEGF is expressed by cancer cells after the activation of hypoxia-inducible transcription factors in response to hypoxia.
We analyzed the VEGF signaling on 17 samples of advancing stage CRC ($T_{3-4}N_{0-2c}M_{0-1}$) compared to normal adjacent tissue by RT-PCR. The VEGF expression level increased more than 3 times in 7 tumors. VEGFR2 was up-regulated in 8 samples (15-1000 times), VEGFR1 - in 14 samples (5-200 times). Co-expression VEGF/VEGFR1 and VEGFR2/VEGFR1 were observed always. High level of HIG2 (hypoxia-inducible gene 2) strongly correlates with VEGFR1 expression. Also we observed up-regulation the hypoxia-inducible CA9 (carbonic anhydrase 9) expression in 8-1300 times which correlated with VEGFR1. Up-regulation of VEGFR2 closely associated with availability of nodal metastasis or peritoneal carcinomatosis.
The expression of VEGF/VEGFR on tumor might be a prognostic marker for successful use anti-VEGF therapeutics.

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P11.092
The EMT gene expression signature in advancing stage colorectal cancer

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Colorectal cancer (CRC) has been considered to be a molecularly heterogeneous disease. Investigation of gene expression signature in CRC is an effective approach for prognosis of disease and its metastatic status. The analysis of EMT (epithelial- mesenchymal transition) program classifies colon tumors on two molecular subtypes: epithelial and mesenchymal.
The gene expression signature (45 genes) was investigated on 17 samples of colon and rectal cancers T3-4 N0-2c M0-1 by RT-PCR. 5 of 17 samples were characterized as mesenchymal subtype: high level of ZEB1, ZEB2, VIM and low level of CDH1. High level of PDGFR and TH were strongly correlated with this gene profiling. The EMT signature was found on 2 of 3 tumors with nodal metastases. 3 of 3 cancers with peritoneal carcinomatosis were demonstrated the EMT profiling also. Whereas the sample with MLH1 germline mutation (p.R100X) showed the epithelial gene signature.
Our data confirm the notion that the mesenchymal subtype of tumor is associated with poor prognosis, metastatic CRC and peritoneal carcinomatosis.

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P11.093
3-phosphoglycerate dehydrogenase polymorphism in male patients with colorectal carcinoma

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/>Introduction:. Colorectal cancer (CRC) is one of the most common malignant tumors and the second leading cancer in Serbia. Recent studies suggest important role of both environmental and genetic factors in cancerogenesis. 3-Phosphoglycerate dehydrogenase (3-PHGDH) gene overexpression is assosiated with patogenesis of human cancer and contributes to cell proliferation.
Aim: The objective of our study was to assess the association of PHGDH gene polimorphism in group of males with colorectal cancer and control group of healthy men.
Methods: The survey was carried out in the Department of Human Genetics-Medical School, University of Belgrade. The study has encompassed 63 man diagnosed with colorectal cancer in The First Surgical Clinic, Clinical Center of Serbia and 100 health males volonteers. The DNA was isolated from the periferal blood with solting out method. The genotypes 3-PHGDH polimorphism were determined by Polimerase Chain Reaction (PCR) and Restriction Fragment Lenght Polimorphism (RFLP). Gel-electrophoresis was used to separate DNA fragments.
Results: There was a statistically significant difference between frequences for genotypic distribution of rs541503 polymorfism in patiens with colorectal carcinoma and healthy volonteers ($p=0.00013$, $p<0.01$).
Conclusion: In the present study we found TT genopype as the most frequent in both of group of patients with colorectal carcinoma and control group. The results of our study also suggest that C allele might be factor of risk associated with colorectal cancer. It is necessary to undergo futher testing with more adequate test groups in order to get firm results.
Key words: PHGDH gene, colorectal cancer, PCR, RFLP

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P11.095

Colorectal Cancer Inherited Susceptibility in the Brazilian population: the first replication study in Latin America

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Background:
Colorectal cancer (CRC) is one of the most common and fatal cancers worldwide, including in developing countries. Genome-wide association studies have uncovered part of the CRC inherited susceptibility through genotyping common low-penetrant variants in individuals with European ancestry. Replication studies in genetic heterogeneous populations are scarce.
Aim: to identify SNPs previously known to be associated with CRC risk in the Brazilian population.

Methods:
Ten SNPs were genotyped in 1,467 individuals (727 cases and 740 controls) using TaqMan assays. SNPs with significant Hardy-Weinberg disequilibrium and genotypes with either poor clustering or call rates <95% were excluded. Effect sizes were calculated by logistic regression and p-values were corrected for multiple analyses.

Results:

Models SNP	Risk allele	Frequency	Allelic OR [95%C.I.]	p	Dominant OR [95%C.I.]	p	Recessive OR [95%C.I.]	p	Additive OR [95%C.I.]	p
rs4939827	C	0.47	**1.43 [1.23 - 1.66]**	**2.12 x 10^{-5}**	**1.67 [1.33 - 2.11]**	**0.0001**	**1.51 [1.18 - 1.93]**	**0.011**	**1.54 [1.20 - 1.98]**	**3.49 x 10^{-5}**
rs10411210	T	0.17	0.83 [0.68 - 1.00]	0.080	**0.75 [0.60 - 0.94]**	**0.040**	1.19 [0.70 - 2.01]	0.749	0.71 [0.56 - 0.90]	0.083
rs4444235	C	0.51	**1.26 [1.09 - 1.45]**	**0.011**	1.26 [1.00 - 1.60]	0.097	**1.45 [1.14 - 1.83]**	**0.012**	**1.38 [1.07 - 1.78]**	**0.012**
rs3802842	A	0.26	**1.29 [1.09 - 1.53]**	**0.010**	1.26 [1.02 - 1.56]	0.072	**1.85 [1.23 - 2.78]**	**0.009**	**1.69 [1.10 - 2.60]**	**0.011**
rs16892766	C	0.08	**1.39 [1.07 - 1.82]**	**0.038**	**1.49 [1.11 - 1.99]**	**0.035**	0.96 [0.40 - 2.27]	0.920	**1.55 [1.14 - 2.10]**	**0.049**

Conclusions:
We found significant association with half of SNPs in the first replication study carried out in Latin America. This study will proceed to increase sample size and stratify our population by ancestry in order to avoid type I and II errors. It is worth to replicate GWAS in admixed populations in order to help to uncover the missing heritability of CRC and narrow the boundaries of the genetic architecture of CRC susceptibility.

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P11.096

HER2 gene status in KRAS positive/negative colorectal cancers

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Mutation at codons 12 and 13 of the KRAS gene in patients with colorectal cancer has been shown to be predictive of cetuximab response in colorectal cancer. Fifty three paraffin-embedded colorectal cancer specimens were analyzed for KRAS mutation and HER2 overexpression/amplification. A high-resolution melting (HRM) assay and single-nucleotide polymorphisms (SNPs) were used to detect somatic mutation in exon 2 notably condons 12 and 13 of the KRAS gene. HER2 overexpression was detected using monoclonal antibody and confirmed by fluorescence in situ hybridization (FISH) analysis.
KRAS mutations for codons 12 and 13 were identified in 19/53 (35.8%) by SNP and in 17/53 (32.0%) of colorectal cancer by HRM technique. Colorectal cancers showed mainly heterozygous 35G>A and 38G>A KRAS gene mutations.
The concordance rate between the two methodologies was high at 89.4%. KRAS mutation was more frequently observed in poorly differentiated tumors and adenocarcinomas than in other histological types.
HER2 overexpression was observed in 42/53 (79.2%) of all colorectal cancers and in 62.1%, 61.5% of KRAS mutation-positive cases detected by SNP and HRM techniques respectively. HER2 overexpression was accompanied by amplification of HER2 gene.
In conclusions, the presence of HER2/KRAS positive immunophenotype of colorectal cancers suggests that HER2 might be the candidate gene for target therapy of patients with colorectal cancer.

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P11.097

KRAS, BRAF and PIK3CA mutations and KRAS-LCS6 polymorphism in the binding site of let-7 microRNA to the 3´UTR region of KRAS gene in Czech patients with colorectal cancer

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Background: Personalised cancer medicine based on mutation profiling of tumours is now reality in the treatment of metastatic colorectal cancer (mCRC). KRAS mutations represents a predictive biomarker for resistance to anti-EGFR therapy. However, up to 65% of patients with KRAS wild-type tumours do not respond to this therapy. This fact suggest that additional markers (in the RAS-RAF-MAPK or PI3K-AKT-mTOR signaling pathways, functional polymorphism in the binding site of microRNA) may also contribute to resistance to anti-EGFR therapy.
Aim: Mutation profiling of selected mutations in KRAS, BRAF, PIK3CA gene and SNP (rs61764370) KRAS-LCS6 in the binding site of let-7 microRNA to the 3´UTR region of KRAS gene.
Method: In this study 503 DNA samples of tumours tissues of mCRC patients were tested for presence of selected mutations of KRAS,BRAF and PIK3CA gene. Analysis was performed by PCR, reverse hybridisation, real-time PCR and primer extension method. SNP (rs61764370) was analysed in 164 samples and was performed by PCR+RFLP method.
Results: Activating mutations were detected in 40% (KRAS), 5,6% (BRAF) and 9% (PIK3CA) of the tumours. Coexistence of mutations were detected in 4,8% (mutations in KRAS and PIK3CA gene) and 0,6% (BRAF and PIK3CA). T/G genotype of the SNP (rs61764370) was identified in 11% of 164 mCRC patients.
Conclusion: Frequency of mutations of KRAS, BRAF and PIK3CA is similar to frequency reported by other authors, respectively. KRAS and BRAF mutations were mutually exclusive. Additional predictive biomarkers may aid in the selection of mCRC patients which could profit from targeted cancer therapy.

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P11.098

The evaluation of miRNA expression levels in LGR5 and SOX2 positive colorectal cancer tissues

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Colorectal cancer (CRC) is one of the leading causes of cancer deaths in the world. Cancer stem cell (CSC), suggested to responsible for tumor initiation, growth, and metastasis in CRC. The search for a reliable marker to identify these CSCs is ongoing as current markers, such as *LGR5* and *SOX2*. Additionally, according to recent studies, microRNAs (miRNAs), may also contribute to preserving stemness of human CSCs. However, the knowledge about the regulation of *LGR5* and *SOX2* positive CRC cells via miRNA expressions is very limited. Therefore, the aim of this study was to evaluate the significance of expression levels of CRC related miRNAs in *LGR5* and *SOX2* positive CRC tumors.
The expression profiles *LGR5* and *SOX2* genes and 38 CRC related miRNAs were analyzed in tumor tissues of 30 early-onset CRC patients, using RT-qPCR assays and miRNA PCR arrays respectively. Statistical analyses were performed using SABioscience PCR Array Data Analysis Software.
The expression level of miR-143 almost 2-fold reduced in normal tissues in compare to tumor tissues. Furthermore, reduction level of miR-143 was significantly increased in tumors with over-expressed *LGR5* and *SOX2* (P = 0.045 and P = 0.040, respectively).
Although miR-143 associated with *SOX2* previously, its association with *LGR-5* determined first time in this study. Even, further studies needed, we suggest that, miR-143 may has a potential to be candidate of new therapeutic target in advance therapy protocols of stemness CRC.

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P11.099

Easy-to-use online referral test detects most patients with a high familial risk of colorectal cancer

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Introduction: Currently, only 12-30% of individuals at high risk for Lynch syndrome, the most common hereditary colorectal cancer (CRC) syndrome, are referred for genetic counselling. We developed a new online referral test based on current guidelines, aimed at improving referral for genetic counselling, of those at high familial risk of CRC. This study focuses on sensitivity, usability and user experiences of this test, which is also available as cell phone App.

Patients and Methods:

Sensitivity was assessed by entering pedigree data from high-risk individuals (i.e., Lynch syndrome mutation carriers) into the referral test to determine whether genetic counselling was recommended. For usability, we assessed non-medical staff members' ability to determine referral according to guidelines in seven fictive clinical cases using the referral test after minimal training. Referral was also determined by medical specialists (surgeons, gastroenterologist, clinical geneticists) **NOT** using the referral test.

Results: Sensitivity of the referral test was 91% for mutation carriers with CRC (n = 164) and 73% for all mutation carriers (n = 420). Non-medical staff members (n = 20) determined correct referral according to guidelines in 84% of cases using the referral test. Without the use of the referral test Medical Specialists (n=312) correctly determined referral in 65%.

Conclusions: The referral test has a high sensitivity for detecting individuals with a high risk of Lynch syndrome and is suitable for use in clinical practice by cell phone. Widespread use of the online referral test will improve recognition of patients with high familial risk of colorectal cancer.

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P11.100

Over expression of CK20 may has a potential to be a prognostic marker in MSS tumors of early-onset colorectal cancer patients

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The association between microsatellite instability (MSI) status and gene expression profiles in tumorigenesis pathways causing early-onset sporadic colorectal cancer (CRC) has not clearly established. Since molecular targeting therapeutics are being used in clinical settings and trials, identifying differentially regulated molecular target genes depend on MSI status in early-onset CRC is required. The aim of this study was to evaluate the altered gene expressions depend on the MSI status of early-onset CRC to identify specific biomarkers that could provide novel therapeutic molecular targets in this population.

5 MSI markers were investigated in tumor and normal tissues of 36 early-onset CRC patients whose expression profiles of 114 CRC associated genes already examined using mRNA PCR arrays. Statistical analyses were performed to clarify the potential influence of MSI status and altered gene expression to clinical features. Prognosis was estimated with Kaplan-Meier analysis.

The frequency of MSI tumors was 41.66 % and microsatellite stabile (MSS) tumors were 58.33 %. The expression levels of *IMPDH2, CK20* and *MAP3K8* were up-regulated in MSS in compared to MSI patients ($P < 0.05$). Up-regulation of *CK20* was significantly associated with lymph node metastasis ($P = 0.026$), recurrence/distant metastasis ($P = 0.0008$) and short survival ($P = 0.0137$) in MSS.

Although validations are required, our findings supported that over expression of *CK20* gene might provide novel therapeutic molecular target related with MSI status for treatment of early-onset CRC, as well as new directions for the development of anticancer drugs.

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P11.101

MUTYH mutations and variants in Jews of Moroccan origin with or without minimal colorectal adenomatous polyposis

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Background: Israeli-Jews of North-African origin were a low-risk population for colorectal cancer (CRC). This is changing. Little is known of contributing genetic susceptibility. **Objectives:** Evaluate frequency and types of *MUTYH* mutations and variants in a defined population of North-African Jews. **Methods:** Patients were of Moroccan origin having colonoscopy 2007-12 and volunteering for genetic evaluation. Excluding Familial Adenomatous Polyposis and Lynch Syndrome, patients with ≥3 colorectal adenomas and non-adenoma controls were examined for 6 *MUTYH* mutations & variants. **Results:** Seventy-five patients, 52 with adenomas, had *MUTYH* analysis; 17 (32.7%) had mutations or variants identified. In adenoma patients there were 8 (15.4%) Y179C, G396D homozygotes or compound heterozygous; all had >10 adenomas and 4 had familial neoplasia. Six others were heterozygotes, 4 with <10 and 2 with >10 adenomas; 5 had familial neoplasia and 3 a neoplasm. Other mutations were two S512F heterozygotes with <10 adenomas and familial neoplasms and one had a neoplasm; another had 1186_1187ins GG and >10 adenomas. Four heterozygotes had the Q324H variant. In 23 non-adenoma controls: 1 had the S512F another the L417M variant, another the R509C variant and 16 the Q324H variant. **Conclusions**: Jews of Moroccan origin can have *MUTYH* mutations associated with only few adenoma, family and personal history of cancer similar to sporadic CRC. The S512F mutation appears to be pathogenetic, the Q324H a frequent variant. The findings suggest *MUTYH* evaluation in these patients with even few adenomas, and need to follow-up heterozygotes and Q324H carriers for CRC.

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P11.102

Identification of potentially functional single nucleotide polymorphisms (pfSNPs) associated with fluorouracil / platinum drug response in colorectal cancer patients

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Colorectal cancer (CRC) ranks among the most common causes of cancer death worldwide. Approximately a third of patients are diagnosed at the metastatic stage (stage IV) and a third of curatively resected cases (stages I-III) relapse. Thus a substantial proportion of patients require drug treatment for metastatic/relapsed CRC. Two common combination drug regimes for this cancer are CAPOX (capecitabine and platinum-based oxaliplatin) and CAPIRI (capecitabine and topoisomerase-I inhibitor) but positive response to these regimes is only ~40-45%. Availability of a reliable early predictive biomarker of drug response in metastatic CRC can enable appropriate individualized tailoring of drug regimes for patients, moving personalized medical care closing to reality. Patients predicted to be non-responders to specific drug regimens can avoid ineffective drug treatments and their associated side-effects, and instead opt for novel treatments. Currently, no reliable tests exist for early prediction of response to chemotherapy in these patients. Single nucleotide polymorphisms (SNPs) in some genes have previously been shown to be associated with differences in drug response. A novel pathway-based approach was used to test potentially-functional SNPs (pfSNPs) in candidate genes that may serve as predictive biomarkers of drug response. Of >100 patients examined, three pfSNPs in strong LD with each other showed presence of the minor alleles only in non-responders. Their minor alleles are predicted to disrupt nonsense mediated decay, an exon-splice enhancer site, and miRNA binding. These 3 SNPs could thus serve as a useful biomarker for early response to standard anti-CRC drug regimens.

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P11.103

Carriership of pathogenic *BLM* alleles is associated with early-onset colorectal cancer

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Around 5% of all colorectal cancer (CRC) cases are caused by known monogenic aberrations, but much of the genetic heritability of CRC is still unexplained. The known genetic causes of CRC predisposition include rare recessive RecQ-helicase deficiency syndromes: Bloom (*BLM*) syndrome and Werner (*WRN)* syndrome. Previous studies have led to conflicting conclusions as to whether carriership of pathogenic *BLM* and *WRN* alleles predispose to CRC. Here we assessed the carriership of pathogenic *BLM* and *WRN* alleles in early-onset CRC (<45 years of age) patients.
By performing whole exome sequencing in a cohort of 57 unexplained early-onset mismatch-repair proficient CRC patients we detected two carriers of a pathogenic *BLM* mutation and one carrier of a pathogenic *WRN* mutation. Screening of an additional cohort of 183 young CRC patients from the Netherlands and Germany resulted in 3 additional patients with a pathogenic mutation in *BLM* and one patient with a pathogenic *WRN* mutation. A comparison with data from 1,302 exomes in our in-house database (non-CRC) as well as with 6,503 exomes from a public database (http://evs.gs.washington.edu/) revealed a significant enrichment of pathogenic *BLM* mutations (both p-values <0.05), but not *WRN* mutations, in our CRC cohort. Moreover, in 1 out of 2 analyzed tumor samples from *BLM* carriers somatic loss of the wild-type allele was found.
In conclusion, we found that carriership of pathogenic *BLM* alleles is enriched in early-onset CRC patients suggesting that these alleles contribute to an increased CRC risk.

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P11.104

The first mutations in the MYH gene reported in Moroccan colon cancer patients

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Background: Biallelic germline mutations in the *MYH* gene cause MYH-associated polyposis (MAP) disease, an autosomal recessive form of inherited colorectal cancer.
There have been many investigations into MAP that have been conducted in many different countries. Currently there is limited data on MAP in Morocco, and it is reasonable to think, that the prevalence of this form of genetic predisposition is as high as other autosomal recessive genetic diseases found in countries with high rates of consanguinity.
The aim of this study is to examine the frequency of MYH mutations in colorectal cancer and/or attenuated polyposis in Moroccan patients.
Patients and methods: We carried out DNA analysis in 62 patients to screen for the three recurrent mutations c.494A>G, c.1145G>A and c.1185_1186dup, whereas 40 subjects were screened for germline MYH mutations in the whole coding sequence of the MYH gene by direct DNA sequencing.
Results: Three patients with colorectal cancer and attenuated polyposis carried biallelic mutations in the MUTYH gene. One patient with 25 adenomas without colorectal cancer carried the c.1145 G>A mutation at a homozygote state and one patient with 3 polyps was heterozygote for the mutation c.1145 G>A.
Conclusion: We report the first biallelic MYH mutations in four Moroccan patients with clinical criteria of MAP. Despite the relatively small sample size of the current study, our findings suggest that the MAP is not a frequent cause of colon cancer in Morocco as we had expected.

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P11.105

Investigation molecular and epidemiologic of polymorphisms Tyr113His gene EPXH1 and DraI-CYP2E1 in xenobiotics metabolism for treating patients with sporadic colorectal cancer.

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Colorectal cancer (CRC) is neoplasm malignancy that occurs in the large intestine (colon) and rectum and has a high incidence among Brazilian patients. The etiology of colorectal cancer includes age over 60 years and lifestyle habits (smoking and alcohol consumption). To analyze the frequency of polymorphism Tyr113His and DraI-CYP2E1 and the association between them and clinicopathological characteristics of the patients compared with controls. The study included a total of 231 individuals (57 patients and 174 controls) Coloproctology Service, Base Hospital, University Hospital, Faculty of Medicine of São José do Rio Preto - FAMERP. The variables analyzed were age, sex, primary site of occurrence. Molecular analysis was performed by Real Time. For the statistical analysis we used chi-square test, Hardy-Weinberg and multiple logistic regression. Statistical analysis showed that there significant differences between patients and controls in relation to sex (p = 0.001; OR = 0.27; CI95%= 0.12 to 0.60) and age (p= 0.035; OR= 3.16; CI95%= 1.08 to 9.25). Also found relation for the DraI-CYP2E1 polymorphism (p≥ 0.000; OR= 6.78; CI95%= 3.19-14.39). In the present study we observed a high frequency of males and in the older age of patients group in the control group. Only the polymorphic allele of *Dra*I-CYP2E1 appears to be associated with susceptibility to CCR. According to preliminary results, male gender, older age and the presence of the polymorphism DraI-CYP2E1*6 are being ratified as a risk factor. Results led us to extend for further study of these polymorphisms and others to a better understanding of RCC carcinogenesis.

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P11.106

K-ras gene - possible correlations of its mutational status with the histopathological findings in metastatic colorectal carcinomas

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Background: Gain-of-function K-ras point mutations, present in 30-50% of the metastatic colorectal carcinomas (CRC), maintain the active form of the ras p21 protein and lead to epidermal growth factor receptor (EGFR) independent activation of intracellular signaling pathways, making the anti-EGFR tumor therapy ineffective.
The aim of this study is to identify possible correlations between the mutational status of the K-ras gene and the histopathological findings in patients with CRC.
Methods: We studied 45 patients with CRC, 39 primary tumors and 6 metastases. The formalin-fixed paraffin-embedded tissue samples were analyzed using an indirect bistadial immunohistochemical (IHC) technique, performed with a Dako EnVision+ Dual Link System-HRP, with antibodies for the EGFR and the ras protein. Mutations in exon 2, codons 12 and 13 of the K-

ras gene were detected by PCR-Restriction Fragment Length Polymorphism analysis.

Results: K-ras mutations were present in 46, 66 % of the cases (21 cases), 19 adenocarcinomas and 2 metastases. EGFR was positive in 10 cases in tumor cells and in 11 cases in the vessels, with 3 cases of double positivity. Immunohistochemical overexpression of ras protein was detected in 14 samples. The relationship between the positivity of the K-ras mutation and the positive immunohistochemical reaction for EGFR and the ras protein did not reach statistical significance.

Conclusions: There were no significant correlations between the mutational status of the K-ras gene and the IHC reactions for EGFR and ras p21 protein, proving once more the major role of the molecular analyses in CRC anti-EGFR therapy.

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P11.107

Delineating the PMS2 cancer risk

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Introduction Lynch syndrome is an inheritable cancer syndrome caused by mutations in one of the mismatch repair (MMR) genes. The clinical phenotype for mutations in the MLH1, MSH2 or MSH6 is comprehensively described, but the consequences of a PMS2 mutation are less well understood. We aim to establish the cancer risk for PMS2 mutation carriers in the largest cohort reported so far.

Methods Data were used from 107 PMS2 families, including approximately 378 mutation carriers and more than 2500 family members, assembled by our centre in cooperation with Dutch university hospitals and several genetic clinics in other European countries. A Kaplan Meier analysis calculating cancer risk in proven or obligated PMS2 carriers will be done.

Results The result Kaplan Meier analysis will be shown during the presentation.

In the 378 mutation carriers analyzed, CRC was most frequently diagnosed (n=112). In 208 female mutation carriers endometrial (n=29) and breast cancers (n=11) (after CRC) were most frequent. Other cancers than the colon or endometrium were reported in a total of 47 cases.

Discussion In this study we analyze the cancer risks in a large cohort of PMS2 mutation carriers.

The tumor spectrum in total group of family members is diverse and families seem less severely affected than other MMR deficient families, suggesting a lower penetrance for PMS2 mutation carriers.

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P11.108

DNA methylation changes in Lynch syndrome associated normal colonic mucosa

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Colorectal cancer (CRC) develops via multiple steps which involve genetic changes, such as mutations in growth-regulatory genes, and epigenetic alterations, such as CpG island hypermethylation. These changes accumulate over time in the normal tissue, for example due to many environmental factors such as diet, and may result in pre-cancerous field defects. Lynch syndrome (LS) is associated with inherited defects of the DNA mismatch repair genes which together with other genetic and epigenetic changes accelerate tumorigenesis. The aim of this project is to gain new insights to the molecular mechanisms through which DNA methylation can affect colorectal carcinogenesis in predisposed LS mutation carriers and to define how CpG island methylation of CRC risk genes varies with age, mutation, CRC diagnosis and different colorectal regions.

Normal colonic mucosa biopsies from different colorectal regions were gathered from 38 Finnish LS mutation carriers during regular surveillance in addition with 11 matching normal-polyp pairs. Patient information was obtained from a nation-wide registry. CpG island methylation was studied with the methylation-specific multiplex ligation-dependent probe amplification test (MS-MLPA) which detects CpG methylation of selected genes. Of 15 CRC risk genes tested so far at least two show increased methylation already in normal mucosa which further increases in the vicinity of polyps and in polyps themselves. Our preliminary findings suggest that increased DNA methylation in normal colonic mucosa may indicate higher risk for developing CRC.

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Constitutional epimutation of *MLH1* is linked to the variants c.-27C>A and c.85G>T present on an ancestral haplotype

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Lynch syndrome, caused by germline heterozygous mutations of the DNA mismatch repair genes *MLH1*, *MSH2*, *MSH6* and *PMS2*, or deletions affecting the *EPCAM* gene upstream of *MSH2*, is characterized by a predisposition to early-onset colorectal and additional extracolonic cancers. An alternative but rare cause of Lynch syndrome is a constitutional epimutation of *MLH1*, which is characterized by promoter methylation and transcriptional silencing of a single allele in normal tissues.

Worldwide, five families with autosomal dominant transmission of a constitutional *MLH1* epimutation linked to an *MLH1* haplotype with two single nucleotide variants (c.-27C>A and c.85G>T) have been identified. To examine whether these families share a common ancestry, we performed SNP array-based genotyping in four of the families. The genotyping revealed a shared haplotype across a ≤2.6 Mb region of chromosome 3p22 encompassing *MLH1* and additional surrounding genes, indicating common ancestry.

This finding has two important implications. First, the candidate region for the genetic defect of this epimutation is now defined by the ≤2.6 Mb minimal region of haplotype overlap between the four families. Furthermore, regardless of the exact underlying mechanism, which might be driven by either the c.-27C>A or c.85G>T variants, these variants can serve as markers for this disease-causing genetic haplotype. Additional carriers of this founder haplotype could be spread across the globe. Our results can therefore improve the identification and diagnosis of (yet unexplained) Lynch syndrome cases.

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P11.110

Screening for constitutional *MLH1* epimutation in the routine diagnosis of Lynch syndrome

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Lynch syndrome is characterized by genetic predisposition to colorectal, endometrial and other cancers due to germline mutations of the mismatch repair genes *MLH1*, *MSH2*, *MSH6* and *PMS2*. In a subset of patients without

genetic mutation, constitutional *MLH1* epimutations (i.e. germline *MLH1* promoter hypermethylation) have been identified as the cause of Lynch syndrome.
We first performed a retrospective study in a cohort of 134 unrelated patients (*Hum. Mutat. 2012; 33:180-8*), and identified the epimutation in two probands, with transgenerational transmission of the epigenetic defect in one case.
Starting fall 2009, we implemented *MLH1* promoter methylation testing on a systematic basis with *MLH1* gene analysis in our lab. Thus 160 patients were screened for germline epimutation, and 119 additional DNA samples from patients referred to other French institutions and tested negative for genetic mutation were sent to our lab for methylation analysis. Altered MLH1 and/or PMS2 protein expression was documented for 158 patients without deleterious genetic mutation. An epimutation was found for 8 patients (5 %, 8/158).
Somatic *MLH1* promoter hypermethylation is frequently observed in microsatellite unstable sporadic cancers and is commonly used to discriminate sporadic from hereditary cancers. However it can also reveal the presence of a germline epimutation, emphasizing the importance of methylation analysis in germline DNA, especially for patients with multiple or young-onset cancers. Additionally some patients with germline epimutation where not tested for somatic hypermethylation due to maintained MLH1 protein expression or lack of available tumor sample. Systematic constitutional epimutation testing proved to be useful in these cases.

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P11.111
Functional identification of splicing mutations in the exon 10 of MLH1, a gene implicated in hereditary colorectal cancer

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It's currently known that sequence variations identified in patients can be deleterious by affecting mRNA splicing: either by changing splice sites or by modifying splicing regulatory elements. Here, we analyzed the effect on splicing of all single-substitutions reported on the Leiden Open Variation Database in the exon 10 of *MLH1* (n=22), a gene implicated in hereditary colorectal cancer (also called Lynch Syndrome). *Ex vivo* splicing assays with representative minigenes revealed that only 5 out of the 22 tested mutations had no effect on splicing. Among the splicing alterations induced by the remaining 17 mutations, we detected the following events: (i) creation of internal splice site, (ii) increased exon inclusion, and (iii) increased exon skipping. Mutations located outside splice sites but inducing exon inclusion or skipping were further analyzed by using a completely heterologous minigene particularly sensitive to the presence of exonic splicing regulatory sequences. Results suggest that indeed those mutations alter splicing regulatory elements. We could confirm the drastic impact on splicing of one of these mutations by analyzing a RNA sample from a patient. Interestingly, in this case, we observed not only increased skipping of exon 10 but also of exons 9-10 and 10-11. Our results indicate that the exon 10 of *MLH1* is very sensitive to splicing mutations. Moreover, this study contributes to the molecular diagnosis of hereditary colorectal cancer.

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P11.112
Elimination of pseudogene interference in a PMS2 mutation screen. Validation in patients at risk for Lynch Syndrome in Ontario, Canada.

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Lynch Syndrome (LS) is characterized by mutations in the DNA mismatch repair genes MSH2, MLH1, MSH6, and PMS2. However the identification of PMS2 specific mutations is challenged by a wealth of pseudogenes, along with the allelic diversity of the 3' region of PMS2, a complexity which has probably lead to under-reporting PMS2 gene mutations as a cause of LS. We describe a technique to characterize the PMS2 gene which combines MLPA analysis utilizing SNP-specific probes, along with methodology, adapted from Clendenning et al, utilizing genomic LR-PCR enabled, and direct specific sequencing, of both PMS2 and PMS2CL exons.
The identification of LS in a panel of 30 patients, at risk of this disease by clinical history, was approached by examination of their tumour tissue/DNA (where available) using immunohistochemical analysis of the 4 MMR proteins, along with assessment of microsatellite instability of the tumour DNA. Using the PMS2 technique outlined above on leukocyte-derived genomic DNA, PMS2 gene mutations were identified in 6 unrelated individuals in the panel of 30 patients: (c.736_741delins [four unrelated families], c.1882C>T, and a deletion containing exons 5-7, i.e. 6/30). All 6 individuals demonstrated isolated imunohistochemical loss of PMS2. No PMS2 mutations were identified in 2 further individuals also showing isolated imunohistochemical loss of PMS2, and no PMS2 mutations were identified in any individuals (14) showing imunohistochemical loss of both MLH1 and PMS2.
Immunohistochemical analysis of the MMR proteins followed by blending MLPA with sequence analysis can effectively identify deleterious PMS2 gene mutations in individuals at risk of LS.

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P11.113
Lynch syndrome due to PMS2 mutations: one clinic's experience

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PMS2 is a mismatch repair gene that forms a dimer with MLH1. Whereas MLH1 mutations are found in almost 50% of Lynch syndrome cases, PMS2 mutations are a rare cause of this condition. Information regarding the spectrum of disease due to PMS2 mutations is limited. In our hereditary cancer clinic we have 3 PMS2 families from a total of 44 mutation-proven Lynch families. We discuss each case to show the variability of presentation. These cases add to the limited clinical information about Lynch due to PMS2 mutations. We also review some of the basic science evidence to support reduced penetrance of heterozygous PMS2 mutations.

Case	Proband's cancer	Criteria by pedigree	PMS2 mutation
EC	Ovary (endometrioid) 56 y.o. Cecum adenoca 58 y.o	Amsterdam II	c.730C>T p.Gln244X (exon 7)
KA	Sigmoid adenoca 29 y.o.	None	c.1788delA p.lys593fs
ES	Endometrial 46 y.o.	Amsterdam I	c.132_134delAAA P.Glu44_Asn45delinsAsp

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P11.114
Lynch Syndrome among Ashkenazi Jews

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Lynch Syndrome (LS) is caused by mutations in DNA mismatch repair genes. Diagnosis is not always trivial and may be costly. Knowledge of incidence, genotype-phenotype correlation, spectrum of mutations and genes involved in specific populations, facilitates the diagnostic process and contributes to clinical work-up. Aim: To report the unique features of LS, gene distribution, mutations detected and co-occurrence of related syndromes in Ashkenazi Jews. METHODS: Patients were studied in high risk clinics. Diagnostics followed a multi-step process. It included testing for founder mutations, tumor testing, gene sequencing and MLPA. LS was defined by positive tumor analysis and/or positive mutation testing. RESULTS: We describe a cohort of 120 carriers from 75 Ashkenazi families with LS. Mutations were identified in 51/75 (68%) families. 36 families harbored mutations in *MSH2*, 9 had mutations in *MSH6*, and 6 had mutations in *MLH1*. 37/51 (73%) families carried one of the 3 ‚Ashkenazi' founder mutations in MSH2 or MSH6. Each of the other 14 families carried a private mutation. 3 (6%) were large deletions. Only 25/75 (33%) families were Amsterdam Criteria positive, 62 (83%) were Bethesda positive and 13 (17%) did not comply with any clinical criteria. We report C-MMRD and co-occurrence of BRCA and LS in our cohort. CONCLUSIONS: Mutation spectrum and gene distribution among Ashkenazi

Jews are unique. Three founder mutations cause LS in 73% families with known mutations. *MSH2* and *MSH6* cause the majority of cases, while *MLH1* comes third. These features affect the phenotype, the diagnostic process, risk estimation, and genetic counseling.

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P11.115

One next generation sequencing panel for all patients suspected of hereditary cancer

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Phenotypes of many hereditary tumour syndromes are known to overlap. In addition, different genes may underlie one syndrome. Therefore the number of genes to examine in a particular clinical case can be large. In current diagnostic and reimbursement settings, the number of genes that can be analysed for one patient through Sanger sequencing is limited. Next generation sequencing offers novel possibilities to analyse large numbers of genes in parallel at relatively low cost. We developed a kit based on Agilent Sure Select Target Enrichment for simultaneous mutation detection in 73 genes that have previously been associated with tumour syndromes. The kit has been validated by two series of twelve patients with known variants identified through Sanger sequencing of the BRCA or the Lynch syndrome genes. In addition, a set of twelve breast or colorectal cancer patients was analysed anonymously for all 73 genes.
The samples were sequenced using 151 bp paired-end reads on an Illumina MiSeq sequencer and analysed using the Next gene software and Cartagenia. All pathogenic mutations, unclassified variants and polymorphisms previously detected were identified. Furthermore, 40 novel variants that were identified in twelve anonymous patients could be confirmed by Sanger sequencing.
The Cartagenia software allows blocking the analysis of certain genes. For diagnostic purposes genes within the panel have been defined for filtering, e.g. depending on the availability of preventive options. The genes to be analysed will be decided during genetic counselling prior to testing.

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P11.116

Influence of VEGF polymorphism -634 G/C on *VEGF* mRNA expression and susceptibility to sporadic colon cancer

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Angiogenesis is one of the six postulated hallmarks of cancer and Vascular endothelial growth factor (VEGF) is one of the key mediators of this very complex process. Thusfar no prognostic and predictive markers for *VEGF* expression have been established and therefore the prediction of VEGF production based on individual genetic background might be useful in the prediction of disease progression and/or the efficacy of anti-cancer treatment. *VEGF* gene is highly polymorphic, with many single nucleotide polymorphisms (SNPs) that may affect VEGF production and/or activity and possibly lead to inter-individual differences in tumor development and progression. In this study we have examined the influence of *VEGF* -634 G/C (rs2010963) polymorphism on *VEGF* mRNA expression as well as susceptibility to sporadic colon cancer.
VEGF mRNA expression analysis in tumour tissue was performed by real-time RT-PCR. VEGF -634 G/C genotyping was performed in 250 patients with sporadic colon cancer and 250 unrelated healthy volunteers by real-time PCR-SNP analysis.
A statistically significant correlation between *VEGF* mRNA expression and *VEGF* -634 G/C genotypes was found (p=0.019). Genotypes carrying the G allele (GG+CG) were associated with high *VEGF* mRNA expression, while the CC genotype was associated with low *VEGF* mRNA expression. However, there was no statistically significant association between *VEGF* -634 G/C genotypes and susceptibility to sporadic colon cancer.
Finally, we can conclude that although in our study VEGF -634 G/C polymorphism was associated with *VEGF* mRNA expression in sporadic colon tumours it was not associated with susceptibility to this type of cancer.

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P11.117

Copy Number Alterations in Small Intestinal Neuroendocrine Neoplasms Determined by Array Comparative Genomic Hybridization

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Small intestinal neuroendocrine neoplasms (SI-NENs) are typically slow-growing neoplasms that have metastasized already at the time of diagnosis. To further define regions of recurrent copy number (CN) alterations (CNA), we applied array CGH on SI-NENs including 18 primary tumors and 12 metastases. The most frequent abnormality was loss on chromosome 18 observed in 70% of cases. CN losses were also frequently found on chromosomes 11 (23%), 16 (20%), and 9 (20%), with regions of recurrent CN loss identified in 11q23.1-qter, 16q12.2-qter, 9pter-p13.2 and 9p13.1-11.2. Gains were most frequently detected in chromosomes 14 (43%), 20 (37%), 4 (27%), and 5 (23%) with recurrent regions of CN gain located to 14q11.2, 14q32.2-32.31, 20pter-p11.21, 20q11.1-11.21, 20q12-qter, 4 and 5. Quantitative PCR analysis confirmed most CNAs detected by a-CGH as well as revealed CNAs in an extended panel of SI-NENs. Unsupervised hierarchical clustering of recurrent regions of CNAs revealed two important tumor groups and 5 chromosomal clusters. Loss in chromosomes 20, 18, 16 and 11 were found in both tumor groups. Tumor group II was enriched for alterations in chromosome cluster D, including gain on chromosomes 4, 5, 7, 17 and gain on 20 in chromosome cluster B. Gain on 20pter-p11.21 was associated with short survival. Statistically significant differences were observed between primary tumors and metastases for loss on 16q and gain on 7.In conclusion, our results revealed several CNAs in recurrent candidate regions with a potential role in SI-NEN development. Distinct genetic alterations and pathways are involved in tumorigenesis of SI-NENs.

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P11.118

Gallbladder Cancer Predisposition: A Multigenic Approach involving Inflammatory, Xenobiotic and Steroidal receptor genes

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Introduction: Gallbladder cancer (GBC) is a multifactorial disease with complex interplay between multiple genetic variants. We analyzed 15 polymorphisms in 9 genes involved in inflammatory, xenobiotic and steroidal receptor pathways to find out combinations of genetic variants contributing to GBC risk.
Methods: The genes included in the study were (MMP-2,7,9) TIMP-2, CYP1A1, CYP1B1, PLCE1, LXR-alpha and LXR-beta. Genotypes were determined by PCR-RFLP and Taqman probes. Statistical analysis was done by SPSSver16. Multilocus analysis was performed by Classification and Regression Tree Analysis (CART) and MDR to identify genetic variants in modifying GBC risk. In-silco analysis was performed using Bioinformatics tools (F-SNP, FAST-SNP).
Results: Single locus analysis by logistic regression showed association of MMP2 (-735 C>T, -1306 C>T), MMP7 -181 A>G, MMP-9 (P574R, R668Q), TIMP2-418 G>C, CYP1A1-MspI, CYP1A1-Ile462Val, PLCE1 (rs2274223 A>G, rs7922612 T>C) and LXR-beta T>C (rs3546355 G>A, rs2695121 T>C) polymorphisms with GBC risk (pA, rs2695121 T>C), MMP-2 (-1306C>T) and MMP-9 (R668Q) and PLCE1 rs2274223A>G to be key players in GBC causation (p<0.001, CVC=7/10). The results were further supported by independent CART analysis ((p<0.001). Sub-group analysis based on gender and gallstone status showed distinct subsets of genetic signatures in GBC susceptibility. Insilico analysis revealed variable change in splicing or transcriptional regulation.
Conclusion: The multigenic approach showed the importance of PLCE 1, LXR-beta and MMPs genetic variants and their interactions in contributing to GBC risk.
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P11.119

Whole genome and whole exome sequencing of gastric cancer samples

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Gastric cancer is diagnosed in 8% of all cancer cases and causes 10% of the cancer deaths worldwide (Jemal et al., 2011). To further analyze the genetic basis of gastric cancer, we sequenced the whole genome of a microsatellite stable and a microsatellite unstable gastric carcinoma and the genome of the matched normal tissue samples on the Illumina HiSeq with an average coverage of 49x. In order to increase the read depth in the coding regions we performed whole exome capture using the Agilent SureSelect Human All Exon v2 kit followed by sequencing on the Solid 4 and received an additional average coverage of 55x.
Using a comprehensive filter strategy for single nucleotide variants (SNVs) and structural variants (SVs) we were able to identify a multitude of novel potentially damaging mutations that are followed up by functional assays. Recurrence is assessed in an independent cohort of gastric cancer patients. With help of the databases OMIM, HGMD and GWAS we could further detect a great number of known cancer associated SNVs. Pathway (KEGG) and functional (GO) analysis of SNVs and SVs were performed using 1087 samples from the 1000 Genomes project as a normal variant matrix.
The results highlight a strategy of an exemplary tumor genome analysis that combines both exome and whole genome sequence information with two different NGS platforms, uses population-based whole genome resources as a novel pathway-based filter and integrates SNV as well as SV analysis.

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P11.120

Gastric cancer as a part of BRCA1-related syndrome

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The risk of cancer disease in BRCA1 mutation carriers is believed to be restricted mainly to breasts and ovaries. We screened 65 patients with gastric cancer (GC) for Russian BRCA1 5382insC founder allele, and identified 2 (3%) heterozygotes. Both mutation-positive patients demonstrated unusually pronounced and durable response to platinum-containing therapy, and reported personal or family history of breast or ovarian cancer. Patient 1, 60 years old female, was treated by cisplatin/5-fluorouracil, and showed 66% reduction of tumor size with a time to progression 10.7 months; her mother was affected by ovarian cancer. Patient 2, 62 years old female, was treated by doxorubicin/cisplatin/5-fluorouracil; the reduction of tumor size was 93%, and the time to progression was 21.9 months; this patient had a personal history of successfully cured breast cancer at age 44. In accordance with the "two-hit" mechanism of inactivation of hereditary cancer gene, both BRCA1-associated gastric carcinomas showed the somatic deletion of the wild-type BRCA1 allele. The involvement of BRCA1 germ-line mutations in GC development may be somewhat related to regional attitudes, which until recently included frequent use of strong alcohol, low intake of fresh fruits and vegetables, excessive consumption of fat and salty food, etc. Thus, based on (1) the elevated frequency of BRCA1 mutations in GC patients, (2) the pronounced sensitivity of mutation-positive GC to platinum containing therapy, and (3) the somatic loss of the wild-type BRCA1 allele in the tumor tissue, we conclude that some cases of gastric carcinoma may be linked to BRCA1 syndrome.

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P11.121

Somatic alterations of the E-Cadherin gene predict survival of gastric cancer patients mainly those presenting with familial history

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The prognosis of gastric cancer (GC) is poor, the treatment ineffective, and the molecular pathogenesis players vastly unknown. As E-cadherin (*CDH1)* deregulation and Microsatellite Instability (MSI) define two major paths involved in GC, we characterized and stratified 246 GC cases according to alterations in these pathways.
We systematically analysed a GC series comprising distinct clinical settings (sporadic, familial) and tumour histological types (intestinal, diffuse), for the presence of *CDH1* structural alterations (mutations, LOH) and promoter hypermethylation. Independently of clinical setting and histotype, ~30% of all cases displayed *CDH1* somatic alterations. Familial intestinal GC (FIGC) and both histological types of sporadic GC showed methylation and structural *CDH1* alterations, whilst tumours from hereditary diffuse GC HDGC showed exclusively *CDH1* methylation. Importantly, the worst patient survival rate was seen for FIGC patients carrying *CDH1* structural alterations. MSI phenotype was significantly different between sporadic (19%) and familial (39%) GC and between intestinal (31.2%) and diffuse (12.8%) GC. MSI phenotype occurred preferentially in FIGC families. Notably, among FIGC families, we identified the group with the worst survival who presented MSS tumours and *CDH1* structural alterations (85.7%), and the group presenting the best prognosis with tumours carrying *CDH1* epigenetic alterations and MSI phenotype (77.8%).
In conclusion, this work pinpointed a somatic molecular landscape that determines clinical outcome in GC patients. Particularly for FIGC families, stratification at diagnosis, based on somatic *CDH1* alterations and MSI phenotype, may significantly improve clinical management.

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P11.122

Molecular classification of gastric cancers using an extended panel of gene amplifications

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Introduction: Gastric cancer represents the third most common malignancy of the gastrointestinal tract in the Czech Republic. Recent advances in biological anticancer therapy revealed importance of understanding of tumor biology underlying its clinical behavior. The standard histopathology categorization into intestinal or diffuse type (lauren classification) is useful for assessing general prognosis, especially, uncovering hereditary origin of the tumor for subsequent CDH1 testing. It is clear, however, that in order to estimate therapy response, further evaluation of molecular profiles is highly desirable.
Aims: In this project we evaluated a large panel of gene amplifications in an attempt to classify gastric cancer tumors.
Methods: Formalin-fixed paraffin-embedded (FFPE) sections from a total of 85 patients were subjected to molecular analysis. Gene amplifications were analyzed by Multiplex Ligation-dependent Probe Amplification (MLPA). The final panel consisted of a total of 70 common oncogenes and tumor supressors. The complex patterns were statistically processed by hierarchical data clstering using Ward and farthest neighbor method.
Results: The overall success rate of DNA extraction from the gastric FFPE samples was at a level of 85%. Both statistical approaches delivered similarities in data clustering. The resulting dendrograms revealed comparably constituted subclusters of samples. As expected HER2, MYC, p16 and b-CAT genes were among the most frequently amplified.
Conclusions: Presence of specific gene amplification may indicate aberrant activation of various signaling pathways, hence, define characteristic tumor behavior. A correlation to monitoring parameters such as progression-free survival or overall survival is ongoing.
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P11.123

Abnormal methylation of tumor-related genes in biopsies of the gastric mucosa from patients previously operated on gastric cancer.

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Gastric cancer (GC) is one of the most common malignant tumors in the world. GC develops through the accumulation of genetic and epigenetic alterations. Epigenetic silencing of tumor-related genes, due to hypermethylation of the CpG sites in the promoter regions, has emerged as one of the main genetic alterations in cancer development. Aberrant methylation of tumor-related genes is one of the early events in carcinogenesis, it can be determined before the clinical manifestation of the tumor.
We investigated the clinical and prognostic importance of CpG-island hypermethylation in 6 tumor-related genes (*CDH1, RASSF1A, MLH1, N33, DAPK, RUNX,*) for gastric cancer patients. We examined hypermethylation of 6 tumor-related genes in 35 frozen biopsies of the gastric mucosa, obtained during endoscopic research, from patients previously operated on gastric cancer.
There was no hypermethylation of the CpG sites of *CDH1, RASSF1A, MLH1, N33, DAPK, RUNX3* genes in 29 biopsies. In 6/35 samples we have identified aberrant methylation of *CDH, N33, RUNX3* genes, in 1 patient we have identified aberrant methylation of three genes (*CDH1, N33, RUNX3*), in 2 patients - two genes (*CDH1, N33*), in 3 patients - of one methylated gene (*N33* or *RUNX3*).
The current study shows that the hypermethylation of tumor-related genes may be used as additional marker for postsurgical monitoring of gastric cancer patients.

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P11.124
Foveolar cells phagocytose apoptotic neutrophils in chronic active Helicobacter pylori gastritis.

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The recognition and removal of apoptotic inflammatory cells by tissue macrophages and non-professional phagocytes, in a process called efferocytosis, is required for resolution of inflammation and is actively anti-inflammatory. Biopsy specimens from 28 subjects with or without H. pylori infection and active inflammation were examined and graded according to the updated Sydney system. Light microscopy, electron microscopy, and Terminal Deoxynucleotidyltransferase-Mediated UTP End Labeling staining were used to identify apoptosis. H. pylori infection was detected by histology and by molecular assay in 16 out of 28 cases. DNA from paraffin-embedded gastric biopsies was amplified using primers specific for cagA, for the cag „empty site" as well as for the s and m alleles of vacA. The more virulent cagA-positive strains were found in five out of nine patients with chronic active gastritis. The vacA s1/m1 and s2/m1 genotypes were more common in nine patients with chronic active gastritis, while the vacA s2/m2 genotype was more frequent in seven patients with chronic inactive gastritis. Apoptotic neutrophils were also detected within the cytoplasmic vacuoles of the foveolar cells of nine cases with chronic active gastritis. Transmission electron micrographs revealed further apoptotic neutrophils within spacious phagosomes of foveolar cells in a similar manner to those described in late-phase efferocytosis both in vivo and in vitro. These new observations expand the morphological spectrum of gastritis in patients infected with more virulent H. pylori strains, compatible with an anti-inflammatory role for the gastric epithelial cells in their removal of apoptotic neutrophils during active chronic gastritis.

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P11.125
O-6-methylguanine-DNA methyltransferase variant in gastric cancer samples from Kashmiri population

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Background and aims: The Kashmiri population under investigation is a non-migrant population with special social, personal and dietary habits. Gastric cancer along with esophageal cancer accounts more than 60% of all cancers in the region. O6-methylguanine methyltransferase (MGMT) the enzyme of our interest repairs, O6-Methylguanine (O6-meG), a DNA adduct. Genetic alterations and variants of MGMT gene impair the protein's ability to remove alkyl groups from the O6-position of guanine, thereby raising the mutation rate and increasing the risk of cancer.
Methods: We have analyzed fresh frozen gastric cancer samples for MGMT variation using conventional method followed by PCR product sequencing.
Results: Out of 30 samples we studied, 13 neoplastic tissue harbored somatic missense MGMT mutations in Exon 5, leading to S151I, which according to *in silico* analysis reduced MGMT function.
Conclusion: In summary, we found that there is variation in MGMT exon 5 in neoplastic tissue which may lead to impaired DNA repair and contributing to underlying molecular mechanisms in progression of cancer.

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P11.126
Chromogenic In Situ Hybridization, a viable alternative to FISH, for detection the HER2neu gene amplification in gastric cancer

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Gastric cancer is one of the most common solid tumours, being the second leading cause of cancer death worldwide, after lung cancer.
Detection of Her 2 neu amplification, its useful to guide therapy for patients with gastroesophageal cancer, as patients with HER2 amplification may be candidates for Herceptin therapy and to confirm the presence of HER2 amplification in cases with 2+ HER2 protein overexpression by immunohistochemistry.
Methods: we collected 55 paraffin-embedded tissue sections of gastric cancer, for which the HER2 status had been predetermined using immunohistochemistry, for this cases determined HER2 gene and chromosome 17 copy numbers using the dual-color PathVysion HER2 DNA probe set (Abbott Molecular), respectively CISH and the scoring system and interpretation of CISH was from - Zytovision.
Immunohistochemistry alone is not sufficient, for determining HER2 status in gastric cancer, detection of Her 2 neu amplification it is a useful methodology for confirming ambiguous immunohistochemistry results.
CISH is a method for detection of gene amplification using a peroxidase reaction, is more practical alternative, due to lower cost, no requirement of fluorescence microscope, use of existing bright-field microscopy, archivable and quantitative results, it s easy to observe both the tissue morphology and the gene amplification evaluation
The CISH results were compared with dual-colour fluorescence in situ hybridisation (FISH) data, there was 98% concordance between CISH and FISH
The high concordance, indicates that CISH is a viable alternative to FISH, for detection of HER 2 NEU gene amplification in gastric cancer.

M. Neagu: None.

P11.127
The correlation between polymorphism of Toll-Like Receptor 4 and cytokines TNFα, TLR4, TGFβ, CD14 AND CCR2 and bacterial infections in patients with pancreatic cancer in Polish population.

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Introduction: Toll-Like Receptors, antigen CD14, pro- and anti-inflammatory cytokines and chemokines with their receptors are involved in the mechanism of innate response during bacterial infections. Polymorphism in these genes can cause that their products may be functionally inefficient and consequence of this may be tendency of some patient to the development of extensive inflammation and sepsis. The aim of study was to research markers of bacterial infections, surgical wound healing disorders or fistula anastomosis in patients operated on pancreatic cancer. Material and methods: Genetic polymorphisms was surveyed polymerase chain reactions - restriction fragment length polymorphism analysis technique in 62 patients with pancreatic cancer. A control group of 130 blood donors was ethnically matched to the study and randomly selected to comparing the study data. Results: We did not note statistical importance of polymorphic genotypes in TNFα (-308G/A), TLR4 (1363C/T), CCR2 (190G/A) and CD14 (-159C/T) genes. Compared with healthy volunteers we observed that polymorphisms of TLR4 (1063A/G) gene was statistically significant. Conclusions: Up to now we still searching for specific genetic marker for bacterial infection during pancreatic cancer. Despite of this we are able to determine that TLR4 (1063A/G) gene is crucial to pancreatic bacterial infections.

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P11.128

Constitutional mismatch repair deficiency syndrome in Israel caused by founder mutations in the MMR genes

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Heterozygous germline mutations in one of the four mismatch repair (MMR) genes, *MLH1, MSH2, MSH6* and *PMS2*, cause Lynch syndrome (LS) an autosomal dominant cancer predisposition syndrome conferring a high risk of colorectal, endometrial and other cancers in adulthood (HNPCC). Offspring of couples, both having LS, have a high risk to inherit biallelic MMR gene mutations. These cause constitutional MMR deficiency (CMMRD), a severe recessively inherited childhood cancer syndrome with a very broad tumor spectrum including mostly hematological malignancies, brain tumors and childhood colon cancer. Most of CMMRD children also present with café au lait spots and axillary freckling mimicking Neurofibromatosis type 1.
We describe our experience in Israel with five CMMRD families. The clinical presentation included: brain tumors at ages 2-19 years, colon cancer at ages 9-20 years and one patient with lymphoma at age 12 years. In two non-consanguineous Ashkenazi families, the common founder Ashkenazi mutations were detected: one family was homozygous for c.1906G>C in *MSH2* and the second family was compound heterozygous for c.3984_3987dupGTCA and c.3959_3962delCAAG in *MSH6.* In the three other consanguineous families, different homozygous mutations were identified: c.2192T>G (p.L731X) in *PMS2*, a recurrent mutation among Iranian Jews, *PMS2* c.686_687delCT and *MSH6*3606_3603 delAGTG were found in large pedigrees of Bedouin and Arab ancestries, respectively.
Given the prevalence of these mutations among the Israeli population, we want to raise the awareness of CMMRD syndrome and open a discussion regarding screening for MMR founder mutations among spouses of LS patients for the purpose of prenatal diagnosis.

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P11.129

Increased frequency of allele C of rs7074064 in patients with colorectal cancer.

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Colorectal cancer (CRC) is the fourth most common cancer in the world, and it is also considered as the third most common cause of cancer-related deaths. On average, it affects 5-6% of people, and the risk depends on both factors related to heredity and lifestyle.
It is assumed that in 20-30% of cases, it is potentially possible to define genetic background and 3-5% develop genetically determined bowel syndromes associated with a high risk of CRC. The search for new genetic factors that increase the risk of the disease is the subject of extensive research in many laboratories. In our study we analyzed the frequency of polymorphisms in the BMPR1A gene in 200 patients with sporadic colorectal cancer and we compared them with their frequency in the control group. Rs7074064 showed a statistically significant increased frequency of allele C and genotype CC in patients with CRC. The frequencies of genotypes for the group of patients and for the control group were respectively TT (31%), TC (62%), CC (7%) and TT (65%), TC (33%), CC (2%). The different distributions of genotypes in both groups were statistically significant at P <0.0001. Odds ratio for occurrence of the disease for people with variant C of rs7074064 was estimated at 2.621. The study was financed by the Ministry of Education and Science, Poland, grant number N402431438

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P11.130

NCL/NPM and BRAF gene structure in metastatic cutaneous melanoma(CM)patients

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Background. Nucleolin/C23 and nucleophosmin/B23 are major nucleolar argyrophilic proteins involved in carcinogenesis and CM progression. There are none data on C23/B23 encoded genes NCL/NPM in CM. Material and methods. DNA samples from 23 CM patient (CMP)metastatic nodes were analyzed by PCR for NCL and NPM genes. Alterations in NCL/NPM gene structure(23 CM)and 2 human CM xenografts(X)+ BRAF mutations in exon 15(7/23 CM)were studied. Results. Genetic alterations in NCL/NPM were grouped in several types: (1) two simultaneous nucleotide substitutions (NS) IVS5-31G/A+IVS6+42G/A in NPM gene - in 13/23 (56%) CMPs and 1/2 X; (2) NS IVS8-63A/G in NPM - in 9/23 (39%) CMPs + two NS from the group (1); (3) germinal deletion p.D255delGAT (c. 763-765delGAT) + common polymorphism p.E149E in NCL gene + intron structure variant in 1 CMP with 4 CM relapses; (4) 1 NS IVS2+31G/A in NCL gene. Somatic mutation V600E BRAF (70% in CM cases) and K600E BRAF + AGT/AAA BRAF were revealed in 3/7 and 1/7 CMPs with 2-5 relapses, consequently. Mutation V600E BRAF in 1CMP was accompany by NS of the group (1) + group (2) in NPM gene; V600E BRAF in other 1CMP was accompany by analogous NS, except IVS6-39A/G+-42G/A. Conclusion. Some certain types of NPM and/or NCL gene alterations, including NS in NPM and germinal deletion in NCL were found in 17/23 CMPs. The data obtained indicate that alterations in NPM/NCL/BRAF genes likely to associate with numerous relapses in CMPs.

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P11.131

A novel mutation in 3'-UTR of the growth factor independence 1 (Gfi-1) gene in the cutaneous T-cell lymphoma

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Background: The growth factor independence 1 (Gfi-1) has recently attracted a lot of attention attention due to its pivotal effects in lymphopoiesis and myelopoiesis. The aim of the study was to investigate possible associations of variability in the Gfi-1 gene with cutaneous T-cell lymphomas (CTCL).
Methods: 63 patients with CTCL (30M/33F, median age 62y, 26-101y) diagnosed and treated at the 1st Department of Dermatology of the St Ann's Faculty Hospital Brno were sequenced for the Gfi-1 gene and the sequences were then compared to the database sequence and the sequency of sex- and age-matched controls (30M/33F, median age 58, age range 26-80y) without personal or family history of skin diseases and without signs of malignancy.
Results: A novel polymorphism 1765C/T in 3´-UTR region of the Gfi-1 gene was identified in several CTCL patients that was not present in the controls. No association of the identified polymorphisms with disease severity and/or survival of the patients was observed.
Conclusion: Our study indicates that a novel polymorphism 1765 C/T in 3'-UTR of Gfi-1 gene might be involved in CTCL ethiopathogenesis, adding Gfi-1 to the short list of potential CTCL susceptibility genes. More research into the role of this variation as well as replication of the observations on larger populations with different ethnicity is imperative.

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P11.132

Update of recent cytogenetic findings in the LeMon5 study

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Introduction: In the ongoing German multicenter Lemon5-study, the safety of lenalidomide monotherapy with special focus on leukemic transformation in IPSS low risk MDS patients with isolated 5q deletion is examined. Here we report on the current cytogenetic results from this study.
Methods: Cytogenetic investigations were performed using chromosome banding and FISH analyses of bone marrow aspirates and CD34+ peripheral blood cells. FISH analyses on blood cells are carried out every 2 to 3 months using 8 to 13 DNA probes to detect cytogenetic changes during treatment.
Results: We have already analyzed 146 suspected MDS patients. Only 84 patients could be enrolled in this study due to cytogenetic and/or morphologic screening failures in 62 patients. To date, follow-up data for 53 patients are available. A complete cytogenetic response could be observed in 35 patients (66%) after a median follow-up of 15.5 (2-36) months. In eleven patients (20%) 5q- clone size did not show any changes. Cytogenetic response could be observed within a median of 6 (2-12) months after start of therapy, lasting for a median duration of 10 (2-25) months.
Conclusion: FISH analysis on CD34+ pb cells allows a reliable frequent and relevant genetic monitoring of treatment response to lenalidomide and to identify cytogenetic non-response or progression as early as possible.

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P11.133
Dog, a spontaneous model for human cancers: example of histiocytic sarcomas

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Dogs spontaneously develop many cancer types presenting the advantage to be homologous to human cancers, breed specific and frequent in the predisposed breeds. These features reflect the fact that predisposing genes could be tracked in these genetic isolates more easily than in humans. Faced to limitations in the use of classical rodent cancer models, Dog who offers a physiopathology closer to humans, gives the opportunity to decipher the natural steps of tumorigenesis from genetic predispositions to acquired abnormalities and to investigate new therapies.
We illustrate the power of this original model with the study of canine histiocytic sarcomas (HS). These tumours present striking breed specificity: HS mainly affects Bernese Mountain dogs (> 25%), while in Human, HS are extremely rare and poorly known.
Thanks to the collection of over 3000 DNA samples, we proposed an oligogenic transmission for this cancer (Abadie et al. 2009) and performed genome wide association studies which allowed us to identify 2 major loci involved in HS predisposition. These loci are under investigation: one of them is orthologous to the human region 9p21 (Shearin et al. 2012). To get a complete view of pathways involved in the tumorigenesis, CGH and RNA sequencing analyses are ongoing to identify tumour progression alterations. Recurrent alterations have been identified among which deletions of CDKN2a and PTEN (Hedan et al 2011).
All together, these genetic analyses are thought to gain a better understanding of the physiopathology and genetics of HS in dogs but also in humans.

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P11.135
Detecting mutations in the EGFR gene in lung adenocarcinomas in order to guide personalized therapy with tyrosine-kinase inhibitors.

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The EGFR gene codifies the Epidermal Growth Factor Receptor, a transmembrane tirosine-kinase glycoprotein, involved in cell growth and differentiation. Mutations in exons 18-21 of the EGFR gene - identified in 10-30% of Caucasian patients with non-small-cell lung carcinomas (NCSLC) - trigger constitutive activation of the EGFR receptor, playing a crucial role in tumor progression. The EGFR mutational status is a predictor of benefit for the new personalized therapy with anti-EGFR tyrosine-kinase inhibitors (TKI).
The aim of our study was to analyse the EGFR mutational status in exons 19 and 21 (cca 90% from all mutations) in NCSLC patients, in order to contribute to the selection of patients for the anti-EGFR TKI therapy.
DNA was extracted from formalin fixed, paraffin embedded lung adenocarcinomas sampled from 139 patients. The EGFR mutational status was analyzed by ARMS-PCR and sequencing.
Activating mutations were detected in the EGFR gene in 24 tumors (17,26%), of which 17 tumors with deletions in exon 19 (2 of them being less-common) and 7 tumors with L858R mutation in exon 21; the amount of tumor cells was variable, between 90% and 1%. Patients were between 49 and 86 year old, sex distribution was 1:1,66 (9M:15F); 18 of them were non-smokers.
Our results were in accordance with international data, contributing to an effective selection of patients with NSCLC for the anti-EGFR personalized therapy.
Further evaluation of the significance of some *EGFR* mutations is needed, including assessment of the functional importance (clinical and/or predictive significance) of the less-common activating mutations.

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P11.136
The importance of the P53, P16, CDH1 and PPP2R1A genes analysis in grade III endometrioid carcinomas

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Endometrial carcinomas are classified in two groups based on clinical and pathologic characteristics: endometrioid (EC) (grade I, II and III) and no endometrioid (NEC) (grade III). EC usually show mutations in PTEN (35-50%), PIK3CA (36%), BRAF (5-20%), KRAS (15-30%) and CTNNB1 (20-40%) genes whereas there are other genes more associated with NEC but sometimes mutated in EC as well: P53 (10-20%), P16 (10%), CDH1 (10-20%) and PPP2R1A (5%) genes. We have analyzed these genes in a group of ECs in order to determine if the mutation frequency is associated to the tumor grade.
We extracted DNA, RNA and total proteins from 53 ECs: 23 grade I, 23 grade II and 7 grade III. We analyzed these 9 genes by PCR, CSGE-heteroduplex and sequencing. Moreover, we performed *in sílico* analysis, allelic pertenence and expression protein studies to characterize the novel mutations found.
Our results are showed in the annexed table. We found that 14.28% of grade III EC cases carry 4 or more mutated genes and that all cases show at least one of these genes mutated.
In conclusion, we report that there is a correlation between the number of mutations and the kind of mutated genes and EC tumor grade. Therefore, we suggest that P53, P16, CDH1 and PPP2R1A genes analysis could help in EC grade discrimination.
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	EC associated genes				NEC associated genes				
	PTEN	**PIK3CA**	**BRAF**	**KRAS**	**CTNNB1**	**P53**	**P16**	**CDH1**	**PPP2R1A**
G. I	65.21%	34.38%	0%	17.39%	34.78%	8.69%	0%	0%	4.34%
G. II	60.86%	4.34%	0%	21.73%	13.04%	17.39%	0%	0%	8.69%
G. III	71.42%	28.57%	0%	28.57%	0%	28.57%	14.28%	0%	14.28%

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P11.137
High-grade endometrial stromal sarcomas show distinct molecular features compared with the other subtypes of endometrial stromal tumors

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Endometrial stromal tumors (ESTs) are the second most common mesenchymal tumors of the uterus. The current classification of ESTs divides these

tumors into three modalities: the most benign endometrial stromal nodules, low-grade endometrial stromal sarcomas (ESS) and undifferentiated uterine sarcomas (UES), which are characterized by the most aggressive clinical course. Over 50% of classical ESS cases carry characteristic chromosomal translocations that involve genes encoding polycomb group (PcG) proteins. On the other hand, UES do not show the evidence of specific chromosomal aberrations. Recently, a new t(10;17)(q22;p13) which results in YWHAE-FAM22A/B fusion formation has been reported in cases described as "high-grade ESS", due to clinicopathological features intermediate between classical low-grade ESS and UES.
In the present study, we performed gene expression microarray experiments on 11 ESTs cases using the Agilent Technologies platform, in order to investigate the genetic profiles of distinct ESTs subtypes. Our cohort included 4 classical low-grade ESS cases with typical translocations, 4 UES cases and 3 cases which were classified as high-grade ESS based on the histological features and the presence of YWHAE-FAM22A/B fusion. Hierarchical clustering analysis of our microarray data revealed that high-grade ESS formed a separate sub-cluster with a distinct molecular profile as compared with the low-grade ESS and UES cases. High-grade ESS cases were characterized predominantly by decreased expression level of a subset of genes related to immune response and cell adhesion. Our results support the notion that the current ESTs classification should discriminate high-grade ESS as a distinct subtype.

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P11.138

Refining the breakpoints in translocations including the 3q26-28 chromosomal region using Bacterial Artificial Chromosomes.

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Background: Myelodysplastic Syndromes (MDS) and Acute Myeloid Leukaemia (AML) share common translocations, involving the 3q21-26 region, and suggesting that EVI1 and/or *MDS1/EVI1* rearrangements may be associated with an adverse prognosis. **The objective** of our study was to refine the chromosomal breakpoints in the 3q26-28 region with the use of Bacterial Artificial Chromosomes (BACs), and to determine whether the *EVI1* and *MDS1* genes were included in the breakpoints. **Material and Methods**: In 9 patients, with either AML(n=5), MDS (n=3) or chronic myelomonocytic leukaemia (CMML)(n=1), the following translocations: t(2;3)(p21;q26)(n=2), t(2;3)(p21;q27)(n=1), t(2;3) (p23;q26)(n=2), t(3;7)(q26;q21), t(3;8)(q27;q24), t(3;10)(q28;q23) ,t(3;12) (q26;p13) were observed. A set of 5 BACs were used to cover the 3q26.2 region, including the *EVI1*, *MDS* and *MYNN* genes, and the BAC RP44B17 to test the 2p21 region.
Results: The breakpoints were precisely established only for one translocation, t(2;3)(p21;q27), within the BAC RP11-415B12 at the 3q26.2 region, including the MDS gene and within the BAC RP 44B17 at the 2p21region with no genes described. The breakpoints in the 2p21 region for the other 4 translocations were located in regions more centromeric than the BAC RP 44B17.The breakpoints in the 3q26.2 region for the other 8 translocations included BACS involving the *MDS1* gene (n=2) the *MYNN* gene (n=1), regions more centromeric than the EVI1 gene (n=2) and regions more telomeric than the *MYNN* gene (n=3). **Conclusion:** BACs covering the 3q26.2 region revealed that the *EVI1* gene was not involved in any of the translocation breakpoints, while the *MDS1* gene was involved in 3.

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P11.139

Analysis of Ewing Sarcoma NGS Transcriptome Data Highlights Mechanisms of Cancer

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The Ewing Sarcoma family of tumors is a category of cancers that mostly affects adolescents of age 10 to 20. To understand the molecular mechanisms of tumorigenesis and metastatic processes in Ewing Sarcoma (ES), gene expression profiling of samples from Ewing Sarcoma patients was performed using Next Generation Sequencing (NGS) technology. In this study we present the results of combined statistical and biological analyses that demonstrate the differences between samples from primary and metastatic tissues from one patient with ES. Ingenuity Pathway Analysis (IPA) was used to analyze biological processes and to identify molecular mechanisms participating in the development of ES. We identified differentially expressed isoforms (CAV1, STAT3) and highlighted their potential roles in tumor progression and/or metastatic processes. We identified contributors to tumorigenesis, such as fatty acid metabolism and highlighted important enzymes in ES tumor progression (CDK1, AKT1). Subsequent IPA Upstream Regulator Analysis enabled prediction of the activity state of several other transcription factors (such as SREBF1, PPARGC1B) as well as activity of cytokines (IL1RN, IL6, EDN1) and growth factors (BMP4, FGF10) that altogether may contribute to the pathological context observed in this patient. We identified sets of connected upstream regulators (such as MBTPS1 that connects to SREBF1 and SREBF2) that can work together to elicit the gene expression changes observed in this dataset and induce key molecular changes for metastasis. The data presented here is from one patient, therefore similar studies are needed to understand if these insights are supported by data from other Ewing Sarcoma patients.

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P11.140

A novel full promoter 1B deletion of APC gene in Bulgarian Familial Adenomatous Polyposis Family

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Familial adenomatous polyposis (FAP) is an inherited condition causing multiple intestinal polyps and predisposition to colorectal cancer. Most cases are caused by mutations in the adenomatous polyposis coli (APC) gene. Over 1500 mutations have already been identified, however routine genetic testing fails to detect causative mutations in about 10% of classic FAP cases. Recently, it has been shown that a proportion of mutation negative FAP cases bear molecular changes in deep intronic and regulatory sequences, warranting targeted genetic testing of these regions.
In this study, we used direct sequencing, followed by MLPA of genomic DNA from a proband and his father, referred for genetic testing as both of them were affected by classic FAP. We found peak area decrease in two of the MLPA probes, corresponding to promoter 1B deletion in both patients. In order to determine the boundaries of the deletion, we conducted a whole-genome oligonucleotide array and long range PCR. The exact breakpoints of the above deletion were identified by direct sequencing as follows - genomic position: chr5: g.112,061,394_112,083,285del21,890 insTTGCTCTATGACCAATT. We performed Real-time PCR assay and Allele specific expression analysis to investigate the total APC expression levels. Thus, the APC promoter 1B deletion was associated with reduced expression of total APC mRNA levels in the patient's blood samples.
In this family, we found a novel deletion affecting the full sequence of promoter 1B. To the best of our knowledge such mutation has not been reported yet. Furthermore, the APC gene expression was clearly reduced, which indicates causative relationship.

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P11.141

Identification and characterization of miRNAs and mRNA targets in Glioblastoma multiforme

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MicroRNAs (miRNAs) are small 20-22 nucleotide-long members these act as regulators of gene expression, and have been implicated in the carcinogenesis oncogenetics of a variety of cancers. Glioblastoma multiforme (GBM) is the most common and most aggressive malignant primary brain tumor in humans. Molecular screening for gene amplification revealed frequent amplification of the EGFR, observed in about 35-70% of glioblastomas.
In this study, we analyzed miRNAs and mRNAs expression in glioblastoma multiforme into different groups according the status of EGFR amplification.

Forty human biopsies (GBM) were analyzed histopathological, and inmunohistochemical characteristics (GFAP, Ki-67, EGFR) and the status of *EGFR* amplification by FISH and differential PCR. We have used Genechip Human Gene Array containing 28.000 gene-level probe sets and Genechip miRNA Array containing 6703 miRNAs to profile mRNA and miRNA expression in tumors.Microarrays studies were obtained and normalized with the use of Gene Chip Operating Software and miRNA QC Tool and analyzed using Partek Genomics Suite 6.4 software.
We determined the expression levels of 145 filtered miRNAs in 40 GBM (16 GBM with amplification, 15 GBM with low amplification and 9 no amplification) . El 46, 2 % of miRNAs showed up-regulation and 53,8% of miRNAs were down-regulated in the relative analysis. Results indicate that miRNAs expression profiles distinguish between GBM into different groups according the status of EGFR amplification.
Our findings indicate that miRNAs are deregulated and changed their levels expression in the different groups suggesting a potential role for these molecules in the pathogenesis of this tumor.

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P11.142
Exon wise deletion status of tumor suppressor P16 gene in high grade glioma in the Indian population

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Since its discovery as a CDKI (cyclin-dependent kinase inhibitor) in 1993, the tumor suppressor p16 (INK4A/CDKN2A) has gained widespread importance in cancer. High frequency of p16 gene alterations were observed in many primary tumors. In human neoplasms, p16 is silenced in at least three ways: homozygous deletion, methylation of the promoter, and point mutation. Gliomas are most common brain tumors occur in the central nervous system. Homozygous deletion of p16 gene deletion has been observed in different grades of glioma especially in high grade glioma. But, the detail Exon wise analysis is not done in correlation with patient survival.
Our study aimed to perform Multiplex PCR in 50 high grade glioma samples, to see status of homozygous deletion in Indian patients. 20% of the patients showed deletion of p16 as compared to world reports (33-74%) and in consistent with Asian reports (17-46%). The exon wise deletions status for 1, 2 and 3 is 10, 4 and 8 percentage respectively. Exon 1 and 3 deletion is found to be higher in the Indian patients, as compared to the world literature (exon 2 deletion is predominant) . We recommend to screen all 3 exons of p16 for Indian patients. The survival status of these patients in relation to the p16 deletions is underway to understand the prognostic value of our findings.

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P11.143
Genomic changes in 295 patients with diffuse gliomas

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Diffuse gliomas form a heterogenous group of primary neuroepithelial brain tumors with non-random mutations, loss of heterozygosity, deletions and/or amplifications of chromosomal regions found in glial tumor cells. Identification of specific genomic changes by combination of I-FISH and SNP-A methods could help in assessment of diagnosis and predict of tumor progression.
During 2004-2012, we examined fresh non-fixed tissue specimens of 295 patients with histologically confirmed glial tumors (WHO grade I-IV). For detection of the most frequent cytogenetic changes (deletion of *TP53*, *CDKN2A, RB1*, 1p36/19q13.3, amplification of *EGFR*, trisomy 7 and monosomy 10) we used I-FISH with specific DNA probes (Abbott Molecular). Unbalanced changes in 60 cases were specified by whole genome SNP-A (HumanCytoSNP-12 BeadChip; Illumina). Results of molecular-cytogenetic analyses were correlated with morphological and clinical findings.
In WHO Grade II and III tumors, the risk of malignant progression was significantly higher in cases with specific aberrations (*EGFR* amplification, *CDKN2A* and *RB1* deletion, monosomy 10 and trisomy 7) compared to patients without these changes. In glioblastoma, monosomy 10 was linked to worse clinical course and shorter OS (8.4 months). In oligodendroglial tumors, a combined deletion 1p36/19q13 was shown as a powerful favorable marker, however prognosis was influenced by additional chromosomal aberrations (OS 80.6 vs. 60 months).
Analyses of genomic aberrations significantly contributed to diagnosis and prognosis of the disease in our patients. Therefore, detailed molecular cytogenetic analysis of tumor cells is recommended and should be an integral part of all gliomas examinations.
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P11.144
Utility of OCT3/4, TSPY and β-catenin as biological markers for gonadoblastoma formation and malignant germ cell tumor development in dysgenetic gonads

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Gonadoblastoma (GB) is regarded as an in situ form of germ cell tumor in dysgenetic gonads (type II GCTs). This type of tumor is thought to be a precursor to seminoma/dysgerminoma tumors. It almost exclusively affects a subset of patients with disorders of sex differentiation (DSD). In 35% of GB cases, overgrowth of the germinal component leads to dysgerminoma/seminoma . The TSPY gene (testis-specific protein, Y encoded) localized within the GBY locus (gonadoblastoma locus on the Y chromosome) has been shown to be involved in the multistep transformation of germ cells to GB. However, the precise role that TSPY plays in GB development and its involvement in the malignant transformation are not clear . OCT3/4 has been implicated in the GB oncogenic process, but the molecular details of OCT3/4 deregulation are still unknown. Analysis of OCT3/4, E-cadherin and β-catenin showed that the proliferation of immature germ cells in GB may be due to the interaction between OCT3/4 and accumulated β-catenin in the nuclei of the immature germ cells, leading to the development of invasive behavior and the progression of GB into dysgerminoma/seminoma in dysgenetic gonads. In the present study, to determine whether TSPY participates in the OCT3/4-β-catenin pathway in the dysgenetic gonad and whether OCT3/4 and β-catenin are expressed in the dysgenetic gonad, we analyzed 18 dysgenetic gonads from DSD patients with mixed gonadal dysgenesis and compared them with GB and dysgerminoma/seminoma tumors.

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P11.145
No association between gr/gr deletion and susceptibility to Testicular Germ Cell Tumor in a Norwegian population

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Introduction: Testicular germ cell tumor (TGCT) is the most common malignancy in young men, but the etiology is unclear. Development of TGCT is most probably multi-factorial and an underlying genetic predisposition has been suggested by family studies. The current age-adjusted incidence rate of TGCT in Norway is 11:100 000 male persons per year, and is the highest worldwide. In epidemiological studies male infertility has been shown to be associated with TGCT. Nathanson et al. (2005) found that presence of gr/gr deletion, a 1.6 Mb deletion on the Y chromosome strongly associated with infertility, was associated with a twofold increased risk of TGCT. Our aim was to investigate a possible association with gr/gr deletion and TGCT in our Norwegian dataset.
Material and methods: We investigated 616 Norwegian Caucasian men with TGCT, divided in 304 seminomas and 312 non-seminomas. 814 healthy blood donors were included as controls. Multiplex PCR with three different markers (SY1191, SY1206 and SY1291) in the AZFc- region were used to determine the presence of the gr/gr deletion.
Results: Preliminary results show that the prevalence of gr/gr deletion in TGCT patients was 2.6% (3% seminoma and 2.2% non-seminoma) compared to 2.9% in controls. No statistically significant difference in frequency of gr/gr deletion between patients and controls (OR=0.88, 95% CI=0.46-1.67,

p=0.69), or between seminoma and non-seminoma (OR=1.00, 95% CI=0.46-2.19, p=0.99 and OR=0.76, 95%CI=0.32-1.77, p=0.52, respectively) were found.

Conclusion: The presence of gr/gr deletion does not seem to influence risk of TGCT in a Norwegian population.

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P11.146

Ffrequency and spectrum of chromosome aberrations and micronuclei in peripheral blood lymphocytes of patients with head and neck tumors and relapse of breast cancer during the course of neutron therapy

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Neutron therapy is an effective treatment for patients with radioresistant tumors, however cytogenetic monitoring of cancer patients during the course have not been performed. Cytogenetic aberrations in peripheral blood lymphocytes were investigated in 17 patients with head and neck tumors and 12 patients with relapse of breast cancer during the treatment by neutron therapy. The treatment course specified 5.5-8.4 Gy by 4 fractions with 1.3-2.4 Gy per first fraction for patients with head and neck tumors and 4.8-8.0 Gy by 3-4 fractions with 1.4-1.6 Gy per first fraction for patients with relapse of breast cancer. There were three time points: before treatment, 24 h after the first fraction and at the end of the neutron therapy. Chromosomal aberration analysis was performed according to standard protocol in the first mitosis of PHA-stimulated lymphocytes. Cytokinesis-blocked micronucleus test was performed in combination with FISH using a pancentromeric DNA probe. It was shown that chromosome-type aberrations were prevalent among cytogenetic abnormalities both before and after neutron therapy. Level of chromosome aberrations and micronuclei significantly increased in lymphocytes of patients from both groups during neutron therapy ($p<0.05$). This increase was mainly due to chromosome-type aberrations and centromere-negative micronuclei. The prevalent types of aberrations are in agreement with expected results based on theoretical mechanisms of neutron effects on cells. This research is supported by the Federal Target Research Program «Scientific and scientific-educational personnel of innovative Russia (2009-2013)» (grant N 14.132.21.1319), grant of President of Russia Federation (MK-6806 .2013.4) and grant for young scientists of "OPTEC" company.

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P11.147

A Benign Vascular Tumor with a New Fusion Gene: EWSR1-NFATC1 in Hemangioma of Bone

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Abstract: The *EWSR1* gene in chromosome band 22q12 is a promiscuous fusion partner involved in a vast array of tumors characterized by gene fusions. In this study, we report the finding of a new fusion gene, *EWSR1-NFATC1*, in a hemangioma of bone; genetic rearrangements have not previously been described in this tumor type. Chromosome banding analysis showed a t(18;22)(q23;q12) as the sole change. FISH mapping suggested the involvement of each of the two partner genes and RT-PCR revealed an in frame *EWSR1-NFATC1* transcript. *NFATC1* has not previously been shown to be involved in a fusion chimera. However, *NFATC2*, encoding another member of the same protein family, is known as a fusion partner for *EWSR1* in a subgroup of Ewing sarcoma. Thus, our findings further broaden the spectrum of neoplasms associated with *EWSR1* fusion genes, add a new partner to the growing list of EWSR1 chimeras, and suggest that chromosomal rearrangements of pathogenetic, and possibly also diagnostic, significance can be present in benign vascular bone tumors.

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Hepatocellular carcinoma in a 16-year-old patient associated with familial adenomatous polyposis

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Hepatocellular carcinoma (HCC) is an aggressive hepatic neoplasm that most commonly affects adults. In contrast to adults, most paediatric HCCs arise without liver abnormalities or pre-existing cirrhotic diseases. So extremely rare, liver tumours such as HCC and also slightly more frequent hepatoblastoma, could occur in familial adenomatous polyposis (FAP), an inherited autosomal dominant disorder due to germline mutations in the adenomatous polyposis coli (*APC*) gene. To our knowledge, only 10 cases of HCC were so far reported in FAP patients. Because of its rarity, the pathogenesis of liver tumours in FAP patients remains undefined although biallelic inactivation of *APC* was already described unlike in sporadic cases. We report the case of a 16-year-old patient with an 18.5 cm encapsulated HCC occurring without underlying hepatic disease. Right hepatectomy was performed following by chemotherapy and sorafenib. A germline *frameshift APC* mutation (codon 1280) was identified in the proband, actually inherited from his mother suffering from a duodenal polyposis after coloproctectomy at 37 but with no contact anymore. Whereas an activating beta-catenin somatic mutation was found in the tumour, we discuss the role of APC/beta-catenin pathway in the hepatic carcinogenesis in this patient after sequencing *APC* and LOH analysis in the tumour. This report revived the debate of the mutually exclusive nature of *APC* and beta-catenin mutations.

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Different pattern of RET proto-oncogene mutations in Iranian Patients with hereditary medullary thyroid carcinoma

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Introduction: Thyroid cancer is the most common endocrine cancer and Medullary thyroid carcinoma is one of the most malignant thyroid tumors which occur in both hereditary (25%) and sporadic (75%) forms. Mutations of the RET proto-oncogene in MTC development have been well demonstrated.

Material and methods: To investigate the spectrum of the RET germline mutations in exons 3, 5, 10-16 in hereditary MTC in Iranian population, 319 participants were studied. Genomic DNAs were extracted from the leukocytes using the standard Salting Out/ProteinaseK method. Mutation detection was performed through direct DNA sequencing.

Results: Totally, from 319 participants (182Female, 137Male) 224individuals were patients (179MTC, 5MEN2A, 3MEN2B, 2Pheochromocytoma) and 95 individuals were their relatives. The germline RET mutations were found in 83.4% of participants (58.4%patients and 25% relatives), including exon 10, exon 11, exon 13, exon14, exon 15, and exon 16. The most common mutation in our population was C634Y (4.1%) on the other hand, C618R, C618S, C620G, L887L (0.3%) had rare allele frequency. Also the allele frequency of G691S/S904S haplotype was 36% in patients and 16.6% in their relatives. Moreover, a novel germline mutation R886Q was detected in exon 15 in two members of a family affected with MTC: 0.62%. The most frequent mutation in our population is haplotype G691S/S904S, and C634Y, whereas in the most population the frequent mutation is C634R. Altogether, further analysis needs to demonstrate the disease progress in individuals harboring multiple risk alleles.

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Whole-exome sequencing of four patients with criteria of familial hyperplastic polyposis

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Background: Hyperplastic polyposis (HP) is a rare condition characterized by multiple or large hyperplastic polyps in the large intestine, typically with

proximal localization, and associated with increased risk of colorectal cancer *via* the evolution of lesions through the serrated pathway of carcinogenesis. Some cases of familial hyperplastic polyposis have been described, but the familial transmission of this entity has not been elucidated by now.
Purpose: We sought genes potentially responsible for familial transmission of HP using whole-exome sequencing strategy.
Methods: Four patients were selected, who fulfilled the diagnostic criteria of hyperplastic polyposis according to the WHO Classification of digestive tumours (2000). They showed either family history of HP and/or colorectal cancer, or early onset at disease. We assessed the carcinogenesis pathway involved by morphological characterization and molecular biology analysis. Whole-exome sequencing was conducted on DNA derived from blood samples. Genomic DNA was enriched for coding region using SureSelectXT Human All Exon v4 (Agilent technologies, Inc., Santa Clara, USA), and paired-end sequencing was realized with Genome Analyzer IIX (Illumina, Inc., San diego, USA).
Results: We used KGGSeq software, that integrate pathways involved in diseases and pubmed references as filters. This analysis selected more than 300 genes, including 13 genes mutated in one patient only, which are in tight relation with the pathways of APC, STK11, MUTYH and BMPR1A, previously associated with forms of familial polyposis. This study enabled us to identify 13 candidate genes in the familial hyperplastic polyposis, and suggested a possible genetic heterogeneity in this syndrome.

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P11.151

Exome sequencing characterise the somatic mutation spectrum of early serrated lesions in a patient with BRAF negative hyperplastic polyposis syndrome

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Background. Hyperplastic polyposis syndrome (HPS) is a yet poorly defined colorectal cancer (CRC) predisposition characterised by the occurrence of multiple and/or large serrated lesions throughout the colon. A serrated polyp-CRC sequence (serrated pathway) of CRC formation has been postulated, however, to date only few molecular signatures of serrated neoplasia (BRAF, KRAS mutations, CpG Island Methylation, MSI) were described in a subset of HPS patients and neither the etiology of the syndrome nor the distinct genetic alterations during tumorigenesis have been identified. Methods. To describe the mutational landscape of serrated polyps and the involved pathways we sequenced the exomes (Illumina HiSeq platform) of 11 early hyperplastic polyps without the BRAF V600E mutation obtained from a 41 year-old female patient with clinically confirmed HPS. For data analysis the VARBANK pipeline was used. Somatic mutations were identified by comparison with leukocyte DNA and are currently validated by Sanger sequencing. Results. We identified 25 unique rare somatic alterations of 25 different genes in the 11 serrated tumours. All variants are single basepair substitutions; all but two variants are present in one tumour only. The predominant mutation type are missense mutations (92%) caused by G>T transversions (75%). No known cancer genes are affected. Conclusions. Somatic mutations seem to be rare events in early hyperplastic lesions of HPS patients without BRAF mutation. No frequently affected genes or enrichment of specific pathways have been observed. Thus, other alterations such as epigenetic changes might be the major driving force of tumour progression.

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P11.152

Modulation of CDX2 expression by the RNA-binding protein MEX3A: impact on intestinal differentiation and stemness

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Introduction: The homeobox transcription factor CDX2 is the key molecular mediator of intestinal development and homeostasis. Furthermore, multiple evidences substantiate its involvement in tumorigenic processes of the digestive tract encompassing intestinal reprogramming. The CDX2 regulatory network is intricate and it has not yet been fully uncovered. **Materials & Methods:** Through genome-wide screening of a 3D culture system, the RNA-binding protein MEX3A was identified as putatively involved in CDX2 regulation, so its biological relevance was addressed by setting up cell-based assays together with expression studies in murine intestine. **Results:** We demonstrate that MEX3A has a translational repressive function by controlling CDX2 levels in gastric and colorectal cellular models. This is dependent on the interaction with a specific binding determinant present in *CDX2* mRNA 3`untranslated region. We have further determined that MEX3A impairs intestinal differentiation and cellular polarization, affects cell cycle progression and promotes increased expression of intestinal stem cell markers, namely *LGR5*, *BMI1* and MSI1. Finally, we show that MEX3A is expressed in mouse intestine in a pattern that correlates with CDX2 modulation and stem cell properties. **Conclusion:** The recent identification of MEX3A as being part of an intestinal stem cell signature, together with our observations, support an association of MEX3A with the intestinal stem cell phenotype and a role in fine-tuning CDX2 expression. We describe a novel CDX2 post-transcriptional regulatory mechanism, through the RNA-binding protein MEX3A, with a major impact in intestinal differentiation, polarity and stemness, likely contributing to gastrointestinal homeostasis and carcinogenesis.

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P11.153

Coexistence of inv(16) and Cryptic BCR/ABL1 rearrangement in the patient with AML

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Coexistence of inv(16) and t(9:22) has been described in chronic myelogenous leukemia in blast phase (CML-BP) and de novo acute myeloid leukemia (AML). Cryptic *BCR/ABL1* rearrangements were reported in 5% of CML patients, which are not visible under conventional cytogenetic analysis and so are detectable only with fluorescence in situ hybridization (FISH) or reverse transcriptase polymerase chain reaction (RT-PCR). Here we report the case with this rare coincidence of coexistence of inv(16) and cryptic *BCR/ABL1* rearrangement. A 65-year-old man presented with leukocytosis (18.2×10^9/L) with 72% blasts. Bone marrow blast cells showed cytogenetic abnormality with 46,XY,inv(16)(p13.1q22)[15]/46,XY[5], and Philadelphia chromosomes were not found. Metaphase FISH detected both the *CBFB* and the *BCR/ABL1* rearrangements, located on chromosome 9. Additional FISH study for *BCR-ABL1-ASS* gene showed no apparent deletion of flanking material of ASS gene on chromosome 9. These results prove that *BCR/ABL1* fusion signal resulted from an insertional translocation from the small part of *BCR* gene on chromosome 22, to the proximal of *ABL1* gene on chromosome 9. *CBFB/MYH11* (A type) and minor *BCR/ABL1* (e1a2, P190) fusion transcripts were both detected by real-time quantitative RT-PCR. The patient was treated with standard AML chemotherapy. Molecular remission was achieved at the end of the first chemotherapy and he remained in remission until the last follow-up (6 months after diagnosis). He wasn't received imatinib and stem cell transplantation. To our knowledge, this is the first reported case of de novo AML with inv(16) that has cryptic *BCR/ABL1* rearrangement.

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P11.154

The efficacy of Anagrelide and Antiplatelets Agents in TE JAK2-V617F positive patients with major thromboembolic events

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Background: All non-CML MPN's have in common the presence of JAK2-V617F mutation. This is associated with leucocytosis, elevated platelet count and higher risk for thromboembolic events.
Objectives: To demonstrate the efficacy of Anagrelide and Antiplatelets Agents in TE JAK2-V617F positive patients with major thromboembolic events.
Patients and Therapy: 40 patients, ages between 33 and 45 years, no cardio-vascular risk, followed between 2010-2013; 26 (65%) women and 14 (35%) men; 24patients (60%) were JAK2-V617F positive. 8 patients(20%) arrived with major thromboembolic event: 3 patients(37,5%) had portal vein thrombosis,3 patients (37,5%) had ischemic stroke and 2 patients(25%) had acute myocardial infarction. They started Anagrelide (1,5-3 mg) +75mg Acetylsalicylic acid and they were followed monthly; 3 patients also received VKA's.
Results: During follow up-3 years, no other thromboembolic event occurred and the platelet count became in normal ranges. No bleeding events also.
Conclusions: The association between Anagrelide and Antiplatelets agents is a very effective treatment in TE- JAK2+ patients with major thromboembolic events.
Comment: Too few cases and too little follow up period; it needs to be studied further more.

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P11.155

Profile of DHFR gene expression in laryngeal carcinoma cell line (HEP-2) administered with different MTX chemoterapy doses

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Introduction: Studies have found varying degrees of success in patients with laryngeal cancer treated with methotrexate (MTX) chemotherapy. The varying degree depends on the dose received by patients and may lead to resistance mechanisms and collateral effects. MTX acts inhibiting dihydrofolate reductase (DHFR), key enzyme that is codified by DHFR gene involved in folate metabolism. **Objectives:** To investigate *DHFR* gene expression for MTX response in laryngeal cancer cell line (HEP-2) treated with three different concentrations of MTX chemotherapy. **Methods**: The 0.25 µM, 25 µM, and 75 µM concentrations of MTX chemotherapy were added separately in HEP-2 cell line plated in six-well culture plates for 24 hours at 37 °C. The Real-time quantitative PCR technique was performed for quantification of gene expression with TaqMan Gene Expression Assay. **Results:** We found that expression level for DHFR gene was higher in cells treated with 75 µM of MTX chemotherapy. There were significant effects of the expression in *DHFR* gene between control group and the three different concentrations evaluated. There was significant association between the control group and increased expression of the *DHFR* gene in 75 µM MTX application group. **Conclusions:** Chemotherapy treatment for laryngeal cancer with higher MTX dose may be associated with increased expression level of *DHFR* gene. These results on the mechanisms involved in the generation of drug resistance by gene expression opening new perspectives in pharmacologic regimens to the chemotherapy treatment.
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P11.156

Analysis of *HOX* genes in patients with laryngeal squamous cell carcinoma

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Laryngeal squamous cell carcinoma (LSCC) is one of the most aggressive neoplasms among head and neck cell carcinoma (HNCC). Besides advances in therapeutic treatment, the survival of HNCC patients wasn't significantly improved. Therefore, understanding of the molecular mechanisms involved in LSCC is important to identify predictive biomarkers. We investigated global gene expression patterns of 32 laryngeal HNSCC patients versus 13 samples of normal tissues surrounding tumors, using the Agilent microarray platform. The comparison of normal versus tumor tissues, revealed 81 differentially expressed genes ($p \leq 10^{-7}$), among these, 35 genes were overexpressed in tumor tissues and, within these; eight genes belonged to the *HOX* gene family. *HOX* genes are expressed during embryogenesis, but its re-expression has been reported to be associated with some tumors. The *HOX* genes overexpressed were *HOXA10*, *-A11-AS1*, *-C8*, *-C9*, *-C10*, *-C13*, *-D10* and *-D11*. The qPCR confirmed the overexpression of *HOX* genes in tumors samples and their almost absent expression in the normal tissue. We also investigated HOTAIR expression, a lincRNA located within *HOXC* locus associated with metastatic tumors. HOTAIR has an increased expression in tumors as compared to normal tissue. Also, there was a significant correlation (Spearman test) between the expression of HOTAIR and the expression of *HOXC13*, *-C10* and *–C9*. There was no significant difference in the expression of these *HOX* genes between the two different cancer stages, although there is trend for a higher expression of *HOX* genes in the early stage. These findings suggest an important role of *HOX* family in LSCC tumorigenesis.

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Exome Sequencing Identifies Recurring *FLT3* N676K Mutations in Core Binding Factor Leukemia

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The t(8;21) and inv(16)/t(16;16) rearrangements affecting the core-binding factors, RUNX1 and CBFB, respectively, are found in 15-20% of adult *de novo* AML cases and are associated with a favourable prognosis. Since the expression of the fusion genes *CBFB/MYH11* or *RUNX1/RUNX1T1* alone is not sufficient to cause leukemia, we performed exome sequencing of an AML sample with an inversion 16 to identify mutations, which may collaborate with the CBFB/MYH11 fusion during leukemogenesis. We discovered an N676K mutation in the ATP-binding domain (TKD1) of the *fms-related tyrosine kinase 3* (*FLT3*) gene. In a cohort of 84 *de novo* AML patients with a *CBFB/MYH11* rearrangement and in 36 patients with a *RUNX1/RUNX1T1* rearrangement, the *FLT3* N676K mutation was identified in 5 and 1 patients, respectively (5/84, 6%; 1/36, 3%). The FLT3-N676K mutant alone leads to factor-indpendent growth in Ba/F3 cells and confers resistance to the FLT3 PTK inhibitors PKC412 and AC220 together with a concurrent *FLT3* internal tandem duplication (ITD). Genes differentially expressed in AML patient samples with an inv(16) and a *FLT3* N676K mutation are signficantly enriched for genes involved in ubiquitin-mediated proteolysis, adherence junction and the JAK/STAT signaling pathway. Ours is the first report of recurring *FLT3* N676 mutations in CBF leukemias and suggests a defined subgroup of CBF leukemias. Although *FLT3* is a well known mutational target in AML, it appears that the spectrum of *FLT3* mutations is still not fully understood. Unbiased mutation screening by exome sequencing allows the detection of novel sequence variations even in extensively studied genes.

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P11.158

ERG deletion exerts a CD2-dependent positive prognostic impact on *IKZF1*-deleted childhood ALL

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B-cell precursor (BCP) acute lymphoblastic leukemia (ALL) is a heterogeneous disease consisting of subgroups with different and partly unknown genetic background as well as heterogeneous prognosis.
Deletions of ERG (ERGdel) were recently described as a recurrent abnormality in childhood ALL. However, a systematic evaluation of this aberration in the context of large clinical trials is missing. Therefore, we designed a multiplex PCR assay to screen ERGdel in 1323 childhood ALL patients enrolled into trial ALL-BFM 2000 and analyzed clinical/biological features of positive patients.
We identified 60 cases with ERGdel, exclusively within BCP-ALL. In more than 1/3 of cases the deletion was bi-/oligoclonal. The deletion was significantly associated with higher age, CD2-positivity and deletion of the IKZF1 gene. We found only a weak association of ERGdel alone with prognosis. However, when combined with CD2 status, the ERGdel/CD-positive patients demonstrated superior outcome. This effect was significant even within IKZF1-deleted cases, known to have a poor prognosis on ALL-BFM 2000. In addition, we analyzed the stability of ERGdel from diagnosis to relapse in all six ERGdel/CD2-negative patients. In 3/6 cases the ERG deletions were lost and in 3 cases the relapse deletions differed from those found at diagnosis.
In summary, we describe a high incidence of ERGdel in BCP ALL, suggesting that the ERG locus is prone to deletion in childhood ALL. However, the deletion does not seem to play a major driving role here. Nevertheless, combined with CD2-positivity, ERGdel confers superior prognosis overcoming the negative impact of IKZF1 deletions.
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P11.159

Cytogenetically unrelated clones in hematologic malignancies: ten years of experiences in a single institution.

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We evaluated the frequency and clinical significance of cytogenetic polyclonality in patients with various hematologic malignancies. The study includes 2683 patients who had been diagnosed with: Acute myeloid leukemia (AML), Acute lymphoid leukemia (ALL), Myelodisplastic syndrome (MDS), Myeloproliferative disorders (MPD) and Chronic myeloid leukemia (CML). During the past ten years, karyotypically unrelated clones were found in 20 patients (0.75%): 1.11% (7/631) of AML, 1.01% (2/195) of ALL, 1.16% (3/257) of MDS, 0.25% (3/1200) of MPD and 1.25% (5/400) of CML. In four AML and one MDS patient, the presence of unrelated clones was confirmed by FISH analysis. Patients diagnosed with AML, ALL and MPD had various structural and numerical aberrations of which the most common were trisomies, affecting chromosomes 8, 9, 12, 18 and 22. Translocations [t(8;21), t(15;17), t(2;5), t(14;16)] and monosomies (-Y,-7, -15, -17) were observed in 4 patients, each, while deletions (del(6q), del(7q) and del(11q)) were detected in 3 patients. Del(5q) and +8 were detected in unrelated clones of one MDS patient, del(5q) and del(8q) were present in the other one, while the third patient with MDS had unrelated clones with del(20q) and t(7;20). Four patients with CML, treated with imatinib mesylate, had unrelated clone of +8 in Philadelphia negative cells. In one patient, diagnosed as CML de novo, del(7q) and der(20) were detected in the unrelated Ph negative clone. Karyotypically unrelated clones are rare events and may originate from the common malignant clone which could be revealed by molecular methods, needed to identify hidden primary aberrations.

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P11.160

SPRED1, the gene involved in a neuro-cardio-facial-cutaneous syndrome, Legius syndrome, is a leukemia predisposing gene

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Germline loss-of-function mutations in SPRED1 located on chromosome 15q14, have been reported to originate Legius syndrome, belonging to the overlapping neuro-cardio-facial-cutaneous (NCFC) syndromes, all caused by germline mutations in the Ras-mitogen-activated protein kinase (MAPK) pathway and are associated with predisposition to cancer in children, notably leukemia.
Until now, only one case of an 11-month-old boy with a SPRED1 germline mutation who developed an acute myeloblastic leukemia, had been reported.
Here, we present a girl who had multiple café-au-lait spots since the age of 2 years and developed an acute lymphoblastic leukemia at age of 3 years. Other members of the family in her paternal lineage had multiple café-au-lait spots too, but none of them developed any cancer. The patient was shown to have a germline loss-of-function frameshift mutation in the SPRED1 gene (c.395dupA NM_152591.2; p.Asn133fs P010194), when she was 11 years old. Going back to her hematologic file, we could find result of a medullar karyotype she had as part of the leukemia diagnosis : a somatic mosaicism of a heterozygous deletion on chromosome 15q was found in medullar leukemia blasts [46,XX(30)/45,XX,der(5),del(15)(q11q25),-20(3)] including the SPRED1 locus. At the time of remission, a normal karyotype was observed.
This is the first case where a loss of heterozygozity of SPRED1 is shown in leukemia, supporting the hypothesis of the involvement of this gene in a few forms of leukemia, as a tumor suppressor.
Furthermore, this observation highlights the usefulness of cancer cells karyotyping to detect somatic chromosomal anomalies.

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P11.161

Polymorphisms in microRNA-binding sites and risk of childhood acute lymphoblastic leukemia and myeloid leukemias in adults

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microRNAs (miRNAs) regulate diverse cellular processes by binding to the 3' untranslated regions (3'UTRs) of messenger RNAs. Polymorphisms in miRNA-binding sites (miRSNPs) may alter the strength of miRNA interaction with target transcripts thereby deregulating gene expression.
In this study we analysed with specialized algorithms (PITA, Partocles, polymiRTS, dbSMR, miRANDA) the 3'UTRs of 138 leukemia-associated genes and identified 111 putative miRSNPs. For further investigation 11 miRSNPs were chosen based on the accordance of at least three algorithms. Using TaqMan allelic discrimination and PCR-RFLP we genotyped patients with childhood acute lymphoblastic leukemia (ALL, n=101), acute myeloid leukemia (AML, n=86), chronic myeloid leukemia (CML, n=153) and healthy controls (n=471). Variant alleles of ETV6_rs1573613 and TLX1_rs2742038 were associated with increased ALL risk in recessive model (OR=1.9264, 95% CI=1.1762-3.1551 and OR=3.9893, 95% CI=1.4377-11.0692, respectively), while the PML_rs9479 variant allele was linked to decreased ALL risk in dominant model (OR=0.5470, 95% CI=0.3464-0.8639). In adult myeloid leukemias we found significant associations between variant alleles of PML_rs9479 and BCL11B_rs1152781 and decreased AML risk (OR=0.6010, 95% CI=0.3669-0.9846, dominant model and OR=0.2557, 95% CI=0.0781-0.8371, recessive model, respectively) and between the variant allele of IRF8_

rs10514611 and increased CML risk in recessive model (OR=2.8128, 95% CI=1.3470-5.8735). Differential allelic expression (DAE) showed that the variant alleles of ETV6_rs1573613, PML_rs9479, and BCL11B_rs1152781 are expressed at a significantly lower level than the wild-type alleles, while the variant allele of IRF8_rs10514611 is expressed at a higher level compared to the wild-type allele. The results suggest that these miRSNPs are a potential risk factor in leukemias.

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P11.162

Novel somatic mutations in large granular lymphocyte leukemia

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Large granular lymphocyte (LGL) leukemia is a clonal disease characterized by expansion of mature cytotoxic T-cells. Our recent findings suggest that 40% of LGL-leukemia patients harbor mutations in STAT3 (Koskela et al. NEJM 2012). To identify additional novel somatic mutations in STAT3-mutation negative LGL-leukemia patients, we performed exome sequencing from leukemic cells of three untreated patients.

Patient 1 harbored a missense mutation in PTPRT with a variant frequency of 14%. PTPRT has previously been found to reverse Tyr705 phosphorylation on STAT3, a modification associated with STAT3 deactivation. This novel V995M mutation occurs within the tyrosine-protein phosphatase 1 domain and may thereby affect STAT3 activity by reducing dephosphorylation of Tyr705, thus increasing the expression of STAT3 target genes.

Patient 2 had a missense mutation in ANGPT2 (variant frequency 34%). K436E occurs within the TIE2-binding domain and is likely to affect the binding of TIE2 by ANGPT2. Over-expression of ANGPT2 has previously been shown to confer an adverse prognostic factor in other forms of leukemia.

Exome sequencing of patient 3 revealed a H126R mutation in BCL11b (variant frequency 51%). BCL11B is required for T-cell survival and overexpression could effectively increase T-cell activation and proliferation.

While these mutations appear biologically relevant and exciting, they have not been found to be recurrent in the screening of large cohort of LGL-leukemia patients (n=170).

Somatic mutations in PTPRT, ANGPT2 and BCL11b may represent rare genetic causes for LGL-leukemia. Inactivating mutations in PTPRT may have the same functional consequence as activating mutations of STAT3 in LGL-leukemia patients.

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P11.163

Expression profiling with microarrays method in human leukemic cells after coumarins and furanocoumarins exposition

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Coumarins are chemical compounds which can be found naturally in some plants. The subject of the study was to find the coumarin inhibitors of the pathogenetic mechanisms of human leukemic cells. We used Affymetrix GeneChips and quantitative RT-PCR technologies for the genes expression profiling in J45.01 human leukemic cells exposed on coumarins in Scan'R system. The apoptotic and cytotoxic effects of coumarins on leukemic cells were observed in FlowSight flow cytometer.

Moreover, we selected two coumarins with the lowest IC50 values. The analysis of transcriptomes with Ingenuity Pathways Analysis (IPA) showed differences in the genes expression in particular cells exposed on coumarins. There were some changes found in genes expression in the cells which were exposed on these coumarins. The genes with the highest coefficient of P were functionally classified as tumor markers, cell death, cell cycle and cell growth regulators and were associated with the cell organization.

The transcriptomes analyses revealed some specific features of the therapy responses depending on the exposition agents, which has increased our knowledge on leukemogenesis.

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P11.164

Cytogenetic analysis of human liposarcomas by array-based comparative genomic hybridization

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Liposarcomas are the most common soft tissue sarcomas accounting for 20% of all mesenchymal malignancies. Morphologically, WHO classifies liposarcomas into four main subtypes consisting of: myxoid/round cell (M/RLPS), dedifferentiated (DDLPS), well-differentiated (WDLPS) and pleomorphic liposarcomas (PLPS). Characteristic cytogenetic features of WDLPS and DDLPS are supernumerary ring giant chromosomes and dmin (*double-minute chromosomes*), while MLPS are characterised by the presence of specific translocation: t(12;16)(q13;p11).

The aim of the study was to analyse genomic profile of 65 liposarcomas (WDLPS n=21, DDLPS=24 and MLPS n=20) using array-based comparative genomic hybridization at unprecedented resolution of ~10kb (*NimbleGen, Roche*). Additionally we analysed genomic profile of subsequent samples of tumors resected from an individual patient during disease progression.

DNA copy number aberrations were found in 60 tumors (92%). Loss of chromosomal segments was observed in 29% of WDLPS (n=6), 60% of MLPS (n=12) and in 71% of DDLPS (n=17). In MLPS the most frequently involved chromosomal region was 1q (30%) with minimal overlapping region of gain 1q25.1-q32.2 (34 Mbp). 42 (93%) of WDLPS and DDLPS had 12q gain, with minimal common region at 12q14.1-q21.1. In WDLPS, the other common aberration was gain at chromosome 5 with minimal common region of overlap 5p15.2-p14.1. In contrary to WDLPS, in DDLPS we identified aberrations at chromosome 13q.

The observed patterns of genomic aberrations in particular liposarcoma subtypes reflect biological differences between these tumors.

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P11.166

Influences of Mitochondrial Uncoupling Protein 2 Variants on Lung Cancer Risk

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Objectives: Lung cancer is often a multistep, multifactorial disease involving both genetic and environmental components. Mitochondrial uncoupling protein 2 (UCP2), a negative regulator of mitochondrial reactive oxygen species (ROS) generation, was observed to be abundantly expressed in components of the immune system such as spleen, lung, and isolated macrophages. The purpose of this study was to investigate the relationship between the UCP2 gene variants and lung cancer susceptibility.

Methods: Seventy five lung cancer patients and 75 healthy controls were included in the study. The UCP2 gene variants (-866G>A and 45 bp insertion/deletion (I/D)) were determined by polymerase chain reaction and/or restriction fragment length polymorphism method.

Results: Distribution of genotype and allelic frequency of UCP2 45 bp I/D variant were significantly different between lung cancer and control groups. The distribution of II genotype was found significantly higher in the lung

cancer group (54.7%) than in the control group (8%) (p< 0.0001). In addition, the I allele frequency (68%) was higher than in controls (23.3%) (p< 0.0001). On the other hand, no statistically significant difference was found between UCP2 -866G>A variant and lung cancer susceptibility.
Conclusion: We suggest that both I allele and II genotype of UCP2 45 bp I/D variant are risk factors for susceptibility to lung cancer. Additional studies are needed to confirm the exact role of UCP2 variants on lung cancer.

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P11.167
EGFR mutation analysis in non-small cell lung cancer

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Background: Lung cancer is a leading cause of death in Bulgaria. Somatic gain-of- function mutation in the epidermal growth factor receptor (EGFR) detected in patients with non-small cell lung cancer (NSCLC) has significance for assigning optimal disease treatment. 29 EGFR somatic mutations are associated with either sensitivity or resistance to gefitinib or erlotinib treatment.
Methods: We performed DNA analysis on 98 patients with NSCLC. All cases are histologically confirmed as NSCLC. Tumor sample materials are extracted from formalin-fixed paraffin-embedded NSCLC samples. DNA extraction was performed by QIAamp DNA FFPE Tissue Kit for genomic DNA purification. The samples are analysed by polymerase chain reaction (PCR), using EGFR RGQ Kit on Rotor-Gene Q instrument, and the results were processed by Rotor-Gene Q Series Software.
Results: Analyses were processed according to manufacturer's recommendation. EGFR-mutations were discovered in 10% of samples. The two most common EGFR somatic mutations are deletions in exon 19 (8 patients, 8.16%) and L858R point mutation on exon 21 (2 patients, 2.0%). These two mutations define NSCLC as sensitive to treatment with the EGFR tyrosine kinase inhibitors (EGFR-TKI) gefitinib and erlotinib.
Conclusion: Our results are in accord with data for European populations. Patients with NSCLC and EGFR mutation, either exon 19 deletions or L858R, have a longer survival following treatment with gefitinib or erlotinib.
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P11.168
Allelic imbalance in non-small cell lung cancer.

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Lung cancer is the leading cause of cancer deaths worldwide. Non-small cell lung cancer (NSCLC) comprises 80% of all lung cancers and is characterized by multiple genetic alterations such as loss of heterozygosity (LOH) and microsatellite instability (MSI).
We performed analysis of molecular-genetic alterations in tumor and in surrounding tissue in different histological types.
The study group included 70 patients with NSCLC. From each patient 3 samples were analyzed: tumor, 2 samples from surrounding normal lung tissue at 2 cm and 5 cm from tumor. Microsatellite analysis was evaluated by PCR using 7 polymorphic markers of chromosomal regions: 12p23.3, 2q35, 3p14.2, 3p22.2, 3p26.3, 9p22.1 and 17p13.3.
Our results demonstrated allelic imbalance (AI) in NSCLC samples with the following frequencies: D2S405 32/65 (47%), D2S164 - 26/60 (43%), D3S1300 - 40/67 (60%), D3S1768 - 33/63 (52%), D3S1539 - 34/65 (52%), D9S925 - 30/64 (47%), D17S938 - 31/63 (49%). LOH and/or MSI in surrounding tissue were not detected. AI in D9S925 (p = 0.005), D17S938 (p = 0.002) was strongly associated with squamous cell lung cancer. LOH and/or MSI in D3S1768 (p = 0.004) and D17S938 (p = 0.03) were linked to smoking status. Frequent genetic alterations in D2S405 (p = 0.01) correlated with early stage cancer (I-II), whereas alterations in D3S1300 (p = 0.0197) were associated with advanced stages of disease (III-IV).
Observed genetic alterations can be used as molecular markers of squamous cell lung cancer in difficult diagnostic cases and can be considered as prognostic markers.

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The analysis of genetic alterations in the NSCLC patients using hybridization with diagnostic biochips

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Specific epidermal growth factor receptor (EGFR) inhibitors (gefitinib and erlotinib) and anaplastic lymphoma kinase (ALK) inhibitor (crizotinib) are used for the treatment of non-small-cell lung cancer (NSCLC). Somatic mutations in EGFR gene and key effectors along EGFR signaling pathway (KRAS, BRAF, PIK3CA), as well as ALK rearrangements are associated with sensitivity to these drugs.
A biochip has been developed which allows detecting 13 mutations in 12, 13 and 61 codons of KRAS; 5 mutations in 858, 719, 790 codons and 13 deletions in exon 19 of EGFR; V600E of BRAF; 5 mutations in 542, 545, 546 and 1047 codons of PIK3CA gene. The technique of LNA (locked nucleic acid)-clamp PCR was used to increase the sensitivity of the assay followed by allele-specific hybridization of fluorescently labeled target on biochip. To determine the ALK rearrangements a biochip analyzing 11 breakpoint variants of EML4-ALK translocations has been designed. The reverse transcription PCR with chimeric EML4-ALK mRNA was used prior to hybridization.
To prove the feasibility of the method clinical samples from 84 unselected patients with NSCLC were analyzed. We identified EGFR mutations in 13 (15%), KRAS mutations in 14 (17%), PIK3CA mutations in 8 (10%) and EML4-ALK translocations in 2 (2%) of patients. The data on mutation analysis were verified by direct sequencing. We consider the biochip-based approach to be simple and useful diagnostic tool for identification of NSCLC patients which will respond to target therapy.
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P11.170
Analysis of Mannose Binding Lectin and Macrophage Inhibitory Factor Gene Variants in Lung Cancer Patients

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Objectives: Lung cancer is caused by genetic and epigenetic changes leading to uncontrolled cell growth and metastasis. Macrophage migration inhibitory factor (MIF) and mannose-binding lectin2 (MBL2) implicated in the pathogenesis of several pathological disorders including cancer. The aim of this study was to examine the relationship between the MBL2 and MIF gene variants and lung cancer risk.
Methods: Eighty six lung cancer patients and 100 healthy controls were recruited in the study. Codon 54 A/B variant in MBL2 gene and -173 G/C variant in MIF gene were analysed by PCR-RFLP method.
Results: In the MBL2 variant, BB genotype was found significantly more frequent in lung cancer than controls (p<0.0010). Conversely, AB genotype was found significantly more frequent in controls than lung cancer (p<0.0098). The allele frequency of A and B was 0.779, 0.221 in cases compared with 0.815, 0.185 in controls. On the other hand, the distribution of G/G, G/C and C/C genotypes for MIF variant was 55.8%, 43% and 1.2% for cases and 55%, 41% and 4% for the controls. The allele frequency of G and C was 0.773, 0.227 in cases compared with 0.755, 0.244 in controls. No significant differences in allele/genotype distribution of the MIF variant were found between patients and controls.
Conclusion: Our data show that homozygous variant B allele of the MBL2 codon 54 polymorphism may be a risk factor for susceptibility to lung cancer. Further studies are required to confirm the exact role of MBL2 and MIF variants on lung cancer.

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P11.171

Common Genomic Variations Identified in Maffucci Syndrome Tissues by Molecular Karyotyping

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Maffucci syndrome (MS) is a rare congenital disorder characterized by multiple central cartilaginous tumors (enchondromas) that are accompanied by cutaneous spindle-cell hemangiomas. These patients have a high incidence of malignant transformation. No familial case is known and the ethiopathogenic cause remains to be unraveled. In enchondromatosis (Ollier's disease, OD), which is comprised of enchondromas only, four mutations in the PTHR1 gene have been identified in four patients; three were somatic and one was germline. No PTHR1 mutation has been detected in MS. On the other hand, in 77% of patients with MS and 81% of patients with OD, somatic IDH1, and more rarely IDH2, mutations have been observed. These changes are shared with other tumors, including glioblastomas, leukemias and thyroid cancers, and may thus be markers of cellular transformation.
To search for underlying somatic genomic causes, we screened MS tissues using Affymetrix SNP-chips. We looked for copy number variations (CNV), loss of heterozygosity (LOH) and uniparental (iso)disomy (UPD) by performing pairwise analyses between allele intensities in tumoral DNA versus the corresponding blood-extracted DNA. While common chromosomal anomalies were absent in constitutional DNA, several shared CNVs were identified in tumors. The most frequently observed is a large somatic alterations not present in CNV databases was a gain of 14q11.2. In the single chondrosarcoma, large chromosomal amplifications /deletions were observed in chromosomes 3, 6, 9, 10, 12 and 13. No loss of heterozygosity was observed in any Maffucci tissue. In conclusion, this study suggests new candidate gene for Maffucci syndrome located.

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P11.172

Over-Expression of FAT10 causes Malignant Transformation

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The FAT10 gene is overexpressed in hepatocellular carcinoma (HCC) and other cancers. High levels of FAT10 protein lead to increased mitotic nondisjunction and chromosome instability (CIN). Here, we demonstrate that when NehepLxHT, an immortalized non-tumorigenic liver cell line, is stably transfected with high levels of *FAT10*, these cells not only demonstrate higher cell proliferation rate and increased resistance against camptothecin-induced apoptosis, it also exhibits transformation potential by its ability to grow on soft agar. Its role in malignant transformation is evident from anchorage independent growth, increased adhesion to collagen IV, increased cell migration and invasion in cells over-expressing *FAT10*. Through genome-wide expression analyses, genes involved in proliferation, transformation, migration as well as invasion were found to be significantly differentially expressed in cells expressing *FAT10*. The most significant network associated with *FAT10* over-expression was found to be "Cell Death, Inflammatory Response and Cancer" where the deregulated genes converge on the NFκB complex as the key nodal hub. Overexpression of *FAT10* leads to downregulation of IκB protein and activation of NFκB activity with consequent upregulation of *CXCR4* and *CXCR7* expression which was found play a role in facilitating the invasive property of *FAT10*-expressing cells.

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P11.173

Melanoma in dogs: spontaneously occurring models for genetics and therapies of human melanoma

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Melanomas spontaneously occur in dogs, as in humans, due to genetic and environmental factors.
To characterize the homologies and differences between dog and human melanomas, we analyzed epidemiological data from 2350 melanocytic tumours and we analyzed in depth epidemiological, clinical and histopathological data from 150 canine melanocytic tumours. We found that melanomas occur in the same anatomical locations than humans and interestingly, specific dog breeds present higher risk to develop oral or cutaneous melanoma, strongly reflecting genetic predispositions.
To tentatively propose a histogenetic classification, the analysis of genes presenting frequent somatic alterations in human melanoma, was performed in dogs.
To decipher the genetic causes of melanoma in dogs, we set up, through the Cani-DNA dedicated canine biobank, a collection of cases and controls in high risk breeds. We collected over 500 blood samples and 200 melanoma tumour samples in different breeds. We first performed a genome wide association study, using DNA samples from 80 affected and 100 healthy poodles, a breed nearly exclusively affected by oral melanomas. Finally, an RNAseq analysis of matched healthy/tumour samples had been undertook on 20 melanoma cases.
Canine melanomas thus appear good naturally occurring models for studying human mucosal melanoma, and for melanoma of dermal origin and constitute specific models to investigate the non-UV pathways, otherwise poorly studied. In conclusion, predisposed dog breeds are invaluable models to identify novel genes and pathways in order to develop new therapies that will be beneficial both for dogs and humans.

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P11.174

Genetic variation in immuno-modulatory genes as markers of melanoma recurrence-free and overall survival.

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The involvement of immune-related genes in melanoma progression has been demonstrated, suggesting the potential of these pathways in the prediction of disease susceptibility and prognosis. In this study, we performed an analysis of germline variants in immuno-modulatory genes for their association with melanoma survival in a well-characterized cohort of 817 melanoma patients. Germline DNA was genotyped for 94 SNPs tagging 55 immuno-modulatory genes using Sequenom iPLEX. Cox models were used to test associations between each SNP and recurrence-free and overall survival (RFS, OS), with adjustments for age, gender, subtype, thickness, ulceration, and anatomic site. ROC curves were constructed combining SNPs with clinical covariates and the area under the curve (AUC) was used to assess their utility in the classification of 3-year melanoma recurrence. The SNP rs2796817 in TGFB2 had strong associations with both RFS (HR=3.8, CI 95%: 1.3-11, p=0.02) and OS (HR=5.5, CI 95%: 1.6-19, p=0.029). Other interesting associations with OS came from IRF8 (rs4843861, HR=0.62, CI 95%: 0.39-0.99, p=0.017) and CD8A (rs3810831, HR=2.4, CI 95%: 0.91-6.2, p=0.048). A multivariate model including stage, subtype and rs3810831 from CD8A was shown to improve the AUC when compared to a model including only stage and subtype in classifying 3-year recurrence (0.77 vs. 0.79). The findings in this study provide evidence that the genetic variants in immuno-modulatory genes may represent novel biomarkers of melanoma prognosis. The validation analysis is currently underway with an expanded set of immune target genes as well as an additional independent cohort of melanoma patients.

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P11.175

Prevalence of the MITF p.Glu318Lys germline mutation in the EPIC and E3N prospective cohorts

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A recently identified recurrent germline mutation in the MITF gene, p.E318K (rs149617956), was shown to increase the risk of melanoma in highly-predisposed patients ascertained through the clinics. MITF encodes for the micropthalmia-associated transcription factor that plays a key role in the pigmentation pathway and acts as a melanoma oncogene. Codon 318 is located in a small-ubiquitin-like modifier (SUMO) consensus site and this missense substitution severely impairs the SUMOylation of MITF and leads to differential regulation of downstream targets. In the studied populations (France, England, Australia), the frequency of the mutation was less than 1% and 2-5 times more frequent in patients with a family history of melanoma and/or multiple malignant melanomas.
To further investigate the importance of the p.E318K mutation in European populations, we determined its frequency in 1,177 melanoma cases and 1,584 controls from two population-based prospective cohorts, EPIC (European Prospective Investigation into Cancer and Nutrition) and the French E3N (Etude Epidémiologique auprès de femmes de la Mutuelle Générale de l'Education Nationale).
The frequency of p.E318K was significantly higher among cases (0.5% vs. 0.2% in controls; Fisher Exact test p-value 0.04; OR 2.98; 95% confidence interval (CI), 0.95-10.96). These findings from two large population-based cohorts confirm an increased risk of melanoma in carriers of the MITF p.E318K mutation in European populations. We are now carrying out a full mutation screening of the coding sequence of MITF to investigate the possibility of other rare, likely deleterious mutations within this melanoma susceptibility gene.

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P11.176
Detection of EGFR, 1p36, 14q32 genomic copy alterations in Meningiomas

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Meningiomas are mostly benign, slow-growing tumors of the CNS that originate from arachnoidal cap cells and account for about 30% of all primary brain tumours. Chromosome 22q12.2 deletion, a region encoding the tumor suppressor gene *merlin*, represent the most common genetic alterations in early meningioma formation. Malignant meningioma progression, however, is associated with increased genetic instability and complex genetic alterations, including numerous genomic losses, gains, and amplifications. For the detection of genomic copy number aberrations of EGFR gene, 1p36/1q25 and IGH (14q32.3) loci, we conducted fluorescence in situ hybridization (FISH) analysis on histopathologically analysed fresh meningioma tissues from 30 cases (23 females and 7 males). Of all analysed tumors, 26 were benign whereas the remainings were atypical meningiomas. EGFR/CEP7 polysomy was detected in 26,9% of benign tumors while polysomy and EGFR gene amplification were seen in all atypical meningiomas. Chromosome 1p36 locus deletions were detected in 84.4% and 50% of benign and atypical meningiomas, respectively. The IGH locus deletions were seen in both benign (92,3%) and atypical (50%) tumors. Our data also confirmed the involvement of chromosomes 1p36 and 14q32 deletions in malignant meningioma progression

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P11.177
Copy number changes and hypermethylation of tumor suppressor genes in meningiomas

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Meningiomas are the second most common intracranial tumors. They arise from the leptomeningeal brain and spinal-cord covering. In meningiomas, clinical malignancy is mainly predicted by histological grading based on World Health Organization (WHO) criteria, being grades II-III biologically more aggressive than grade I. However, an important problem in these tumors is that as grade I meningiomas are the most frequent, in absolute numbers they are also the most recurrent. In this work we study copy-number changes and methylation status of a set of tumor suppressor genes (TSG) in order to establish a relationship between genetic status and biological behavior.
Samples studied were 40 tumors, surgically removed and diagnosed in the Clinic University Hospital of Valencia. Formalin fixed, paraffin-embedded tissue tumor samples were available and representative areas of each were selected and punched for DNA extraction. Methylation-Specific Multiplex-ligation Probe Amplification (MS-MLPA) with ME001-C1 kit was performed according to manufacturer instructions, PCR products were separated by capillary electrophoresis and data was analyzed with Excel-based software. All cases showed deletions in at least one TSG. Most frequently deleted genes were ERS1 and TP73. Promoter hypermethylation was detected in TP73, PTEN, RASSF1 and HIC1 genes. Grade I tumors showed more frequently deletions, while in higher grade tumors hypermethylation was more patent. These results suggest that both mechanisms are implicated in meningioma biology and that a relationship may exist between an increased hypermethylation pattern and a more aggressive behavior in human meningiomas.
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P11.178
O6-methylguanine-DNA-methyltransferase (MGMT) is a surgery-activated gene

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Genetic status of the MGMT gene encoding the DNA repair protein O6-methylguanine-DNA-methyltransferase is among the most important prognostic factors for glioblastoma patients and predicts response to treatment with alkylating agent temozolomide. It is shown that using temozolomide improves both overall and progression-free survival. Currently in clinical practice assessment of genetic status of MGMT is most commonly performed either as a study of the MGMT promoter methylation, or the determination of its mRNA expression level. It is known that surgical manipulations can lead to an increased expression of a number of so-called 'surgery-activated' genes. Since the current standard of treatment for glioblastoma patients includes maximal feasible surgical resection followed by radiotherapy and chemotherapy, the objective of this study was to investigate the effect of surgical procedures on the MGMT expression level in the blood of patients operated for glioblastoma. Peripheral blood samples taken 1 hour before surgery, during or within 1 hour after surgery and 8 days after surgery, were obtained from 10 glioblastoma patients. The assessment of the MGMT expression, performed by real-time PCR, has shown that all patients had in average a 2 times increased MGMT expression level in the first hours after the operation and a more than 5 times increase in 8 days after surgery. Thus, we can assume that MGMT is a surgery-activated gene. Obtained results may raise the question about adequacy of using of investigation the MGMT expression level for determining genetic status of this gene in clinical practice.

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P11.179
First experience with array-CGH in 19 monoclonal gammopathy of undetermined significance (MGUS) patients

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The incidence of clonal chromosomal aberrations in plasma cells (PCs) is

considered as one of the most important and independent prognostic factors in patients with myeloma. Also in the premalignancy MGUS, there are chromosomal changes in PCs f.e. del(13)(q14), IGH translocation, 1q21 gain and hyperdiploidy, but it is not known much about them and their significance in relation to malignant transformation. Unfortunately, MGUS research is complicated by small amount of malignant cells that can be obtained from patients.

We analysed 19 MGUS (13M/6F; median age 64) using array-CGH, Agilent platforms "Human Genome CGH, 4×44K" (N=12) and "SurePrint G3 CGH+SNP, 4×180K" (N=7). DNA was isolated from separated PCs (using CD138, CD19 and CD56 markers) and amplified by MDA whole genome amplification.

We found chromosomal abnormalities in 58% (11/19) MGUS. In 26% (5/19) cases we found hyperdiploidy, thus gains of minimally two of following chromosomes: 3, 5, 6, 7, 9, 10, 11, 15, 17, 18, 19, 21. The most frequent were gains of chromosomes 9 (32%; 6/19) and 11 (26%; 5/19). Moreover, gains were found at 1q, 3q22.3-q29, 6p, 11q13.3-q25, 16p, Xq23-q28 and losses were found at 6q, 6q23.3, 8q24.21-q24.22, 13q13.3-q34, 16p11.2-p11.1, 16q11.2-q13, 16q21-q24.3, 16q23.1-q23.2.

In our study we optimized protocol of array-CGH from amplify DNA and we use it in first MGUS patients. We found there are various chromosomal changes in more then half of patients, mainly gains of whole odd chromosomes, what is also typical for myeloma, in which MGUS often develops.

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P11.180

TaqMan-based miRNA profiles classify expression in breast cancer and leukemia

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MicroRNAs are small endogenous RNA molecules that play an important role in the regulation of developmental and physiological processes. Studies indicate that microRNAs are involved in the multilevel regulation of gene expression targeting a battery of mRNA genes. Researchers have discovered that microRNAs are efficacious biomarkers for the classification of tumors and prediction of outcome for many diseases because of their evolutionary conservation, unique expression signatures, relative stability, and abundance. To identify potential candidates involved in tumor progression in humans, we examined the expression of 750 microRNAs using Applied Biosystems TaqMan® MicroRNA Assays in five human breast cancer cell lines, and two human leukemia cell lines. Data from leukemia cell lines were compared to two cell lines of normal peripheral blood mononuclear cells, and breast cancer cell lines were compared to normal breast tissue. In addition, the two groups of cancer samples were examined relative to the expression levels of 38 different normal tissues. We confirmed several microRNAs that were previously identified to be associated with cancer. We also determined a group of microRNAs that were consistently differentially expressed relative to the normal samples in all cancer cell lines. In addition, we identified microRNAs that are uniquely expressed in breast cancer but not in Leukemia cell lines. Further, microRNA families that are represented frequently in the list of significant microRNAs in both types of cancers were determined. These findings provide insight into possible common and distinct pathways between breast cancer and leukemia and the role of microRNAs in human cancer.

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P11.181

miRNA clusters in cromosome 7 including MIR29, MIR25, MIR93 and MIR106 genes associate with gastric cancer in Europeans: Results from the EPIC-EURGAST study

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MicroRNAs (miRNAs) are post-transcriptional gene regulators involved in a wide range of biological processes including tumorigenesis. Although deregulation of miRNA pathways has been associated with cancer, their genetic contribution to this complex disorder is still unravelled. We used logistic regression and gene-based permutation tests to analyse the genetic association of gastric cancer (GC) and its anatomical localization (cardia/noncardia) and histological subtypes (intestinal/diffuse), with 133 SNPs tagging 11 individual miRNAs and 26 miRNA clusters potentially involved in cancer, in 365 incident GC cases and 1284 matched controls (European Prospective Investigation into Cancer and Nutrition (EPIC) cohort). Various SNPs significantly associated with different subtypes of GC under the log-additive model, being the most significant ones those in two miRNA clusters on chromosome 7: three tagSNPs of the miR-29a/miR-29b-1 cluster associated with the diffuse phenotype (minimum p-value=1.7x10-4; OR(95% cl):1.72(1.3-2.3)) and one tagSNP of the miR-25/miR-93/miR-106b cluster associated with the cardia localization (OR(95% cl):0.56(0.37-0.86), p-value=5.378x10-3). We predicted several target genes for these miRNAs, some of which have been functionally validated and are implicated in cancer-related processes such as methylation (DNMT3A, DNMT3B), cell cycle (E2F1, CDKN1A, CDKN1C) and apoptosis (BCL2L11, VEGFA). Furthermore, we identified significant interactions between variants in some of these genes and in the chromosome 7 miRNA clusters. Deregulation of the expression of these miRNAs in gastric tumours also supports our findings, altogether suggesting that genetic variation in MIR29, MIR25, MIR93 and MIR106b genes may have a critical role in the genetic susceptibility to GC, probably through modification of their expression.

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P11.182

Detection of miRNA Expression in Glial Tumors

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MicroRNAs (miRNA) are single strand, non-coding RNA molecules with the length of 18-25 nucleotides and play major roles in the regulation of gene expression. Mature miRNA molecules are partially complementary to one or more messenger RNAs and cause tumor suppressor or oncogenic mRNA degradation or inhibition of the protein translation.

In this study it is aimed to investigate the expression profiles of miRNAs in 50 cases diagnosed with brain tumor (32 glioblastoma cases (GBM)-grade-IV, 10 diffuse astrocytoma cases (DA)-grade-II, 8 anaplastic oligodendroglioma cases (AO)-grade-III) and in brain tumor cell lines as control group (U-87MG, U-118MG and LN18). Expression profiles of miRNAs were studied by microarray method. Putative target genes of miRNAs that showed significant changes were identified by using 7 databases.

In GBM cases, miR-495 and miR-432 expression showed significant 2-fold increase and miR-708-3p, mir-339-5p and miR-4286 expressions 4-fold, mir-331-3p, miR-625-3p, and miR-20a-3p showed 5-fold reduction compared to control group. mir-204 expression was significantly decreased 6 times in AO cases compared to control group. There was a significant increase in 18 miRNAs and decrease in 7 miRNAs in DA cases compared to control group. When miRNA expressions of DA, AO and GBM cases compared with each other, significant differences were found in 29 miRNA. In all cases miR-21 expression level was high and no mir-124 expression was detected.

Ultimately, novel genes could be effective in tumor development supposed to be regulated by miRNAs. Regulation of miRNA expression may affect specific gene targets in brain tumors.

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Identification by next-generation sequencing of an unexpected nonsense mutation in the MSH2 gene in a family suspected to have a BRCA mutation.

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The new generation of sequencing strategies technologies is changing genetic diagnosis because of its huge sequencing capacity and decreasing cost. Using this new approach, challenge for physicians will be to apprehend clinical uses of the genetic data generated, even in gene panels. So far genetic testing in inherited diseases is targeted, relying on recognition of a well-known clinical presentation evocative of a targeted predisposition. As an ethical issue, patients will have to be counselled about potentials of the technology to reveal other genetic alterations than those initially searched, that may be relevant for their risk of future disease. We report the case of a woman suspected to have a *BRCA* mutation because of the occurrence of an ovarian seropapillar adenocarcinoma at age 62 and breast cancer in her sister at age 62. Next-generation sequencing (NGS) analysis reveal no mutation for the *BRCA* genes but an unexpected nonsense mutation in the *MSH2* gene further confirmed by direct Sanger sequencing method. Although Amsterdam II criteria were not fulfilled, pedigree data had noted two cases of colon cancer respectively in a paternal aunt (age 69) and cousin (age 54), but among a large family. Intriguingly, neither microsatellite instability nor loss of MMR protein expression were observed in the ovarian tumour. Given the paucity of data on ovarian tumour in Lynch syndrome, classically described as non serous and at a younger age, we discuss such unplanned results. This report highlights importance of anticipation of such unexpected results with NGS introduction in clinical daily practice.

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Somatic cancer mutations and NGS panel diagnostics - translation to the clinic.

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Knowledge of tumor biology and especially cancer genetics is growing fast. Novel therapeutic strategies are continuously being developed (notably monoclonal antibodies and small molecules). This process was highly accelerated with the advent of Next-Generation-Sequencing. So-called panel analyses allow parallel examination of multiple genes on DNA-level within days including detection of low level mutation mosaicism by deep sequencing. The results were proven to be of prognostic value and may predict drug response. We used a commercially available hotspot panel (48 genes, Illumina) to analyze a total of 43 different tumor samples. The analysis encompassed both FFPE material and native tissue. Sequencing quality and results were comparable for both tissue types with an average number of reads above 1000000 and a depth at 100x of above 97 %. Each mutation was validated by a second method and proven to be somatic by comparison to a reference sample (e.g. blood, normal tissue). A total of 24 somatic mutations were identified in 19 different samples (19/43 = 0.44). We found well-known mutations in important cancer genes like KRAS, TP53, CTNNB1, PIK3CA and ERBB2, some of them of therapeutic relevance. An interdisciplinary oncogenetic tumor board discussed the results. In addition two germline variants were found and classified as variants of unknown significance (APC, MET). These two findings may be relevant for the families and genetic counseling was offered to both patients. On the basis of our current results we are extending our gene panel to a set of ~ 180 genes.

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P11.185

MicroRNAome profiling in benign and malignant neurofibromatosis type 1-associated nerve sheath tumors: evidences of PTEN pathway alterations in early NF1 tumorigenesis

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BACKGROUND: Neurofibromatosis type 1 (NF1) is a common dominant tumor predisposition syndrome affecting 1 in 3,500 individuals. The hallmarks of NF1 are the development of peripheral nerve sheath tumors either benign (dermal and plexiform neurofibromas) or malignant (MPNSTs).
METHODS: To comprehensively characterize the role of microRNAs in NF1 tumorigenesis, we analyzed 377 miRNAs expression using the 384-wells microfluidic TaqMan human microRNA Low Density Array in a large panel of 9 dermal and 41 plexiform neurofibromas, and 15 MPNSTs.
RESULTS: The most significantly upregulated miRNA in plexiform neurofibromas was miR-486-3p that targets the major tumor suppressor gene, *PTEN*. We confirmed *PTEN* downregulation at mRNA level. In plexiform neurofibromas, we also report aberrant expression of four miRNAs involved in the RAS-MAPK pathway (miR-370, miR-143, miR-181a, and miR-145). In MPNSTs, significant deregulated miRNAs were involved in *PTEN* repression (miR-301a, miR-19a, and miR-106b), RAS-MAPK pathway regulation (Let-7b, miR-195, and miR-10b), mesenchymal transition (miR-200c, let-7b, miR-135a, miR-135b, and miR-9), *HOX* genes expression (miR-210, miR-196b, miR-10a, miR-10b, and miR-9), and cell cycle progression (miR-195, let-7b, miR-20a, miR-210, miR-129-3p, miR-449a, and miR-106b).
CONCLUSION: We confirmed the implication of *PTEN* in genesis of plexiform neurofibromas and MPNSTs in NF1. Markedly deregulated miRNAs may serve for the basis of potential diagnostic and predictive biomarkers and could represent novel strategies for effective pharmacological therapies of NF1 tumors.

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P11.187

Mother-to-offspring inheritance of MLH1 promoter hypermethylation

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MLH1 promoter methylation analysis is a relatively inexpensive test used to help distinguish between sporadic and non-sporadic colon cancers with loss of expression of the proteins **MLH1** and PMS2 by immunohistochemistry. However, in a subset of Lynch Syndrome (LS) cases a constitutional MLH1 epimutation has been identified. Until recently, constitutional MLH1 epimutations were thought not to be passed on to the next generations, but cases of mother-to- offspring inheritance of constitutional MLH1 epimutations have been reported a few times in the literature.
We present a case that regards a deceased female suspected constitutional MLH1 epimutation carrier with three LS- associated cancers (endometrial cancer age 60, colon cancer age 61 and rectal cancer age 75). There are no other LS-associated cancers in the pedigree. All cancers and a dermal lipofibroma from the deceased female were found to be hypermethylated in the C-region of the MLH1 promoter. Screening of the MLH1, MSH2, MSH6 and PMS2 genes in the three children of the proband revealed no pathogenic mutations. Preliminary studies suggest the presence of hypermethylation of the C-region of the MLH1 promoter in blood lymphocytes of all three children. Further investigation is ongoing and includes searching for a second hit in the proband's three LS-associated tumors and testing of the offspring and proband for epigenetic changes in other parts of the genome.
Hypermethylation is the most plausible cause of LS in the proband.
The above indicates that an epimutation can be transferred to the offspring.

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P11.188

Involvement of the MLL gene in adult T-lymphoblastic leukemia

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While the *MLL* "recombinome" is relatively well characterized in B-cell precursor acute lymphoblastic leukemia (BCP ALL), available data for adult acute T-lymphoblastic leukemia (T-ALL) are scarce. We performed fluorescence in situ hybridization (FISH) for an *MLL* split signal on 223 adult T-ALL samples obtained within the framework of the German Multicenter ALL 07/2003 therapy trial. Three biphenotypic leukemias (T-ALL/AML) were also included in the analysis. Samples showing any alteration by FISH were further investigated to characterize the *MLL* aberration. In addition, they were investigated for common genetic lesions known in T-ALL. Twenty-two cases (9.5%) showed an abnormal *MLL* signal by FISH analysis. Most of these appeared to be deletions or gains but in five cases (2.1%) a chromosomal translocation involving the *MLL* gene was identified. The translocation partners and chromosomal breakpoints were molecularly characterized. Three T-ALLs had an *MLL-AF6*/t(6;11) and two biphenotypic leukemias had an *MLL-ELL*/t(11;19). The chromosomal breakpoints in two of the *MLL-AF6*-positive cases were located outside the classical *MLL* major breakpoint cluster known from BCP ALL. In conclusion, the spectrum of *MLL* translocation partners in adult T-ALL much more resembles that of AML than that of BCP ALL and thus the mechanisms by which *MLL* contributes to leukemogenesis in adult T-ALL appear to differ from those in BCP ALL. Proposals are made for the diagnostic assessment of *MLL* fusion genes in adult T-ALL

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The partial tandem duplication of *MLL* gene in patients with AML and cytogenetic abnormality of chromosome 11

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The partial tandem duplication of the *MLL* gene (PTD) has been described in bone marrow cells of 6 - 10% of adult patients with acute myeloid leukemia (AML) with normal karyotype and in most cases with trisomy 11 as a sole cytogenetic abnormality. We investigated 210 patients with AML at diagnosis by RT-PCR and the *MLL* PTD was proved and confirmed by DNA sequencing in 50 of them (23%). All 210 patients were examined by conventional cytogenetics and FISH with a locus-specific probe for the *MLL* gene (11q23.3). *MLL* PTD were detected with: normal karyotype (n=21), 0 mitosis (n=3), t(8;21)(q22;q22)/inv(16)(p13q22) (n=4), complex karyotype (n=5), and with other chromosome changes (n=17). Duplication of the exones 2 to 6 was the most frequent ones. Chromosome 11 abnormality was identified in 12 patients: trisomy 11 (n=2), partial trisomy 11q (n=1), alteration of 11p (n=1), balanced rearrangement of 11p/11q (n=1), and *MLL* gene rearrangement (n=7; mostly t(9;11)(p22;q23)).

In conclusion, patients with *MLL* rearrangements or *MLL* PTD constitute the groups of AML patients with specific clinical and biological features. We proved that these two different disrupting mechanisms can co-exist together. It is not clear, if one *MLL* gene allele is carrier of both mutations or two *MLL* alleles are affected or if they originated simultaneously or consequently. Therefore, further analyses on the larger cohort of patients focused on molecular, biological and clinical relationship of these rearrangements in malignant processes are needed.

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P11.190

Multiplex Ligation-Dependent Probe Amplification (MLPA) assay as a diagnostic tool to detect genomic rearrangements in myelodysplastic syndromes (MDS).

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Approximately 50% of MDS patients present bone marrow cytogenetic abnormalities at diagnosis, with an important role on prognosis and tailored therapy. Conventional cytogenetics (CC) with fluorescence in situ hybridization (FISH) are performed to detect chromosome abnormalities. MLPA assay simultaneously detect 40 different chromosomal rearrangements(deletions/duplications) in a single reaction. The study aim was to validate the MLPA assay in MDS samples comparing the results with the CC approach. Cell cultures and total DNA were obtained by bone marrow samples from 62 MDS patients (M:40, F:22, median age 73 years, range: 44-88 years). For each sample at least 20 metaphases were examined according G-banding protocol and two MLPA reactions were performed according to the manufacturer's recommendations (MRC-Holland). Our study revealed a good correlation between CC and MLPA, since 46 cases (74.2%) showed identical results with both techniques. For the other 16 cases (25.8%), we found different results.

In 5/16 samples (8%) CC analysis failed while the MLPA assay showed in one sample three chromosome deletions and no anomalies in the other samples. In the remaining 11/16 samples (17.8%) we found discrepancies. First group included three samples (4.9%) in which MLPA detected separately two different deletions and one trisomy, while CC did not show any alteration. The second group included 8 samples (12.9%) in which MLPA did not identify any rearrangement. Our data suggest that MLPA, used in conjunction with CC, can improve diagnostic and prognostic assessment, particularly in MDS cases with karyotype failure or with small rearrangements.

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P11.191

Epigenetic changes in relation to asbestos exposure in malignant pleural mesothelioma

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Malignant pleural mesothelioma (MPM) is a rare and aggressive tumor strongly associated with asbestos exposure. Only 5-17% of individuals exposed to asbestos develop MPM, suggesting the involvement of other environmental, genetic or epigenetic risk factors.

DNA methylation is an important mechanism of gene silencing in human malignancies. The relationship between aberrant DNA methylation and inflammation has been documented in many types of cancers, including MPM. Asbestos exposure may contribute to MPM onset through this relationship.

We conducted an epigenome-wide scan searching for differentially methylated regions (DMR) in 40 MPM cases and 40 controls and high-exposed versus low-exposed. Methylation status was measured for ~470k CpG sites in DNAs from lymphocytes, using the HumanMethylation450 BeadChip (Illumina, S. Diego, CA).

Logistic regression analysis after adjustment for age, gender, center of recruitment showed no significant methylation change of single CpG site associated with MPM. However, a regional analysis showed multiple significant signals in several genomic regions. In particular, a significant decreased methylation ($P<10^{-7}$) has been identified for *BLCAP* (tumor suppressor gene) and *NNAT* (expressed in non-small cell lung carcinoma) genes in exposed subjects compared with non exposed, whereas no significant difference has been found between in the overall cases and controls at the regional analysis. These results suggest that different DNA methylation profiles can related to asbestos exposure induced tumorigenesis of MPM and that epigenetic changes are detectable also in blood DNA.

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P11.192

First case of Muir-Torre syndrome caused by a germline mutation in PMS2

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The autosomal dominant inherited Muir-Torre syndrome (MRTES, OMIM #158320) is defined by the co-occurrence of internal malignancies with sebaceous gland tumours or keratoacanthomas. The most common internal malignancies are gastrointestinal and genitourinary cancers. MRTES is a clinical variant of the Lynch syndrome (HNPCC, hereditary nonpolyposis colorectal cancer) which is caused by mutations in one of the mismatch repair genes *MLH1*, *MSH2*, *MSH6* or *PMS2*.
To date, germline mutations in *MSH2*, *MLH1,* and *MSH6* have been reported for MRTES. Contradictory studies exist about the predominance of *MSH2* mutations in comparison to the frequency of *MLH1* mutations. Only a few cases of *MSH6* mutations have been published so far, whereas no *PMS2* mutation in MRTES has been described.
The patient presented here was diagnosed with right-sided colon cancer and nasal sebaceous gland adenoma at the age of 34. Her sister was also diagnosed with a nasal sebaceous gland adenoma at the age of 37, however she had no pathological findings in colonoscopy. The parents of the siblings were apparently healthy. The brother refused any examination. The maternal grandmother suffered from breast cancer.
No mutation in *MLH1*, *MSH2* and *MSH6* was identified. Analysis of *PMS2* revealed the heterozygous missense mutation c.53T>C, p.Ile18Thr in exon 2, which has not been described yet. All four *in silico* prediction programs applied (SIFT, PolyPhen-2, AGVGD, MutationTaster) classified the mutation as pathogenic with highest scores.
As this is the first description of a *PMS2* mutation in a MRTES patient, *PMS2* mutations should also be considered in this condition.

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P11.193

Changes of multidrug resistance genes expression in human leukemic and myeloma cells after exposure to resveratrol derivatives

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Multidrug resistance is a mechanism in which many of cancers develop the chemotherapy resistance. The phenomenon of multidrug resistance (MDR) is the ability to actively pump in chemotherapeutics through the membrane transport proteins in the cellular and nuclear membranes.
The aim of this study was to determine some genes expression levels changes regulating the process of multidrug resistance that occured in the leukemic cells under the influence of resveratrol and six derivatives (stilbenes).
Quantitative RT-PCR analysis demonstrated some significant differences in the gene expression levels regulation. All the stilbenes caused a very high MDR1 gene expression level reduction in the H9 lymphocytes.
The MRP1 gene expression was the lowest one in 1301 and J45.01 cell lines after the exposure to some stilbenes. The MDR1 gene expression was the lowest one in the EOL1 cell lines after exposure to one stilbene. Two stilbenes decreased MVP gene expression level in J45.01 cell line.
Our results have provided some preliminary data suggesting that the resveratrol and derivatives play an important part in the leukemia chemotherapy.
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P11.194

Multiple myeloma: importance of cytogenetic study

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Introduction
Multiple myeloma (MM) is a clonal bone marrow disease characterized by the neoplastic transformation of differentiated B cells. In many newly diagnosed patients, the abnormal clones have a low proliferative activity and, therefore, most of the analyzable metaphase cells are derived from normal hematopoiesis. As a result, only 30-40% of the new patients have an abnormal karyotype by conventional cytogenetics. Fluorescence *in situ* hybridization (FISH) increases detection rate to 90%. Both numerical and structural abnormalities are found and complex karyotypes are common.
Material and methods
The authors reviewed the cytogenetic results of 128 samples, referred to the Cytogenetic Laboratory of Centro Hospitalar de Trás-os-Montes and Alto Douro, with a presumptive diagnosis of MM, between January 2008 and February 2013.
Cytogenetic and G-banding analysis were performed according to standard techniques. In some cases, FISH technique was applied, with probe panel for del(13)(q14), del(17)(p13), t(4;14) and t(11;14) (Vysis).
Results and discussion
Metaphases were obtained in 95% cases. Of these, 44 cases had chromosomal abnormalities, mostly associated with MM [aneuploidies, del (13q), del (17p)]. FISH technique allowed confirming the results obtained by conventional cytogenetics and enhancing detection rate in 3% of cases.
Despite the small number of cases, the results obtained are consistent with those described in the literature and revealed the importance of conventional cytogenetics in the study of MM.

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P11.195

Expression of copy number-dependent genes in patients with Multiple Myeloma

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Whole genomic methods represents effective tool for studying genomic changes in cancer cells. Aim of this study was to find and describe genes whose expression is dependent on the DNA copy number (gene dosage) in patients with multiple myeloma. Total of 57 patients with MM were simultaneously examined by arrayCGH for DNA copy number variations (gain/losses) utilizing Agilent Human Genome CGH Microarray 4x44K Arrays and for gene expression utilizing Affymetrix GeneChip Human Gene 1.0 ST. Gene dosage-dependent genes were defined by Spearman correlation [R>0.5, p(FDR)<0.05] of CNV status and expression level and analysed using DAVID Bioinformatics software.
Total of 852 from all 27391 transcripts were strongly and significantly dependent on gene dosage. Cytogeneticaly, majority (25%) of all 852 genes were located on chromosome 1 (with 19 genes mapped to 1q21 locus). Other involved genes were mostly located on chromosomes 15 (8.7%), 19 (8.7%), and chromosome 13 (8.6%). Pathway analysis showed most genes to be involved in PDGF pathway, ubiquitin proteasome pathway, Ras pathway and TNFR1 signaling pathway.
Although almost all chromosomes have been at least once affected by either gain or loss of genetic material, number of genes with affected exrrpession is relatively low. We anticipate two mechanism for expression level compensations: i) increase of related supressors activity in case of gains ii) impact of second allele in case of losses.
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P11.196

Comprehensive screening of the incidence of LOH/UPD and copy number abnormalities in cohort of newly diagnosed multiple myeloma patients from Czech centers: first results

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Multiple myeloma (MM) is the second most common hematological malig-

nancy. It is characterized by malignant transformation of clonal proliferation of B-lymphocytes and their accumulation in bone marrow. Our previous results showed large genetic heterogeneity among MM patients when genome-wide analyses were done. In order to obtain more detailed description of genetic lesions in MM we utilized novel high-density CGH+SNP microarrays for detection of LOH/UPD regions and copy number abnormalities (CNAs) and in cohort of 48 MM patients. Overall, we found 16 regions affected with LOH/UPD in 23% (11/48) of cases. The most frequently was affected chromosome 6p in 8.3% (4/48); other regions in single cases were found in chromosome 4, 7, 9, 11, 14, 16, 17, 18, 20 and 22.
The most common observed genetic lesions were trisomies of odd-numberded chrmosomes (3, 5, 7, 9, 11, 15, 19 and 21), gain 1q, and monosomy 13. We observed loss of genetic material in loci for genes associated with adverse prognosis for MM patients in 1p32.3 (FAF1, CDKN2C), 11q22 (BIRC2, BIRC3), 14q32.32 (TRAF3, AMN) and 16q12.1 (CYLD), which are associated with deregulations of important signaling pathways such as NF-κB (TRAF3, CYLD, BIRC2, BIRC3), or essential for cell cycle (CDKN2C, RB1) and apoptosis (WWOX, FAF1).
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P11.197
Multiple primary malignant tumours: Clinical comparison of patients with and without detected germline mutations in cancer susceptibility genes.

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Multiple primary malignancies (MPM) represent a significant proportion of overall cancer diagnoses. Individuals diagnosed with a primary cancer are more likely to develop a (second) malignancy than the general population, which can be explained in part by inherited genetic predisposition. In addition to MPM in itself, predisposition can be suggested by clinical factors such as positive family history and early age of diagnosis.
Individuals diagnosed with two or more malignancies at a relatively young age (≤60) may be more likely to harbour germline mutations in cancer susceptibility genes. 210 such patients were identified among referrals to a UK cancer genetics service, 157 of whom had no demonstrated mutation despite undergoing germline genetic testing in 71 cases.
Clinical parameters including tumour diagnosis age, Manchester score and revised Bethesda criteria (cases involving breast and colorectal cancers respectively) were compared between groups with and without detected mutations. A multiple tumour score based on Manchester score but incorporating all malignancies in a single lineage was also applied.
Results are shown in the table. Of note was the similarity in tumour diagnosis age and the fact that around a fifth of patients without mutations had a multiple tumour or Manchester score higher than the mutation group median. These data suggest that any MPM might be a useful clinical indicator of germline mutations in established or novel cancer susceptibility genes.

Comparison of clinical measures between groups with and without detected mutation

All cases	**Mutation group N = 53**	**No mutation group N = 157**
Mean age of tumour diagnosis	44	46
Mean multiple tumour score	20.7	13.2
Median multiple tumour score	17	12
% cases with multiple tumour score higher than mutation group median	N/A	21
Cases involving breast cancers	**N = 25**	**N = 107**
Mean Manchester score	25.3	13.9
Median Manchester score	20	14
% cases with Manchester score ≥ 15	80	38.3
% cases with Manchester score higher than mutation group median	N/A	20.5
Cases involving colorectal cancers	**N = 25**	**N = 41**
% cases with ≥ 1 Revised Bethesda criteria for MSI testing	84	58.5

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P11.198
Identification of a patient with atypical MUTYH-associated polyposis through detection of the *KRAS* c.34G>T mutation in liver metastasis

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MUTYH-associated polyposis (MAP) is an autosomal recessive colorectal adenomatous polyposis syndrome due to bi-allelic germ line mutations in the *MUTYH* gene that encodes a DNA glycosylase involved in base excision repair. A deficiency of MUTYH function results in somatic G:C>T:A transversions that can be observed in *APC* and *KRAS* genes.
We report the case of a 33-year-old woman with atypical MAP, diagnosed via detection of the somatic *KRAS* c.34G>T mutation. The patient was admitted to our institution with stercoral peritonitis and liver nodules diagnosed initially as abscesses upon imagery. Further investigation revealed hepatic metastasis of an occult colonic cancer. Given her young age, she was sent to the Oncogenetics Unit even if family history was negative. The hypothesis of Lynch syndrome was excluded by the tumor phenotype (microsatellite stable phenotype and maintenance of expression of MMR proteins). Rather, the diagnosis of MAP was suspected on the basis of the presence of the somatic *KRAS* c.34G>T mutation in liver metastasis. *MUTYH* gene sequence analysis identified two germ line mutations: c.[494A>G];[1395_1397delGGA] (p.[Tyr165Cys];[Glu466del]) (refseq NM_001048171.1) confirming the diagnosis of MAP. Twelve months later, total colectomy was finally performed, which showed the presence of an adenocarcinoma of the sigmoid colon and more than 30 adenomatous polyps.
This case was interesting in view of the unusual presentation of MAP, with stercoral peritonitis revealing metastatic colon cancer in a 33-year-old woman. Moreover, it highlights the potential value of the somatic *KRAS* c.34G>T mutation for identifying patients with atypical MAP.

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P11.199
The t(2;11)(p21;q23) without MLL rearrangement - a possible marker of good prognosis in MDS patients

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Chromosomal abnormalities are detected in approximately 50% of patients with *de novo* myelodysplastic syndromes (MDS) and are of great value for the diagnosis, classification and prognostic stratification. However, some non-random chromosomal changes, which can be found in approximately 10% of *de novo* MDS patients, still don't have clearly defined prognostic value. The t(2;11)(p21;q23) translocation has an overall frequency of approximately 1% and belongs to the group of abnormalities that are characteristic for *de novo* MDS. Patients with the t(2;11)(p21;q23) have been shown to share some common clinicopathological features like lower age compared to the median age of MDS patients at diagnosis (mostly under 60) and strong male predominance (3M:1F). Cytological or histological assessment of bone marrow usually reveals only mildly presented MDS with marked dysplasia in megakaryopoiesis. Recently, it was proved that t(2;11)(p21;q23) without *MLL* gene rearrangement results not in direct oncogene activation but in up-regulation of miR-125b-1. The over-expression of miR-125b has been shown to target several important genes for hematopoietic development such as *JUN*, *STAT3*, and *BAK1*. However, most of the clinical data dealing with the t(2;11)(p21;q23) has been collected without known status of *MLL*. Contrary to other studies, this is the first report with evaluation of the clinical outcome of patients with the translocation without *MLL* being affected. Despite the low number of cases in our study (7 patients), our results indicate that the t(2;11)(p21;q23) could be associated with a good prognosis for MDS patients.
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P11.200
Oncologic phenotype of Peripheral neuroblastic tumours associated with PHOX2B mutations.

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PHOX2B gene encodes for a homeobox transcription factor involved in autonomic neuron development, and regulates the transcription of key enzymes of the catecholamine synthesis, including the Tyrosine hydroxylase (TH). Germline mutations in PHOX2B predispose to peripheral neuroblastic tumors (PNT), frequently in association with other neurocristopathies such as Hirschsprung disease (HSCR) or congenital central hypoventilation syndrome (CCHS). These syndromic presentations are commonly thought to have a good prognosis. Although some in vitro data suggest PHOX2B mutations impair neuronal differentiation, their physiopathological role in PNT remains speculative.
We have analysed eight patients from six different families presenting with peripheral neuroblastic tumors associated with non-polyalanine expansion mutations in PHOX2B. We describe clinical features, somatic arrayCGH profiles, and histological pattern including INPC (International Neuroblastoma Pathology Classification) and immunohistochemistry with PHOX2B and TH antibodies.
Four patients had hypoventilation syndrome and/or HSCR, four had no neurocristopathy. We found three ganglioneuromas and two ganglioneuroblastomas, hence illustrating that PHOX2B mutations are found in predominantly differentiated tumors. All tumours showed a retained nuclear expression of PHOX2B protein, while TH was variably expressed. Among the syndromic forms, we report two patients with stage 4 disease and one patient with stage 3 disease. Segmental chromosomal rearrangements, associated with aggressive disease, were found in four tumours by arrayCGH profiling. One patient died of the tumour, one died of hypoventilation and six patients were alive, with a median 14 months follow-up.
Our series finally suggests that PHOX2B mutations preclude neither tumour differentiation nor TH expression, but may be associated with aggressive genomic patterns.

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P11.201
PHOX2B over-expression as a pathogenic mechanism in neuroblastoma

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Neuroblastoma (NB) is an embryonic tumor derived from impaired neural crest cells differentiation. The heterogeneous genetic etiology is suggested by the identification of gain of function mutations of genes encoding for the receptor tyrosine kinase Anaplastic Lymphoma Kinase (ALK) and the transcription factor Paired-like Homeobox 2B (PHOX2B) in a limited proportion of NB patients.
A role for *PHOX2B* in NB is strengthened also by the following observations:
1. *PHOX2B* and ALK genes over-expression in NB cell lines and samples;
2. PHOX2B-mediated *ALK* transcriptional regulation.
For all these reasons, and in particular for the early role of PHOX2B in development, we are investigating mechanisms that may underlie *PHOX2B* expression.
We have planned to perform a drug repositioning approach to identify pathways responsible for driving such over-expression.
We have produced IMR32 NB cell line (characterized by high *PHOX2B* expression) stably transfected with the *PHOX2B* promoter upstream the *Luciferase* gene and have performed the following studies:
1. By a cell-based approach, a little set of molecules, selected on the basis of their effects in other diseases models, has been tested for their role on *PHOX2B* promoter activity, gene expression and protein amount;
2. By a high throughput approach, a library of 43 molecules specifically acting on epigenetics mechanisms has been added to the NB cell line and analyzed for their effects of *PHOX2B* transcription.
Luciferase activity has revealed several molecules effective in down-regulating *PHOX2B* gene expression, actually under investigation to identify regulatory pathways through which they act on the PHOX2B promoter.

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P11.202
Transduction of Neurofibromin corrects the mechanosensoric blindness in cultured NF1 haploinsufficient fibroblasts

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Recently, we found a new function of neurofibromin in the sensoric mechanism to submicron topographies. In cultured NF1 (Neurofibromatosis type 1) haploinsufficient fibroblasts a partial mechanosensoric blindness was characterized. Here, we investigated whether transduction of neurofibromin corrects the mechanosensoric blindness in NF1 haploinsufficient fibroblasts. The partial mechanosensoric blindness was measured as reduced orientation of the cells on defined submicron topographies. To overcome the common problem of endosomal entrapment of transduced proteins, combination treatments of transduction and endosomolytic treatments were performed. In these experiments, the orientation of NF1 haploinsufficient fibroblasts increased by inducing an uptake of functional recombinant neurofibromin. The partial mechanosensoric blindness is reduced by these treatments. These data suggest that dysregulations in cultured NF1 cells can be corrected by a cellular replacement of the lacking protein.

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P11.204
New markers of susceptibility to osteosarcoma: microRNAs

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Purpose
Osteosarcoma is the most common primary bone cancer in children and young adults. Susceptibility to osteosarcoma is due, at least in part, to complex and multiple genetic factors.
MicroRNAs (miRNAs) are non-coding RNAs that act as negative regulators of the expression of other genes. Deregulated miRNAs have been reported in osteosarcoma, showing their importance in the disease. Recent studies have provided evidence that single nucleotide polymorphisms (SNPs) in miRNA-related genes can affect miRNA levels and function. These SNPs have been associated to risk in different cancers, but such studies are lacking in osteosarcoma.
The aim of this study was to evaluate the role of miRNA-related SNPs in susceptibility to osteosarcoma.
Materials and Methods
We analyzed 99 blood samples from osteosarcoma patients and 387 healthy controls. We studied 118 SNPs in pre-miRNAs and in miRNA biogenesis pathway genes.
Results
For the first time, six polymorphisms in miRNA related genes were significantly associated ($p<0.05$) with osteosarcoma risk. Of them, 3 were located in genes involved in miRNAs biogenesis (CNOT1, CNOT4 and SND1) and 3 in miRNA genes (mir-585, mir-612 and mir-499).
Conclusion
Our results suggest that SNPs in miRNAs and miRNA biogenesis pathway may affect osteosarcoma susceptibility.
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P11.205

Could triaging family history of cancer during palliative care enable earlier genetic counselling intervention?

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Background. Patients are commonly referred to cancer genetics services when all affected family members are deceased. This makes genetic testing and risk assessment more difficult, reducing the benefit from screening and prophylactic treatment.
Methods. Observational, retrospective, cohort study of 508 randomly selected patients referred to a regional cancer genetics unit, using review of case notes to explore whether a simple clinical '3, 2, 1' family history rule could have been used to improve timely and appropriate referrals for genetic assessment. The '3, 2, 1' criteria are: *three* affected relatives with the same/associated cancers, across *two* generations, with at least *one* person affected aged under the age of 50 years.
Results. Most (71% [362]) genetic risk assessment referrals were in unaffected individuals and 22% (80) of these were referred after all affected family members had died, including 24% (19) who lost their last remaining affected relative in the previous year. Most (59% [301]) referrals met all '3, 2, 1' criteria, and 67% of these could have been made earlier in clinical practice. A further 23% (115) met two of the three criteria.
Conclusion. Using a simple clinical '3, 2, 13, 2, 1' family history rule in cancer care and particularly in palliative care could enable earlier cancer genetic risk assessment for unaffected relatives, improving the potential to benefit from targeted screening and intervention.

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P11.206

Altered interphase FISH profiles of chromosomes 4, 8q24 and 9q34 in pancreatic ductal adenocarcinoma are associated with a poorer patient outcome.

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Introduction. Pancreatic ductal adenocarcinoma (PDAC) is a fatal disease with an overall survival (OS) of almost 5-years. Currently, no consistent tumor markers exist which contribute to predict patient prognosis and/or survival. Here we analyze the cytogenetic profile of PDAC tumors and its act on patient survival.
Patients and Methods: The frequency and prognostic impact of the copy number alterations of chromosomes 1, 4, 7, 8, 9, 17, 18, 20 and Y, were analyzed by iFISH (n=21probes) in 55 PDAC patients. The prognostic value of chromosomal alterations showing an impact on OS were validated in a external cohort of PDAC patients analyzed by comparative-genomic arrays from public databases (n=44 cases).
Results: Overall, for all alterations identified only alterations of chromosomes 4 and 9q34 as well gains of chromosome 8q24 (p=.04; p=.01and p=.01, respectively) were associated with a shorter patient OS. The prognostic impact of 8q24 chromosome gains and changes at chromosome 9q34 were confirmed in the external dataset (p=.05 and p=.03, respectively). Based on these cytogenetic variables a scoring system was built and patients were classified into 3 different risk groups: low-(no adverse cytogenetic features), intermediate-(one adverse feature) and high-risk (>1 adverse features) with OS rates at 4-years of: 80%, 16% and 0%, respectively (p<.001).
Conclusion: Here we show that alterations of chromosomes 4, 8q24 and 9q34 in PDAC tumors are associated with a poorer patient outcome. Additional prospective studies in larger series of patients are necessary to confirm the clinical utility of the new prognostic markers here identified.

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P11.207

Gain-of-function HIF2A mutations in patients with paraganglioma or pheochromocytoma

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HIF2A germline mutations cause polycythemia but recently *HIF2A* somatic mutations were described in several patients with polycythemia and paraganglioma (PGL) or pheochromocytoma (PH). Herein, we report *HIF2A* mutations in three different patients with PH or PGL. Patient 1 was a woman suffering from congenital polycythemia diagnosed at 16 years, operated on for a PH at 45 years and for abdominal PGL at 64 years. She was also diagnosed for a somatostatinoma. Patient 2 was operated on PH at 24 years and was then lost to follow-up. Patient 3 was a child suffering from polycythemia from infancy and operated on for non-functional adrenal PGL at 11 years. No germline mutations on the main PGL susceptibility genes nor in *PHD2* gene were identified. We then sequenced *HIF2A* gene in the DNA extracted from paraffin-embedded tumors of the three patients. We identified a somatic *HIF2A* heterozygous mutation in PH, PGL and somatostatinoma of patient 1 (c.1586T>C; p.Leu529Pro) and in the PH of patient 2 (c.1591C>T; p.Pro531Ser) but both mutations were absent in the corresponding germline DNA. In patient 3, we found a *HIF2A* heterozygous mutation (c.1625T>C; p.Leu542Pro) at somatic level while the mutation was also present at mosaic stage at germline level. All mutations disrupt the hydroxylation domain of HIF-2α protein. Altogether, these reports demonstrate that *HIF2A*-related syndrome is due to a postzygotic genetic event occurring at an early developmental stage that can affect males or females. A germline mosaicism should always be considered during the genetic counseling of a newly identified patient.

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P11.208

Next generation sequencing (NGS) for pheochromocytoma/paraganglioma genetic testing

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BACKGROUND: Pheochromocytomas and paragangliomas (PPGL) are rare sporadic or familial endocrine tumors. Currently, germline mutations at different genes have been identified in around 35% of the cases. The major susceptibility genes are *SDHB*, *SDHD*, *VHL*, *RET*, *SDHC* whereas *NF1*, *TMEM127*, *MAX*, *SDHA*, *SDHAF2* and *HIF2A* are mutated in <10% of the cases. Germline mutations in *SDHB*, *SDHD*, *SDHC* and *VHL* explain more than 80% of inherited PPGL.
OBJECTIVE: To develop and implement a NGS-based workflow for a one-step assay PPGL genetic screening.
METHODS: Together with Multiplicom, we designed and optimized the SDH MASTR™ assay, enabling the amplification of the coding regions of *SDHB*, *SDHD*, *SDHC* and *VHL* (step 1) and then to incorporate molecular barcodes (Multiplex Identifiers - MIDs) in each amplified product (step 2). NGS was performed on a Roche GS Junior system. Twenty-eight DNA samples containing various variants and mutations, previously identified by Sanger method or MLPA, were used. The sequencing data were analysed with AVA (Amplicon Variant Analyzer, Roche) and SeqNext software (JSI Medical Systems).
RESULTS: All the variants previously identified by Sanger sequencing of the 28 patients were found with the new NGS PPGL workflow. The validation is yet ongoing on a set of new patients blindly analysed in comparison with our routine direct Sanger sequencing strategy.
CONCLUSION: Our first results suggest that this strategy is a cost-effective option for PPGL genetic testing. *RET*, *MAX* and *TMEM127* will be further added in the assay in order to test seven PPGL susceptibility genes in a single run.

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P11.209

Preliminary data on AIP and AHR genotyping in apparently sporadic acromegalic patients.

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Context: Germline mutations of the *AIP* (aryl-hydrocarbon receptor interacting protein) gene are associated with a predisposition to pituitary adenomas. Such mutations are found in about half of patients with familial acromegaly, but penetrance is incomplete.
Objective: This is a preliminary study concerning genotyping of AIP and AHR genes in a small group of acromegalic patients with sporadic pituitary adenoma.
Patients and Methods: The entire coding sequence of the *AIP* and *AHR* genes were screened for germline mutations in 18 patients (M= 8, age 57.5±12.8 yrs±SD) with diagnosis of acromegaly in adult age. Genotyping was performed by direct automated sequencing.
Results: A missense AIP mutation, R304Q, whose pathogenic role has already been confirmed, was found in 1 (5.5%) of 18 patients. Furthermore, in all patients we found 2 polymorphic AIP variants, rs641081 (c.682C>A) and rs4930199 (c.920A>G) in homozygosity, with uncertain pathological role. Concerning AHR gene, 15 (83%) out of the 18 patients showed a major polymorphic variant rs2066853 in heterozygous state, 1 (5.5%) showed the rs2066853 (c.1661G>A) and the rs4986826 (c.1708G>A) variants in compound heterozygosity and in 1 patient (5.5%) showed both variants in homozygosity and heterozygosity state, respectively. The pathogenic role of AHR gene variants is still unconfirmed.
Conclusion: On the basis of this preliminary data, we can conclude that AIP mutations with potential pathogenetic role are present in 5.5% of subjects with adult onset acromegaly due to apparently sporadic GH-secreting pituitary adenoma. On the contrary, no AHR gene mutations have been detected in this small group of acromegalic patients.

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P11.210

New Gene Tests for Familial Colorectal Cancer Patients; *POLE, POLD1* and *GREM1* Analysis.

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Many colorectal cancer patients who undergo genetic testing for mutations in known colorectal cancer susceptibility genes do not have a pathogenic variant identified. Recently Jaeger *et al* and Palles *et al* reported variants in three genes associated with an inherited polyposis / adenoma phenotype in colorectal cancer patients.
Jaeger *et al* identified a 40kb duplication at 15q13.3 encompassing the region 3' of the *secretogranin-5* (*SCG5)* gene to upstream of *Gremlin1* (*GREM1).* The duplication is located on a specific haplotype and has only been found in Ashkenazi Jewish patients to date. The phenotype associated with this variant is hereditary mixed polyposis syndrome type 1, characterised by multiple polyps of different types including; serrated, juvenile, Peutz-Jegher, adenomas and carcinomas.
Palles *et al* identified variants in the proof reading domains of the *polymerase D1* (*POLD1)* and *polymerase E* (*POLE)* genes. The variants are associated with a phenotype similar to MutYH associated polyposis (multiple adenomas) or Lynch syndrome (large adenoma / early onset carcinoma). Patients with *POLD1* mutations are also at risk of developing endometrial tumours and brain tumours.
We have screened a cohort of patients who have previously had no variants identified in appropriate colorectal cancer susceptibility genes, for variants in *POLD1* and *POLE* or the *SCG5-GREM1* duplication, as dictated by their phenotype.
We present our results and discuss whether these molecular analyses should be considered as a routine reflex test for patients who have no mutation identified in the polyp predisposing or mismatch repair genes.

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P11.211

Molecular-genetic investgation of hereditary predisposition to different forms of large intestine`s polyposis

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Familial Adenomatous Polyposis (FAP) - is an important inherited CRC syndrome. It is caused by a germline mutation in the adenomatous polyposis coli
(*APC*) gene and it is most often
inherited in an autosomal dominant manner. Inherited mutations in *MYH* gene can also provide these diseases. Biallelic *MYH* mutations are the genetic reason of an autosomal
recessive mode of inheritance but we also observed risk of developing FAP in monoallelic *MYH* gene mutation carriers of some populations. The object of our
research is to investigate
germline mutation in specific gene-supressor *APC*
and *MYH* genes as well. We study DNA
of 17 patients with classic form of FAP and 5 patients with attenuated FAP. We found
11 mutations (5 nonsense, 4 deletions and 2 splice acceptor) in *APC* and 1 heterozygous missense mutation
in *MYH* genes in 17
patients with classic form of FAP (70,5%). Also 2 heterozygous *MYH* missense
mutations has been found at attenuated form in 5 patients (40%). 5 of all 14 mutations (35,7%) have been found for the first
time.

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P11.213

The interaction network of the *ARLTS1* gene and risk for prostate cancer

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Prostate cancer (PCa) is a complex trait and several susceptibility genes have been implicated by genome-wide linkage or association studies. The genomic region 13q14 is frequently deleted in sporadic and familial PCa. It is widely recognized as a possible locus of tumor suppressor gene(s) as it has been found to be deleted also in other cancers. We have previously shown that homozygous carriers for *ARLTS1* (ADP-ribosylation factor-like tumor suppressor protein 1 or *ARL11*, located at 13q14) T442C variant are associated with an increased risk for unselected and familial PCa. The role of the variant was further strengthened with the observation of greater frequency among malignant tissue samples, PCa cell lines and xenografts. In this study we performed *ARLTS1* interaction studies, as the functional network of *ARLTS1* is poorly known. First differences of *ARLTS1* expression status between PCa cases (n=84) and controls (n=15) was studied. Statistically significant (p=0.0037) difference in *ARLTS1* expression between the groups encouraged to eQTL analysis to detect regulatory variants. Altogether fourteen significant *cis*-eQTLs affecting *ARLTS1* expression levels were found. Interestingly, two of the most significant eSNPs located in *PHF11* and *SPRYD7* genes, which are functioning in immune system processes. This is in concordance with our previous results in which *ARLTS1* co-expression was highly connected to expression of genes functioning in immune system processes. Two additional eQTLs located in histone methyltransferase *SETDB2* gene which modulates the expression of its target genes epigenetically. In conclusion, this study identifies novel regulators for *ARLTS1* gene expression and PCa risk.

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P11.214

Fine-mapping of chromosomal region 11q13.5 in Finnish prostate cancer

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The identified prostate cancer (PrCa) associated single-nucleotide polymorphisms (SNPs) explain only a part of PrCa heritability. Chromosomal region 11q13-14 is associated with PrCa. Previously we screened *EMSY* at 11q13.5 for mutations in Finnish PrCa patients and identified a rare intronic mutation IVS6-43A>G that increases risk of aggressive PrCa and associates with familial PrCa. No causal factor(s) predisposing to PrCa has been identified from 11q13-14.

The aim of the study was to characterize the genetic structure of 11q13.5 and to asses if there are additional PrCa risk variants. The study included a total of 4034 PrCa patients and 908 controls of Finnish origin. We imputed SNPs and structural variants on 0.9 Mb at 11q13.5 and validated the associations by genotyping. Imputation was performed with IMPUTE2 using 1000 Genomes reference population. Haplotype analysis was conducted to study haplotype blocks, linkage disequilibrium and haplotype associations.

Two correlated SNPs at *EMSY* were associated with PrCa. SNPs were located in consecutive introns in close approximation to IVS6-43A>G. *EMSY* variants formed a common predisposing haplotype and a rare protective haplotype. In addition, five correlated intergenic SNPs and a one-nucleotide insertion associated with PrCa. Both a predisposing and protective haplotype was identified. The findings indicate that two regions at 11q13.5 contribute to PrCa predisposition with complex genetic structure. Further studies are needed to determine the genetic mechanism of susceptibility and functionality of the regions.

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P11.215

Determination of GADD45γ methylation level in prostate cancer with high resolution melting analysis and evaluation of the possible correlation with apoptosis

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Prostate cancer (PCa) is the second most common cancer type worldwide in men. It is believed that deregulation of important cellular mechanisms is involved in the pathogenesis of PCa although the exact mechanism of its pathogenesis has not been clearly elucidated. "Growth Arrest DNA Damage-Inducible 45" (GADD45) is one of the most important gene family in these mechanisms and consists of 3 members: GADD45α, GADD45β, GADD45γ.

The aims of this study are to detect the protein expression and methylation profile of GADD45γ in benign prostate hyperplasia (BPH) (60 patients) and PCa (56 patients), to examine the correlation between protein expression and methylation profile, and to evaluate whether apoptosis is related to these two parameters.

We found that the methylation frequency for GADD45γ was quite low in PCa compared with BPH (p=0.000). This was consistent with the literature with the reported frequency of the gene in solid tumors was lower than in hematological malignencies. In both groups, there was no correlation between GADD45γ methylation and protein expression, but there was a significant difference between Bcl-2 and Bcl-xL expressions in both groups (p=0.000 and p=0.003, respectively). A statistically significant relation between GADD45γ expression and apoptosis was not found although GADD45γ expression was higher in PCa than that in BPH.

In summary, we found that the methylation of GADD45γ was not common in PCa, the regulation of apoptotic pathways is more complex than we expected and GADD45γ is not the only key factor for the cells to direct into the apoptotic process.

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P11.216

Association analysis of polymorphisms in candidate genes on 11p15 locus with prostate cancer in Bulgarian patients

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Introduction More than 70 susceptibility loci associated with prostate cancer (PC) have been identified. PRACTICAL consortium has recently found association of 11p15 locus with PC. To investigate in detail the region associated with PC in the Bulgarian sub-sample we analyzed polymorphisms in several of the plausible candidate genes *IGF2, INS-IGF2, TH* and *ASCL2,* located in the vicinity of the most strongly associated marker rs7127900.

Materials and Methods Using TaqMan® method 176 PC samples and 169 controls were genotyped for polymorphic variants rs11603378, rs2239681, rs10770125 in *IGF2*, rs3842756 in *INS-IGF2*, rs2070762 in *TH*, rs11564710, rs7127900 and rs55930300 in *ASCL2* located on locus 11p15. We have preformed allele, genotype and haplotype analysis.

Results Allele and genotype frequencies of the variants rs11603378, rs10770125, rs3842756 and rs55930300 did not show significant differences between PC patients and controls. In contrast, the following genotypes and alleles were more common in patients than in controls: GG (OR=1.69, p=0.041) for rs2070762, CA (OR=2.12, p=0.0007) and A allele for rs11564710, GA (OR=1.7, p=0.018) and A allele for rs7127900. Association with aggressive disease showed the genotype CA (OR=3.76, p=0.003) of rs11564710. The haplotype T-G-G-C-G-C-G-A (rs11603378-rs2239681-rs10770125-rs3842756-rs2070762-rs11564710-rs7127900-rs55930300) was more common in patients with high Gleason score (p=0.026).

Conclusions In Bulgarian patients markers rs11564710 and rs2070762 showed stronger association with PC than the initial finding with rs7127900. One of the variants (rs11564710) showed correlation with aggressive PC. Additional study in larger cohort and different approach is needed to elucidate the association and the potential causal variants on 11p15 related to prostate carcinogenesis.

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P11.217

Epistatic interactions between prostate cancer susceptibility loci: evidence from Serbian population

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Prostate cancer (PCa) is the most common non-skin cancer among males in western populations and the sixth leading cause of cancer death worldwide. Still, molecular factors involved in PCa etiology remain largely unknown. Studies of genetic association of PCa have identified over 30 PCa susceptibility loci. To date, no conclusive evidence of epistasis between these loci in PCa has been obtained. The aim of this study was to test for epistasis between genes at 8q24, 17q12, 7q36 and 19p13. The study population included 175 PCa patients, 161 patients with benign prostatic hyperplasia (BPH) and 115 healthy controls derived from general population. Genotyping of ten selected single nucleotide polymorphisms (SNPs) was performed by PCR-RFLP method and by Taqman® SNP genotyping assay, while PLINK software was used for statistical analysis of obtained data. Case-only analysis of epistasis in PCa showed interaction between rs3918226 (7q36) and rs7501939 (17q12) (P=0.023). Case-control analysis of epistasis, in which BPH patients were considered as a control group, also showed interaction between these two loci (P=0.038; OR=2.875). This analysis yielded evidence of epistatic interaction between rs1799983 (7q36) and rs3787016 (19p13) (P=0.042), as well as between rs6983287 (8q24) and rs3760511 (17q12) (P=0.021). Case-control analysis of epistasis in the dataset that included 10 SNPs in PCa patients and controls derived from general population showed epistatic interaction between rs2070744 (7q36) and rs7837688 (8q24) (P=0.045), as well as between rs7501939 (17q12) and rs6983287 (8q24) (P=0.032). These results suggest possible epistatic interactions between genes located at 8q24, 17q12, 7q36 and 19p13 in PCa.

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P11.218

Simultaneous interpretation of combinative molecular analysis with histological and clinical data in prostate cancer patients

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Early detection of prostate cancer (PCa) can increase the chances of patients for a favorable outcome in the fight with this social significant disease. The aim of the study was to interpret simultaneously the results of DD3 marker, the TMPRSS2-ERG gene fusions, hypermethylation of GSTP1 gene promoter and to use this data for early diagnosis of PCa patients. An attempt to correlate the molecular data with histology (Gleason score and Tumor Node Metastasis, TNM) is made. Altogether, 16 individuals with PCa, 14 benign prostate hyperplasia (BPH) and 6 suspected for a prostate malignancy are studied. The following specific biological samples are used: 20 "tru-cut" prostate biopsies; 8 prostate tissues from radical prostatectomy; 17 blood samples, 6 urinary sediments, obtained after DRE (digital rectal examination). Patients with aggressive PCa were found to be positive for DD3 marker, for GSTP1 gene promoter hypermethylation and for TMPRSS2-ERG fusion, which correlates with Gleason score (5-7 moderate-differentiated tumors) and Gleason score (8-10 low-differentiated tumors). The molecular testing might help to clarify invasive, fast progressive, androgen-independent subtype cancer with high potential to form metastases. Our results suggest that positive molecular status in patients with borderline histological result might help for better estimation of high risk for development of PCa and strict monitoring. A combinative study of DD3, TMPRRS2-ERG fusions and the GSTP1 promoter hypermethylation provides a useful molecular arsenal in monitoring of PCa by the achievement of greater sensitivity and accuracy.

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P11.219

Increased fitness in PTEN mutation carriers due to increased cancer survival is rationale for childhood PTEN testing.

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PTEN mutation carriers may develop cancer from early childhood onwards. Mutation carriers are rare, mutation carrying families are small, and *de-novo* mutations have been demonstrated. All these observations fit the model of an early-onset autosomally dominantly inherited disorder being infrequent due to reduced fitness, and prevalence maintained by *de-novo* mutations (Lynch et al. Am J Hum Gen 1997, 1254).

We have examined the filed data from PTEN mutation carriers in four clinical genetic centres. Fifty-nine mutation carriers were identified, of whom 27 (46%) had developed cancer, 14 (52%) of whom had had two or more cancers. Total survival years after cancer was 403 years (mean 14.9). After inclusion, patients with cancer were followed for 199 observation years. One died 64 years old from breast cancer (annual death rate cancer patients after inclusion = 0.5%). Four had had thyroid cancer before 20 years. Youngest age at diagnosis of breast cancer was 24 years. She was alive and 54 years at end of study. No patient less than 20 years had been subjected to genetic testing due to cancer. Mutation carriers had an average number of children.

The results were that all early onset cancers associated with PTEN mutations had been cured in the last generation, and through that the cause of previous reduced fitness probably removed. Young patients with thyroid or breast cancer should be offered PTEN mutation testing. Children in PTEN mutation carrying families are at risk for curable childhood cancer and should be offered predictive genetic testing.

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P11.220

Differential expression of glycolysis pathway genes in renal and lung cancer

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Glycolysis is the major pathway of glucose catabolism accompanied by a synthesis of ATP. There are more than 30 genes in the glycolysis pathway that can be considered as housekeeping genes because of the high conservatism and the evolutionary antiquity of the process. Using bioinformatic analysis of transcriptomic databases and methods of quantitative real time PCR, we investigated the expression of these genes in papillary renal cell carcinoma and squamous cell lung cancer. Quantitative analysis of mRNA levels showed that only some of the glycolytic pathway genes remained relatively stable; in papillary renal cell carcinoma, these genes included *HK1, ADPGK, GPI, PGK1, PKM2,* and in squamous cell lung cancer these genes included *ADPGK, ALDOA, GAPDH, PGK1, BPGM, ENO1, PKM2*. For the first time we found frequent increases of mRNA expression of genes *PFKP, ALDOA* and *GAPDH* in renal cancer, as well as *GPI* gene in lung cancer using qPCR. For the remaining genes, differential expression was shown - we found both gains and losses of mRNA levels. Thus, we identified several genes that can be used as reference for qPCR transcriptome analysis in renal and lung cancer, and a number of differentially expressed genes that could be potential oncomarkers.

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P11.221

The development of clear cell renal cancer prognostic markers based on gene co-expression.

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Despite of a number of microchip expression data, there are no recognized prognostic gene expression profile for clear cell renal cancer (CCRC) at the present time. We used an approach consisting in bioinformatics analysis of accumulated data followed by quantitative investigation of gene expression. Several databases (Oncomine: www.oncomine.org, GEO: www.ncbi.nlm.nih.gov/geo, CGED: http://lifesciencedb.jp/cged/) and 4000 publications, selected by keywords in PubMed, were screened. There were 200 genes selected with expression two times more than in normal renal tissue observed in more than 50% of CCRC samples. Expression profiles of these genes were determined in CCRC in comparison with normal renal tissue by quantitative RT-PCR. Near 130 genes were expressed two times above normal in 40% of samples. The cluster of 22 co-expressed genes was found. Analysis of transcriptional regulation showed that most of the genes in the cluster are regulated by a single gene - HIF1A. This may explain their co-expression. The grade score of anaplasia was correlated with a number of highly expressed genes of the cluster. The relationship of a number of co-expressed genes with the TNM classification was identified.

In summary, the identified genes, on their function and role in the development of cancer, can be markers of prognosis, reflecting the propensity for aggressive behavior of the tumor.

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P11.222

Gene-gene interactions of GSTM1, CYP1A1, IL4, IL16, VDR, PON1, VEGF genes contribute to renal cell carcinoma susceptibility in populations of Bashkortostan Republic of Russia

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Unraveling the nature of genetic interactions is crucial to obtaining a complete picture of complex diseases. Renal cell carcinoma (RCC) is the most common neoplasm affecting the adult kidney. It's known that a predisposition to RCC due to a number of polymorphisms of genes involved in the metabolism of xenobiotics, anti-tumor immunity, angiogenesis and transcription.

The goal was to investigate the association of SNPs in seven genes with RCC and to evaluate gene-gene interactions in the development of RCC. A case-control association study of twelve SNPs in the genes GSTM1,CYP1A1,IL4,IL16,VDR,PON1,VEGF included 200 RCC patients from Bashkortostan Republic of Russia and 250 controls. Statistical analysis was held using logistic regression model and GMDR method.
The analysis showed genotype rs662*T/T and allele rs662*T of rs662 in PON1 gene (OR=3,23(95%CI 1,40-7,65);OR=1,49(95%CI 1,07-2,08), respectively) and genotype rs731236*G/G of the VDR gene(OR=2,42(95%CI 1,07-5,77)) to be markers of the increased risk for RCC development. The genotype rs2228570*T/T of rs2228570 in VDR was the marker of low risk for RCC (OR=0,61(95%CI 0,39-0,93)). We identified the most informative model of gene-gene interactions for RCC development in population of Russians (rs2243259(IL-4))-rs4778889(IL-16)). The combination of genotypes rs4778889*C/C(IL16)-rs2243250*T/C(IL4) was the marker of the decreased risk for RCC in Russians (OR=0,47(95%CI 0,22-1,01)), whereas the combination of genotypes rs4778889*T/C(IL16)-rs2243250*T/C(IL4) was the marker of the increased risk for RCC(OR=4,50(95%CI 1,33-16,78)). The analysis gene-gene interactions in Tatar ethnic origin found the statistically significant model (rs2243259(IL-4))-rs22432250(VDR)-rs731236(VDR)) predisposing to RCC development. Detecting interactions among disease associated SNPs may reveal basic biological mechanisms critical for the disease development.

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P11.223

Clinical evaluation of Myelodysplasia using a SNP microarray

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Over the past 1½ years we have evaluated over 1000 patients, to delineate the utility of a SNP microarray (Affymetrix Cytoscan HD) approach for the study of myelodysplasia. Results have shown that: (1) Over 23% of chromosomally normal patients with myelodysplasia were found to have an abnormality detectable by the SNP array; (2) Approximately 120 different cases of segmental aUPD were observed, representing approximately 15% of the patients; (3) This technology has been especially useful in cases equivocal for MDS, where in approximately 25% of cases the SNP array was informative when flow cytometry and pathology studies could not provide a definitive diagnosis; (4) Even when cytogenetics and/or FISH demonstrated an abnormality, the array provided additional results in 59% of patients; (5) In addition to the abnormalities routinely detected by cytogenetics and FISH, the array detected numerous abnormalities including: deletions and aUPD of 4q (TET2); 21q (RUX1); 11q (Cbl); 17p (TP53), 9p (JAK2), 17q (NF1), 1p (likely MPL or NRAS) and aUPD of 7q (EZH2); (6) Of these alterations, involvement of TET2 and RUNX1 are the most commonly detected; (7) The precision and breakpoint analysis suggest two different genes on 7q may be important, not only EZH2 (distal), but also SERPINE1 which has been implicated in several submicroscopic 7q22.1 deletions and also when ASXL1 was included in the 20q deletion; (8) Overall this work clearly illustrates the additional important diagnostic and prognostic information detected by a SNP array analysis and the involvement of significant genes not detectable by routine analysis.

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P11.224

Novel mutations in spliceosomal gene *PRPF8* show ring sideroblast, proliferative, and missplicing phenotype in patients with myeloid neoplasms

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The pathogenesis of myelodysplastic syndromes is complex and associated with vast heterogeneity in histomorphology and molecular somatic lesions. To understand the molecular somatic lesions in MDS, we applied whole exome sequencing in 352 patients. Commonly mutated gene was *PRPF8*, found on 17p13.3, coding for an essential protein in the spliceosomal core. Germline mutations in *PRPF8* have been shown to cause the autosomal dominant retinitis pigmentosa type 13. More than 80% of mutated *PRPF8* cases showed presence of ring sideroblasts in their pathologies. Furthermore, when we analyzed additional 23 cases using SNP arrays that had 17p deletion, 65% of cases contained ring sideroblasts. The knockdown of *PRPF8* showed increased colony formation. Since PRPF8 is extremely well conserved between humans and yeast, we created homologous mutants in yeast. All mutants showed the same phenotype--defect in the second step of splicing. These results suggest that the mutant PRPF8 might activate incorrect splice sites leading to frame shifted mRNAs. To understand the global effects of PRPF8 splicing defects, we analyzed RNA-seq data for 2 mutant cases of *PRPF8* and 17 cases of del17p or low PRPF8 expression. Misspliced genes included genes involved in progression, metabolism, and retinitis pigmentosa. Novel somatic mutations of PRPF8 are associated with myeloid malignancies with increased ring sideroblasts, followed by the evidence of similar pathogenesis by haploinsufficiency. Somatic events constitute loss of function, resulting in the change of splice site recognition function which is associated with proliferating potential and could be a considered as a new therapeutic target.

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P11.225

High Resolution Melting analysis as a rapid and efficient method for molecular diagnostics of patients with Peutz-Jeghers syndrome

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Peutz-Jeghers syndrome (PJS) is a rare, hereditary predisposition characterized by the occurrence of hamartomatous polyps in the gastrointestinal tract, mucocutaneous pigmentation and increased risk of cancer in multiple internal organs. The incidence of the syndrome, depending on the studied population, has been estimated to range from 1:300 000 even up to 1:25 000 births. PJS is an autosomal dominant disease and, in most of the cases, it is caused by mutations in the STK11 gene.
The majority of causative DNA changes identified in patients with PJS are small mutations and, therefore, developing a method of their detection is a key aspect in the advancement of genetic diagnostics of patients suffering from PJS. In our study, we designed 13 pairs of primers which amplify in the same temperature and enable the examination of all coding exons of the STK11 gene by the HRM analysis.
In our group of 41 families with PJS, small mutations of the STK11 gene were detected in 22 families (54%). In the remaining cases all of the coding exons were sequenced. However, this did not allow detection of any additional mutations. Therefore, the developed methodology is a rapid and cost-effective tool of searching for small mutations in PJS patients and made it possible to detect all the STK11 gene sequence changes occurring in our group.
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P11.226

Comprehensive genetic characterisation of pediatric T-cell acute lymphoblastic leukaemia

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Several genetic events need to accumulate in a single cell in order for clo-

nal expansion of a malignant T-lymphoblast to occur. To elucidate further the genetic aberrations resulting in T-cell acute lymphoblastic leukaemia (T-ALL) conventional chromosome banding, fluorescence *in situ* hybridization (FISH), single nucleotide polymorphism (SNP) array, expression, and large scale sequencing analyses were preformed in a consecutive series of 47 pediatric T-ALL patients. Cytogenetic analyses were informative in 41 cases, displaying a normal karyotype in 54% of cases. Recurrent cytogenetic aberrations comprised T-cell receptor (TCR) rearrangements (*TRA@/TRD@* at 14q11 and *TRB@* at 7q34) and deletions of 6q and 9p. FISH for TCR rearrangements resulted in aberrant hybridization patterns in 26% of investigated cases of which 25% were cytogenetically cryptic. The median copy number alteration displayed by SNP array was 3 (range 0-10), with the majority being less than 10 Mb. Six genes were recurrently targeted by deletions: *CDKN2A/CDKN2B*, *STIL*, *PTEN*, *LEF1*, and *RBI*. Eight paired diagnostic-relapse samples were analysed with SNP array; two samples displayed the same aberrations at diagnosis and relapse, three cases harboured additional changes in the relapse, two cases shared some aberrations but also harboured unique aberrations, and one sample had completely different aberrations between diagnosis and relapse. The expression and sequencing data are currently being analysed. Taken together, the presented data illustrate the multistep genetic process required for T-ALL to occur.

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P11.227

Effect of siTRF2 knockdown, a shelterin protein, on the radiosensitivity of telomerase-immortalised human mesenchymal stem cells

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Imperfect capping of telomeres can lead to tumor formation. The cap structure is preserved by shelterin complex where TRF2 is an important member of it. Telomerase-induced bone-marrow derived human mesenchymal stem cells (hMSC-telo1) were used to assess the role of TRF2 knockdown in radiosensitivity. Radiation response and transformation efficiencies of these cells were previously well documented (Serakinci et al. 2008).
siTRF2 knockdown efficiency was found to be 78.53% vs 81.8% for non-radiated (NR) and radiated (R) groups (vectors was kindly provided by Drs Zaffaroni & Marco) respectively, in time point of 48 hours after application of radiation, whereas they were 72% vs 62% after 72 hours (2.5 Gray).
The siTRF2 knockdown cells at 48th time point showed a significant increase in the levels of SA-β-gal (senescence) after exposure to IR, but that difference diminished in 72 hours. Previously we have shown that SA-β-gal ratio was 30% and 76% before and ten days after irradiation, for hMSC-telo1(Serakinci 2007). In this study siTRF2 was found to be highly toxic after 96 hours and senescence difference was much less pronounced in NR and R groups (22% vs 33%).
Colony forming efficiency was found to be decreased in siTRF2 knockdowns following 48 hour incubation, but this difference also did not extend to 72 hours.
Conclusion: We found that knockdown of TRF2 increase the short time cellular effect of irradiation, thus, our data suggest that knockdown of TRF2 might a tool for increasing radiation induced senescence and radiosensitivity of cells for therapeutic purposes.

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P11.228

Telomere length in histologically free margins of oral squamous cell carcinoma

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Telomere shortening over a critical length leads to chromosomal instability, which might be a key event in the initiation of carcinogenesis. Telomere length as well as different genetic alterations that could be found in tumor free margins of oral carcinoma may be of great importance for estimation of oral pathogenesis.
The aim of this study, was to measure relative telomere length (RTL) in histologically free tumor margins of patients with oral squamous cell carcinoma (OSCC), using real-time PCR. RTL was evaluated by comparing telomere/single copy gene (T/S) ratio in 36 samples of tumor margins and T/S value in reference cell line (293T). The estimated RTL values in margins according to tumor stages were: 1.63 in stage I (25%), 1.37 in stage II (28%), 2.45 in stage III (28%), and 1.67 in stage IV (19%). Also, mean telomere length values of adjacent mucosa in correlation to lymph node status were as follows: 1.67 and 2.12, in cases with negative (68%) and positive (32%) local invasion, respectively.
Overall, a statistically significant increasing of telomere length in free margins of patients with advanced stages was observed (p=0.032). The same trend toward longer telomeres in cases with lymph node metastasis was found, but there was no a significant association. However, our data indicate that telomere length in tumor adjacent, histopathologically confirmed as free of neoplastic cells, might be a valuable tool in prognosis of oral carcinoma.

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P11.229

A novel case of TEL/AML1-positive acute lymphoblastic leukemia with t(2;12;21): Is translocation involving 2p13 the secondary genetic change for leukemogenesis in TEL/AML1-positive acute leukemia?

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TEL-AML1 (ETV6-RUNX1) fusion is the most common in childhood B-cell progenitor acute lymphoblastic leukemia (BCP-ALL) and is related with favorable prognosis. Complex chromosomal translocations, involving three or more different chromosomes, have been rarely observed in leukemia with t(12;21)(p13;q22), and to our knowledge, only three cases involving chromosome 2 were reported with different breakpoints. This is the first TEL/AML1 positive BCP-ALL case with novel three-way molecular variant involving 2p13. The patient was 3-year-old female with lethargy and severe leukocytosis at the time of onset. The leukemic cells were CD34 negative immature B cells. Cytogenetic analysis showed that 45,X,-X,t(2;12;21)(p13;p13;q22) on chromosome study and one TEL-AML1 fusion signal with a single TEL signal on FISH analysis. The patient is alive with complete remission for 14 months although she had histories of invasive fungal and bacterial infections after induction chemotherapy. TEL-AML1 fusion interferes with normal function of the transcription factor RUNX1 and has been considered as necessary but not sufficient for the developmenet of leukemia. Many researchers suggest that secondary genetic event in addition to TEL-AML1 fusion is necessary for leukemogenesis. Regarding 2p13, several cases of acute or chronic B cell leukemia with t(2;14)(p13;q32) were reported as sole chromosomal abnormality. Furthermore some authors suggested that t(2;14)(p13;q32) could be recurrent genetic abnomality with mention of BCL11A gene on 2p13 as a strong candidate for leukemogenesis. This case contributes to further delineate the role of 2p13 rearrangement in leukemogenesis and the clnical course of BCP-ALL with this rare chromosomal aberrancy.

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P11.230

New germline JAK2 mutations resistant to current JAK2 and HSP90 inhibitors are responsible of a hereditary thrombocytosis in two pedigrees

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Thrombocytosis are rare entities characterized by an abnormal proliferation of the megakaryocytic lineage resulting in overproduction of platelets. The majority of thrombocytosis are sporadic essential thrombocythemia classified as myeloproliferative neoplasms (MPNs) due in half cases to mutations in JAK2 (*JAK2 V617F*) or in molecules affecting the thrombopoietin receptor (MPL)/JAK2 axis. Familial cases of thrombocytosis exist and are usually transmitted by autosomal dominant inheritance. They are subdivided as MPN-like disorders with incomplete penetrance or as pure thrombocytosis with a complete penetrance of hematological abnormalities.
We identified two families with hereditary thrombocytosis presenting three novel heterozygous germline mutations of *JAK2*. One family carries the *JAK2*

R867Q mutation located in the kinase domain, while the second family revealed double mutations in cis, one in the pseudokinase and the other in the kinase domains (*JAK2 S755R/R938Q*). These substitutions are gain-of-function mutations exhibiting spontaneous cell growth and constitutive signaling in Ba/F3-MPL cell models. Moreover, these mutations required MPL to be in active dimeric orientations to signal. In contrast to JAK2 V617F, both JAK2 R867Q and S755R/R938Q half-lives were significantly increased which correlated with an increased cell surface expression of MPL. Consequently, these mutations conferred a resistance to classical JAK2 inhibitors and HSP90 inhibitors compared with *JAK2 V617F*.
These results suggest that mutations in the kinase domain of JAK2 might confer only a weak activation of oncogenic signaling specifically dependent on MPL, indicating that their identification in familial cases of thrombocytosis might be underestimated.

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P11.231

The prevalence of polymorphic variants I157T (c.470T> C, rs17879961) and R145W (c.433C> T, rs137853007) in CHEK2 gene and its putative correlation with the risk of differentiated thyroid cancer in Polish population.

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Thyroid carcinomas are the most often carcinomas of endocrine system. Most often occurs papillary and follicular thyroid cancer: tumors with well prognosis, low benignity and slow progress but often giving recurrences and regional or remote metastasis and as well progression from well differentiated thyroid cancer to malignant anaplastic carcinoma.
The aim of this study was to analyze the prevalence of polymorphic variants I157T (c.470T> C, rs17879961) and R145W (c.433C> T, rs137853007) CHEK2 gene and its putative correlation with the risk of causing differentiated thyroid cancer (DTC).
Group of 623 patients 574 females and 49 males) with differentiated thyroid cancer and 500 individuals (297 females and 203 males) from population group were examined. The analysis of gene variants, using the method of pyrosequencing, showed no variation in response to changes in R145W, and confirmed diversity of the I157T in both group of patients with DTC in 4,36% and 2,6% in population. Performed statistical analysis allowed to demonstrate the relationship between the presence of the I157T variant and the occurrence of thyroid cancer. Odds ratio for the rare allele C against the wild one T, amounted 0,5855 at P = 0,0310. Obtained data proved as well correlation between the C allele and higher risk of DTC morbidity for women in Polish population. There has been also observed the increasing impact of age on disease morbidity in Polish population. Project supported by Polish Ministry of Science and Higher Education grant N N402 287436.

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P11.232

Variant c.723A in cyclin D1 gene (CCND1) as a risk allele in differentiated thyroid cancer occurring.

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Polymorphisms in the genes critical to cell cycle control are strong candidates for association with elevated risk of cancer. The CCND1 gene (11q13) encodes the cyclin D1, playing an important role in the transition from G1 to S phase of cell cycle during cell division. Mutations, amplification and over expression of this gene, which alters cell cycle progression, are observed frequently in a variety of tumors and may contribute to tumorigenesis.
We examined common CCND1 gene polymorphisms in cohort of Polish patients with differentiated thyroid cancer (DTC) and Polish population. Analysis concerned the CCND1 c.723G>A polymorphism (rs9344; p.Pro241=), which is located in exon 4, and increases the frequency of alternate splicing and c.669C>T (rs3862792; p.Phe223=).
DNA was extracted from whole blood lymphocytes of 652 patients diagnosed with differentiated thyroid cancer. The population group included 824 subjects. To perform genotyping we used the pyrosequencing technique (PSQ96).
We observed statistically higher frequency of allele A in rs9344 locus in thyroid cancer patients (p=0,0173). We observed also statistically significant differences between genotypes in subjected group. Genotypes AA and GA occurs more frequently in patients with DTC (p=0,0467) than in population group.
We did not observed any statistically important differences in frequencies of genotypes or alleles in rs3862792 locus in subjected groups.
Our findings indicates that A allele in locus rs9344 in homozygotes and heterozygotes as well, may be a risk allele in the development of differentiated thyroid cancer in Polish population. Research funded by grant no. N N402287436.

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P11.233

TNF siRNA as modulators of apoptosis and angiogenesis, in cell migration and invasion in triple negative breast cancer cells (TNBC)

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Tumor necrosis factor-alpha (TNF-α) is a proinflammatory cytokine that has been linked to breast cancer development. Estrogen metabolic pathway is also involved in breast carcino-genesis and DNA adducts formation. A particular case is the TNF- α modulation in TNBC (triple negative breast cancer). Currently there are several TNF-α antagonists applied in clinical use and these agents have provided significant benefits for a variety of cancers. In the last years a special interest have received triple negative breast cancer which is a highly aggressive subtype that observed particularly in young patients with a poor overall survival rate and no target therapy.
In our experiments, we used as an *in vitro* model for triple negative breast cancer - the cell line Hs578T - for evaluation of gene expression profiling of signaling networks involved in apoptosis using PCR-array genes. Treatment with special designed siRNA molecules to target TNF-α mRNA has been done for angiogenesis modulation and most relevant genes were evaluated by qRT-PCR
Knockdown of *TNF-α* gene was connected with activation of apoptosis processes and inhibition of cell migration and invasion, as monitored using the xCELLigence RTCA System and by *in vitro* matrigel angiogenesis assay. There are down-regulated genes involved in cell survival and angiogenesis, whereas the up-regulated ones with pro-apoptotic role.
TFN-α represents a central molecule in the modulation of processes, apoptosis and angiogenesis. TFN-α gene silencing offers an alternative therapeutic strategy in triple negative breast cancer.

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P11.234

The association of apoptotic protein expressions SAG, p73 and p53 with prognosis in cervical carcinoma

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Radiotherapy, applied on advanced stage cervical carcinoma, can only be effective in low resistance cells. Thereby the tumor response and survival rates are highly variable among individuals with different tumor radiosensitivity.
Tumor growth is intimately associated with apoptosis in tissues and apoptosis seems as a likely candidate to estimate prognosis of a treatment.
To further elucidate the roles of apoptotic factors, we determined mRNA expression levels of proteins which are important in apoptotic pathways, especially focusing on a recently identified sensitive-to-apoptosis protein (SAG). In this study, pairwise comparison of expression levels for SAG / Bcl-X_L, Bcl-X_L / Bak and p73 / p53 showed significant correlation with each other. Bak expressions were far from significance in all analyses, probably as a result of joint action of Bax and Bak.
Patients with high levels of SAG expression had a low spontaneous apoptotic index, as expected. Besides, these two factors were clear indicative of better survival rates. Local free, disease free and overall survival have all indicated a higher profile in patients who had lower levels of expressions for both SAG and Bcl-X_L proteins. Overall survival rates after five-years follow-up period have indicated to a better survival in group with low SAG and low Bcl-X_L expressions (P =0.014 and 0.002 respectively).
Conclusion: Our findings approves that SAG is a strong candidate to estimate survival in cervical cancers and shows signifcant correlation with apoptosis and better survival. However we have not observed such a correlation neither with p53 nor p73 expressions.

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P11.235
The TP53 gene Arg72Pro polymorphism is associated with the increased risk of cervical cancer in Russians

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The P53 tumour-suppressor protein (encoded by the TP53 gene) has been called the guardian of human cells against cancer. Recent studies have shown that the heterozygosity for TP53 gene Arg72Pro polymorphism is protective against different types of cancers. The current consensus is that TP53 Arg72 allele is more effective at inducing apoptosis and protecting stressed cells from neoplastic development while 72Pro allele is more effective at inducing cell cycle arrest and senescence. The aim of the study was to examine the association of the TP53 genotypes with the odds ratio (OR) for cervical cancer among women. TP53 gene variants were determined in 123 women with cervical cancer (all Caucasians and citizens of Russia). Cases were then compared with ethnically matched healthy controls (n=467). The TP53 Arg72 allele frequency was not different between cases and controls (67.5% vs. 69.8%). However, those women with homozygous genotypes (Arg/Arg, Pro/Pro) were at 2.6 times the risk for cervical cancer [OR, 2.587; 95% CI, 1.595-4.195] compared to women with the heterozygous (Arg/Pro) genotype (frequency of Arg/Pro genotype: 19.5% vs. 38.5%; P<0.0001). In conclusion, the heterozygosity for TP53 gene Arg72Pro polymorphism is protective against cervical cancer in Russian women.

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P11.236
Magnetic resonance imaging screening in Li Fraumeni Syndrome: An exploratory whole body MRI study (the SIGNIFY study)

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Li Fraumeni Syndrome predisposes individuals to a range of malignancies with a lifetime cancer risk of up to 90% in women and 70% in men. Current UK national screening recommendations are for breast screening with mammography and MRI in women. Some centres employ family specific screening tailored to malignancies found within a family, most centres have an "open-door" policy. Recent evidence suggests there may be a survival benefit for more intensive screening, including whole body MRI, but there are no published data on the psychological impact of such screening programmes. The primary end-point of this study is to assess incidence of malignancies diagnosed in asymptomatic *TP53* mutation carriers using whole body MRI without contrast, against general population controls. The secondary end-points are to evaluate any incidental findings and assess the psychological impact of whole body MRI screening. Recruits must be aged between 18 and 60. Cases must carry a germline *TP53* mutation and must not have been diagnosed with a malignancy in the last 5 years; controls must be non-related individuals with no personal history of malignancy. *TP53* mutation carriers and population controls will undergo conventional and diffusion weighted MRI, reported independently by two radiologists blinded to their mutation status. An intersite virtual MDT will be held at regular intervals to discuss the diagnosis and management of incidental findings. A series of questionnaires will be used from time of recruitment up to a year after the MRI scan to assess the psychological impact of screening. Initial results will be shown.

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P11.237
TP53 p.R337H is a conditional cancer-predisposing mutation: further evidence from a homozygous patient

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BACKGROUND: Adrenocortical carcinomas (ACCs) are among the most common cancers in children affected with the Li-Fraumeni/Li-Fraumeni-like (LFS/LFL) syndromes, caused by germline mutations in the TP53 gene. In Brazil, a particular common mutation, TP53 p.R337H, is due to a founder effect and is strongly associated with ACC. We describe for the first time, the molecular and clinical follow-up of a patient diagnosed with ACC and homozygous for p.R337H. CASE PRESENTATION: At age 11 months, the female child was diagnosed with a virilising anaplastic ACC, which was completely excised without disturbing the adrenal capsule. Family history was consistent with LFL. Genotyping identified the p.R337H mutation in homozygosity in genomic DNA from lymphocytes and fibroblasts. Haplotype analysis confirmed the occurrence of the mutation in the Brazilian founder haplotype previously described. No other TP53 germline or somatic or rearrangements were identified. At age 9 years, the child was asymptomatic and had no evidence of endocrine derangements. Full body and brain magnetic resonance imaging failed to detect any suspicious lesions, and cardiopulmonary exercise testing results were within the normal reference for the child's age, ruling out a major exercise capacity deficiency. CONCLUSION: Our results support the hypothesis that p.R337H, the most common TP53 mutation ever described in any population, is a conditional mutant. Observations over a long period of clinical follow-up suggest that p.R337H homozygotes don't have a more severe disease phenotype than heterozygotes. Homozygotes for p.R337H will require careful surveillance for lifetime cancer risk and for effects on metabolic capacity later in life.

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P11.238
Impact of polymorphisms in genes of p53 pathway on clinical manifestations of the Li-Fraumeni-like syndrome

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Li-Fraumeni Syndrome (LFS) and its variant, Li-Fraumeni-like Syndrome (LFL), are autosomal dominant disorders characterized by increased predisposition to multiple early-onset cancers, caused by a germline mutation in the *TP53* tumor suppressor. The core tumors of the syndrome are soft tissue and bone sarcomas, brain tumors, breast cancer, and adrenocortical carcinoma (ACC). In Southern Brazil, a germline mutation (*TP53* p.R337H) with incomplete penetrance is present in 0.3% of the general population and has been associated with a broader cancer spectrum than that observed in LFS. The environmental and genetic factors underlying cancer risk and tumor patterns in LFS/LFL patients are not completely understood. In this study, the single-nucleotide polymorphisms (SNPs) *MDM2* SNP309T>G (rs2279744), *MDM4* C>T variation (rs1563828) and *HAUSP*

G>A (rs1529916) were genotyped using TaqMan assays in 59 p.R337H *TP53* mutation carriers with different tumors and 100 *TP53* wild-type healthy controls. The *HAUSP* SNP showed a borderline effect (*P*=0.051) as genetic modifier of tumor type in cancer affected mutation carriers. The genotype in heterozygosity was observed in 7/9 (77.8%) mutation carriers with ACC as compared to 6/21 (28.6%) of those diagnosed with breast cancer. In contrast, we did not observe any statistically significant association between *MDM2* and *MDM4* SNPs and clinical manifestations. Our results suggest that there may be an effect of the *HAUSP* polymorphism rs1529916 on the type of tumor developed by *TP53* p.R337H carriers. Further studies with a larger sample size and possibly, other SNPs in the *HAUSP* gene should be undertaken to confirm this observation.

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P11.239

20 years of Li-Fraumeni syndrome in France

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The Li-Fraumeni syndrome (LFS) represents a remarkable genetic predisposition to cancer characterized by a wide tumour spectrum including sarcomas, premenopausal breast cancers, brain tumours and adrenocortical carcinomas. Twenty-three years after the demonstration that LFS results from TP53 germline mutations, we have identified 188 unrelated French LFS families harbouring TP53 alterations, thanks to the Chompret criteria elaborated by the French LFS working group to facilitate the clinical recognition of the syndrome. The update of clinical data performed in 280 affected mutation carriers who had developed 478 tumours revealed that the median age of first tumour onset was 27 years (17y in males versus 28y in females), that 44% of patients developed 2 to 6 multiple primary tumours, and that at least 20 of secondary tumours developed within the radiotherapy field of a previous tumour. The most frequent tumours observed were breast carcinoma (110), soft-tissue sarcoma (85+7 breast sarcoma), osteo/chondrosarcoma (52), brain tumours (40) and adrenocortical carcinomas (35). Among 102 patients with adrenocortical carcinoma referred for TP53 testing, 51% of children and 22% of adults carried a TP53 mutation. Germline TP53 alterations were in 61% missense mutations and in 5% genomic rearrangements. Patients harbouring dominant negative missense mutations developed first tumour 16 years earlier than patients with null mutations (22y versus 38y). We found that TP53 mutation carriers harbouring the MDM2 285-309 G-G haplotype developed tumours 5 years earlier than patients harbouring other haplotypes (p=0.043), indicating that the MDM2 285-309 G-G is a higher risk haplotype in patients with germline TP53 mutations.

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P11.240

Germline copy number variation of genes involved in chromatin remodelling in families suggestive of Li-Fraumeni syndrome with brain tumours

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Germline alterations of the tumour suppressor *TP53* gene are detected approximately in 25% of the families suggestive of Li-Fraumeni syndrome (LFS) characterised by a genetic predisposition to a wide tumour spectrum, including soft-tissue sarcomas, osteosarcomas, premenopausal breast cancers, brain tumours, adrenocortical tumours, plexus choroid tumours, leukaemia, and lung cancer. The aim of this study was to determine the contribution of germline copy number variations (CNVs) to LFS in families without detectable TP53 mutation. Using a custom-designed high-resolution array CGH, we evaluated the presence of rare germline CNVs in 64 patients fulfilling the Chompret criteria for LFS, but without any detectable *TP53* alteration. In 15 unrelated patients, we detected 20 new CNVs absent in 600 controls. Remarkably, in 4 patients who had developed each brain tumour, the detected CNV overlap the *KDM1A, MTA3, TRRAP* or *SIRT3* genes encoding p53 partners involved in histone methylation or acetylation. Focused analysis of *SIRT3* showed that the CNV encompassing *SIRT3* leads to SIRT3 overexpression and that in vitro SIRT3 overexpression prevents apoptosis, increases G2/M and results in a hypermethylation of a subset of genes involved in cancer. This study supports the causal role of germline alterations of genes involved in chromatin remodelling in genetic predisposition to cancer and, in particular, to brain tumours.

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P11.241

Mutations in the von Hippel-Lindau gene, a graduate tuning on the way to cancer

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Over the past 30 years, the genetics of inherited cancer syndromes associated with germline mutations in tumor suppressor genes (TSGs) has been dominated by Knudson's two-hit model. This model implied that onset of malignant transformation resulted from full inactivation of the TSG. Recently, a new "continuum model" has been proposed, which introduces more versatile concepts such as gene dosage-sensitivity, specific phenotype of heterozygote cells and tissue specificity. Mutations in the von Hippel-Lindau (VHL) tumor suppressor gene are associated with a complex spectrum of conditions from no symptoms or polycythemia to predisposition to several types of tumor. We report here an atypical family bearing two VHL gene mutations in cis (R200W and R161Q), together with structural modeling, functional and transcriptomic studies of these mutants. We demonstrate that the complex pattern of clinical manifestations related to distinct VHL mutations is perfectly correlated with a gradient of VHL dysfunction in hypoxia signalling pathways, which supports the new quantitative "continuum model" of tumor suppression.

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P11.242

A synonymous mutation on VHL gene causes a pheochromocytoma and paraganglioma history through three generations

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The index-case was a 12-years old girl, operated on for a pheochromocytoma (PH). Her father and grandfather were also affected by PH or paragangliomas (PGL) several years ago. Genetic counselling was organized in a specialized oncogenetic multidisciplinary consultation, including systematic psychological approach. In this family, the diagnosis of PH was delayed for the child, although her father and grandfather were aware of their medical history, illustrating the denial as a defensive mechanism against guilt

of disease transmission. In this context, testing three generations required reflexion about the temporality of the test.
VHL, *RET*, *SDHB*, *SDHD*, *SDHC*, *MAX* and *TMEM127* were first investigated in the index-case. A single neutral variation of unknown significance (UV) was identified on *VHL* gene (c.414A>G; p.Pro138Pro). That UV was inherited from the paternal branch and was present on tumor DNA associated with loss of heterozygosity. The *VHL* gene is composed of 3 exons encoding a 4.7 kb mRNA and a minor *VHL* transcript deleted from exon 2 (*VHLΔ2* isoform). The UV was absent in Exome Variant Server but ESEfinder analysis suggested the creation of a new cryptic splicing site. Further investigations on RNA extracted from leucocytes of each patient and from the tumor, compared with RNA from similar samples of controls showed a high expression of *VHLΔ2* isoform in patients' leucocytes and the absence of main *VHL* transcript in the PH. The diagnosis of von Hippel Lindau disease was made on those biological data and this family now benefits from a specific follow-up of the disease.

C. Simian: None. **L. Heidet:** None. **K. Bouchireb:** None. **N. Abermil:** None. **V. Nau:** None. **M. Menara:** None. **J. Favier:** None. **S. Irtan:** None. **J. Mazzella:** None. **K. Lahlou-laforet:** None. **A. Gimenez-roqueplo:** None.

P11.243
Mutational analysis of the Wilms' tumor gene (WT1) in Greek patients presenting with nephroblastoma

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Wilms' tumor (WT) or nephroblastoma is a childhood tumor of the kidney. It affects children below the age of 5 and has an incidence of 1 in 10000 children. Most cases are sporadic, unilateral and not associated with other symptoms of systemic disease. The average age of diagnosis is 42-47 months for children with unilateral tumors and 30-33 months for children with bilateral tumors. WT displays a high heterogeneity. Mutations in genes such as *WT1, β-catenin, WTX, TP53* and *CTNNB1* as well as deletions in the chromosomal region of *WT1* (chr11p.13) are implicated in the pathogenesis or progression of the WT. Heterozygous *WT1* mutations account for 20% of WT. In this study, we examined 8 sporadic cases and 1 familial case with nephroblastoma. Mutation analysis of the *WT1* gene (exons 2-9) in patients (and their parents) revealed alterations in 5/8 sporadic cases. De novo mutations were found in exon 8 (the novel p.Q374P and p.R362X), exon 9 (p.R390X and p.R394W/N) and intron 9 c.1432+4C>T. Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) indicated that the p.Q374P (legacy name) is probably damaging with a score of 0.992. Thus, 5/8 cases presented with mutations in *WT1* indicating that molecular investigation of this gene is useful to support the definitive diagnosis. Concerning the non-characterised patients' mutational analysis will be continued in the other exons of *WT1* as well as in the other genes implicated to be involved in the presentation of WT.

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P11.244
Targeted resequencing of BRCA1 and BRCA2 in familial breast cancer

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Breast cancer affects about 13,000 women every year in Australia. Inherited loss-of-function mutations in *BRCA1* and *BRCA2* predispose to high risk of breast and/or ovarian cancer. Since the discovery of breast cancer susceptibility genes *BRCA1* and *BRCA2*, there have not been any other genes identified that play a significant role in predisposition to inherited breast cancer. A large proportion of individuals with inherited breast cancer are negative for *BRCA* mutations and despite numerous research efforts, further breast cancer susceptibility genes still remain elusive.
We hypothesize that genetic anomalies are present in the *BRCA1* and *BRCA2* genes in a subset of individuals with familial breast cancer where no genetic anomalies where identified using Sanger sequencing. This study aims were to identify genetic anomalies in *BRCA1* and *BRCA2* by completely re-sequencing 200kb surrounding *BRCA1* and *BRCA2* using next-generation sequencing.
This pilot study involves 10 individuals with familial breast cancer, had previously been screened and found to be negative for *BRCA1* and *BRCA2* coding region mutations. Targeted next-generation paired-end sequencing of regions containing *BRCA1* and *BRCA2* was performed using Illumina GAIIx. Genetic differences in the form of single nucleotide variants and insertions/deletions were identified in most individuals tested in regions that had previously remained unexplored, such as the non-coding regions of BRCA1 and BRCA2, the 5'UTR, 3'UTR and promoter sites. Variants in these sites are being further analysed for effect on expression of transcript.
This study has comprehensively investigated *BRCA1* and *BRCA2* and surrounding genomic regions in a mutation-negative inherited breast cancer population.

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P12.01
Discrepancies between prenatal aCGH on cell cultures and postnatal aCGH on blood DNA: two case reports.

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Chromosomal microarray (CGHa) is becoming the first-tier diagnosis test for prenatal diagnosis allowing detection of small chromosomal rearrangement. We report two cases of discrepancies between aCGH performed using DNA obtained either from chorionic villus culture or from amniotic fluid cell culture and aCGH performed using DNA obtained from blood after birth.
Case 1:
aCGH, performed on a chorionic villus culture performed because of nuchal translucency at ultrasound examination, evidenced two duplications (95Mb 6q11q25 duplication and 1,5Mb 15q21 duplication). Both of these abnormalities were absent on aCGH performed on DNA obtained after birth.
Case 2
aCGH, performed on amniotic fluid culture at 33weeks of gestation because of abnormalities of foetal corpus callosum at ultrasound, evidenced two duplications (54Mb 1q22q32 duplication and 4,8Mb 11q24q25 duplication) and associated with one deletion (38Mb 1q32q44 deletion). None of these abnormalities were present on aCGH performed on DNA obtained from blood after birth.
Discrepancies between aCGH performed using DNA obtained from culture cells and DNA directly extracted from tissue have been reported in few cases in the literature.
We discuss the reasons of such discrepancies and we recommend caution in the interpretation of copy number variation evidenced on prenatal cell cultures. Finally, we recommend when possible to perform aCGH using DNA directly extracted and not DNA obtained from cell cultures.

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P12.02
A whole-genome reference panel enriched in Italian lower-frequency variants.

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Genetically isolated population cohorts have contributed to large-scale discovery efforts of genetic loci associated with complex traits shared between different European populations. Additional opportunities for novel discoveries of complex trait loci arise from exploiting the population specific enrichment in low frequency variants characteristic of these populations.
The Italian Network of Genetics Isolates (INGI) has collected up to 3000 individuals sampled for a large set of phenotypes from several isolated populations along Italy. We sequenced a subset of 110 individuals randomly selected from the Val Borbera cohort (VBI), and a second subset of 250 individuals from Friuli Venezia Giulia (FVGI) maximally representative of ancestral chromosomes, using low-coverage whole-genome sequencing.
After genotype calling and refinement, accurate quality controls steps were applied to obtain a reliable set of rare and common variants.Through comparison between INGI cohorts, with other Italian (e.g. the Tuscany population from 1000G[1]) and European populations (in 1000G or in the WTSI-led UK10K project[2]) we have produced a full catalog of genetic variation, common and rare, in each population.
With this approach we expect to produce an imputation reference panel specific for Italian/Southern European populations, containing a complete

set of genome-wide low frequency and rare variants that could be exploited to search for causative variants for a large set of phenotypes.
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P12.03
Array CGH and fetal pathology : pitfalls of cell culture.

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aCGH has proven its usefulness to detect Copy Number Variation (CNV) in malformed fetuses. Currently, this technique is either performed on DNA extracted from fetal tissue or cell cultures. Here, we report two fetuses with discordant pathogenic CNV depending on the nature of the sample studied.
The first fetus presented a spina bifida and termination of pregnancy (TOP) was performed at 21 weeks of gestation (WG). aCGH, performed from DNA extracted from fibroblast culture after 3 passages highlighted three pathogenic CNV: 2q12.3 duplication, 22q11.2 deletion and a 14q12-q21.3 mosaic deletion (50%). Interstitial 14q deletion wasn't confirmed by FISH in the subculture. Given this discrepancy, a second aCGH was performed from DNA extracted from frozen liver of the fetus showing only the 2q duplication and 22q deletion.
For the second polymalformed fetus a TOP was performed at 25 WG. aCGH was performed on DNA extracted from amniocytes culture (9 passages), highlighted three CNV : 7p22.3-p22.1 duplication, 2q23.3q31.1 and 16q24.3 deletions. FISH studies on frozen thymus only confirm the interstitial 2q deletion, while the three anomalies were confirmed on amniocytes subculture. A second aCGH performed on the DNA extracted from the thymus only confirmed the 2q deletion.
These observations emphasize the possibility of occurrence of unbalanced chromosomal rearrangements in cultured cells possibly favoured by hyper stimulation and chromosome abnormality. These observations emphasize the importance to check identified CNV on fetal tissue. Also, we proposed to perform aCGH from DNA extracted from fetal tissue.

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P12.04
Comparative study between single strand sequencing and double strand sequencing in breast cancer predisposition.

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Currently, next generation sequencers are of the top of sequencing technologies. But Sanger sequencing is still keeping as reference method for genetic testing in clinical. However, there's no consensus on methodology as many publications validated this method without evaluating the analytical sensitivity between sequencing with double strand as matrix versus sequencing single strand.
The aim of this study was to compare sensitivity between sequencing single strand (Sss) and sequencing double strand (Sds) for evocative patients of breast cancer and ovary predisposition.
We took runs of 50 patients for whom *BRCA1/BRCA2* genes were tested using 3130/3500 (ABi) sequencers and Sds for diagnosis to have routine variation detection rate. Analysis was done by Seqscape V.2.7 (ABi). For our study reading was done by one biologist who analyzed once only one strand (forward or reverse) and several months later two strands together.
656 variations were identified for our 50 patients:
-34 were missed in Sss (27) even in Sds (7): respectively, detection rate was about 95% for Sss and 99% for Sds but after verification of differences between the 2 methods in Seqscape we realized that errors were only dues to operator reading and not technologies. Lacks appeared when quality sequences wasn't good enough.
-3 were identified in Sss whereas they were only artifacts not confirmed by resequencing.
Our results indicate that Sss and Sds sensitivities were similar with required conditions of quality. Improvement of our methods by quality standards and resequencing allows us to use Sss instead of Sds for diagnosis.

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P12.05
Presence and potential of cell free DNA in forensic casework

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Cell free nucleic acids (CNAs) have been found to exist in many biological media, including blood, saliva, semen and urine, and have been subject of research in oncology and non-invasive prenatal diagnosis. The origin of CNAs remains obscure, although necrosis, apoptosis and active secretion have been suggested as potential mechanisms by which CNAs are released.
To date, several studies have been performed on the potential of extracellular mRNA profiling in forensic science to identify the biological origin of forensic stains. Less is known about the potential value of cell free DNA in forensic casework. Recent studies suggest that this cell free DNA is a contributing factor to DNA recovered from touched items and state that it is likely that a substantial proportion of cell free DNA is being discarded with the supernatant during standard extraction processes. This would imply that potentially valuable information would be discarded as well.
To investigate the presence and potential of cell free DNA in forensic casework, DNA profiles of cell pellet and concentrated supernatant from 30 artificial case like samples and from 100 real forensic samples were compared. Presence of cell free DNA was shown in all investigated sample types. Moreover, in some samples additional alleles, not detected during analysis of the cell pellet, were detected. In 16% of the samples, the cell free DNA had an added value.
The results presented here indicate that cell free DNA deserves further consideration since it has the potential to increase the DNA yield in forensic casework samples.

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P12.06
Metabochip-based copy number variants (CNVs) calling and association with body mass index (BMI) in adults: benchmarking and meta-analysis

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Many single nucleotide polymorphisms influence common disease susceptibility but, to date even cumulatively, they explain only part of the heritability. As large and rare CNVs were also found to be associated with common diseases/traits, it was suggested that part of the missing heritability could be accounted for by rare variants with intermediate penetrance. The extent of this contribution remains however largely unknown. To address this question, we are collecting cohorts genotyped on Metabochip and other Illumina platforms to identify new rare or short CNVs associated with BMI and other complex traits.
We assessed CNV detection sensitivity, specificity and optimal filtering parameters of PennCNV calls on 300 unrelated adults genotyped on both Metabochip and OmniExpress. Assuming that CNVs called in high probe-density regions of the Metabochip are genuine, we examined the concordance with results of the OmniExpress platform. We assessed how different filtering parameters, such as length, number of probes, confidence score, influence true positive and false discovery rates (TPR, FDR). We then defined thresholds that gave an optimized CNV call reliability. TPR and FDR typically decreased as the minimum length threshold increased, revealing the difficulty in detecting CNVs smaller than 20kb (TPR=0.034, FDR=0.775) on OmniExpress chip.
We performed a preliminary genome-wide CNV association meta-analysis (N=8295) based on our filtering. As a proof-of-concept we confirmed the well-known *SH2B1*-BMI CNV association and found several promising hits to be replicated in additional Metabochip-genotyped cohorts. To the best of our knowledge, this is the first effort to discover small CNVs associated with adult BMI.

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P12.07

Key elements for implementation of NGS-based diagnostics - it is the people, not the machines

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The scope of next-generation DNA sequencing (NGS) is transitioning from research to diagnostics (and beyond), but the conditions for routine clinical application have not been clearly defined. Well-defined technical standards and guidelines are obvious prerequisites, but the clinical genetics centres in the Netherlands went beyond performance criteria; they auto-assessed the performance of NGS as a diagnostic tool in their laboratories. All centres participated in a pilot project to analyse the internal organisation and process of NGS-based diagnostics, from sample intake to reporting. We sent a request to all laboratories to provide an NGS-based diagnosis for the same patients with cardiomyopathy. Instead of identifying a 'winner', we constructed a systematic overview of the key elements for transition of NGS from research to diagnostics. Despite the variety in approaches (targeted, exome; WES, whole genome; WGS) and performance criteria, the laboratories produced high-quality data and generally identified the same known clinically relevant variants. Subsequent steps – uniformly interpreting results, exchange of data, reporting the diagnosis – appeared more challenging. Although some of the bottlenecks are due to technological barriers – e.g. transfer speed for data exchange – the major limitations are inherent to the general organisation of diagnostic care. We present the practical outcomes of the pilot, along with proposals for dealing with the emerging role of research in a diagnostic context, for responsibly managing patient data, and for appropriately informing physicians and patients. We thereby provide a practical contribution to the debate about the role of NGS in future diagnostics.

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P12.08

Sequencing of sodium bisulfite converted DNA as a method to discriminate different patterns of methylation in human genome

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Many regions in human genome are differentially methylated (DMRs) on maternal or paternal copy and this helps genes to work properly. Methylation changes along these DMRs are associated with different pathologies. Beckwith-Wiedemann syndrome (BWS) is an overgrowth disorder, mainly caused by epigenetic alterations in one of the two imprinting control regions (ICR1 and ICR2) on chromosome 11p15. The most common defect in ~50% of BWS cases is loss of methylation at KvDMR (ICR2). Here, we introduce sequencing of sodium bisulfite converted DNA as an alternative technique for determining the methylation status of KvDMR in BWS patients. In order to be able to compare the results with those, obtained by MS-MLPA (methylation specific-multiplex ligation probe amplification), PCR primers were designed over the region to cover two of the MS-MLPA specific probes. Altogether, 4 patients with KvDMR demethylation showed loss of methylation in the analyzed by sequencing CpG sites. The obtained sequencing results in patients and normal controls are totally in accordance with the MS-MLPA data.

The same technique was applied to analyze methylation status in the FMR1 gene promoter, hypermethylated in Fragile X syndrome. The results showed very good discrimination between the normal unmethylated promoter in healthy males and hypermethylated promoter in Fragile X full mutated boys.

In conclusion, sequencing of bisulfite converted DNA can be applied to study other DMRs, as for example H19DMR (ICR1) locus in Beckwith-Wiedemann/Silver-Russell syndromes.

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P12.09

Evaluating laboratories' interpretation of genetic test results through a EuroGentest External Quality Assessment survey

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Interpretation of test results is a key component of a genetic laboratory report, with potential implications for the patient and the family. In order to evaluate laboratory performance in this area, a EuroGentest survey among External Quality Assessment (EQA) programmes, from four European providers (CEQA, CF Network, EMQN & ERNDIM) was undertaken, with the aim of determining the interpretation elements that were missing in reports, in regard to existing guidelines and recommendations.

EQA reports from 2011 representing 519 participations in six programmes: cystic fibrosis, Friedreich ataxia, hereditary deafness at the DFNB1 locus, postnatal microarray, prenatal diagnosis of chromosomal abnormalities on amniotic fluid and metabolic diseases, were evaluated using criteria derived from the OECD Guidelines.

Mean interpretation scores varied between cases, ranging from 1.61/2 to 1.98/2. Despite heterogeneity among schemes in the scoring systems and in assessment, the data analysis revealed the following: 1) although the answer to the clinical question was provided in most reports, the survey emphasized the need to clearly restate the indication of testing; 2) items related to follow-up testing were the most often omitted, e.g. recommendation to search for a second mutation or to test the parents. 26-36% of molecular genetics and 29% of cytogenetics reports did not mention the recurrence risk of disease and/or the possibility of prenatal diagnosis for future pregnancies.

This first and preliminary survey emphasizes the need to find effective training and communication processes, including harmonization of assessment between EQA programmes, so that laboratories can improve the interpretation content of their reports.

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P12.10

FISH probes expiration date in constitutional cytogenetic laboratories

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Background: the use of FISH probes with expired validity date is a recurrent dilemma in cytogenetic diagnostic laboratories and became more complex with accreditation process.

Some companies provide FISH probes with very short expiration date (6 months) but the procedure used for the determination of the expiration date of commercial probes is not clear. Many laboratories are impacted by this short "expiration date" as FISH probes are expensive and their use is not predictable since cytogenetic abnormalities are rare and various. When producing home-made probes the problem is similar: how to establish the expiration date after labelling, required for accreditation ISO 15189 ?

Method: We did a pilot studies in 10 cytogenetic laboratories. All Laboratories tested their oldest home-made and commercial FISH probes in order to obtain an overview of the possible longevity of probes. The commercial probes originated from various providers, with various dyes. Home-made probes had been labelled by different protocols mostly by nick translation. The hybridisation was considered satisfactory when observed in at least 20 metaphases and 50 nuclei.

Results: **Commercial probes**: 31 probes expiring between 1998 and 2012

were tested from october 2012 to 2013 and gave satisfactory signals in all cases except one.
Home-made probes: 45 probes labelled from 2002 to 2011 were tested recently, 44 showed visible satisfactory signal. The four oldest probes labelled 10 years ago gave perfect signal.
This pilot studies shows that the labelled probes have longer validity period than expected, over 10 years for some of them when tested.

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P12.11
Comparison between standard and novel PCR-based method for the detection of mutation in the FMR1 gene

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Fragile X syndrome is the most common inherited form of mental retardation. Fragile X carriers are known to be associated with premature ovarian failure in females and fragile X-associated tremor/ataxia syndrome in males. The pathogenic mutation is mostly caused by CGG repeat expansion in the FMR1 gene. Normal allels contains from 6-50 CGG repeats. Premutated alleles have expansions between 55 and 200 repeats, but CGG repeats over 200 are indentified as full mutations.
Rutine testing for FMR1 mutation rely on conventional PCR and Southern blotting (PCR/SB), however, analysis is costly and time-consuming, requires large amounts of DNA sample and may have poor sensitivity to detection of lower abundance mosaic alleles.
Aim of this study was to compare the novel FMR1 PCR-based method to the previously used PCR/SB method.
DNA samples were extracted from different specimen sources: whole blood, culture of amniotic fluid and chorionic villi. Additionaly, bucal swab, uncultured chorionic villi and amniotic fluid samples were tested using FMR1 PCR-based method. Cohort of 50 samples contained 12 full mutations, 1 premutation/full mutation, 23 premutations, and 14 normal samples (PCR/SB results).
Novel FMR1 PCR-based metod identified normal alleles within 0-3 repeats of previously reported allele sizes. Results have shown a mosaic premutation pattern in 8/23 samples previously recognized as non-mosaic premutation. In one sample previously reported as premutation, mosaic premutation/full mutation pattern was identified.
We concluded that the novel FMR1 PCR-based method is more sensitive, compatible with uncultured chorionic villi and amniotic fluid as well significantly reducing testing time.

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P12.12
A novel assay for automated electrophoretic analysis of genomic DNA

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The success of any study in the field of genomics depends primarily on the quality of starting material available, like genomic DNA. The integrity and quantity of genomic DNA clearly affects experimental workflows such as aCGH microarrays or next generation sequencing library construction. With these experiments being expensive in time, effort and reagent consumption, an initial quality control (QC) check of genomic DNA is recommended.
The novel Agilent genomic DNA ScreenTape has been developed specifically for the electrophoretic separation, sizing and quantification of genomic DNA samples. This ready-to-use device, which runs on the Agilent 2200 TapeStation instrument, provides a reproducible QC method for analyzing the integrity and quantity of genomic DNA combined with the convenience of an automated system. The genomic DNA ScreenTape requires only one microliter of sample, minimizes sample preparation efforts, and suits variable throughput needs with individually contained separation channels. The TapeStation system provides automated, reproducible sample loading and returns digital electrophoretic data in less than 2 minutes per sample. Results are presented as gel image, data tables and in an electropherogram view. The ability to overlap and compare electropherograms enables a clear discrimination of sample quality across different degradation states, sample types and concentrations.
This study presents data on key performance characteristics of the novel genomic DNA ScreenTape, like molecular weight size range, accuracy and reproducibility of sizing and quantification results, as well as detection sensitivity. In addition the genomic DNA ScreenTape was used to characterize genomic DNA prepared with different extraction protocols.

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P12.13
Recurrent chromosomal abnormalities in human pluripotent stem cells and their derivatives

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Due to their original properties, human pluripotent stem cells (hPSC) and their progenies are highly valuable not only for regenerative medicine but also as tools to study development and pathologies or as cellular substrates to screen and test new drugs. However, their unlimited self-renewal property bears the risk of tumor formation, which could potentially be exacerbated by the presence of genomic abnormalities. Quality control of hPSC is, therefore, essential for the reproducibility of research experiments and for the safety of clinical applications based on these cells. As hPSC are often propagated for extended period of time, these necessary controls include monitoring and controlling of the integrity of the genome of these cells.
Chromosomal abnormalities such as gain of chromosomes 12, 17 and X occur non-randomly and quite commonly in hPSC lines. Using high-resolution analysis we identified a region of the genome that is highly unstable in hPSC, located on chromosome 20 (20q11.21). We have also identified a systematic abnormality that affects neural derivatives of hPSCs namely jumping translocation of chromosome arm 1q. All these genomic aberrations may have negative consequences on all stem cell applications.
These chromosomal defects, observed in culture, reflect tumorigenic events that occur in vivo, particularly in testicular germ cell tumors (trisomies 12 and 17), in early stage of cervical cancer (20q11.21) and in pediatric brain tumors with poor clinical outcome (duplication 1q).
These results raise the importance of understanding structural variations of chromosomes in hPSC, as they will have a significant impact on future medical applications.

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P12.14
Selection of reliable housekeeping gene for qRT-PCR analysis on head and neck squamose cell carcinomas (HNSCC)

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Quantitative real time reverse transcriptase-PCR (qRT-PCR) is used in the study of gene expression of cancer researches. Selection of reliable reference genes as an internal control has a critical importance. The objective of this study was to evaluate a set of housekeeping genes (HKGs) to be used in the normalization of gene expression in HNSCC. Malignant and non-malignant tissue samples were obtained from 12 patients with primary untreated larynx and tongue squamose cell carcinoma. Reference genes used were glyceraldehyde-3-phosphate dehydrogenase (GAPDH), actin beta (ACTB), tubulin alpha 1 (TUBA1), 18S rRNA (18S), and 28S rRNA (28S). Widely used softwares, geNorm and NormFinder, were utilized in the evaluation of the data. Results from geNorm showed that average expression stability values (M) of all candidate genes were smaller than 1.5 (accepted M value for geNorm) indicating that all the evaluated genes can be used as HKGs although 18S and 28S were found to be the most stable HKG (followed by ACTB, TUBA1, and GAPDH). Based on NormFinder, 28S was the most stable and GAPDH and 28S were considered to be the best combination in the studies evaluating the specific gene expression in tissues obtained from various HNSCC.

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P12.15

Comparison of different DNA binding fluorescent dyes for applications of high-resolution melting analysis

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High-resolution melting (HRM) analysis of PCR amplicons is a flexible tool adaptable for a wide range of applications, such as mutation screening, genotyping and methylation studies. As several commercial HRM chemistries have been released, together with chemistries used for other applications, we have decided to test twelve different green fluorescent DNA binding dyes for their suitability to use in HRM based mutation screening, genotyping of single nucleotide changes (through both unlabelled probes and small amplicons) and genotyping of a large in/del polymorphism. We also compared some basic properties of the dyes, such as fluorescence intensity enhancement in the presence of dsDNA, general applicability in melting curve analysis, effect on dsDNA melting temperature (Tm) and PCR inhibitory effect. The studied dyes were LCGreen Plus (LC), EvaGreen (EG), ResoLight (RL), SYBR Green I (SG) and eight different green fluorescent dyes from the SYTO family (S11, S12, S13, S14, S16, S21, S24 and S25). In general, the dyes designed for HRM applications (LC, EG and RL) performed very well in our study, as it was expected. SG, S12, S21, S24 and S25 had less suitable properties for HRM applications. S11, S13, S14 and S16 allowed us to obtain results of similar quality (especially S13 and S16) as the HRM dyes, with some properties even exceeding them. Our results suggest, that some, but not all of the SYTO family members, may represent suitable tools for a variety of different HRM applications.

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P12.16

Indian Hedgehog (IHH) and LRBA: New genes for Hirschsprung disease

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Hirschsprung is characterized by the absence of enteric ganglia in a variable length of intestinal tract. A large, multi-generational Dutch family with five affected family members with HSCR, revealed linkage to 4q31.3-q32.3. As the family shows an autosomal dominant mode of inheritance with incomplete penetrance, we assume that the mutation in the linkage region is necessary but not sufficient to cause disease development. To identify the mutation in the linkage region, but also mutations elsewhere in the genome, we exome sequenced two patients. One variant rs140666848 (T > C) in exon 20 of the *LRBA* gene was found in the linkage region. In addition, missense mutations in *RET* (P398L) and *IHH* (Q50K) were identified in each patient, respectively. Functional analysis of the RET and IHH variants showed that both mutations give rise to a non-functional protein. The *LRBA* variant is located downstream of *MAB21L2*, a gene which plays a role in the proliferation of enteric neural crest cells during enteric nervous system (ENS) development in Zebrafish. We hypothesize that this variant might regulate *MAB21L2* expression in ENS. Luciferase assays showed that the region containing the variant does show enhancer activity. Currently we are testing whether the variant, when compared to wild type sequence, show significant differences on gene expression level, using luciferase assays. Our data shows that combination of mutations, as expected, cause autosomal dominant disease with reduces penetrance. Furthermore, we show for the first time that *IHH* mutations can contribute to Hirschsprung disease.

Y. Sribudiani: None. **R.K. Chauhan:** None. **M.M. Alves:** None. **C. Kockx:** None. **T. van Essen:** None. **R. Brouwer:** None. **M. van den Hout:** None. **W. van Ijcken:** None. **I.T. Shepherd3:** None. **A. Brooks:** None. **R.M.W. Hofstra:** None.

P12.17

Production of CE-marked reference materials and WHO international standards for *in vitro* genetic diagnoses: quantitative controls for *JAK2* V617F and beyond

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NIBSC produces reference materials for human nucleic acid-based diagnostics where there is strong evidence of need, increasingly in the quantitative monitoring of cancer biomarkers. These control materials are produced as either CE-marked reference materials or World Health Organization international standards, suitable for *in vitro* diagnostic use in Europe or worldwide, respectively. In 2009, the 1st WHO International Genetic Reference Panel for *BCR-ABL* Translocation Quantitation for the monitoring of chronic myeloid leukaemia established NIBSC's role in improving the quality of cancer molecular monitoring.
In 2013, production began on the *JAK2* V617F Tumour Genotyping Reference Panel. The *JAK2* V617F mutation (c.1849G>T) is commonly present in chronic myeloproliferative disorder patients, making the JAK2 protein an obvious candidate for drug-targeting. It is therefore important to identify V617F-positive patients and to monitor treatment response by mutant clone quantitation. The panel of sensitivity controls will comprise a dilution series of *JAK2* V617F genomic DNA (UKE-1 cell line-derived) in a wildtype genomic DNA background (MRC-5 cell line-derived) at 10, 5, 1, 0.1 and 0%. The samples will be stored at ambient temperature in the highly stable matrix of GenTegra tubes. Dilutions will be confirmed by deep sequencing and material performance independently verified with commonly used techniques. The panel is intended as a CE-marked *in vitro* diagnostic device for *JAK2* V617F assay validation, including specificity and sensitivity determination. Tumour genotyping standards are also proposed for *BRAF*, *KRAS* and *PIK3CA*. NIBSC seeks input from clinicians and scientists worldwide to ensure the developing needs of cancer genetic diagnostics are met.

J. Boyle: None. **C. Lockie:** None. **M. Hawkins:** None. **E. Gray:** None. **P. Metcalfe:** None. **J. Hawkins:** None.

P12.18

Implementation of a Diagnostic Pipeline for Marfan und Long QT Syndrome with Next Generation Sequencing (NGS) using Illumina TruSeq Custom Amplicon Panels

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Amplicon panels for Marfan syndrome (MFS, FBN1) and LongQT syndrome (LQTS, KCNE1, KCNH2, SCN5A, KCNQ1 and KCNE2) were designed using Illumina's TSCA design studio. To evaluate a diagnostic pipeline based on NGS, we analyzed a total of 53 patients whose Sanger sequencing data were already available. Using a single Illumina MiSeq run we sequenced 25 samples for MFS and 28 samples for LQTS. Bioinformatic data analysis was carried out using CLC Genomics Workbench 6.0. A first variant call was performed by using the following parameters in the CLC software: A probability of 70% that the variant is true and a coverage cut-off of 20 reads. Broken pairs and non-specific matches were ignored.
Results: All true positive variants were observed with an average per base quality of more than 20. Using these criteria, all point mutations from Sanger sequencing could also be detected in the MiSeq data. 8 of 11 deletions (3 exons were not covered in all samples), all indels and 3 of 5 insertions were detected.
Based on these results the filters were refined for causal mutation detection: Variants which were found in more than 20% of the samples were filtered out. Variants which showed an average quality lower than 20 and a frequency lower than 5% were sorted out. Variants were annotated with special created tracks for each subpanel (gene names, exon numbers, and amino acid changes), HGMD biobase annotation and dbSNP.

I. Vogl: None. **S. Eck:** None. **S. Eck:** None. **C. Heberle:** None. **S. Küçük:** None. **C. Marschall:** None. **K. Mayer:** None. **H. Klein:** None.

P12.19

Microarray testing for urgent paediatric referrals: audit of six years clinical experience

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Rapid reporting of urgent results for babies up to three months of age forms a clinically important part of our service. Rapid FISH (for 13, 18 and 21 aneuploidy testing/confirmation of chromosomal sex) and karyotyping +/- targeted FISH is now complemented by microrray. In April 2010, chromosomal microarray (Roche-Nimbelegen, 12x135K) was introduced as a first line test for all appropriate referral categories at Great Ormond St Hospital for Children an in January 2011 for all users.We have audited the last six calendar years for this patient referral group (babies =< 3 months) (n=3163) and the results are tabulated below by test type selected.Target turnaround times/success rates/mean days to report/percentage reported within turnaround time for the above test categories are: **Rapid Aneuploidy (13,18 and 21)/Sexing**: 1 day; 100%; 1.1 days; 100%; **Urgent Karyotyping +/- FISH**: 7

days; 94.8%; 5.7 days; 94.9% and **Urgent Microarray**: 7 days; 100%; 7.6 days; 86.1%. (2011-12 financial year audit data).
Rates of clearly significant abnormalities (details will be presented) were comparable across the audited period, probably reflecting the fact that babies with overt dysmorphic features are more likely to be tested at this time. Ordering and selection of the appropriate test continues to involve education of clinicians, sample availability and clinical judgement on the part of laboratory staff.

Sample numbers by test category

	2007	2008	2009	2010	2011	2012
Rapid anaeuploidy/ sexing (interphase FISH)	149	153	148	116	111	131
Urgent Karyotyping +/- FISH	383	349	351	315	240	127
Urgent microarray	0	6	11	48	236	289

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P12.20
Simplifying diagnostic mitochondrial genome analysis with HaploGrep and Mitomaster online algorithms

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Mitochondrial genome analysis is complicated by the abundance of polymorphisms as every individual carries, on average, 30 sequence variants when compared to the revised Cambridge Reference Sequence (rCRS). Additionally, haplogroup-specific variant frequencies are lacking, thus making the assignment of variant pathogenicity problematic. To simplify our lab analysis time we chose to implement two online algorithms: HaploGrep, and Mitomaster, following sequence analysis with both Seqscape™ (Life Technologies) and Seqpilot™ (JSI Medical Systems). Using our new process, those variants that do not fall into the patient's predicted haplogroup, are further examined by Mitomaster to obtain frequencies, species conservation and general variant information relevant to assigning pathogenicity. These algorithms were validated using 40 full mitochondrial genomes of various haplotypes and permitted successful identification of likely pathogenic variants. Therefore, this new mitochondrial variant analysis workflow has reduced our overall analysis time and improves specificity by permitting the categorization those variants that are more likely to be pathogenic.

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P12.21
The MLPA-dHPLC procedure for NF1 gene analysis.

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The identification of mutations in the NF1 gene causing neurofibromatosis type 1 (NF1) is still presenting a considerable amount of work mainly because of the large size of the gene (350 kb - 60 exons) and the restricted number of recurent mutations. The high frequency of NF1 which is affecting 1 in 3500 individuals lead us to choose two complementary methods to perform NF1 gene analysis : the multiplex ligation-dependant probe amplification (MLPA) first and then the automated denaturing high performance liquid (dHPLC) screening method.
The sensitivity was evaluated in a MLPA-dHPLC analysis of a panel of unrelated french NF1 patients with at least two consensus diagnostic criterias (global mutation detection rate to 96%).
Our results and the high detection rate confirm that the association of the MLPA and dHPLC techniques provides an accurate, fast and automatizable method for the identification of NF1 mutations.

S. Pinson: None. **P. Combemale:** None. **S. Giraud:** None. **A. Calender:** None.

P12.22
Massive targeted re-sequencing for the diagnosis of neurological diseases: a 200-gene panel

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Today, neurological diseases have become a major health concern worldwide, with more than 600 pathologies affecting the central- and peripheral-nervous system. The incidence of these pathologies among the population is not negligible and it is estimated that diseases such as Parkinson affects ~ 0.3% of the E.E.U.U. population. The Diagnosis of neurological diseases is not straightforward, mainly due to the presence of nonspecific overlapping clinical symptoms, and genetic testing becomes essential to confirm clinical diagnosis. Nevertheless, conventional techniques have been insufficient to provide a comprehensive molecular diagnosis, given the high number of genes implicated.
We designed a NGS targeted re-sequencing panel for 200 genes associated with neurological diseases, including: i) syndromic and non-syndromic ataxias (>80 genes) ii) Charcot-Marie-Tooth disease (34g) iii) syndromic and non-syndromic myasthenia (40g) iv) syndromic and non-syndromic paraplegia (28g). The panel includes a total of 1.3 Mb comprising coding exons, splice sites and 5' and 3' untranslated regions (UTR) of these 200 genes. To test this panel, all these regions were fully sequenced in 7 control patients with known mutations and in two HapMap cell lines (NA12144 and NA12813). Enrichment of the exonic regions and sequencing were carried out using *SureSelect Enrichment System* (Agilent) and *SOLiD 5500* (Life Technologies), respectively.
Here, we present the results obtained during the validation of our panel, showing its high level of efficiency. Our targeted re-sequencing system offers massive analysis of 200 genes involved in Neurological diseases, making the comprehensive molecular diagnosis of these disorders feasible.

A. Romera López: None. **V. Volpini:** None. **A. Matilla:** None. **C. Collado:** None. **R. Rodríguez-De Pablos:** None. **V. Fernández-Pedrosa:** None. **D. Cantalapiedra:** None. **L. Pérez-Cabornero:** None. **J.M. Rosa-Rosa:** None. **J.C. Triviño:** None. **S. Zúñiga-Trejos:** None. **R. Miñambres:** None. **M. Gil:** None. **S. Santillán:** None.

P12.23
Personalised clopidogrel therapy by teststrip-based CYP2C19 genotyping

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Introduction: Clopidogrel (Plavix™) is widely prescribed to reduce recurrent ischaemic complications in patients with acute coronary syndromes and/or post-percutaneous interventions. High interindividual variations in and non-responsiveness to clopidogrel-induced platelet inhibition often complicate the treatment. Genetic polymorphisms in the CYP2C19 gene, which encodes the principle enzyme responsible for the bioactivation of this prodrug, lead to reduced enzyme activity and reduced levels of clopidogrel's active metabolite. Patients carrying defective CYP2C19 variants have a higher risk for major adverse cardiovascular events than noncarriers, whereas individuals with increased enzyme activity are more at risk for developing bleeding.
Methods: A genetic test (StripAssay) was developed for the detection of the CYP2C19 loss-of-function alleles *2, *3, *4, *5, *6, *7 and *8, as well as for the gain-of function allele *17. The StripAssay is based on multiplex PCR, followed by reverse-hybridisation of biotin-labeled amplification products to a parallel array of allele-specific oligonucleotides immobilised on membrane teststrips.
Results: Genotyping for functionally enhanced (*17) or defective (*2 - *8) CYP2C19 variants allow the classification of patients into ultrarapid, extensive and poor metabolisers for clopidogrel. Favourable properties, such as the rapid DNA extraction protocol, ready-to-use reagents and teststrips, as well as the potential for automation of the hybridisation/detection step, make the StripAssay convenient and easy to perform within less than six hours.
Conclusion: A simple and reliable diagnostic tool was developed for predicting the response of patients to clopidogrel treatment. The CYP2C19 StripAssay will assist clinicians to achieve a more individualised antiplatelet therapy.

H. Puehringer: None. **B. Rauscher:** None. **C. Oberkanins:** None.

P12.24
Evaluation of GWAS chip-genotyping of fetal DNA extracted from umbilical cord tissue and WGA-DNA extracted from filter paper bloodspots

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The InterPregGen consortium is searching for maternal and fetal pre-

eclampsia susceptibility genes by genome-wide association screening (GWAS). We examined suitability for chip-genotyping of fetal DNA extracted from umbilical cord tissue and filter paper (Guthrie) blood spots.

Methods:

Cord-DNA was extracted from 50-100mg of umbilical cord. DNA extracted from Guthrie spots was whole genome amplified (WGA) using Illustra GenomePhi-V2.

DNA quality was assessed by agarose electrophoresis and Sequenom genotyping. 36 cord-DNA samples and 18 WGA-DNA samples were genotyped using Illumina HumanOmniExpress BeadChips. Genotyping quality was assessed by subject call rate (CR) and heterozygosity (HET) and by comparing fetal GWAS P-value distributions with distributions for case-control association (ASSOC) and Hardy-Weinberg Equilibrium (HWE) in 2000 pre-eclamptic mothers and 5000 controls already rigorously QCed.

Results:

ASSOC and HWE distributions for cord- and WGA-DNAs with CR>95% were similar to corresponding maternal-case distributions implying absence of frequent, widespread errors in genotypes of cord or WGA subjects. However, CR<95% occurred in 22% of WGA- versus 11% of cord-DNAs and lower CRs were associated with lower HET which was significantly lower in WGA-DNA (0.277; SD0.010) than cord-DNA (0.289; SD0.007) (P<0.0001). Lower CR was also associated with higher genotyping error (lower genotype concordance) in 6 DNA replicates genotyped twice. Cord-DNAs with CR <0.95 had passed initial QC checks; only 1/18 degraded samples had CR <0.99.

Conclusions:

Cord- and WGA-DNA are suitable for GWAS when CR>95%. However, WGA-DNA yields more samples with CR<95%, lower HETs and likely higher genotyping error. Checks of cord-DNA quality are poor predictors of chip-genotyping success.

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P12.25

Higher quality standards should be aimed for: feedback from EQA for *KRAS* mutation testing in colorectal cancer

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KRAS mutational status has become an essential biomarker in personalized medicine. Since 2009 the ESP *KRAS* EQA Scheme evaluates the reliability and accurateness of *KRAS* testing on a yearly basis.

In the ESP *KRAS* EQA Scheme 2012, 105 laboratories from 26 different countries participated. Participants were asked to test samples from eight invasive colorectal carcinomas and two external quality controls, using routine testing procedures and submitting results within 14 days after sample receipt. Performance was evaluated on both genotype results and written reports.

Only seventy-five out of 105 participants made no genotyping errors (71%). Nineteen laboratories genotyped one out of 10 samples wrong (18%), 11 laboratories made two or more errors (10%). Both false positives and negatives occurred, as well as incorrectly genotyped mutations and technical failures. One sample, mimicking a tumor cell content of 10%, resulted in a high number of false negative results, which uncovers weakness in test performance for samples with less optimal characteristics. The errors and genotyping scores will be studied over the years. Written reports were evaluated for the presence and correctness of 29 elements, of which nine were marked to determine a reporting score. Results indicate that several essential elements are frequently missing, including clinical interpretation, method sensitivity, and use of a reference sequence. Correct nomenclature according to HGVS guidelines is not sufficiently integrated.

The ESP *KRAS* EQA Scheme is an important tool in assessing quality in *KRAS* testing. Results indicate that performance can be improved, both in mutation analysis and result reporting.

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P12.26

Using Lean Six Sigma tools to improve the clinical genetic laboratory

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Clinical genetic laboratories are under increasing pressure to provide rapid results on increasingly complex tests, while maintaining low test costs and high levels of quality. In 2012 the Division of Molecular Genetics at the Hospital for Sick Children in Toronto, Canada began a multi-part project to apply Lean Six Sigma methodology to improve efficiency of the hospital's divisional clinical Cytogenetic and Molecular Genetic laboratory operations. Lean Six Sigma methods are continuous improvement tools based on the scientific method to solve chronic process and quality problems. Molecular Genetic and Cytogenetic laboratory operations were analyzed to define highest needs for initial Lean Six Sigma projects, which included 1) Lean facility design for merger of separate Molecular and Cytogenetic laboratory services into a combined Genome Diagnostic laboratory, 2) Process improvement methods to increase throughput of genomic sequence variant reporting and 3) Definition of metrics to manage laboratory workflow and turnaround time. Methods used include Value Stream Mapping to define current and future states, defining wastes and use of 5S to standardize work areas, defining key performance measures for current state and impact of changes, and use of DMAIC (define, measure, analyze, improve, control) cycles as a data drive strategy to solving operational problems. To date the project has achieved improved turnaround time for variant reporting (Molecular Genetics, 20% improvement), and better utilization of space and personnel matched to sample flow in the new Genomic Laboratory.

T.L. Stockley: None.

P12.27

Ten year experience with the use of real-time PCR in the detection of ΔF508

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Objective: Cystic fibrosis is the most common autosomal recessive genetic disorder in the Caucasian population. About 1400 different genetic mutation, deletion, etc. were detected until now in the cystic fibrosis transmembrane regulator gene (CFTR). This makes the molecular diagnosis difficult, while about 60 % of the cases is caused by ΔF508 in Hungary. We started to use the real-time PCR in the clinical genetic practice 10 years ago.

Materials and Methods: We isolated the DNA from 188 samples (High Pure PCR Template Preparation kit; Roche, Germany) from different tissues (97 blood, 14 amnioticfliud cells and 77 CVS). Quantitative real-time PCR with melting curve analysis was performed to detect ΔF508 by using hybridization probe system (TIB MolBiol, Germany).

Results: We detected 112 normal healthy, 65 heterozygous and 9 ΔF508 homozygote samples. We observed recognizable difference in the melting point in the normal (56ºC) and ΔF508 (49ºC) PCR products.

Conclusion: Based on our ten years experience it seems that real-time PCR and melting curve analysis is a reliable and sensitve method for the detection of ΔF508. The source of the sample does not have effect on the results. The applied primer and probe set design gives additional information for the presence of other mutations in the ΔF508 region.

B. Nagy: None. **R.G. Nagy:** None. **J. Rigo Jr.:** None. **L. Lazar:** None.

P12.28

Flexible Throughput RNA Sample QC using the Agilent 2200 TapeStation system

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The Agilent TapeStation system provides a flexible solution for automated analysis of up to 96 samples using pre-packaged reagents and minimal manual handling. Here we present a new assay - the RNA ScreenTape assay - to enable robust quantification and quality analysis of Total RNA samples from both eukaryotic and prokaryotic sources from 100 pg/µl to 500 ng/µl. The new assay additionally benefits from the ability to provide separation of contaminant genomic DNA allowing more accurate purity assessment of sample material.

This study compares the performance of the RNA ScreenTape and High Sensitivity RNA ScreenTape assays against the "gold standard" Agilent 2100 Bioanalyzer and NanoDrop for RNA quality and quantity determination. We conclude that the new RNA ScreenTape and High Sensitivity assays provide

correlated data to these existing technologies, as well as exceeding these technologies in terms of flexibility or quality of data generated.

M.T. Connelly: A. Employment (full or part-time); Significant; Agilent Technologies. **A. Inche:** A. Employment (full or part-time); Significant; Agilent Technologies. **D. Boland:** A. Employment (full or part-time); Significant; Agilent Technologies. **A. Padmanaban:** A. Employment (full or part-time); Significant; Agilent Technologies. **E. Graf:** A. Employment (full or part-time); Significant; Agilent Technologies. **C. Tissott:** A. Employment (full or part-time); Significant; Agilent Technologies.

P12.29
Transcriptome Analysis on the Ion Proton™ System

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RNA-Seq technology has become widely utilized as a tool to understand the transcriptome of a given experimental system. This method utilizes next generation sequencing platforms to sequence a cDNA library in order to gain information about the RNA content and transcriptional status of a sample of interest. Profiling the transcriptome of a system in this way has become an invaluable tool in many genomic studies.
The newly launched Proton™ system utilizes the same simplified chemistry first introduced with the Personal Genome Machine (PGM™). The increased sequencing depth of the Proton™ instrument and the Ion PI™ chip now lends this simplified sequencing technology to true whole transcriptome evaluation including sequencing analysis of polyadenylated RNAs, long non-coding RNAs, and non-adenylated transcripts.
Here we report transcriptome sequencing of ribosomal RNA (rRNA) depleted control RNAs analyzed on the Proton™ instrument with the Ion PI™ chip. The transcriptome profiles of two well-studied RNAs utilized in the historic Microarray Quality Consortium (MAQC) were analyzed by Proton™ sequencing. External RNA Control Consortium transcripts (ERCCs) were spiked into the control RNAs to provide a known set of RNA sequences useful in monitoring sample preparation, sequencing and data analysis. Differential expression profiles between two control RNAs generated from transcriptome sequencing on the Proton™ compare well to profiles generated during the MAQC study. This study demonstrating over 50M mapped reads from each Proton™ transcriptome sequencing run and good sensititivity and dynamic range, solidifies the Proton™ sequencing system as a viable platform for complex transcriptome analysis.

K. Bramlett: A. Employment (full or part-time); Significant; Life Technologies. **Y. Sun:** A. Employment (full or part-time); Significant; Life Technoloies. **J. Schageman:** A. Employment (full or part-time); Significant; Life Technologies. **L. Qu:** A. Employment (full or part-time); Significant; Life Technologies. **C. Hinahon:** A. Employment (full or part-time); Significant; Life Technologies. **B. Setterquist:** A. Employment (full or part-time); Significant; Life Technologies.

P12.30
Reproducible mRNA and small RNA sequencing across different laboratories

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RNA-sequencing is an increasingly popular technology for genome-wide analysis of transcript structure and abundance. However, the sources of technical and inter-laboratory variation have not been assessed in a systematic manner. To address this, seven centers of the GEUVADIS consortium sequenced mRNAs and small RNAs of 465 HapMap lymphoblastoid cell lines (incl. large numbers of replicates). The variation between laboratories appeared to be considerably smaller than the already limited biological variation. Laboratory differences mainly manifested in differences in insert size and GC content. The randomized study design allowed nearly full correction of these laboratory effects. In small RNA sequencing, the miRNA content differed widely between samples due to competitive sequencing of rRNA fragments. This did not affect relative quantification of the miRNAs. We conclude distributed RNA-sequencing is well feasible when proper standardization and randomization procedures are used. The combined sequencing data from this project significantly extended our understanding of the genetic basis of transcriptome variation and generated an unprecedented resource of novel transcripts and eQTLs.

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P12.31
High-throughput sample identification and tracking for exome and custom targeted sequencing projects

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New sequencing technologies combined with capture-enrichments methods enable large-scale re-sequencing of whole exomes or targeted gene panels. To ensure quality and traceability in these sequencing efforts we developed a custom SNP panel. It includes 32 highly informative SNPs across European, Asian, and African populations. Most SNPs have a minimum allele frequency of at least 0.3, and are distributed across the genome with one or two SNPs per chromosome. In the latter case we selected one SNP on the P arm and one on the Q arm. The SNPs were selected from regions that are well covered by the different exome capture chemistries and can be reliably genotyped from exome sequencing data. The Sequenom® MassArray® iPlex® Gold - SNP genotyping platform was used for genotyping around 200 samples that have been exome sequenced. Our custom panel allowed the identification of samples swaps and the retrieval of the right samples. Parents-siblings could be easily discriminated, sharing only 50% of genotypes. Our panel provides a highly accurate and robust method for sample tracking and identification. By including the SNP loci in new designs of gene panels, this iPLEX assay can be used for all targeted re-sequencing samples.

E. Souche: None. **J.K.J. Van Houdt:** None. **K. Herten:** None. **S. Jackmaert:** None. **G. Matthijs:** None. **J.R. Vermeesch:** None.

P12.32
About sequence quality: impact on clinical applications

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Advance of sequencing technologies is accelerating with a surprisingly fast rhythm, and several new sequencing platforms aimed at small laboratories and the clinical diagnostic market are released. Some of laboratories are already started to test these machines in clinical situations.
Nevertheless, it doesn't necessary mean the end of Sanger sequencing: comparative studies show that performance of next-generation sequencing (NGS) platforms varies considerably and they produce different types of errors. Most annoyingly these machines are based on very dissimilar technologies (a semiconductor technology for the Ion Torrent PGM, a sequencing-by-synthesis technology for Illumina MiSeq) and it makes practically impossible to conceive a standardized clinical platform.
Therefore Sanger sequencing remains the sequencing "gold-standard" for the accreditation of clinical laboratories according to the major international standard ISO 15189.
However, even in Sanger sequencing the sequence quality definition remains fuzzy and too empirical for clinical applications. Available guidelines describing all important parameters to insure the sequence quality didn't give clear advices on how to use them.
We realized a heuristic analysis of more than 31 000 sequence traces generated in the clinical diagnosis and demonstrated that the sequence traces qualities are inherently variable in routine practices and the most commonly used criterion "average quality value" alone is too inaccurate and not sufficient for clinical uses.
We demonstrated that with a combination of three parameters (average quality value, relative sequence intensity and electropherogram profile) and applying our decision making diagram, it is possible to determine accurately the quality of any sequence.

T. Noguchi: None. **V. Bourdon:** None. **H. Sobol:** None.

P12.33

Rapid genotyping for the prevalent mutation of XPC gene: a useful tool for genetic counseling

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Xeroderma Pigmentosum (XP) has a higher prevalence in countries where consanguinity is frequent such as North Africa, where the disease represents a major health concern. Among the 8 defective NER subtypes XP-A to XP-G, type C is the most frequent, with a common frame shift mutation of XPC gene present in more than 90% of the patients. We set up a simple assay based on fragment size analysis which permitted accurate determination of the 3 possible genotypes on an automatic sequencer. Compared to classical sequencing, the method allows substantial lower cost, higher throughput, improved resolution and time saving. We tested 50 DNA samples from 20 unrelated families from Algeria and quickly confirmed the presence of the common frame shift at the homozygous or heterozygous state in affected children and obligate carriers, respectively: 17 unrelated patients (85 %) were accurately diagnosed as XP type C; 3 patients did not harbor the common mutation of XPC gene, 2 had the common nonsense mutation described in XPA gene, and the last one was investigated for XPV gene. Sequencing needs were drastically reduced by using the new assay as a screening test. This work describes a genetic test readily available for screening and diagnostic purposes in XP which may greatly facilitate genetic counseling in exposed families from low-income countries, and help promoting early management of this severely cancer prone and devastating photosensitive disease.

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P13.01

Diagnostic Whole Exome Sequencing in patients with clinically suspected, genetically heterogeneous syndromes

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Identification of disease causing mutations in genetically heterogeneous conditions by Sanger sequencing is time-consuming, costly and often unsuccessful. Targeted next generation sequencing (NGS) techniques of limited disease specific gene panels enable very deep sequencing likely ensuring low false negative results for the genes investigated, but is not useful if the clinically suspicion was not correct or the affected gene was not in the panel. We therefore investigated the diagnostic power of whole exome sequencing as a mutational screening tool in patients with clinically suspected, genetically heterogeneous syndromes. In order to establish an optimized work-flow for diagnostic whole exome sequencing using the SOLiD 5500XL platform we used DNA of 7 patients with a variety of known disease causing mutations. In the initial approach with pooling of 8 samples on one flowchip 3 mutations out of 5 were not detected due to low coverage. We therefore changed the procedure to pool only 4 samples resulting in an average 100x coverage and optimized the analysis setting by permutation of the various alignment and filter parameters. We then used an optimized protocol with 16% aberrant allele calling in at least 3 reads and 6x coverage to analyze five patients with suspected genetically heterogeneous syndromic diagnosis and unknown mutation. Using these settings disease causing mutations were identified in all 7 subsequently tested patients for Joubert syndrome, Fraser syndrome, Noonan syndrome, primary microcephaly and others. Our results thus demonstrate that whole exome sequencing is a powerful diagnostic tool in patients with suspected genetically heterogeneous syndromes.

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P13.02

Detection of new variants in ABCB4 gene using NGS 454 Instrument GS Junior.

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ABCB4 gene encodes a canalicular phospholipid translocator MDR3 involved in biliary phosphatidylcholine excretion. Several phenotypes have been described in association with ABCB4 mutations mostly 1) Progressive Familial Intrahepatic Cholestasis 3, a severe liver disease of childhood; 2) Intrahepatic Cholestasis of Pregnancy; 3) "Low Phospholipid Associated Cholelithiasis" syndrome. So far, the genotyping of ABCB4 gene was performed by Sanger technology in all French laboratories involved in this genotyping. Due to a considerable increase of patients to test, Sanger sequencing technology is no longer adapted. Therefore, the aim of the study was to genotype at least 32 patients at the same time using Next Generation Sequencing technology and to compare the most accurate data with those of Sanger technology. Patients were diagnosed with these ABCB4 - related diseases. DNA was extracted from blood using the Puregene kit (Qiagen). Specific PCR of 27 exons and junction introns were performed. Pooling of all PCR was done for each patient and a second PCR was run using multiplexing tag primers (one by patient). After purification of the tag mixture amplification of 32 patients, a pool of the 32 template DNAs was prepared. The library was then submitted to emulsion PCR and pyrosequencing in a picotiter-plate in the 454 Instrument GS Junior (Roche). Several softwares were used to analyze the data. We ran 290 DNAs. The vast majority of sequence variations were confirmed by Sanger technology indicating that this new technology is reliable and time-efficient for the screening of ABCB4 variants on large scale.

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P13.03

Next Generation Sequencing analysis of COL4A3, COL4A4 and COL4A5 genes in 100 unrelated Alport syndrome patients: diagnostic implications and identification of 41 novel mutations.

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Alport syndrome (ATS) is a clinically heterogeneous progressive nephropathy caused by mutations in three large genes: the X-linked COL4A5 gene, thought to be responsible for the majority of cases (85%), and the autosomal COL4A3-COL4A4 genes accounting for a minority of cases (15%). We developed a protocol for the simultaneous analysis of COL4A3-COL4A4-COL4A5 genes using a benchtop Next Generation Sequencer (Artuso et al, 2012 Eur J Hum Genet). Here, this approach is employed to screen a cohort of 100 ATS patients. Overall, 57 mutations are identified in 54 patients, including 41 novel mutations. Unexpectedly, nearly 40% of cases were autosomal, mostly dominant, indicating that autosomal forms are underestimated. The method has proved particularly useful in that fraction of cases (28) that lakked a diagnostic orientation according to traditional criteria and/or formal genetics. In such cases the traditional diagnostic workflow would have led to the analysis of COL4A5 as first, based on mutation frequency prioritization. NGS directly identified a consistent number of autosomal cases (13) with a significant reduction of turnaround time. The most important finding of this study is that a fraction of families (5) that would not have been classified as X-linked, based on traditional criteria relying on the greater severity in males, eventually turned to be X-linked. This last observation shows for the first time X-linked "forme fruste" and highlights the necessity of revaluating the ATS natural history. This in turn might lead to a reassessment of the prognosis that usually is discussed with patients during genetic counseling.

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P13.04

Mutation analysis in patients with Alport syndrome using multiplex PCR and next generation sequencing

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Alport syndrome (AS) is an inherited nephropathy associated with mutations in *COL4A5* (X-linked AS) or in *COL4A3* and *COL4A4* (autosomal recessive and dominant AS). Mutation screening of these 3 large genes is time consuming and expensive. Here we carried out next generation sequencing (NGS) mutation analysis in 70 unrelated patients using a PCR-multiplexing approach allowing amplification of 149 amplicons covering all but 3 exons of the 3 genes. Amplicon-PCR-derived fragments were checked by capillary electrophoresis and then processed for sequence analysis on Ion PGM using the 170-200 bp chemistry, on an Ion 316 chips (Life Tech). Bioinformatic analysis was performed using an in-house web interface. All targeted positions but one were covered at least 15X. We identified 71 mutations (19 in *COL4A5*, 32 in *COL4A3*, 20 in *COL4A4*) thought to be pathogenic in 59 patients. All were confirmed by Sanger sequencing. Genescan analysis of multiplex PCR products allowed the discovery of 5 large rearrangements (3 deletions, 2 duplications) that would not have been detected by conventional sequencing. Sequencing the 3 genes at once was particularly helpful as in 7 cases considered to be likely X-linked (based on genealogy analysis and including one case with weak α5(IV) labelling of skin basement membrane) we found mutations in *COL4A3* or *COL4A4*, and in one case considered to be probably autosomal we identified a *COL4A5* mutation. We conclude that the combined approach of multiplex PCR amplification and NGS is highly efficient, reduces time and cost and strongly facilitates diagnostic mutation analysis in AS.

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P13.05

Challenges and solutions for preparing NGS libraries from clinical samples

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Clinical samples are difficult to prepare for NGS. Plasma, serum, urine, single cancer and fetal cells, formalin-fixed tissue, and even fresh tissue are collected as small samples of variable quantity and quality. To obtain high-quality, reproducible results, the enzymology and chemistry of NGS library preparation need to be highly efficient and robust over wide ranges of DNA and RNA size, quantity, and quality. In addition, the libraries must be prepared with minimal number of steps, sample transfers, and elapsed time.
We will show that careful design and quality control of library preparation can yield high-quality libraries by objective criteria, including a) library diversity, b) sensitivity, c) uniformity of coverage, d) balance and breadth of GC coverage, e) reproducibility over a 100X range of sample input and insensitivity to the number of PCR amplification cycles used, and f) high concordance with NGS results from microgram amounts of undamaged DNA using PCR-free methods. These criteria can be routinely achieved for non-invasive prenatal screening, PGS/PGD, and for mutation detection in formalin-fixed tissue and small numbers of circulating tumor cells.

J. Langmore: A. Employment (full or part-time); Significant; Rubicon Genomics. **E. Kamberov:** A. Employment (full or part-time); Significant; Rubicon Genomics.

P13.06

Exome Sequencing Identifies a Novel *RP1* Mutation in a Belgian Family with Autosomal Dominant Retinitis Pigmentosa

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Purpose: Identifying the genetic cause in a Belgian family with autosomal dominant retinitis pigmentosa (adRP) using genomewide linkage analysis and exome sequencing.
Methods: Five affected and eight unaffected individuals from a Belgian adRP family underwent genomewide linkage analysis using BeadChips (Illumina) and multipoint linkage analysis (Merlin). Exome sequencing was performed in two affected individuals (TruSeq Exome Enrichment Kit, HiSeq, Illumina). The CLC Genomics Workbench was employed for read mapping and variant calling. Linkage regions and RetNet genes were used for filtering.
Results: Genomewide linkage analysis revealed two regions with a maximum LOD score of 1.7. As to the exome data, 11.3/12.7 and 12.9/14.5 million reads could be mapped against the human genome. Integrated linkage- and RetNet gene-based filtering of exome data revealed a heterozygous *RP1* change in both patients: p.Leu749fs and p.Leu750fs respectively. Of note, variant calling failed to correctly call this change. Sanger sequencing confirmed following mutation: c.2245_2248delinsTGAG (p.Leu749X). This mutation co-segregates with disease, one unaffected individual excepted, suggesting incomplete penetrance. It creates a premature stop codon that may lead to a truncated protein. The mutation belongs to the 'Class II' mutations, frequently reported nonsense mediated decay-insensitive truncations, with a net dominant negative effect.
Conclusions: Combined linkage-based filtering of exome data revealed a novel *RP1* mutation in a Belgian pedigree with adRP. Our study expands the spectrum of *RP1* Class II mutations leading to adRP with incomplete penetrance. Interestingly, the findings of this study can be used as a starting point for further research on modifiers influencing *RP1* expression.

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P13.07

Breakpoint mapping in a family with balanced translocation t(1;12) and learning difficulties and cerebral infarctions drawn from the Finnish national registry of balanced rearrangements

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We have established a National Registry of Balanced Translocations and Inversions in Finland by ascertaining all known Finnish carriers (n=2575). Our registry contains medical records of all available carriers in each family including relatives carrying the same rearrangement identical-by-descent. As a pilot study, we have focused on families identified from this resource, which appear to have a phenotype correlating to the balanced translocation. In our first gene mapping pilot we identified a potential positional candidate gene for intracranial and aortic aneurysm. Here, we present a family where all carriers (n=6) of t(1;12) manifest first with learning problems and later with unexplained neurological symptoms, which seem to lead to cerebral infarctions without identified predisposing factor.
Conventional methods for breakpoint mapping are laborious and with insufficient resolution to identify the disrupted loci. The next-generation sequencing paired end approach serves this aim exceptionally well, as it allows focusing on the precise break region from the vast DNA information overload. Here, we employ genome-wide mate pair sequencing using Illumina platform to fine-map and subsequently identify the specific breakpoints in this family.
Disease-associated balanced rearrangements are a unique resource for bridging genotype to phenotype in patients with both early-onset Mendelian disorders as well as complex and late-onset diseases. Our registry facilitates diagnostic purposes, genetic counseling, and subsequent follow-up and, moreover, the identification of asymptomatic carriers in at-risk families. It also provides us momentum towards characterizing more generally the molecular basis of all translocations in Finland and their associated phenotypic effects.

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P13.08

Targeted high-throughput sequencing for mutation detection in Bardet-Biedl Italian patients

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Bardet-Biedl syndrome (BBS) is characterized by truncal obesity, polydactyly, hypogonadism, developmental delay, learning disabilities, progressive retinopathy, renal disease and susceptibility to diabetes mellitus. Although BBS is mainly transmitted in an autosomal recessive manner, few families exhibit a tri-allelic mode of inheritance. To date, 16 different BBS genes (BBS1-BBS16) are known and BBS1 and BBS10 show the highest mutation frequency in BBS patients.
Six unrelated patients evaluated by standard ophthalmologic examination and with a clinical diagnosis of BBS were analysed by targeted re-sequencing of 130 retinopathies-related genes on HiScanSQ Illumina platform (mean coverage 500X). Bioinformatic analysis identified a mean of 1100 sequence variants per sample. Filtering pipeline (exonic function, frequency, prediction and inheritance model) leads to distil a mean of 10 candidate variants per sample. The candidate variants were independently validated by Sanger sequencing and segregation analysis was performed.
We identified two known causative deletions in BBS9 (c.1877_1880del) and BBS7 (c.712_715del) genes and a novel frameshift deletion in BBS10 (c.804_805del). Morever, a patient is compound heterozygote for two novel variants in BBS10 (c.1677_1678insA and c.1220T>C); one patient present a homozygous variant in BBS2 gene (c.209A>G) and one novel heterozygous variant (c.538C>T) in BBS3 gene. The last patient has two variants in BBS12 gene both in homozygous status [c.1044del (novel) and c.61T>C (described)].
NGS approach has enabled us to detect variants in BBS genes. Although for described variants and ins/del frameshif variants the genotype-phenotype correlation is clear, in order to clarify tri-allelic variants' role in BBS-onset, further association studies are required.

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P13.09

Best practice guidelines for the use of NGS applications in genome diagnostics: A national collaborative study of Dutch genome diagnostic laboratories .

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Next-generation sequencing (NGS) methods are advancing in genome diagnostics laboratories worldwide. However, the implementation of NGS based tests according to diagnostic standards is challenging for individual diagnostic laboratories. In order to facilitate the implementation of NGS in the Dutch laboratories the Dutch Society for Clinical Genetic Laboratory Diagnostics (VKGL) initiated a workgroup. The result of this workgroup will be presented. We provide best practice guidelines and criteria for validation and implementation of NGS applications in a clinical setting. We introduce the concept of 'diagnostic yield' as the main performance characteristic to evaluate diagnostic testing for a given genetic disease. We recommend that the laboratory procedures including the tested genes are recorded in a publicly available document describing the complete 'diagnostic routing'. In addition, we propose the use of a 'core disease gene list' for specified genetic diseases. This core disease gene list encloses the significant genes for a defined genetic disorder and these should all be included in a diagnostic test in order to establish a molecular diagnosis. This will ensure a clear and standardized quality of care throughout the genetic diagnostic laboratories. The best practice guidelines and criteria that will be presented are accepted by Dutch Society for Clinical Genetic Laboratory Diagnostics.

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P13.10

Targeted next generation sequencing approach of Brugada syndrome and sudden cardiac death

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Brugada syndrome (BrS) is a heritable primary electrical disorder with typical ECG alterations accounting for up to 20% of sudden cardiac death (SCD) in young patients (<45 yrs) without apparent structural cardiac abnormalities. BrS is genetically heterogeneous and so far a dozen causative genes have been identified. A clinical diagnosis is not always straightforward as expressivity is variable and penetrance is low. Therefore molecular confirmation of the diagnosis is important for further patient management and therapy. The consecutive molecular screening of all BrS genes using conventional methods is expensive and labor-intensive. To shorten the turn-around-timeand to reduce the overall cost of molecular testing, we have developed a BrS gene panel for next generation sequencing of eight BrS genes (*CACNA1C, CACNA2D1,CACNB2,GPD1L,KCNE3,SCN1B,SCN3B* and *SCN5A).*
After optimalisation of multiplex PCR, designed by Multiplicom, sequencing was initially performed on a GS junior (Roche), and later adapted for Miseq (Illumina). To date we have screened 58 patients: 8 patients with unexplained SCD, 14 patients with atypical BrS ECGs and 36 patients with type 1 BrS ECGs. We identified 15 genetic variants, all in the latter group. Twelve variants (10 mutations and 2 variants of unknown significance) were discovered in the *SCN5A* gene, whereas one and two mutations were identified in *CACNA1C* and *CACNB2*, respectively. Our results demonstrate that a next generation panel approach can increase the genetic yield from 33% (if only SCN5A analysed) to 42% in probands with typical BrS ECGs, whereas no mutations werd found in atypical BrS or unexplained cardiac deaths.

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P13.11

Understanding genetics of Brugada Syndrome: NGS leads the charge

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Brugada syndrome (BS) is a cardiac arrhythmic disorder characterized by typical electrocardiographic pattern that can lead to sudden cardiac death at young age.
BS is inherited as an autosomal dominant trait with incomplete penetrance and variable phenotype expressivity; however, genetic bases have been only partially understood. Most mutations are located in the SCN5A gene, encoding the α-subunit of the Na+ cardiac channel, but more than 70% of BS patients remain genetically undiagnosed.
Our study aims to increase the proportion of genetically diagnosed BS patients, identifying new syndrome-related genes and causative variants by target resequencing.
We analysed 100 BS patients for a panel of 158 candidate genes, mainly encoding cardiac ion channels, structural proteins, modulators of ionic flow and regulatory proteins. We used the Agilent SureSelect target enrichment protocol and we performed the deep sequencing on Illumina GAIIx. We also developed an automated bioinformatics pipeline based on BWA aligner to map reads versus the hg19 reference with great accuracy. For each sample, at least 2 million paired-end reads was successfully mapped. More than 98% of the target sequences were covered with more than 230X mean depth by gene and the 90% of the bases are sequenced above a 50X depth.
We detected 87 novel missense mutations, 2 nonsense and 2 splice-site variants, 16 indels in 64 different genes. Almost all of these mutations are privates. In 25 candidate genes we identified also 48 clinical variants that can contribute to phenotype modulation.

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P13.12

Elucidation of genetic basis of hypoplastic left heart syndrome

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BACKGROUND: The hypoplastic left heart syndrome (HLHS), mainly characterized by an underdeveloped left ventricle, accounts for significant mortality and morbidity in infancy. Despite strong evidence of an underlying genetic aetiology, only a minority of cases can be explained on genetic bases. Our aim was to discover novel causative genes using whole-exome sequencing (WES) in combination with functional studies in zebrafish and cellular models.

METHODS AND RESULTS: We have analyzed by WES 15 unrelated parents-child trios. Our analyses lead to the identification of several *de novo* mutations in different genes involved in cardiac development and tissue remodeling in heart. Missense mutations have been detected in genes belonging to HDAC pathway, which regulates mesoderm differentiation into cardiac muscle cells. Other genes are associated to NOTCH signaling pathway, which is involved in impaired endothelial to mesenchymal transition (EndMT). Using morpholino antisense oligonucleotides in a novel candidate gene, we were able to show severely impaired chamber formation. Moreover, our newly established animal model of endocardial fibroelastosis strengthened the role of aberrant EndMT in the development of HLHS.

CONCLUSIONS: Our results confirm an extreme genetic heterogeneity in HLHS pathogenesis, as previously reported by our group. Moreover, our data suggest that different molecular defects interfering with EndMT, development of the atrioventricular valves and ventricular chamber formation are at basis of HLHS. The elucidation of genetic defects of HLHS may lead to a more appropriate classification of patients potentially useful for therapeutic management.

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P13.13

Comprehensive molecular genetic analysis for Arrhythmogenic Cardiomyopathy: conventional and Next Generation Sequencing

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Background: Arrhythmogenic cardiomyopathy (AC) is an autosomal dominant disease characterized by progressive fibrofatty replacement of the working myocardium and an increased risk of sudden death. Mutations in desmosomal encoding genes are accounting for
approximately 50% of AC patients and 10% of AC patients are multiple mutation carriers.

Aim: The aim of the present study was to develop a NGS whole-exome strategy in order to assess its potential diagnostic value in comparison with conventional screening approaches.

Methods: Twenty-eight patients identified by classical clinical screening at the Referential Clinical-Genetic Centre of Arrhythmic Cardiomyopathies underwent genetic evaluation. Nucleotide sequencing was performed both on an Applied Biosystems 3730XL DNA Analyzer and an Illumina Hiseq2100 platform. Novel variations absent in NHLBI GO Exome Sequencing Project (ESP) and dbSNP databases, were considered potentially pathogenetic.

Results: Sanger sequencing identified causative mutations in 82% of the probands, of which 11 were multiple mutation carriers. Novel putative causative nucleotide variations were identified in desmocolin-2 (S868F, T524A, H604L, N629L), plakoglobin (A458T, E654L), desmoglein-2 (N330T, N330H, R292H, I386F), desmoplakin (L2487fsX189, C2259X, E2090K, E1833V) and plakophilin-2 (H179N, K37X, N670fsX683, S70N) genes. Whole-exome sequencing confirmed 68 out of 90 variations, including causative mutations and polymorphisms, with a sensitivity of 80% and a specificity of 100%.

Conclusions: Whole-Exome Sequencing represents a fast, cost-effective and comprehensive approach for mutation screening, however further data are needed to optimize variant detection parameters and therefore to define it as a diagnostic tool for AC.

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P13.14

Adiponectin G276T gene polymorphism is associated with myocardial infarction in south Indian patients with type 2 diabetes: A hospital based study

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Adiponectin has anti-atherogenic properties and reduced serum adiponectin levels are associated with Myocardial infarction (MI). In this study, we examined the relationship between MI and *adiponectin* (*ADIPOQ*) gene G276T polymorphism that is associated with serum adiponectin levels in type 2 diabetic patients. We enrolled 425 subjects with type 2 diabetic (males, 61.1%; age, 54.9 ± 7.9 years old) to determine their genotypes regarding *ADIPOQ* G276T polymorphisms, and evaluated the association between this polymorphism and the prevalence of MI. The prevalence of MI tended to be higher as the number of G alleles increased [GG (9.5%), GT (6.8%), TT (5.6%), p value for trend = 0.0059] and was significantly higher in the subjects with GG genotype compared to those with GT or TT genotype (9.5% vs. 6.6%, p = 0.0060). Multiple logistic regression analyses revealed that the number of G alleles (Odds ratio (OR) = 1.49 with 95%) CI 1.09-2.05, p = 0.0125) and GG genotype (OR = 1.66 with 95%CI 1.13-2.43, p = 0.0098) were significantly associated with MI even after adjustment for conventional risk factors. Interestingly, further the presence of obesity significantly increased the risk of MI in the subjects with GG genotype (OR = 1.67 with 95%CI 1.14-2.44, p = 0.0090) but not in the subjects with TT or GT genotype (OR = 1.17 with 95%CI 0.73-1.89, NS). It is likely that the G allele of the *ADIPOQ* G276T polymorphism is a susceptibility allele for MI in type 2 diabetic patients, especially when they accompany with obesity.

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P13.15

Introduction of NGS in diagnostics for cardiomyopathies results in increased diagnostic yield

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Cardiomyopathies are a group of disorders characterised by extreme genetic and clinical heterogeneity, with >40 causative genes known to date. In the past 10 years, diagnostic testing of affected individuals using serial Sanger sequencing of a limited number of genes has been expensive and time-consuming. The aim of this study was to develop an NGS-based workflow for routine diagnostics to improve genetic testing for a group of cardiomyopathy patients (HCM/DCM/NCCM).

An NGS-based targeted workflow was designed by developing an enrichment array (Agilent Sure Select) for 46 genes, running12 pooled barcoded samples on the Miseq system (Illumina), followed by mapping and variant calling using the SeqNext software package (JSI). Fifteen frequently mutated genes, previously tested by Sanger sequencing, were selected to validate the process. Testing the workflow also showed that it is possible to detect deletions of up to 65 bp and duplications of up to 28 bp.

At present, samples of the first 55 patients have been tested and analysed. Fifteen patients showed a pathogenic mutation and in five of them, the causative mutation would not have been found using the conventional approach. Furthermore, in a young severely affected patient at least two pathogenic mutations were identified. Variants of unknown significance (VUS) were found in 27 patients and 13 patients did not show any pathogenic mutation or VUS.

In conclusion, targeted NGS is an effective approach for cardiomyopathy diagnostics, resulting in increased diagnostic yield. One of the major challenges remaining is to solve the increased number of VUS identified.

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P13.16

Unparalleled outcomes of using Next Generation Sequencing technology in cardiac genetics

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Inherited cardiac diseases, such as cardiomyopathy or inherited arrhythmia, are clinically important genetic disorders, as well as significant causes of sudden death. Genetic testing for these conditions remains limited due to complex genetic pathology. They are often heterogeneous disorders with overlapping clinical symptoms, which may not always be penetrant. This adds to the complexity of determining whether variants identified are pathogenic, influence clinical severity or just normal variation within the genes. In addition some of the associated genes are amongst the largest genes described, which further reduces the practicality of detailed genetic testing and the availability of published information.
We have developed a 100-gene cardiac next generation sequencing [NGS] panel to screen for the arrhythmias, cardiomyopathies, aortopathies and unexplained sudden death conditions. To date we have tested greater than 150 samples. By analyzing the entire gene panel in all samples, we have been able to determine the value of the clinical phenotype to variant analyses, normal variation within these genes, and identified possible new gene associations for these conditions. In addition, we have been able to further determine the strengths and limitations of different forms of NGS technology and the validations required for use of this technology in clinical testing.

Analyses of 96 samples

Panel	Disorder	Pathogenic	Unclassified	Negative
Arrhythmia [28 genes]	LQT	13	3	2
	Brugada	3	-	1
	CPVT	3	1	1
	ARVC	2	-	-
Cardiomyopathy [65genes]	DCM	3	3	-
	HCM	17	4	15
72 gene panel	Sudden Death	15	7	3

Comparison of different NGS platforms

Capture Design Statistics	Overlap capture design	End-to-end capture design	Whole exome sequencing
Capture Design Size (bp) [7Mb]	6051521bp	3681483bp	-
% Target in Capture Design	84.90%	51.63%	5.76%
% Exon Bases Covered by Design	91.34%	81.93%	94.02%
% of genes with >5% exons with zero coverage	1%	6%	42%

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P13.17

Genetic causes of cardiomyopathies identified by Whole Exome Sequencing

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Genetic basis of hereditary cardiomyopathies (CMs) are not yet entirely understood. We studied 12 patients with different form of hereditary CMs, by Whole Exome Sequencing (WES).
Twelve families with early onset CMs were selected: 8 families with hypertrophic cardiomyopathy (HCM), 1 with dilated cardiomyopathy, 1 with arrhythmogenic cardiomyopathy, 1 with unexplained juvenile sudden cardiac death and 1 unclassified form. WES was performed on IlluminaHiScan SQ platform. After bioinformatics analysis, data were filtered and the cosegregation was tested in the family.
We obtained 98% mapped reads against genome with mean coverage on target of 60X. More than 61000 variants were found within each family. Variant calling selected about 18700 of them, both exonic and splice-site. By filtering and prioritization about 20 previously unreported variants were selected for each sample. However, only 4 of them turned out to be potential mutations. Cosegregation analysis, performed in 7 families, identified two potentially disease-causing mutations: the c. 1357C>T (p.Arg453Cys)myosin heavy chain 7 gene variant in a large Italian family with HCM, and thec.878A>T, (p.Glu293Val) desmoplakin gene variant in a family with ARVC.
Whole exome sequencing enable the identification of causative variant in two-third of families with hereditary CMs. These preliminary results underline the promising role of NGS technologies. However, due to the huge amount of data arising from NGS analysis, bioinformatics management becomes crucial and traditional genetic rules are still needed to understand and interpret the real significance of new variants.

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P13.18

A novel strategy for identification of Performance Enhancing Polymorphisms in athletes: new determinants that can modify Hypertrophic cardiomyopathy (HCM) risk

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In the last years, performance enhancing polymorphisms (PEPs) have been described as genetic variants that relevantly influence phenotypic traits related to athletic performance. Hypertrophic cardiomyopathy (HCM), an autossomal dominant genetic disease, is the primary cause of sudden cardiac death in the young and athletes. PEPs have been identified in over 230 genes, both in nuclear and mitochondrial DNA, coding for proteins involved in metabolic pathways, oxygen transport, muscle biogenesis and blood flow. Therefore, it is important to tackle the most relevant genetic features related to physical performance that can influence training adaptation and/or HCM risk being in this regard, considered molecular biomarkers related to athletic performance.
Our aim was to identify PEPs in 20 normal athletes and 20 HCM pacients (athletes) of a Portuguese cohort in 19 genes previously described: ACE, ACTN2, ACTN3, ADRA2A, AGTR1, BDKRB2, CALM3, CKM, EPAS1, FHL1, GLA, MTDN5, NEXN, NOS3, PPARα, PPARGC1A, RAF1, TTR and VCL. Athletes´ DNA was extracted from peripheral blood and genotyped using high throughputt technologies such as High Resolution Melting (HRM) (LightCycler 480®, Roche) and Next Generation Sequencing (NGS) (Ion Torrent PGM platform, Life Technologies®) for which a genomic library was specifically constructed. Using HRM several polymorphisms were identified in ACTN2, AGTR1, GLA, RAF1 and TTR genes. Moreover, several new genetic variations were identified by NGS, nevertheless, due to the high complexity of NGS data analysis, these results are still under validation. Genetic data provided from these studies will allow contributing to establish an association between PEPs, athletic performance and HCM risk.

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P13.19

Massive parallel Sequencing in non-syndromic familial CHD

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The majority (~74%) of Congenital Heart Defects (CHD) occurs as an isolated defect (non-syndromic CHD). Non-syndromic CHD with familial recurrence represent ~4% of all CHD cases. Identification of disease genes involved in non-sydromic CHD is difficult, not only different genes can be involved, also different inheritance patterns exist. Resequencing all genes using massive parallel sequencing technology offers the unique opportunity to identify causative mutations. In order to determine the proportion of non-syndromic familial CHD cases that can be explained by the currently known cardiac genes, we performed targeted sequencing in selected non-syndromic CHD families. For target genes capturing, we used NimbleGen Sequence Capture 385K Arrays and NimbleGen SeqCap EZ Choice library. We included 43 genes and 13 miRNAs in the probe design for the arrays, and 14 additional genes in the in-solution library. The target genes were selected via CHD Wiki (http://homes.esat.kuleuven.be/~bioiuser/chdwiki). The sequencing was initially performed on the 454 FLX platform and subsequently on the Illumina HiSeq 2000 platform. In total we performed targeted sequencing in 39 patients from 15 non-syndromic CHD families. The sequencing coverage is highly reproducible and uniform between multiple samples. The data analysis is ongoing and results will be presented at the meeting. For the families in which no shared mutation in the affected members were found in the target genes, we will perform exome sequencing to search for novel CHD associated genes.

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P13.20

Exome sequencing in research and diagnosis of Congenital Disorders of Glycosylation

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Congenital disorders of glycosylation (CDG) are a heterogeneous group of disorders caused by genetic defects in the protein glycosylation pathway. Most of them have a wide clinical spectrum and biochemical approaches cannot point out to a solely genetic defect, making diagnosis complicated and high-time consuming. Up to now, we have detected over 40 CDG patients but there is still remaining a group of unsolved cases. Here we present the preliminary results obtained by whole exome sequencing in a group of 7 patients clinically and biochemically selected as potential CDGs. The analysis was performed by combination of in solution capture kit SureSelect (Agilent) and subsequent next-generation sequencing with HiSeq (Illumina). On average, coverage was 50 fold and we detected over 270.000 SNVs per patient. We performed an *in silico* capture of 150 candidate genes involved not only in the glycosylation pathway but also in galactosemia, glycogen storage diseases and nucleotide-sugars and dolichol synthesis pathway. We also filtered them out so that their frequency in Exome Sequencing Project (ESP) were lower than 0.5% and focused on pathogenic changes predicted by SIFT and mutations already described in HGMD®professional. The initial results have allowed the detection of pathogenic mutations in DPAGT1, DPM1 and GALT genes. Based on the clinical and biochemical features of the GALT patient, we analyzed and identified two more GALT-deficient patients by Sanger sequencing. In summary, whole exome sequencing is a potential technique that provides an efficient analysis for heterogeneous disorders comprising a large cohort of genes such as CDG.

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P13.21

Profiling circulating miRNAs in plasma samples of Celiac Disease patients

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Celiac disease (CeD) is an autoimmune disorder triggered by gluten in genetically predisposed individuals. Its affects around 1% of the world population and thus far the only treatment available is a gluten-free diet (GFD). Diagnosis of CeD is currently based on symptoms and serology, but vague symptoms can mean that possibly 7 out of 8 patients with CeD are not diagnosed or misdiagnosed. We aim to identify novel biomarkers that can lead to better identification and monitoring of this disease.
MicroRNAs (miRNA) are small non-coding RNAs that are involved in many cellular processes by fine-tuning gene expression. Recently, it was found that circulating miRNAs in plasma can be excellent biomarkers for disease or even the disease-stage, partly because of their unexpected stability in plasma.
We aim to profile circulating miRNAs in plasma samples from CeD patients before and after GFD. To date, we have isolated miRNAs from 10 plasma samples from 5 individuals (5 samples before and 5 after GFD). Next generation sequencing libraries were sequenced on the Illumina HiSeq2000 and the results were analyzed using previously described methods. In a pilot experiment, we found miR-155 to be significantly more highly expressed before GFD than afterwards (*P*-value=0.04). MiR-155 is known to be involved in inflammation and immunity, and it activates the innate immune system and Th1 and Th17 cells. We are now expanding this cohort to 50 patients to validate the results and improve the power to discover novel biomarkers.

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P13.22

Hedgehog acetyltransferase (HHAT) mutation in autosomal recessive chondrodysplasia-pseudohermaphrodism syndrome identified by exome sequencing.

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Chondrodysplasia-pseudohermaphrodism syndrome (MIM 600092) following autosomal recessive inheritance has only been reported in 2 siblings in literature [Nivelon et al., 1992]. The clinical phenotype comprised severe dwarfism with generalized chondrodysplasia, severe microcephaly with cerebellar vermis hypoplasia, facial anomalies, hypoplastic irides, coloboma of both optic discs, myotonia and 46,XY disorder of sexual development with complete gonadal dysgenesis. Until now, the pathogenesis of this striking association was unknown. Here, we report for the first time the involvement of HHAT (*H*edge*h*og *a*cetyl*t*ransferase) in this congenital human disorder using exome sequencing. Within 13 genes with mutations compatible with an autosomal recessive inheritance, we identified a homozygous missense mutation (p.G287V) of HHAT, confirmed by Sanger sequencing. HHAT is a protein catalysing the attachment of a palmitate moiety to hedgehog proteins, thus enabling their long-range action as morphogens during embryonic development. Functional studies showed that the palmitol transferase activity of the G287V mutant HHAT protein is abolished in vitro. Interestingly, previous analysis of *hhat*-deficient mice [Dennis JF et al ., 2012 (PMID:23055936) and Chen MH et al., 2004 (PMID:15075292)] showed early lethality associated to reduced growth, chondrodysplasia and holoprosencephaly. These data suggest that mutation in the HHAT gene is responsible for the Chondrodysplasia-pseudohermaphrodism syndrome by modification of Hedgehog proteins action due to defective palmitoylation.

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P13.23

Gene panels for the rapid diagnosis of connective tissue disorders and familial arterial diseases

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Genetically heterogeneous connective tissue disorders, such as the Ehlers-Danlos syndrome (EDS), Cutis Laxa (CL) or Familial Thoracic Aortic Aneurysms (FTAA) are cumbersome to diagnose at the molecular level due to overlapping clinical features that hamper the selection of the right gene for testing. Next Generation Sequencing (NGS) technology has enabled the development of gene panels which provide a clinically relevant and cost effective solution for the molecular diagnosis of those heterogeneous disorders. Here, we present a highly efficient approach for the diagnosis of a range of genetically heterogeneous connective tissue disorders. We developed 9 panels (*FTAA-panel1-2; EDS-panel1-2; OI panel1-2 (Osteogenesis Imperfecta); CL panel; PXE panel (Pseudoxanthoma Elasticum); ST panel (Stickler))* for a total of 51 causative genes. Using a high-throughput PCR enrichment platform, all coding exons are amplified by a uniform PCR approach followed with paired-end sequencing on a 454 NGS sequencer (Roche), hereby significantly enhancing the efficiency of this approach. With the application of our new FTAA panel 1 containing 7 FTAA genes (*FBN1, TGFBR1/2, ACTA2, TGFB2, SMAD3 and COL3A1*), we identified causative mutations in 10 of 35 patients with a syndromal or non-syndromal form of FTAA. Interestingly, in several of them the nature of the causative gene revealed to be different from the initially suspected gene. Moreover, in some, the mutation had remained undetected with conventional sequencing, illustrating the power of this efficient strategy. Concurrent work is exploring this NGS approach on the MiSeq instrument (Illumina).

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P13.24

Cystic fibrosis and molecular analysis of CFTR locus: comparison of three technical approaches by targeted Next Generation Sequencing (NGS)

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23 years after the discovery of the *CFTR* gene, more than 1900 anomalies are described in the world, the majority of them being single base-pair substitutions or micro-insertions/deletions. Identification of mutations has important implications for genetic counselling, prenatal diagnosis, cascade screening in families, as well as for understanding the genotype-phenotype relationship.
Since a few years, NGS technologies enable us to overcome the classical approaches of whole coding sequence sequencing at single nucleotide resolution.
The aim of our study is to compare 3 different strategies with the Ion Torrent

technology (Life Technologies, LT): Ampliseq (LT), Haloplex (Agilent) and home-made Long Range PCR (LR).
Preliminary results showed that the coverage of the *CFTR* locus by Ampliseq is limited to 51% and includes 86% of exons with a depth generally consistent between amplicons. Haloplex approach gives more heterogeneous depth and coverage. Finally, LR-PCR showed coverage consistent with the design but differences at depth between each LR amplification.
The development of libraries by the two first approaches is rapid (less than 24h) and technically easy. For LR-PCR, It takes about two days to build a library with long and time- consuming manual steps.
Sequencing result for the *CFTR* gene were validated with wild-type samples and DNA carrying known variants (mutations and polymorphisms) previously identified by DHPLC, HRM and sequencing.
In conclusion, the sequencing of the entire *CFTR* locus by NGS is a tool that can quickly respond to issues such as prenatal diagnostic and the most promising approach seems to be the LR strategy.

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P13.25
Development of a comprehensive gene screen for dilated cardiomyopathy using next-generation sequencing.

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Dilated cardiomyopathy (DCM) is a heart muscle disease with an estimated prevalence of 1:2500; it is clinically heterogeneous and appears to be familial in ~30% of cases. The genetic basis of DCM is complex with over 30 genes reported to be involved, few are associated with any differentiating phenotype and the majority of genes account for less than 1% of cases. This clinical and genetic heterogeneity means that traditional analysis strategies, undertaking testing on a gene by gene basis, have had limited utility in a clinical setting. Next Generation Sequencing (NGS) technology means that it is now feasible to develop comprehensive and sensitive genetic tests for DCM, incorporating analysis of the most commonly involved genes in a single test. A diagnostic NGS workflow for the analysis of 28 DCM genes has developed and validated using Haloplex enrichment technology (Agilent Technologies) followed by sequencing on the MiSeq (Illumina). Initial results demonstrate that this is a sensitive and robust assay with an average of 97.5 % of target regions consistently covered to x30 depth. The results of this study will be presented to demonstrate the analytical and clinical sensitivity in our validation cohort; the potential utility of this test strategy in a clinical setting will be discussed.

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P13.26
NADf chip, a two-color microarray mutation detection tool for the diagnosis of deafness in North Africa

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Hearing impairment (HI) is the most prevalent sensorineural disorder. Genetic causes are responsible in two-thirds of prelingual cases. About 70% of hereditary HI cases are non-syndromic (NS); the other 30% are associated to other symptoms. NSHI is exclusively autosomal recessive and highly heterogeneous. Among the most common syndromic forms, Usher and Pendred syndromes are inherited in an autosomal recessive pattern. Autosomal recessive HI is particularly frequent in countries with high rate of consanguinity such as Northern Africans. This population shares several common features, such as history and social behaviors that promote the spread of founder mutations. The most current strategy used for molecular genetic testing in North African countries relies on PCR-RFLP and Sanger sequencing techniques. The extreme genetic heterogeneity of HI renders its molecular diagnosis using these methods time-consuming and very expensive. We designed and optimized a NADf (North African Deafness) chip using multiplex PCR coupled with two-color arrayed primer extension (APEX) for the simultaneous analysis of 60 mutations that are commonly found in Northern African deaf patients. These mutations are distributed over 40 different exons and introns. We used the fluorescent dyes Cy3 and Cy5 that are compatible with the most common scanners. Our array showed 100% sensitivity and specificity in the detection of 40 HI mutants, and ensures robust genotyping. The whole procedure can be performed to completion in a single day. Our miniaturized assay format allows simultaneous genotyping of eight different test samples at minimized reagent costs and is therefore amenable to large scale screening.

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P13.27
Whole-exome sequencing in pedigrees segregating Type 1 Diabetes

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Type 1 diabetes (T1D) is a complex autoimmune disease with high heritability. Genetic association studies identified more than 56 susceptibility loci outside the major HLA locus, but a remarkable portion of heritability (up to 40%) remains unexplained. We thought part of the missing heritability could be attributable to variants segregating in families with closely related affected individuals. With the aim to assess this hypothesis, we sequenced the whole-exome of 32 Sardinian individuals representing 16 parent+offspring affected pairs. Being Sardinia an isolated population, we can not exclude that a founder, highly penetrant variant, shared among those families, is present on the island. Samples were sequenced with the TruSeq Exome Enrichment Kit (Illumina), that provides coverage across 62 Mb of exomic sequence, including 5' UTR, 3' UTR, microRNA, and other non-coding regions. Reads were aligned using Burrows-Wheeler Algorithm (BWA) to the reference human genome (build 37). Alignments for each sample were converted to BAM format, sorted, indexed and processed with MarkDuplicates from the package Picard-tools 1.81 for removing PCR duplicates. The local indel realignment and recalibration was performed according to the GATK (v. 2.2-15) pipeline. On average, mean depth is 52.5x and a total of 98.9% of Q20 bases were observed. We are now performing multisample variant calling of both SNPs and indels using the HaplotypeCaller algorithm in GATK. Our results will elucidate whether an highly penetrant variant shared between families or private variants in genes on the same pathway can explain disease segregation in those trios.

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P13.28
One step molecular diagnosis of 43 forms of monogenic diabetes or obesity, using microdroplet PCR enrichment and next generation sequencing

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Objectives: Whole exome sequencing (WES) and Sanger sequencing can be used for the molecular characterization of monogenic diseases but these techniques are still expensive and labor-intensive. Here, we aimed to develop a cost-effective high-throughput approach for molecular diagnosis of genetic diseases. We assessed a new technology in 45 patients presenting with monogenic forms of diabetes or obesity, of which causal mutations were already known.
Methods: We designed a PCR-based sequence enrichment panel targeting exons of 43 known susceptibility genes for monogenic forms of diabetes and obesity. Each of the 45 DNA samples was processed on the RDT1000 (RainDance). Fragmented DNA mixed with PCR reagents was processed into microdroplets that were fused with microdroplets containing the primer library. The resulting microdroplets were subsequently amplified by PCR. Finally, all 45 amplicon enriched samples were sequenced on the HiSeq2000 (Illumina) at mean coverage depth of 100×.
Results: The mean depth was ≥8× for 99% of targeted regions resulting in a good uniformity of coverage in each sample. The known mutations of 43 patients were confirmed. These included missense, nonsense and splice site muta-

tions as well as deletions. Two proven variants were not retrieved due to issues in Indel detection algorithm and homopolymer sequencing errors. Surprisingly, we found that three patients carry other potentially causal mutations than the expected one.
Discussion: Microdroplet based PCR enrichment combined with next generation sequencing is a promising technique for molecular diagnosis of genetic diseases. It may replace Sanger sequencing as it is far more cost effective and quicker.

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P13.29
Targeted next generation sequencing for identification of genotypes and associated phenotypes in epileptic encephalopathy

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Known monogenic causes for epileptic encephalopathy (EE) explain only a fraction of all patients. To explain more cases, the phenotypic spectrum associated with mutations in known genes needs to be further explored, and new genes for EE need to be identified. In order to do so, we selected 342 genes with a known or possible relationship to EE and performed targeted next generation sequencing of these genes in 64 EE patients. To investigate how common deleterious mutations in these genes are in healthy people, we also performed this sequencing in 64 healthy persons.
After filtering, in the EE patients 144 variants that were novel and unique for the patient group, were found, 0-6 per sample. Of those 34 were in known epilepsy or encephalopathy genes, of which six have been confirmed by Sanger sequencing (at this time), but two were not. In controls 94 unique and novel variants were found, of which 9 in genes previously found to be associated with epilepsy or encephalopathy. Therefore, patients seem to be enriched for mutations in known epilepsy-related genes.
One interesting variant was a non-synonymous mutation in CACNA1A in a patient with moderate intellectual disability, refractory epilepsy and ataxia. CACNA1A is commonly found to be mutated in patients with familial hemiplegic migraine and/or (episodic) ataxia. Another interesting variant was in KCNMA1, which has previously been found mutated in a family with generalized epilepsy and paroxysmal dyskinesia. Follow-up is underway.

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P13.30
Exome sequencing as a diagnostic tool identifies a novel mutation associated with congenital generalized lipodystrophy.

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Exome sequencing has emerged as a diagnostic tool in cases of genetic heterogeneity or when clinical and/or laboratory data from patients is unattainable. We identified a consanguineous Pakistani pedigree with three members, two females and one male, with short and muscular stature, mild mental retardation and acromegalic appearance. The diagnosis remained unclear in the absence of further clinical information and laboratory data. We then performed exome sequencing on affected members and we identified a homozygous novel variant on chromosome 11 in intron 5 of the *BSCL2* gene encoding Seipin. Mutations in the *BSCL2* gene cause congenital generalized lipodystrophy type 2 (CGL2). The transition (c.574-2A>G) identified in this family is predicted to alter the intron 5 acceptor splice site and expression analysis of the mutated allele confirmed skipping of exon 6.
Patients with CGL2 are born without body fat and later develop insulin resistance, hypertriglyceridemia, hepatic steatosis and early onset of diabetes. Clinical re-evaluation of affected family members showed lipodystrophy and axillary acanthosis nigricans. Thus, congenital generalized lipodystrophy was confirmed despite cholesterol and triglycerid levels within normal ranges.
The cost of exome sequencing has become more and more affordable and could represent a universal diagnostic tool for genetic disorders at early stages of investigations or, as illustrated here, when access to clinical information is limited.

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P13.31
Diagnostic application of exome sequencing in Limb Girdle Muscular Dystrophies and Distal Myopathies.

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Exome sequencing offers promising perspectives for molecular diagnosis of genetically heterogeneous diseases, either as a first-tier-approach, or following an initial negative result of targeted single-gene-analysis. In autosomal recessive muscular dystrophies, the latter situation may account of approximately 10-20% of samples screened for a selected gene after clinical orientation.
This includes dysferlinopathies which present mainly as Limb-Girdle Muscular Dystrophy or distal myopathy (Miyoshi myopathy).
Among our cohort constituted during the past 12 years for mutational analysis of the dysferlin gene (DYSF), we included 37 samples initially addressed for diagnostic suspicion of dysferlinopathy, without identified disease-causing mutation (DCM). Exome sequencing (Agilent Sureselect V3 and Illumina sequencing) yielded a mean depth of 68X and an overall coverage of >10% for 98.4% of the targeted exons. Variants were filtered through the "Gene Table of Neuromuscular Disorders" list of genes, disease-group "muscular dystrophy" (http://www.musclegenetable.fr ; 34genes). In six samples, molecular diagnosis could be concluded by identifying compound heterozygous or homozygous DCM in previously known LGMD or distal myopathy genes (including CAPN3, ANO5 and GNE). In three additional samples, respectively one DCM was identified, warranting further targeted exploration (including MLPA/CGH) for the identification of a possible second DCM. For the remaining 28 samples, no DCM were identified among the disease-group "muscular dystrophies", which points to further genetic heterogeneity with regard to the currently known list of genes implicated in muscular dystrophy. Widening of the informatics filter to the entire "Gene Table of Neuromuscular Disorders" list (297genes) identified several mutated candidates which are under further investigation.

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P13.32
Activating mutation of natriuretic peptide receptor B causes extremely tall stature

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Here we describe the combined approach of whole exome sequencing (WES) and subsequent functional studies in the identification and characterization of a novel activating *NPR2* gene mutation in a healthy male with extreme tall stature (221 cm). GH hormone overproduction, known syndromes, and a deletion in chromosome 15q25.2q25.3 containing 8 protein-coding genes had been excluded as the cause of tall stature. WES was performed and analysis of the data with a home-made analysis pipeline elucidated a heterozygous missense mutation in the *NPR2* gene (c.1963C>T p.Arg655Cys) within the NPR2 kinase homology domain as the most likely pathogenic event. This variant was not present as a known variant in any of the available databases. The *NPR2* mutation was not found in the patient's sister or son, who are tall but within the normal range. His parents were not available for analysis. Transfection studies of the mutant NPR2 protein in HEK293 cells resulted in increased basal and CNP stimulated NPR2 activity. Co-expression of wild-type and mutant NPR2 resulted in increased activity, close to the value of mutant NPR2 alone, suggesting a dominant positive effect. Co-immunoprecipitation studies confirmed heterodimer formation of wild-type and mutant NPR2. Studies using skin fibroblasts of the patient showed increased NPR2 activity after stimulation with CNP, confirming that the mutant NPR2 enhances CNP-NPR2-cGMP signalling. This pathway is critically involved in bone development by stimulating growth plate chondrocyte differentiation and proliferation. We conclude that the identified *NPR2* mutation in our patient is responsible for the extremely tall stature.

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P13.33

Performance enhancement of Haloplex target enrichment disease panels using advanced probe design algorithms

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Targeted next-generation sequencing (NGS) is a powerful technology that is transforming the understanding of human disease. The Haloplex PCR method combines target enrichment and sequencing library preparation in a simple one day protocol without the requirement for dedicated instrumentation. While the Haloplex method is highly specific and results in deep coverage across targets of interest for variant identification, specimens such as formalin-fixed paraffin-embedded (FFPE) tissues present challenges due to age, presence of interfering substances, limited material, and DNA template degradation and damage. To make the Haloplex protocol even more robust when using difficult samples, we examined the effect of probe design parameters on enrichment metrics and variant calling performance using several disease-specific panels with various sample types. We focused on targeting shorter fragment sizes, capturing both strands of the target DNA, building redundancy into design, and adjusting stringency settings for targets with high homology to other regions of the genome. We demonstrate how these advanced design considerations improved the performance of the disease panels in terms of coverage, uniformity, and specificity. We also discuss the effect of probe design on coverage of hotspot regions, false positive rates due to formalin-induced damage, and allelic balance in the case of low frequency mutations. Our results demonstrate the utility of Haloplex for targeted next-generation sequencing using challenging samples often encountered during disease research and assay development.

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P13.34

Whole exome sequencing of germline DNA from non-BRCA1/BRCA2 familial breast and ovarian cancer cases

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Genetic etiology for the majority of families with breast/ovarian cancer (BC/OC) is unknown. This could be due to a genetic heterogeneity with multiple loci of unequal frequency and penetrance. Next generation sequencing can be used to search predisposition genes by sequencing the entire protein-coding sequence (exome). In a still ongoing analysis (ISCIII Miguel Servet funded) we applied whole-exome sequencing to multiple affected relatives from eight BC/OC families. Genomic library was prepared from 1 µg of DNA. Exome enrichment was performed using the SureSelect XT HumanAllExon 50Mb (Agilent). Sequencing, paired-end 2x100, was performed on a HiSeq2000 instrument (Illumina). Reads were aligned to the human genome (hg19) using the Burrows-Wheeler Aligner algorithm. Single nucleotide variants (SNVs) and indels were identified using the GATK software and annotated with Annovar. Variants were filtered for confident calls using a ≥30 quality threshold, ≥15 read depth, and ≥ 0.15 allele frequencies. The average read depth for capture target regions was 98.84. Prior to further filtering, variants were assessed for overtly deleterious mutation in known BC/OC genes. Then, variants in the 1000 Genomes Project, NHLBI Exome Sequencing Project, and dbSNP database with minor allele frequency of ≥1% were removed. Next, variants functionally deleterious in heterocigosis (frameshift and nonsense SNVs) were identified. After filtering, an average of 37 deleterious mutations was identified per individual. Only those genes with deleterious mutations shared by the affected relatives were considered. Priority was given to genes implicated in well-established pathways associated to cancer. The variant validation will be done by Sanger sequencing.

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P13.35

Prediction and prioritization of non-coding variants of uncertain significance in heritable breast cancer

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We present an approach to prioritize pathogenic non-coding, variants of uncertain significance (VUS) based on information analysis of changes in DNA and RNA sequences bound by regulatory factors. Complete gene sequences were captured by solution hybridization, enriching for variants in genes that harbor breast cancer risk mutations. Oligo baits for ATM, BRCA1, BRCA2, CDH1, CHEK2, PALB2 and TP53 spanned complete coding and adjacent intergenic regions. A novel probe design captured both repeat-free and effectively single-copy, divergent repeat sequences. After sequencing 21 patient samples, information theory-based sequence analysis prioritized non-coding variants predicted to occur within sequences recognized by proteins or protein complexes. VUS were screened for mutations affecting binding sites in mRNA splicing, by transcription factors (TFBS), and proteins interacting with untranslated regions (UTR). Information theory based models of exon recognition predict the relative abundance of natural, cryptic, and mutant splice isoforms resulting from predicted mutations (Mucaki et al 2013). We introduce a similar approach to detect mutations altering TFBS and UTR binding sites. Information weight matrices were computed by entropy minimization of ENCODE ChIP-seq data for 47 transcription factors expressed in breast cancer. Novel variants in patients were then evaluated for alteration of TFBS affinity. We predict 9 splicing, 10 TFBS, and 6 UTR variants affect gene expression of 6 protein coding genes in the patient samples (from 7,909 variants in 7 genes). This strategy more comprehensively covers non-coding regions in breast cancer genes than repeat masking, and introduces a unified framework for systematic interpretation of VUS that affect expression.

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P13.36

Development and validation of massively parallel sequencing for the routine molecular diagnosis of hereditary breast and ovarian cancer

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In order to optimize the molecular diagnosis of hereditary breast and ovarian cancer (HBOC), we developed a NGS-based routine screening based on the capture of 21 genes (exons and introns) involved in HBOC, pooling of 12 indexed DNA, 2x76bp paired-end sequencing on a GAIIx and confirmation by Sanger sequencing or MLPA/QMPSF. The bioinformatic pipeline includes CASAVA, NextGENe, CNVseq and Alamut-HT. The procedure allows the analysis of 80 patients per run with a 60x coverage. We validated this procedure first by analysis of 60 DNA with deleterious SNV, indels or genomic rearrangements of BRCA1 or BRCA2, then by performing in parallel the NGS and Sanger se-

quencing/MLPA analyses of BRCA1 and BRCA2 in 160 patients. All mutations detected by conventional procedures were detected by NGS. We then analysed 707 new patients for SNV and Indels. We detected 91 variations inducing a premature codon stop or a splice defect, in BRCA1(n=28), BRCA2(n=24) and 39 in the other genes: ATM(n=5), CHEK2(n=5), PALB2(n=5), MRE11A(n=4), RAD51C(n=3), NBN(n=3), RAD50(n=2), BARD1(n=1), CDH1(n=1), MSH2(n=4), PMS2(n=2), PMS1(n=1), MLH3(n=1), RAD51D(n=1) and RAD51B(n=1). Interpretation of these potentially deleterious variants is being performed by segregation studies within the families. We also detected potentially deleterious 21 missense mutations, including 11 within BRCA1 and BRCA2 (Align GVGD score > C65 MAF in ESP sample <0.01). These results demonstrate the efficiency of this NGS procedure to perform molecular diagnosis of HBOC and shows that the fraction of potentially deleterious mutations detected within the other genes than BRCA1 and BRCA2 justifies their analysis.

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P13.37

Ultra-Fast BRCA1/2 Analysis for Clinical Diagnostics of DNA from Blood Samples

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Several studies have indicated that, hereditary breast and ovarian cancer patients who harbor mutations in BRCA1/2 can benefit from new therapeutics, known as poly-ADP-ribose polymerase (PARP) inhibitors. Patients suitable for entry in protocols using PARP inhibitors are typically at a late stage of their cancer disease. If patients without prior knowledge of mutations in BRCA 1/2 should benefit from treatment, very fast screening for mutations in BRCA 1/2 is needed. Therefore, we developed a novel in-house ultra-fast BRCA1/2 mutation analysis, making it possible to screen the coding regions, the intronic regions (33-1881 bp) flanking the exons and the 5' and 3' UTRs of the BRCA1 and BRCA2 genes within two weeks. This allows BRCA1/2 positive patients to be enrolled in clinical trials at an earlier stage, since traditional Sanger sequence based screening of BRCA1/2 may take up to, as long as 3 months, in Denmark.
Method: Genomic DNA was subjected to 13 individual LongRange PCR amplifications (In House primer design and Qiagen LongRange PCR kit). 1 ng of pooled amplicon DNA was used as input DNA for the library preparation (Nextera XT, Illumina). Sample libraries were multiplexed and sequenced on a MiSeq (PE 2x 150 bp, dual indexing). Data analysis was performed using CLC Genomic Workbench. Patient DNA was tested for larger InDels using MLPA P002-C2 and P045-B3.
Results: A total of 60 patient samples have been analyzed since August 2012. The average analysis time is 13.3 days (8 to 25 days), and 6 disease causing mutations have been detected.

A.H. Petersen: None. **A. Bojesen:** None.

P13.38

Screening FFPE archival tissue for mutations in BRCA1 and BRCA2 - A new paradigm in genetic counselling/investigation

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By revealing the BRCA status of deceased relatives using archival Formalin-Fixed Paraffin-Embedded (FFPE) samples, genetic counsellors get an extra tool to make more qualified risk assessment on living family members. The elucidation of a family's BRCA status may also release family members from psychological worries, if the test result is BRCA negative. Importantly, if the test result is positive for a disease causing BRCA mutation, the genetic counsellors can guide the family members about the benefits of genetic testing and screening programs.
The department of Clinical Genetics at SLB-Vejle Hospital, Denmark, is implementing a targeted sequencing strategy of 29 cancer genes including BRCA1/2 for routine analysis of mutations in DNA extracted from FFPE samples < 10 years old. The first study included 7 FFPE samples and 1 blood sample, all with a known germline BRCA mutation, and focused on examining the quality of the chosen method for targeting the cancer genes, and setting up a data analysis pipeline. A second study involves FFPE samples from 20 cancer specimens with a known germline BRCA1/2 mutation and focus on examining the relationship between the age of FFPE sample, quality and amount of DNA required to get a successful result.
Results: All BRCA mutations were identified except from a large deletion. The mean coverage was between 628-1079x, and 74.9-98.5% of the target region had a coverage of at least 30x. Through these studies, expertise will be gained providing the genetic counsellors with an important tool in the battle against inherited cancer disposition.

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P13.40

Targeted high-throughput sequencing of 220 genes identifies a high proportion of causative mutations in 50 patients with undiagnosed intellectual disability

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Over 200 genes have a well-documented implication in monogenic forms of intellectual disability (ID), including 100 located on the X-chromosome. While there is a good coverage for diagnostic demands in patients with evocative syndromic forms, in lesser syndromic patients the diagnostic offer is limited to Fragile-X and CGH-array analyses and most cases hence remain undiagnosed.
We tested a targeted exon-capture strategy of 220 ID genes (100 XLID genes and 120 autosomal genes mostly associated to dominant forms) coupled with multiplexing and NGS. We successfully analyzed 50 patients (mostly males and sporadic cases) with undiagnosed moderate to severe ID.
We identified eight certainly-causative mutations: five maternally-inherited in X-linked genes (DMD/DP71, KDM5C, MAOA, MECP2, SLC9A6) and three truncating de novo in autosomal genes (DYRK1A, RAI1, TCF4). We also reported four likely-causative X-linked mutations in HUWE1, IQSEC2, SRPX2 and FLNA requiring additional validation analyses.
Interestingly, the DMD/DP71 mutation is a very distal frameshift in a patient with no muscular phenotype. The MECP2 mutation is an exceptionally complex rearrangement detected in an 11 years-old boy with severe ID and his unaffected mother. Patients with RAI1 and TCF4 mutations did not present with clear phenotypic traits evocative of Smith-Magenis or Pitt-Hopkins syndromes.
The identification of certainly/likely causative mutations in 16%/24% of the patients proves the relevance of our strategy for the diagnosis of ID, being more cost-effective than trio-exome sequencing while leading to a comparable proportion of diagnostic results (16%/41% in 100 trios, de Ligt et al.; 31%/43% in 51 trios, Rauch et al., 2012).

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P13.41

Using Ion AmpliSeq™ RNA to Detect Fusion Transcripts in Human Oncology Samples

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We have developed a new method of targeted RNA sequencing application for low input and degraded FFPE total RNA samples using the Ion AmpliSeq™ RNA technology. This technology has been shown to be technically reproducible, quantitative and to maintain excellent correlation with qPCR using TaqMan gene expression assays. We have applied this novel targeted RNA sequencing approach to the detection of fusion transcripts in human disease tissues.
Panels of amplicons were designed to all the known fusion transcripts associated with mesenchymal neoplasms (soft tissue tumors). The Ion AmpliSeq™ RNA technology was used to quickly scan through all known chimeric

transcription factors in the COSMIC database that have been observed in soft tissue tumors. This was accomplished by generating a fusion transcript AmpliSeq™ RNA panel that contained oligo primers specific for highly observed fusion transcripts as well as, rare fusion transcripts in a single tube. Thus, if the fusion transcript is expressed in the sample, that amplicon will be present and contribute to the mapped reads on the Ion PGM. If the fusion transcript is not present in the sample, the unused oligonucleotides are destroyed and no reads are produced. With this approach hundreds of fusion transcripts can be screened in a simultaneously in a single tube on the Ion PGM with resolution down to the single nucleotide level. Data from positive and negative control tissues will be demonstrated as a proof of principle for the use of Ion AmpliSeq™ RNA for the detection of fusion transcripts in oncology samples.

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P13.42

Streamlined Ion Torrent PGM-based diagnostics: *BRCA1* and *BRCA2* genes as a model

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To meet challenges in terms of throughput and turnaround time, many diagnostic laboratories are shifting from Sanger sequencing to higher throughput second-generation sequencing (SGS) platforms. Bearing in mind that the performance and quality criteria expected from SGS in diagnostic or research settings are strikingly different, we have developed an Ion Torrent PGM-based routine diagnostic procedure for *BRCA1/2* sequencing. The procedure was first tested on a training set of 62 control samples, and then blindly validated on 77 samples in parallel with our routine technique. The training set was composed of difficult cases, e.g. insertions and/or deletions of various sizes, large-scale rearrangements and, obviously, mutations occurring in homopolymer regions. We also compared two bioinformatics solutions in this diagnostic context, an in-house academic pipeline and the commercially available NextGene software (Softgenetics). NextGene analysis provided higher sensitivity, as 4 previously undetected nucleotide variations were found. Regarding specificity, an average of 1.5 confirmatory Sanger sequencings per patient was needed for complete *BRCA1/2* screening. Large-scale rearrangements were identified by two distinct analyses, i.e. bioinformatics and fragment analysis with electrophoresis profile comparison. Turnaround time was enhanced, as series of 30 patients were sequenced by one technician, making the results available for the clinician in 10 working days following blood sampling.
BRCA1/2 genes are a good model, representative of the difficulties commonly encountered in diagnostic settings, which is why we believe our findings are of interest for the whole community and the pipeline described can be adapted by any user of PGM for diagnostic purposes.

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P13.43

Colorectal cancer predisposition with multiplex PCR enrichment in an Ion Torrent PGM-based routine diagnostic process

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BACKGROUND: Colorectal cancer predisposition can occur either in the Lynch Syndrome (4 genes) and also with Familial Adenomatous Polyposis (2 genes). We developed an Ion Torrent PGM-based routine diagnostic process for sequencing those genes based on the Multiplicom HNPCC and FAP MASTR kit. The goal was to estimate the performance of this system and to extend to the routine diagnostic process.
METHODS: The procedure was first tested on a training set of 16 samples from patients with a suspicion of Lynch syndrome and 16 samples from patients with an attenuated polyposis syndrome. For those 32 patients, a screening with qPCR-HRM method was performed for *MLH1* , *MSH6* , *MSH2* (85 variants) and *MUTYH* (42 variants). The variants were 4 deleterious in *MSH2* , 2 in *MLH1* , 4 in *MSH6* . The variants were 4 deleterious in *APC* and 1 monoallelic and 1 biallelic in *MUTYH* gene. All data were analyzed with NextGene module.
RESULTS: All deleterious variants were detected. The difficulties were observed mainly with homozygous variants, *PMS2*pseudogene and indel variants in homopolymer region. In addition to mutants already identified, two deleterious mutations were identified in *PMS2* and *APC* gene, whereas the screening was pending. The assets and limitation of the approach will be discussed.
CONCLUSIONS: This new approach offers the possibility to propose a widest panel of screened genes, reducing the time lag in the diagnostic process due to unspecific tumor information (attenuated polyposis or no molecular data available).

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P13.44

IonTorrent: 2nd generation sequencing in a diagnostic laboratory

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Molecular diagnosis of complex human genetic diseases is still challenging because in most cases multiple genes harboring putative deleterious mutations have to be investigated. So far in most diagnostic laboratories Sanger sequencing is used but capillary sequencing is excessive time-consuming and expensive at least for the screening of multiple genes. Recently next-generation sequencing (NGS) technologies have started to be applied, but because these technologies are made especially for large sequencing projects it is not easy to scale it down for screening a set of disease causing genes in a diagnostic laboratory. To fill this gap table top NGS systems have been introduced by Life Sciences (Ion Torrent PGM) from Illumina (MiSeq) and from Roche (GS Junior). While Illumina and roche launched sequencer adapted to established technology IonTorrent introduced a sequencing device using a sequencing technology based on the detection of hydrogen ions that are released during the polymerization of DNA.
To validate this technology in respect to usability, software requirements and accuracy we tested several gene panels comprising between 3 and 420 genes covering between 200 and 16000 exons. Regions of interest were enriched in different ways: single PCR, multiplex PCR (AmpliSeq, IonTorrent; GeneRead NGS System, Qiagen) or HaloPlex custom specific kits (Agilent). Our validation showed that for enrichment multiplex PCR is the superior technology and that with the exception of one all tested software packages are either excessive time consuming or did not find the mutations at all and still need urgent improvements.

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P13.45

Whole-genome sequencing in Kallmann syndrome

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Kallmann syndrome (KS) is a rare disorder characterized by absent/defective sense of smell and absent/incomplete puberty resulting from defects in the development of the hypothalamic GnRH neuron population. X-linked recessive, autosomal dominant (AD) and autosomal recessive (AR) inheritance patterns have all been described in KS patients, and di-or oligogenic inheritance of the disorder has also been suggested. Mutations in the many genes already connected with the disorder explain only about 30-40 % of the cases, implying new disease genes are yet to be found. Whole-genome sequencing data of a male KS patient and his healthy parents and brother was analyzed to search for candidate causal variants in the coding regions of the patient's genome. Different filtering criteria were used for different modes of inheritance: 1) AD de novo mutation; 2) homozygous AR mutation; 3)

compound heterozygous AR mutation; 4) AD mutation with sex-dependent penetrance. We only considered novel variants. Scenarios 1 and 2 were ruled out as no candidate variants were left after filtering. No obvious causal variants were found for scenarios 3 and 4. Comparison of the patient's genome against that of his healthy brother revealed variants in several interesting genes involved in cell migration and axon guidance, including ITGB1 and NDNF. Additional analysis of de novo copy number variations and detection of SNPs and indels in genes encoding miRNAs revealed no further candidate variations. These results highlight the many challenges still present in the study of genetically heterogeneous disorders.

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P13.46

A combined NGS and CGH arrays approach identifies novel TRIM32 mutations in an unclassified case of limb-girdle muscular dystrophy

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The EU-funded NMD-Chip project was established to design, develop and validate new sensitive high throughput DNA arrays to efficiently diagnose patients affected with neuromuscular disorders (NMDs). In this context, both CGH arrays to detect copy number variations as well as Sequence Capture (SC) arrays for massive re-sequencing and point mutation detection, have been set up, allowing the simultaneous screening of more than 50 genes known to be involved in NMDs, in addition to hundreds of candidate genes selected from experimental and published data. Thanks to these combined NGS and CGH arrays approaches, we report here the molecular diagnosis of TRIM32 compound heterozygous mutations in a 50 year-old male patient suffering from slowly progressive muscle weakness, characterized by a late age of onset (30y), unspecific limb-girdle muscular dystrophy with scapular winging and moderately elevated CPK (400U/l). For this patient, sequencing of TRIM32 using the custom in-solution DNA SC library revealed first a distal frameshift mutation, c.1603delC (p.Leu535Serfs*21) which appeared at homozygous state. Because there was no history of consanguinity, a possible deletion of the other allele of TRIM32 has been investigated by CGH array, which allowed the identification of a large deletion of chromosome 9 encompassing the whole TRIM32 gene. Defects in TRIM32 have already been reported in limb-girdle muscular dystrophy type 2H (LGMD2H), also known as muscular dystrophy Hutterite type, a rather mild and progressive myopathy characterized by a wide phenotypic heterogeneity. These novel mutations confirm that integrity of the C-terminal domain of TRIM32 is necessary for muscle maintenance.

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P13.47

Identifying the incidence of rare genetic disorders in infants with liver disease using targeted next generation sequencing.

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Background: The incidence of infantile liver disease due to rare genetic disorders, including Niemann Pick type C (NPC), progressive familial intrahepatic cholestasis (PFIC) and citrin deficiency (CD) is unknown. Early genetic diagnosis allows targeted therapy avoiding inappropriate liver transplantation. Traditional testing by sequential sequencing of the genes involved using Sanger sequencing is costly and time consuming. Next generation sequencing (NGS) permits simultaneous analysis of multiple genes whilst reducing time to diagnosis and cost.

Aim: To develop a targeted NGS screen to diagnose genetic disorders in infants with liver disease.

Methods: 14 centres worldwide recruited 230 infants less than 2 years old presenting with cholestasis, acute liver failure or splenomegaly. The 6 genes involved in NPC, PFIC and CD were sequenced using 48.48 Access Array (Fluidigm) and GS Junior (Roche). Variants were classified into likely pathogenic, unknown significance (VUS), and likely non-pathogenic groups.

Results: Testing was complete in 181 cases. A genetic diagnosis was possible in 18 patients (10%, see Table). A further 7 patients (5%) had only one likely pathogenic mutation, and approximately 20% had VUS only with no pathogenic mutations.

	Number of patients	
Gene	Diagnosis	Carrier
NPC1	1	2
NPC2	0	0
ATP8B1	3	1
ABCB11	9	1
ABCB4	5	5
SLC25A13	0	0

Conclusions: The combined incidence of these genetic diseases in infantile liver disease is 10% with PFIC type 2 being the most common. NGS has shown to be an efficient method of analysing multiple genes for these disorders; pros and cons will be discussed.

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P13.48

Massive parallel sequencing in molecular diagnostics: universal tailed amplicon sequencing of lynch syndrome genes

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Lynch syndrome accounts for 3-5% of all colorectal cancers (CRC), making it the most common hereditary CRC syndrome. The disease is inherited in an autosomal dominant manner, and is attributed to mutations in either of the DNA mismatch repair (MMR) genes *MSH2*, *MSH6*, *MLH1* and *PMS2*.

We have one year experience in the use of massive parallel sequencing (MPS) (GS Junior, 454 technology, Roche) for diagnostic detection of germline mutations in Lynch syndrome genes. A time- and cost effective workflow suitable for routine molecular diagnostics has been developed. The GS Junior system is based on emulsion PCR (emPCR) to clonally amplify single stranded DNA molecules to be sequenced. Before emPCR a DNA amplicon library is prepared by two-step PCR based amplification of the regions of interest. With the use of molecular barcodes (multiplex identifier sequences, MIDs), many amplicons from several patients can be pooled and sequenced together. All amplicons are sequenced in parallel by pyrosequencing chemistry. Data analysis is done with the AVA-software supplied by Roche. A well-known problem with Pyrosequencing is incorrect basecalling in homopolymeric regions. Our workflow circumvents this issue by Sanger sequencing amplicons that contain homopolymers.

Implementation of diagnostic MPS at our laboratory has led to decrease in sample turnaround time and costs. We find the technology to be good in terms of sensitivity and well suited for use in molecular diagnostic laboratories.

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P13.49

Effect of a novel intronic FBN1 mutation on splicing revealed by splicing minigene assay.

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A girl presenting severe neonatal form of Marfan syndrome (MFS) was born

to healthy parents. Mutational screening of patient's DNA revealed an intronic change, 20 bp deletion in intron 34 (c.4211-32_-13del) in the *fibrillin-1* gene (*FBN1*). *In silico* predictions of the effect of the deletion on pre-mRNA splicing showed marked drop in both the 3' splice site score and the polypyrimidine tract score. As we were not able to perform RT-PCR directly from patient's RNA, we took advantage of splicing minigene assay. We cloned exon 35 together with neighbouring intronic regions into the vector pET. After transfection of HeLa cell line with the wild type and mutant constructs, we isolated total RNA and performed RT-PCR. Its results clearly showed that the mutation led to exon 35 skipping in the majority of RNA. In patient, this splicing aberration presumably caused the in frame deletion of 126 nucleotides from *FBN1* mRNA.
The splicing minigene analysis is not a standard diagnostic procedure and the results driven from this highly artificial system could differ from the real situation in the patient. We can not be sure about precise RNA aberration that the examined mutation induced. On the other hand, since we have detected that the mutation affects splicing under certain conditions, there is high probability that the mutation disrupts normal splicing to some extent. Furthermore, we have not detected any other mutation neither in the *FBN1* gene nor in other MFS-related genes. Hence, we regard the mutation as causal one in this case.

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P13.50

Comparison of screening for Marfan syndrome using SSCP and NGS

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Background: Marfan syndrome (MFS) and Marfan-like diseases are inherited autosomal dominant disorders that affect connective tissue, the tissue that strengthens the body's structures, mainly involving the cardiovascular, skeletal and ocular systems. The estimated incidence is of about 1:5 000 - 1:10 000 and approximately 30% of cases are associated with *de novo* mutations. MFS is caused by mutations in fibrillin-1 gene (*FBN1*, 15q15-q21.1.) resulting in defective glycoprotein fibrillin-1. Recently, two other genes TGFBR2 (3p22) and TGFBR1 (9q22) have shown to effect MFS.
Aims: In this study we performed analysis of all exons of FBN1 gene, except exon 1, in order to identify pathogenic mutations in unrelated patients with clinical diagnosis of MFS. 758 patients were analyzed by SSCP (single-strand conformation polymorphism) and 56 by NGS (next generation sequencing).
Materials and Methods: DNA was isolated from whole blood, the molecular analysis included SSCP and NGS (GS Junior454, Roche). Exons with abnormal migration patterns and changes in nucleotide sequence were confirmed by direct sequencing. Newly detected sequence variants were subjected to *in silico* predictions.
Results: We identified 129 *FBN1* mutations by SSCP and 16 *FBN1* mutations by NGS.
Conclusions: We have confirmed 129 cases of Marfan syndrome caused by *FBN1* mutation by SSCP analysis, which constitutes detection about 17%, and 16 cases by NGS, which constitutes detection about 29%. We have found that NGS is a more sensitive method for screening MFS than SSCP.

M. Dvorakova: None. **R. Krenkova:** None. **P. Cibulkova:** None.

P13.51

Preventive genetic testing using microarray technology is perspective prophylactic approach in medical practice

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Currently personalized medicine is developing very rapidly. The main focus of such approach is usually concentrated on detection of genetic predisposition to common complex disorders whereas less attention is devoted to preventive genetic testing for monogenic diseases. Our previous work proved that DNA-analysis for monogenic defects can be useful among newborns. Testing program "Gemascreen" includes 6 conditions: cystic fibrosis, phenylketonuria, spinal muscular atrophy, autosomal recessive deafness, Gilbert syndrome, lactase deficiency. From 211 cases the genotyping results of 115 analyses were found to be essential for health maintenance of the examined persons. In 19 cases the results were beneficial to the planning of individuals' future reproductive behavior (table 1).
To improve diagnosticum the microarray "Ethnogene-400" was developed based on APEX technology. The microarray allows to diagnose 80 monogenic disorders, 12 multifactor diseases and includes several pharmacogenetic tests (table 2). During design of the microarray several points were taken into account: Frequencies of diseases in Russian population; Known genetic cause of the disorder together with existence of common mutations in particular genes; Technical capability. Thus using of the microarray "Ethnogene-400" will facilitate the prophylactics of genetic disorders in the families of asymptomatic carriers and increase life quality and longevity in general population.

Genotyping results of 211 newborns for the most common monogenic disorders and conditions

Total amount of analyses: 211	Cases			
	Hetero-zygous	Homo-zygous		Homo-zygous
Disorders			Conditions	
Cystic fibrosis (CFTR)	3		Gilbert syndrome (UGT1A1)	25
Phenylketonuria (PAH)	2		Lactase deficiency (LCT)	89
Spinal muscular atrophy (SMN)	5			
Autosomal recessive deafness (GJB2)	8	1		
Total	18	1		114

Monogenic diseases from Federal list of Russian newborn screening program included into microarray

Disorder	Gene	Disease frequency	Amount of testing mutations	Testing informativity
Cystic fibrosis	CFTR	1/8 500	24	82%
Phenylketonuria (type 1)	PAH	1/10 000	16	87%
Phenylketonuria (type 2)	PTS	1/200 000	3	18%
Galactosemia	GALT	1/20 000	6	70%
Deafness (isolated neurosensory, autosomal recessive)	GJB2	1/5 000	6	91%
Deafness (Pendred syndrome)	SLC26A4	1/10 000	6	50%

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P13.52

Targeted PCR-based enrichment and next generation sequencing for diagnostic testing of Primary Microcephalies (MCPH and Seckel Syndrome).

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Primary Microcephalies (PM) are a group of recessive disorders characterized by reduced head circumference without additional severe brain malformations, normal motor development and mental retardation. PM encompass two phenotypes, primary microcephalic dwarfism (PMDW or Seckel syndrome), with growth retardation and Autosomal recessive primary microcephaly (MCPH), with normal stature. MCPH and PMDW share the same pathogenesis and may result from mutations in at least 15 genes. Due to genetic heterogeneity, exhaustive conventional Sanger sequencing for PM diagnosis is expensive and time consuming and then, 50 to 75% of PM patients from the Caucasian population have no identified gene defect. In order to develop an efficient mutation-screening strategy for diagnosis, we evaluated a multiplex PCR-based approach in combination with next generation sequencing on 96 patients. The coding exons of 24 genes known to cause PM were targeted. 48 samples were screened with a first primer panel design. 95.2% of initially targeted regions were effectively covered. Sequencing resulted in a mean coverage of 301X with 88% of targeted regions being covered more than 25X. In targeted regions, we detected, on average, 163 variants per patient. Filtering and ranking finally yielded to 27 potentially pathogenic mutations still under investigations. The following 48 patients were screened with a second panel of primers designed to improve the coverage of the targeted regions and are still being analyzed. Our study shows the effectiveness of this strategy available to routine laboratories and its optimization will achieve the reliability required before its implementation in the context of the diagnosis.

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P13.53

High Throughput Sequencing Approach Identify MAFA as the fourteenth MODY gene

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Maturity-onset diabetes of the young (MODY) is a monogenic form of diabetes mellitus characterised by autosomal dominant inheritance, a young age of onset and pancreatic β-cell dysfunction. It is both clinically and genetically heterogeneous with mutations at the moment in at least thirteen genes. Genetic testing for MODY has become a routine procedure allowing to set up proper treatment and discriminate from type 2 diabetes (T2D) whose symptoms are often overlapping. Although more than 40 loci associated with T2D or glycemic traits have been reported and reproduced, only a minor part of the genetic component of the disease has been explained, and the causative variants and affected genes are unknown for many of the loci.
To determine the role of low-frequency coding variants in traits reflecting pancreatic β-cell function, insulin sensitivity and glycemia, we performed targeted resequencing technology by high-throughput sequencing in 27 patients with suspected MODY/T2D. They were Sanger sequencing-negative for mutations in the GCK, HNF1A, HNF4A, HNF1B genes. We excluded common, non-coding and synonymous gene variants, and performed in-depth analysis on filtered sequence variants in a pre-defined set of 103 genes implicated in glucose metabolism. We found, in association with known heterozygous SNPs already described in diabetes, rare and pathogenetic variants, demonstrating that this approach led to a genetic diagnosis in most of patients.
Interestingly two novel possible causative variations in MAFA gene, implicated in diabetes in animal models, in two different families could identify this gene as the fourteenth MODY gene.

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P13.54

Validation of a novel high-throughput gene variation identification assay for diagnosis of monogenic diabetes

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Introduction: Maturity-Onset Diabetes of the Young (MODY) is a monogenic form of diabetes. It is a heterogeneous disorder and to date, 13 genes in which mutations may cause MODY have been reported. These distinct molecular etiologies explain the clinical heterogeneity and also differential responsiveness to treatment observed among MODY patients. MODY patients are often misdiagnosed as either type 1 or type 2 diabetes patients, resulting in suboptimal treatment, and the importance of correct MODY diagnosis through genetic testing is thus evident. So far, however, genetic screening has been performed using expensive and time-consuming Sanger sequencing.
Aim: To validate a novel high-throughput, cost-effective gene variation identification assay for diagnosis of monogenic diabetes.
Methods: We generated a novel targeted DNA capture and Illumina next-generation sequencing assay which is applicable to whole genome regions (including exons, introns, and untranslated regions of selected genes). A study sample of 70 Danish patients carrying previously identified causative variants in six of the known monogenic diabetes genes (*HNF4A, GCK, HNF1A, HNF1B, INS* and *KCNJ11*) were screened applying the novel assay.
Results: Among the 70 analyzed disease samples, we identified 63 small size single nucleotide polymorphisms and indels as well as seven larger deletions and duplications as the pathogenic variants. Our results are consistent with previously identified pathogenic mutations based on Sanger sequencing and the use of a Multiplex Ligation-dependent Probe Amplification assay.
Conclusion: The established platform is a cost-effective and high-throughput gene testing method which may be applied in clinical diagnosis of monogenic diabetes.

M. Hollensted: None. **R. Gao:** A. Employment (full or part-time); Significant; BGI Shenzhen, Shenzhen, China. **X. Wan:** A. Employment (full or part-time); Significant; BGI Shenzhen, Shenzhen, China. **Y. Liu:** A. Employment (full or part-time); Significant; BGI Shenzhen, Shenzhen, China. **A.P. Gjesing:** None. **O.B. Pedersen:** None. **X. Yi:** A. Employment (full or part-time); Significant; BGI Shenzhen, Shenzhen, China. **T. Hansen:** None.

P13.55

Whole exome sequencing as genetic diagnostic tool in Myofibrillar Myopathies

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Hereditary myofibrillar myopathies (MFMs) are a group of rare neuromuscular disorders with a great genetic heterogeneity. Currently, the molecular genetics diagnosis is based on a gene-by gene approach (six causative genes identified until now) which is time consuming and expensive. Furthermore, to date, up to 50 % of MFM patients remain devoid of genetic confirmation of their disease. In a selected group of seven MFM patients we adopted high throughput techniques as WES (Whole-Exome-Sequencing) and CGH (Comparative Genomic Hybridization) to identify causative mutations, also supported by gene prioritization based on an algorithm providing candidate genes to interrogate the exome. Except for one with an autosomal dominant inheritance, all cases were sporadic. In all muscular biopsies, focal areas strongly immunoreactive with antibodies against desmin, αB-crystallin and myotilin were present within muscle fibers. Among these MFM patients, we identified two pathogenic mutations in FLNC gene, two variations in the titin (TTN) gene and a genomic duplication in LAMA2 gene. We also identified a variation located within the CAMK2D gene (not known to be involved in muscular diseases) as a novel variation with a possible role in MFM phenotype, to be confirmed in larger patients series and by functional evidence. We are currently studying a further cohort of 12 MFMs cases by WES analysis and pathway-driven candidate genes interrogation. We propose that these approaches may be the ideal tools for gene identification in MFM and other similarly clinically/genetically
heterogeneous muscle diseases.

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P13.57

Targeted "ciliome" sequencing for gene identification in nephronophthisis and associated ciliopathies

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Nephronophthisis (NPH) is an autosomal recessive, clinically and genetically heterogenous renal ciliopathy and a major cause of end stage renal failure in childhood. NPH is frequently associated with extra renal defects, retinal degeneration, cerebellar hypoplasia, polydactyly, cystic liver, situs inversus and skeletal anomalies.
To identify new genes underlying NPH and characterize the genetic bases of the phenotypic variability, we have recently developed a targeted high throughput approach allowing exome sequencing of 1200 ciliary genes (SureSelect Agilent technologies and SOLiD5500 XL Platform). We screened 113 patients presenting with isolated (28 cases) or syndromic (85 cases) NPH, in whom NPHP1 deletion, the most frequent mutational event, had been excluded.
Two pathogenic mutations in known ciliopathy genes were identified in 54 patients (48%). A major result is that all the genes encoding components of the intraflagellar retrograde transport (6 IFT-A genes and DYNC2H1) are frequently mutated (20 patients, i.e. 18%). Moreover, we identified 20 new

candidate genes, based on the presence of homozygous or compound mutations predicted to be damaging (frameshift, stop, missense) in 25 patients (22%). Among the 36 patients without any causative mutation identified, 14 presented with isolated NPH.
In conclusion, this method proved successful for both identifying mutations in already known ciliopathy genes as well as in novel candidate genes and to highlight the phenotypic variability associated with mutations of the same gene. Further analysis of the ciliome data will allow us to characterize the epistatic effect of mutations that could explain the phenotypic variability.

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P13.58
Identification and characterization of a new candidate gene for steroid resistant nephrotic syndrome

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Nephrotic syndrome (NS) is characterized by consistent proteinuria, oedema, hypoalbuminemia, and it can be classified as steroid-sensitive (about 90%) or steroid-resistant (SRNS-about 10%). To date, mutations in at least 15 genes have been found to cause SRNS and although mutations in *NPHS1* or *NPHS2* are frequent causes of children SNSR, mutations in other genes are very rare. In addition, multiple allelism and heterogeneity together with a phenotypic clinical overlap require an extensive mutational analysis effort to identify the molecular aetiology. To identify SRNS by molecular diagnosis has important clinical implication, as this would prevent unnecessary administration of corticosteroids and immunosuppressants.
We performed targeting and whole-exome (re)sequencing in 25 probands with a diagnosis of a paediatric SNSR (including 25 subjects steroid-sensitive). Because mutations in several genes have been demonstrated to lead to familial or sporadic SNSR, we focused our attention on these genes and on those candidates potentially implicated in the pathogenesis of the disorder. We identified a putative new candidate gene that may allow to define a novel clinical entity in the field of genetic disorders (hyperactivation of this gene was described to cause NS in podocyte-specific transgenic mice). Definition of the causative role of these mutations would represent the first case of an defect leading to a disorder where SRNS is part of a more complex clinical syndrome including also immunologic alterations. The results of our work may lead to the identification of a new causative gene for SRNS and provide definition of a previously unidentified clinical syndrome

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P13.59
Next generation sequencing for neurofibromatosis type 1 molecular diagnosis

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Neurofibromatosis type 1 (NF1) is a tumor predisposition syndrome with a worldwide birth incidence of 1 in 2500. NF1 is caused by dominant loss-of-function mutations of the NF1 gene (Neurofibromin 1). NF1 is a large gene containing 60 translated exons over ~280 kb. Most NF1 patients have private loss-of-function mutations scattered along the NF1 gene. Here, we present a NF1 targeted next generation sequencing (NGS) strategy developed at the Cochin Hospital (AP-HP, Paris, France). The custom primers panel targeting the entire NF1 coding exons and the 5' and 3' UTRs were designed using the AmpliSeq Designer. The targeted region (~12 kb) was amplified by 149 Amplicons of 200 bp using 20 ng of genomic DNA. NGS libraries preparation was performed using the Ion AmpliSeq Library Kit 2.0. Template-positive bar-coded samples were loaded on Ion 314 chips and sequenced with an Ion PGM Sequencer (Lifetechnologies). The 314 chip capacity allowed mixing 22 bar-coded samples, providing a 150X average coverage. Data were reanalyzed using the Torrent Suite 3.4. Sequence alignment and extraction of SNPs and indels were performed using the Variant Caller plugin on the Ion Torrent Browser. DNA sequences were visualized using the Integrated Genomics Viewer (IGV) from Broad Institute. Identified mutations were confirmed using Sanger sequencing. We validated this approach by testing 50 NF1 mutated DNA samples previously characterized by means of Sanger sequencing at DNA and cDNA levels. A prospective NGS study of 200 NF1 patients will confirm this method as a reference for routine molecular diagnosis.

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P13.60
Whole exomic next-generation DNA sequencing from dried blood spot DNA

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Efficient DNA sequencing approaches, such as next-generation sequencing (NGS), allows for simultaneous re-sequencing of many genes of interest (up to all genes in the genome) with greater speed and lower cost than by other methods. However, studies of applying this technology to dried blood spots (DBS) have not been completed. Here, we describe a comparative whole exome sequence (WES) analysis of DNA isolated from DBS and peripheral blood from the same individual. DBS DNA was sequenced on MiSeq (Illumina Inc.) platform using the Nextera Enrichment DNA Sample Prep Kit (Illumina Inc.). DNA from whole blood was sequenced on HiSeq 2000 (Illumina Inc.) with SureSelect V4 capture kit (Agilent). Although data obtained from the HiSeq had much higher read output, both sample types generated good quality data with high alignment percentages and the DBS enrichment was possible as seen by the high percentage of mapped reads (in capture region) numbers (97.7%). When assessing regions that were common to both capture kits (~31 Mb), over 95% of a total of 5,000-6,000 identified variants were identical between the studied sample types. Our preliminary data suggests that DNA isolated from DBS can be processed through our NGS system and results in accurate and specific reads. Moreover, the data imply that DBS are potentially a viable option for performing WES experiments with possible application for newborn screening. For our future studies, we propose to use the HiSeq2000 as sequencing platform as it results in a greater depth of coverage greater reliability.

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P13.61
Adult patients with normal intellectual ability experience high satisfaction and low distress after diagnostic whole-exome sequencing: Preliminary results.

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Background: Our Human Genetics department is one of the first offering diagnostic two-step whole-exome sequencing (WES). This study aimed to evaluate acceptance, risk perception and distress of WES in adults.
Patients/Methods: Between August 15th 2011 and June 20th 2012, 213 patients were offered diagnostic WES for colorectal/kidney cancer <40 years (n=51), deafness (n=60), blindness (n=41) or movement disorders (n=61), 177 (83%) accepted. Baseline questionnaires including risk perception and heredity-specific distress (IES) were sent to 141 adults with normal intellectual ability, 111 (79%) were returned. Follow-up after initial WES-results from disease-related gene sets, including satisfaction, is ongoing (currently n=73).
Results: Baseline responders were diagnosed with: 26% cancer, 27% deafness, 12% blindness, 35% movement disorders. Median age was 49 [22-79], 50% women, 83% with a positive family history. At follow-up, diagnostic suggestive mutations were found in 26%. Nearly all responders (94%) were satisfied with WES. Heredity risk perceptions were similar in baseline and follow-up: believing heredity caused their disease (77% and 69%) and expecting WES to find a genetic cause (51% and 46%). However, significantly more patients believed chance likely caused their disease (44% versus 58%, p=0.04) and fewer expected incidental findings (27% versus 19%, p=0.08). Heredity-specific distress was reported in only 17% versus 13%.
Conclusion: Acceptance was high in the group offered diagnostic WES after genetic counseling. Adults with normal intellectual disability reported low distress. After initial WES-results, they were highly satisfied. At follow-up heredity risk perceptions were unchanged, while perceptions of random

causality increased.

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P13.62
Application of next generation sequencing for diagnosis of long QT syndrome

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Next generation sequencing (NGS) is changing genetic diagnosis due to its huge sequencing capacity. The aim of this study was to develop an NGS based workflow for routine diagnostics for congenital long QT syndrome. Long QT syndrome is a genetic disease associated to delayed cardiac repolarization that is reflected by electrocardiographic feature of QT prolongation and T wave abnormalities, its predisposition for syncope, seizure, and sudden cardiac death in young individuals with structurally normal hearts. Mutations in 13 genes have been reported causing this pathology but current diagnostic testing is restricted to a small number of genes and 25% remained unresolved. A NGS-based workflow was designed using Haloplex Agilent libraries followed by MiSeq Illumina sequencing. We will describe our approach to the validation of the entire workflow (assay design, sample preparation, bioinformatic analysis and scientific analysis) of a NGS targetted enrichment of the 13 genes known to cause long QT syndrome. The validation process will include: defining minimum coverage criteria, assigning quality and coverage thresholds for SNP calling, defining criteria for filtering of benign polymorphisms and assessing SNP concordance between next generation sequencing and Sanger sequencing. Preliminary data showed that on a training set of 16 DNA samples of patients containing 16 unique pathogenic mutations, all mutations were detected and all samples were enriched with >70 % specificity, resulting in an average depth of coverage exceeding 100 fold after two runs of Illumina MiSeq sequencing. The results for 30 DNA patients negative for the major genes will be presented.

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P13.63
Molecular diagnosis of monogenic diabetes applied to Next-Generation Sequencing

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Monogenic diabetes (MODY) is characterised by autosomal dominant inheritance of young-onset diabetes due to defective insulin secretion. It is both clinically and genetically heterogeneous. The identification of a mutation has important implications for clinical management in diabetes. We co-developed with Multiplicom, a MASTR assay as front-end amplification for NGS based mutation and CNV detection in 7 MODY genes.
The MASTR assay comprises 99 amplicons enabling the amplification of all coding exons of 7 MODY genes (*GCK, HNF1A, HNF4A, HNF1B, ABCC8, KCNJ11* and *INS*) and 21 control amplicons for CNV analysis, all amplified in 5 multiplex reactions.
To verify the MASTR assay workflow, sixteen patients with know mutations were analysed using the standard MASTR protocol followed by sequencing on the 454 GS Junior instrument. The resulting sequencing data were analysed by AVA2 and SeqNext software tools.
We obtained an average of 100.000 reads per run (8 samples) corresponding to on average of 94X coverage per amplicon. All 17 known mutations (10 substitutions, 7 indels), 1 complete gene-deletion and 196 SNP at homozygous or heterozygous state were identified except one subtitution in a low-coverage amplicon and two indels located in homopolymer stretches. However, both indels were identified by GeneScan analysis of the MASTR assay. Based on the current analysis further optimisation of 10 amplicons is required to reach sufficient (40X) individual amplicon coverage for all amplicons with an average coverage of 100X.
In conclusion, the MASTR assay based NGS workflow seems well suited for the routine diagnosis of monogenic diabetes genes.

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P13.64
Next Generation Targeted Amplicon Resequencing with Long Sanger-Like Read Lengths on the GS FLX+ System

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The Roche 454 GS FLX+ System features the unique combination of long reads, exceptional accuracy and high-throughput, making the system well suited for genomic projects that require large quantities of long reads such as *de novo* whole genome sequencing and assembly of large, complex organisms. With the launch of v2.9 system software, the long read and high accuracy sequencing capabilities will be available for amplicon applications. Here we describe our current development program leveraging an acyclic nucleotide flow pattern, Flow Pattern B, for highly accurate targeted sequencing of amplicons 700-800 base pair and longer. The longer reads allow more direct transition of existing Sanger amplicon designs to massively parallel sequencing with minimal re-design, direct haplotype phasing across longer spans, and improved identification of complex genetic variations including large insertions, deletions and block substitutions. The technique is also promising for metagenomic studies targeting 16S and 18S rRNA subunits by allowing full coverage of 6-7 variable regions in a single uni-directional amplicon read which enables the investigator to generate accurate diversity and abundance profiles. The advancements in long read sequencing will be applied to the GS Junior System as well.

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P13.65
Targeted Next Generation Sequencing (NGS) for the molecular diagnosis of Autosomal dominant polycystic kidney disease (ADPKD)

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenic disorder, with an estimated prevalence ranging from 1/500 to 1/1000.
Mutation-based diagnosis in ADPKD is complicated by genetic and allelic heterogeneity, with 2 genes involved, *PKD1* and *PKD2*. Moreover, *PKD1* is duplicated over 2/3 of its length and is extremely rich in pyrimidic nucleotides.
Since a few years, NGS technologies enable us to overcome the classical approaches of whole coding sequence sequencing at single nucleotide resolution.
The aim of this study was to develop and validate a strategy to analyse both genes by Next generation sequencing (NGS) with the IonTorrent technology (Life Technologies).
As the complexity of the two genes renders impossible the use of target enrichment, we develop a home-made long range (LR) PCR strategy, followed by fragmentation and multiplexing to build our libraries. Thanks to PCR optimization, accurate quantification of our amplicons, we obtained a good coverage of the two loci and a depth generally consistent between each LR amplification for a multiplexing of 16 samples per run.
We used this approach to characterize the molecular defects in a series of DNA from patients with typical ADPKD carrying known variants (mutations and polymorphisms) previously identified by Sanger sequencing.
We identified an average of respectively 14 and 2 variations in *PKD1* and *PKD2* genes and all the molecular abnormalities, with the exception of one mutation affecting an homopolymer repeat were found.
In conclusion, NGS is a promising strategy for ADPKD molecular diagnosis as targeted therapies are under development.

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P13.66

A diagnostic strategy based on Next Generation Sequencing

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Next Generation Sequencing (NGS) technologies are revolutionizing Genetics and Medicine reducing cost and timeline. Nevertheless their clinical utility still remains somewhat limited. Reduced sensibility compared with Sanger sequencing (gold standard), high volume of data and elevated number of incidental findings are some of the main obstacles. Our main objective is to develop a NGS based strategy that fulfills the following requirements: a) high sensibility comparable with Sanger, b) 100% representation of the regions of interest (ROI) and c) reduction of the information without clinical utility.
We have developed a protocol based on the specific amplification by PCR of coding regions of genes with clinical association and sequencing in a MiSeq Personal Sequencer. This protocol has been validated by comparison with the current gold standard sequencing technology, Sanger sequencing, in more than 900 nucleotide variations: substitutions and small indels, in homozygosis, heterozygosis or hemizygosis.
This strategy NGS based shows a specificity and robustness comparable with Sanger sequencing and a higher sensitivity detecting mosaicisms. The specific design, high coverage and sensitivity and the complete representation of the ROIs provide to this strategy a high clinical utility making possible its application at diagnostic level.

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P13.67

CORIMAGEN: Advanced diagnostics of cardiovascular risk and sudden death prevention in paraffin tissue.

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Objectives: To analyze the accuracy of NGS in paraffin tissue for the study of genetics diseases in specific cases. We have developed a conceptual model for a complete genetic test in Sudden Death (SD) prevention.
Methods: NGS analysis was performed sequencing 72 genes related to cardiomyopathy and SD. FFPE spleen tissue and blood samples were collected at the postmortem examination. Autopsy reports indicated Arrhythmogenic Cardiomyopathy (AC) as a tentative cause of death. NGS was performed in SOLiD v4. Bioinformatic analysis was carried out according to our pipeline and pathogenic variants were then confirmed by Sanger.
Results: Firstly studied the correlation between fresh and FFPE tissue and we found a 76% of correlation. In first patient, we found a variant in *TMEM43* in both FFPE tissue and blood DNA. This has been previously described as a pathogenic mutation associated with AC. It was only found in the index case, suggesting its involvement in this cardiomyopathy. Second case we found two uncertain variants in *TTN* and *CACNB2*, which were detected also in both samples. Several healthy members also carried these variants suggesting a) these variants are no pathogenic b) One of them is pathogenic although with incomplete penetrance.
Conclusions:
-NGS in FFPE shows a high concordance with fresh tissue 76%
-We have detected the same variants in fresh and FFPE tissue.
-Additional studies are necessary.
-NGS is suitable for the study of FFPE tissue samples in specific families and circumstances.

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P13.68

Comprehensive massive parallel DNA sequencing strategy for the genetic diagnosis of the RAS/MAPK related syndromes

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The Noonan, Cardio-facio-cutaneous, Costello and LEOPARD syndromes are members of the RASopathies. Mutations in 11 genes have been causally linked to these disorders. Recently, an exome sequencing study also associated the gene *A2ML1*. Due to the genetic and clinical heterogeneity of these disorders it is challenging to define straightforward strategies of gene selection for their molecular diagnosis. Therefore, the aim of this study was to develop and validate a massive parallel sequencing (MPS) based strategy for the molecular diagnosis of RASopathies.
Genomic DNA of 50 clinically defined cases of RASopathies, including 3 suspected cases of prenatal diagnosis were used to optimize, validate and evaluate the new MPS methodology. A multiplex PCR-based strategy for the enrichment of the 12 genes and a dedicated variant prioritization pipeline was established. All samples were studied using both Sanger sequencing and Ion Torrent PGM.
All variants identified by Sanger sequencing were detected with our MPS approach. The most frequent mutated gene was *PTPN11* (n=15). Mutations in less frequent genes, like *CBL* (n=3), *SHOC2* (n=1) and the novel *A2ML1* (n=2) were found. The methodology resulted in an experimental approach with a specificity of 99.7% and a maximum analytical sensitivity ≥97.7% with a confidence of 95%.
In conclusion, here we present a workflow that provides a comprehensive genetic screening tool in patients with RASopathies in a fast and cost-efficient manner. This approach demonstrates the potential of a combined MPS-Sanger sequencing based strategy as an effective diagnostic tool for heterogeneous diseases and for prenatal genetic testing.

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P13.69

A novel nonsense mutation in *EDA* associated with nonsyndromic tooth agenesis

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Mutations in the ectodysplasin-A (*EDA*) gene have been generally associated with X-linked hypohidrotic ectodermal dysplasia (XLHED), which is characterized by skin lesions, sweat gland dysfunctions, and defective morphogenesis of teeth, hair, and nails. Recently, missense mutations in *EDA* have been reported to cause familial nonsyndromic tooth agenesis. In this study, we report a novel *EDA* mutation in an Estonian family segregating nonsyndromic tooth agenesis with variable expressivity. Affected individuals had no associated defects in other ectodermal organs. Using exome sequencing capture, we identified a heterozygous nonsense mutation c.874G>T (p.Glu292X) in the TNF homology domain of *EDA* in all affected females. This protein-altering variant has arisen *de novo* and the potentially causative allele was transmitted to affected offspring from affected mother. Glu292 is a highly conserved residue located on the outer surface of the EDA protein. We suggest that dental phenotype variability described in heterozygous female carriers of *EDA* mutation may occur because of the differential pattern of X-chromosome inactivation, which retains certain level of EDA-receptor signaling in tissues involved in tooth morphogenesis, resulting in tooth agenesis rather than XLHED.
The present study broadens the mutation spectrum for this locus and demonstrates that *EDA* mutations may result in nonsyndromic tooth agenesis in heterozygous females.

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P13.70

Use of next-generation sequencing for diagnostics of connective tissue disorders

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Connective tissue disorders (CTDs) are a candidate for application of next-generation sequencing (NGS) in the DNA diagnostics setting because of the large size and large number of genes involved. The complex spectrum of, often overlapping, phenotypes, makes differential diagnosis difficult. For familial thoracic aortic aneurysms (FTAA), Marfan syndrome (MFS), Ehlers-Danlos syndrome (EDS) and Osteogenesis imperfecta (OI), more than 40 genes have been described. We currently perform approximately 2000 genetic analyses per year for patients suspected of a CTD. To improve diagnostics, we designed a platform for the parallel analysis of a set of CTDs, including all known genes for FTAA, MFS, EDS and OI.
A solution-based target enrichment kit was designed to capture all exons and flanking splice sites of 42 genes involved in CTDs. Sixty CTD patients, previously tested by Sanger sequencing and/or MLPA, were sequenced in multiplex on two separate lanes of a HiSeq2000 run. Subsequently, all samples were analyzed using a home-made pipeline, which specifically analyses six gene panels depending on the phenotype.
In the CTD patients, if coverage met our quality criteria (~97%), all mutations previously identified with Sanger sequencing were detected. Low coverage fragments (~3%) will be analyzed by Sanger sequencing analysis.
We offer the NGS CTD kit as a diagnostic service since January 2013. The NGS methodology offers advantages in terms of time-efficiency, especially in CTD with a complex spectrum of phenotypes and difficult differential diagnosis. Furthermore, this approach offers the possibility to associate mutations in known genes with novel, CTD related phenotypes.

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P13.71

Mutations of PKD1, PKD2 and PKHD1 genes in families with polycystic kidney disease in the Czech Republic

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary renal disorder caused by mutations of *PKD1* and *PKD2* genes and affecting approximately 1 in 500-1,000 births. ADPKD is a systematic disorder causing decline in renal function which often results in renal failure in adulthood.
The autosomal recessive form of polycystic kidney disease caused by mutations in the *PKHD1* gene is less common than ADPKD but it usually presents in early childhood when up to 50% of affected neonates die of pulmonary hypoplasia.
The aim of this work is the optimization of the molecular methods to provide reliable and fast presymptomatic, prenatal and preimplantation diagnostics of polycystic kidney disease. Presymptomatic DNA analysis has been performed in our laboratory for over 20 years by the linkage analysis. Nowadays the analysis performed within research projects also includes detection methods as heteroduplex analysis, Multiplex Ligation-dependent Probe Amplification and high resolution melting analysis. Recently we have added the next generation sequencing as well as a new mutational detection method in the *PKHD1* gene which is too complex for conventional detection methods.
So far, mutations of the PKD genes have been detected in more than 50% of 150 Czech families with ADPKD. Most mutations were unique for Czech population. Determination of localization and type of mutations within the *PKD* and *PKHD1* genes and their genotype-phenotype correlation improves DNA diagnostics together with the assessment of the clinical prognosis of patients.
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P13.72

PhenoVar: a phenotype-driven analysis of exome sequencing data - proof of principle.

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BACKGROUND: Widespread clinical utilization of Exome Sequencing (ES) requires an approach addressing issues such as incidental findings and accurate prediction of causal mutations among massive amount of variations. We propose a phenotype-driven analysis of exome data to predict diagnosis in the clinical setting.
METHODS: Control exome-files, modified to include a previously published pathogenic mutation, were combined to the corresponding published phenotypic data of ten test patients with varied polymalformative syndromes and were used to test the efficiency of our software, PhenoVar, in predicting their diagnoses. For each test patient, a geneticist blinded to the diagnoses reviewed the phenotype and selected 3 traits using terms in Human Phenotype Ontology. Modified exome variants file was input along with selected phenotypic traits in PhenoVar. PhenoVar calculated phenotypic similarity between the test patient and a database consisting of simulated patients for each OMIM phenotype with known molecular basis. Final diagnostic score assigned to a given OMIM entry took into consideration both the patient's phenotype and mutations. Resulting OMIM entries list was sorted according to diagnostic score and filtered using a minimal phenotypic weight threshold to prevent undesired discovery of incidental findings.
RESULTS: PhenoVar predicted the correct diagnosis in 5 / 10 patients, while in 8 / 10 patients the diagnosis ranked within top 5 predicted most likely diagnoses and within top 20 in all. No incidental findings were found using our minimal phenotypic threshold.
CONCLUSION : Results suggest that this "phenotype-driven" approach could render widespread use of ES more practical, ethical and clinically useful.

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P13.73

Homozygous frameshift deletion of *KIAA1279*, encoding for KIF1B-binding protein, results in autosomal recessive polymicrogyria

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Polymicrogyria (PMG) is a clinically heterogenous malformation of cortical development, characterized by a loss of the normal gyral pattern which is replaced by many small and infolded gyri separated by shallow sulci that are partly fused in their depths. Causes of PMG are heterogeneous and include acquired and genetic causes. There are more than 100 syndromes possibly associated with PMG but mutations in specific genes such as *SRPX2*, *GPR56*, *TUBB2B*, *TUBB3, NHEJ1*, *TUBA1A, TUBA8* and *WDR62* have been reported only in a minority of patients.
Nonsense mutations in the *KIAA1279* gene known to encode the KIF1B-binding protein, were initially described in patients with Goldberg-Shprintzen syndrome (GOSHS, MIM 609460).
By combining whole genome genotyping using single-nucleotide polymorphism (SNP)-array and whole exome sequencing, we identified a new pathogenic mutation in *KIAA1279* in two fetuses with severe PMG born from a healthy, consanguineous Pakistani parents. It consists of a frameshift deletion of 2 exons leading to premature termination of the translation. By detailed phenotypic analysis of both fetuses, we found that this homozygous mutation in *KIAA1279* gene causes PMG with microcephaly, but no additional sign suggestive of GOSHS.
This study expands the spectrum of KIF1B-binding protein related disorders and supports its critical role in neuronal migration and organization. Furthermore, the accurate molecular diagnosis of this family exemplifies the power of the combined strategy -SNP-array and exome sequencing- in the diagnosis of Mendelian disorders and allows us to broaden and refine our understanding of *KIAA1279*-related disorders.

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P13.74

Involvement of HYDIN in primary ciliary dyskinesia unveiled by a series of exome pitfalls

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Primary ciliary dyskinesia (PCD) are rare autosomal recessive disorders resulting from structural/functional defects of motile cilia, leading to recurrent respiratory infections. To identify new molecular defects involved in PCD, we combined homozygosity mapping and whole exome sequencing (WES). 3,953 sequence variations were identified in the homozygous regions of a consanguineous patient with a typical clinical PCD phenotype and subtle ultrastructural defects (i.e. abnormal central complex sheath). These include a frameshift (c.11709delT) in exon 69 of HYDIN, a gene that has just recently been involved in PCD. Although already described as SNP, we paid particular attention to this variation, given its presence in the homozygous state in the patient and apparently in the heterozygous state in all controls tested. The existence of a 99%-identical pseudogene of HYDIN (HYDIN2) led us to look for HYDIN variations with a 0.5 frequency and to note that at least one third of the so-called "SNPs" (including c.11709delT) are not true polymorphisms but represent sequence differences between HYDIN and HYDIN2. The apparent homozygosity of those fake "SNPs" in the patient revealed a homozygous intragenic deletion involving exons 68 to 71 of HYDIN (c.11469-750_12126+181del4046bp). As subsequently shown by the analysis of HYDIN transcripts obtained from nasal brushing, this deletion, which results in shorter transcripts, leads to a frameshift and a premature stop codon (p.Phe3824SerfsX20). This study, which underlines the role of HYDIN in the pathogenesis of PCD, illustrates the importance of cautious analysis of sequence variations identified by WES, in order to thwart multiple possible exome pitfalls.

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P13.75

Identity-by-Descent Mapping and Exome Sequencing Reveals a New Candidate Gene for the Primary Congenital Glaucoma locus GLC3E

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Primary congenital glaucoma (PCG) is caused by developmental anomalies of the trabecular meshwork and the anterior chamber angle, resulting in increased ocular pressure and optic nerve damage from early life. PCG mostly displays an autosomal recessive inheritance. To date, four PCG loci are known (GLC3A-D), with two genes identified, *CYP1B1* and *LTBP2*. Here, we aimed to map the disease gene in a four-generation consanguineous family with PCG from Jordan.
Mutations in known PCG genes were excluded. Identity-by-descent (IBD) mapping was performed in six affected members using genomewide SNP genotyping (250K, Affymetrix). Two affected individuals underwent exome sequencing (TruSeq Exome Enrichment; HiSeq, Illumina; CLC Bio).
IBD mapping revealed a common region of 11.5 Mb on 19p13.2-p13.11, being a new candidate PCG locus named GLC3E. Using IBD filtering of exome data and Ingenuity Variant analysis, we found a homozygous missense variant c.304C>T (p.R102C) in a novel candidate gene for GLC3E. This variant is predicted to be deleterious. It was absent in 718 Caucasian control chromosomes and was found in one out of 156 Jordan control chromosomes, being consistent with its reported minor allele frequency. The potential GLC3E might play a role in Toll- and BMP-signalling pathways. In addition, its mutations might dysregulate mitochondrial complex I. Its expression domain in the eye anterior segment is currently being investigated in zebrafish and mouse.
We identified a new candidate gene for the GLC3E locus, confirming the genetic heterogeneity of PCG, and possibly representing the third PCG gene.

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P13.76

Next Generation Sequencing (NGS) for rare hemochromatosis forms: Ampliseq versus Long-Range PCR

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Rare forms of hemochromatosis are associated with private missense mutations in five iron metabolism genes (*HFE*, *HJV*, *HAMP*, *TFR2* and *SLC40A1*). The Sanger sequencing method is commonly used to track these mutations. However, depending of the strategy used by laboratories, this method can be time consuming and quite expensive. Here, we aimed to explore whether next generation sequencing could improve the molecular diagnosis of hemochromatosis. We also considered the *FTL* gene that has been associated with different forms of inherited hyperferritinemia.
We have compared two strategies with using the Ion Torrent platform (Life Technologies, LT): Ampliseq(LT), which was designed to explore the whole coding sequences, the intron/exon junctions and the 5' and 3'untranslated regions (12.6 kb), and a home-made Long Range PCR(LR) method, which allowed us to explore the six entire loci (74 kb). Variants (mutations and polymorphisms) previously identified by Sanger sequencing were used for validation.
The coverage by Ampliseq is limited to 90.3% and only includes 94.9% of CDS, with a depth consistent between amplicons. The LR-PCR method allows analysing of regions not covered by Ampliseq but differences at depth are observed between LR amplicons. Libraries constructs from the LR-PCR method are two-times longer and require more manual steps.
NGS with using the Ampliseq technology could be used as a first screening method to diagnose rare forms of hemochromatosis and inherited hyperferritinemia without iron overload. Advances in technology should rapidly permit to improve coverage of the coding regions and to get an exhaustive and fully accurate reading.

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P13.77

Gene panel for screening of RASopathies using HaloPlex target enrichment and next-generation sequencing

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RASopathies are a group of clinically and genetically related disorders, including Noonan, LEOPARD, cardio-facio-cutaneous (CFC), Costello, Legius and neurofibromatosis-Noonan syndrome and neurofibromatosis type 1 (NF1). These disorders result from mutations affecting the RAS-MAPK signaling pathway, which explains the clinical overlap within this group of syndromes. In the clinical setting, RASopathy-associated genes are traditionally tested sequentially using Sanger sequencing until a likely causative mutation is identified, which is laborious, time consuming and expensive.
We have utilized the advent of next-generation sequencing technology to develop a cost-effective multi-gene sequencing panel for RASopathies by using HaloPlex target enrichment, sample barcoding and sequencing with MiSeq. Our multi-gene panel targets the coding sequences of *NF1, PTPN11, SOS1, RAF1, BRAF, HRAS, KRAS, NRAS, SHOC2, SPRED1, MEK1, MEK2* and *CBL* (about 40 kb).
To validate the gene panel, 10 patients with known disease-causing mutations associated with NF1, Noonan, CFC and Costello syndrome were investigated. Five samples were pooled and run on a single lane of the MiSeq. Obtained sequences were analyzed using commercial data programs. The gene panel was highly specific; ~93% of sequencing reads aligned to targeted regions. Average read depth in the region of interest (ROI) was >2000 and ~98% of targeted bases in ROI had at least 30x coverage. All known mutations were identified, including a 4 bp deletion and a splice site mutation in *NF1*. Screening of additional RASopathy patients is presently underway.
Conclusively, the validated RASopathy-multi-gene sequencing panel presented here enables rapid and cost-effective high throughput screening of individuals with RASopathies.

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P13.78

Massively parallel sequencing identifies the large majority of mutations causing red blood cell membrane disorders.

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Hereditary disorders of the red blood cell (RBC) membrane constitute a major cause of hereditary hemolytic anemia, and are caused by mutations affecting structural proteins of the RBC cytoskeleton and their anchoring to the RBC membrane. Hereditary spherocytosis (HS) and elliptocytosis (HE) are common abnormalities, hereditary pyropoikilocytosis (HPP) and stomatocytosis (HST) are rare. In most cases the mode of inheritance is autosomal dominant. Traditionally, RBC membrane disorders are classified according to morphological appearance on blood smear. Molecular analysis has long been hampered due to the size of the genes involved, but has become feasible with the advent of Next Generation Sequencing (NGS). We set up and implemented an NGS based test to sequence seven genes associated with RBC membrane disorders: *SPTA1, SPTB, ANK1*, *SLC4A1*, *EPB41*, *EPB42* and *RHAG*. After enrichment of gDNA using a custom Agilent SureSelectXT kit (targeted at 650 clinically relevant genes) exons and flanking intronic sequences were determined using a SOLID™-5500XL system (~92% of bases covered with >99% sensitivity). Causative mutations, most of which were novel, were identified in 16/20 patients with defined membranopathies, predominantly in the *SPTA1* and *ANK1* gene. Surprisingly, no mutations were detected in the *SLC4A1* gene, a major cause of HS. NGS based DNA diagnostics has thus been shown to be a highly reliable and effective method to detect mutations in patients with hereditary RBC membrane disorders. The efficacy on these RBC membrane disorders might well be indicative for other disorders like epilepsy, heart and kidney diseases using the same custom enrichment kit.

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P13.79

Next generation sequencing in renal disorders: molecular and clinical aspects of renal tubular acidosis

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Molecular genetics has allowed the identification of the structure and function of the several main transporters and ion channels involved in renal disorders. Between that, distal renal tubular acidosis (dRTA) received increased attention because of advances in the understanding of the molecular mechanism. We studied 90 subjects with dRTA (where possible also their families) analyzing the *SLC4A1/ATP6V1B1/ATP6V0A4* genes and we focused on the clinical features and their correlation with the different types of mutations. Furthermore, in some of our cases we reported the association with medullary sponge kidney (MSK), a rare congenital renal disorder, characterized by diffuse ectasia of the tubules precaliceali collecting duct. In some subjects we have found variants in *ATP6V0A4* and *ATP6V1B1* genes, providing evidence of a possible role of the subunits B1 and a4 apical pump H^+ATPase in the pathogenesis of the MSK. Of all the individuals tested, 35 were negative after molecular analysis of the genes above cited and then we polarized our efforts to characterize from a clinical-molecular point of view families affected by RTA applying next generation sequencing strategy. We decided also to analyze genes encoding for SLC4A4/NBCe1, for ENaC subunits, those responsible for PHA2 and for the other the V-ATPase subunits and other pathway's genes that for us may be responsible of this genetic disorders (SLC26A11 and SLC4A9 subunits, SLC4A7 and SLC4A8 transporters, isoforms of the carbonic anhydrase, various G-protein-coupled receptors activated by protons or divalent cations). We will show the results of this new pilot study.

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P13.80

Next generation sequencing analysis of Inherited Retinal Dystrophies (IRD) using Agilent Haloplex technology.

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Introduction: IRD is a common cause of vision loss (population prevalence ~ 1/3500) with significant genotype and phenotype heterogeneity. Genetic testing in clinical diagnostics has been limited because a similar clinical phenotype may be caused by mutations in numerous genes, all of which could require testing. Targeted gene capture and Next generation sequencing has provided the opportunity to investigate multiple genes per patient. We have designed a targeted retinal panel for 45 common causative genes and one deep intronic splice site associated with retinal degeneration.
Method: Our retinal panel workflow uses Agilent's Haloplex enrichment technology with Illumina's MiSeq platform. A bioinformatics pipeline has been designed to annotate and filter our results; using SoftGenetic's Next GENe as a variant caller and WAnnovar for annotation. Variants identified at a frequency of <5% in EVS and 1000 genomes are taken forward for literature investigation.
Results: Each MiSeq run produces > 4Gb of data and our validation experiments indicate that 96-98% of bases are covered at a minimum of 30 reads. Within the first panel of 10 patients, a pathogenic homozygous frameshift in AIPL1 was found in a Lebers congenital amaurosis (LCA) patient, whilst a second patient, with Usher II, had a pathogenic premature stop in the USH2A gene.
Conclusions: The Haloplex protocol is a fast, simple and efficient workflow that is suitable for use in a diagnostic setting for large gene screens in heterogeneous conditions.

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P13.81

Application of Whole Exome Sequencing (WES) for the genetic characterization of families with an unusual phenotype of autosomal dominant Retinal Dystrophy (adRD)

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Autosomal dominant retinal dystrophies (adDR) are a group of inherited diseases clinically and genetically heterogeneous. Late Onset Retinal Degeneration (LORD, MIM# 605670) is a rare subtype of adDR, which can be caused by the mutation p.Ser163Arg in C1QTNF5 gene. Patients with this mutation present a phenotype characterized by late onset with nictalopia and drusenoid deposits at the beginning, followed by midperipheral deposits, chorioretinal atrophy and electroretinographic reduced b wave.
Three members of family with and initial diagnosis of Retinitis Pigmentosa (RP) and/or Macular Degeneration (MD) and six healthy relatives were studied using Whole Exome Sequecing (WES). adDR common mutations were previously screened and discarded by adDR microarray (Asper Biotech) and Sanger sequencing for the most frequent genes responsible for adDR.
WES analysis identified the mutation p.Ser163Arg in C1QTNF5 gene in heterozygosity in all the affected members of the family.
The further screening of this mutation by Sanger sequencing in a cohort of 200 non-characterized adDR patients enabled the characterization of an additional family with a similar phenotype.
The follow up of patients is important for a precise diagnose on adDR families. The evaluation of new strategies for analysing infrequent adDR genes should be considered on patients with complex or unusual diagnose in order to exclude adDR known genes before performing WES.

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P13.82

Next-generation sequencing blurs phenotype limits

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Inherited retinal diseases (IRDs) are probably the most genetically heterogeneous disorders in man. To date, more than 190 genes have been identified and the number of loci that influence phenotype variability is expanding. In consequence, the diagnosis is not always easily performed due to phenotypic and genetic overlap. Current diagnostic approaches have been focused on the systematic evaluation of a set of known genes for each phenotype but this approach may fail in patients with inaccurate diagnosis. To overcome these limitations, we conducted an exome sequencing approach for the diagnosis of 7 unsolved families clinically diagnosed of autosomal recessive Retinitis Pigmentosa (arRP). In total, 28 samples were sequenced on SOLID 5500xl platform and stringent bioinformatic filters were applied. The most likely disease-causing variants were validated by Sanger sequencing and potential pathogenicity was assessed by functional predictions and frequency in controls. As a result, 10 likely pathogenic variants (6 novel) were detected in 6 out of 7 families. In two presumed arRP families, syndromic retinal disease genes were implicated. Clinical revaluation showed extraocular features that were overlooked at the time of first diagnosis. In other case (a sporadic RP patient) the inheritance pattern assigned was arRP (the most frequent one for sporadic RP cases), explaining why the disease-causing mutations were not found before. Our results reinforce the role of exome sequencing in the diagnosis of retinal diseases as a global approach even when clinical diagnosis or presumed inheritance pattern were not accurate. Supported by ISCIII (PI11-02923), FRA (CIVP16A1856) and CEICE (CTS-03687).

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P13.83

AmpliSeq™ RNA: Targeted sequencing of genes on the PGM ™

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As Next Generation Sequencing matures, it is quickly moving into translational research applications where it has promise to be a useful tool for evaluation of human samples. RNA profiling using NGS (RNA-seq) is one of the applications where this potential is currently being realized. RNA-seq experiments have traditionally started with a whole-transcriptome library preparation that produces a sequencing template from all RNA species in a sample. However, in many cases, only a handful of the genes present are necessary to make a clinically relevant diagnosis.
We have demonstrated new technology that allows for RNA-seq from a panel of directed amplicons using an AmpliSeqTM approach with Ion Torrent semiconductor sequencing. This approach offers many advantages over microarray or qPCR such as faster turnaround and data analysis, sample multiplexing, lower RNA inputs, and ability to use degraded or FFPE-derived samples. In addition, the technique simultaneously provides quantitative gene expression information and gene sequence at the single nucleotide level.
For this targeted RNA-Seq method, cDNA is generated from total RNA, followed by amplification using primers designed for targeted genes. Resulting amplicons are prepared for sequencing using the AmpliSeqTM technology and sequenced on the Ion Torrent sequencing platforms. We demonstrate that the technique produces results that are technically reproducible, quantitative, and have excellent correlation with qPCR using TaqMan® gene expression assays. Employing barcodes, we have also tested multiple samples on a single chip thereby increasing the cost-effectiveness of the tool for higher throughput laboratory settings.

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P13.84

Unraveling the cause for familial sudden cardiac death by whole-exome sequencing (WES) - an approach

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Sudden cardiac death (SCD) in the youth is a quite heterogeneous, mostly genetic disorder. In a large family with six victims of SCD (5 males/1 female, age of death 20 +6 years) WES was performed (Agilent SureSelect capture, Illumina sequencing) in an individual with stress induced ventricular tachycardia. A mean reading depth of the targeted sequences of 130x was reached (coverage: 92% >10x). Overall, 2008 variants were rare (MAF<1% in 1000 Genomes Project) and filtered for presence in 392 selected genes associated with inherited heart disorders. Hereafter, 8 (0.3%) coding variants remained (6x ns, 1x splice-site, 1x del). After analysis for evolutionary conservation, pathogenicity prediction (PP) and presence in the ESP database, 3 out of 8 (RYR2, RYR3, PLEC1) were checked for familial co-segregation. The RYR2 variant c.1082G>A (p.Cys361Tyr) is novel and likely to be causative, since sequence alignment showed high conservation, absence in databases and PP mostly (6:1) as 'deleterious'. Mutations in cardiac ryanodine receptor gene cause catecholaminergic polymorphic ventricular tachycardia (CPVT1). This RYR2 variant was also present in 5 additional family members (one SCD victim). The variants in PLEC1 c.12741C>G, p.Asp4247Glu) and RYR3 (c.7604T>C, p.Leu2535Pro) also affected highly conserved amino acids; PP was not conclusive (PLEC1: 2:3; RYR3: 4:1) and no clear clinical cosegregation for the RYR3 variant (SCD victim negative). The observation that in this large SCD family some relatives also were at cardiac risk may indicate presence of other gene variants causing a confounding overlay. This would have been undetected in a smaller family set.

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P13.85

Spinal Muscolar Atrophy - NGS targeted resequencing of the entire SMN2 gene

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Genetic heterogeneity of individuals highlights the need to enhance personalized medicine to achieve effective treatments of human diseases. Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease characterized by degeneration of α-motorneurons in the spinal cord. The primary SMA-determining gene is SMN1, absent in about 95% of patients. The neighbouring nearly identical SMN2 gene fails to generate adequate levels of full-length SMN protein (FL-SMN). Still, SMN2 copy numbers can vary between 1 and 6, potentially modifying the severity of the disease. However, all SMN2 alleles are not functionally equivalent since they produce FL-transcripts with different efficiencies, most probably due to variations in their sequence.
We established a new method to identify genetic polymorphisms in the complete genomic region of SMN2. We resequenced 40 SMN2 gene copies from 12 SMA subjects with varying SMN2 copy numbers using the Illumina platform and a pooled indexing strategy. We identified a total of 69 SNPs and 40 INDELs out of which 21 and 37, respectively, had not been reported previously. So far, we confirmed by genotyping assays the first fifteen SNPs that were chosen for validation. The estimated variant frequencies are usually indicative of the number of variant gene copies per subject when related to the individual's SMN2 copy numbers.
The method we described is ready to be used to identify variants/haplotypes associated with a particular SMA phenotype.

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P13.86

Proximal monosomy of 13q due to 6;13 translocation

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Chromosome 13q deletion is associated with varying phenotypes, which seem to depend on the location of the deleted segment. Although various attempts have been made to link the 13q deletion intervals to distinct phenotypes, there is still no acknowledged consensus correlation between the monosomy of distinct 13q regions and specific clinical features. Here we describe a case of proximal monosomy 13q in a 8 month-old boy. He was referred for cytogenetic study due to different dysmorphic features involving a brachydactily, beaked nose, high frontal hairline, flat occiput, wide nose bridge, epicantus along with double sided hydronephrosis and non-closed Bottalo's duct. Karyotype revealed the translocation 45,XY,t(6;13)(p25;q12)dn. Firther Affymetrix Genome-Wide SNP Array 6.0 analysis has revealed approximately 6Mb deletion on chromosome 13(13q11-13q12.12)(chr13:19045719-25196912, GRCh37/hg19) in the patient's genome. No deletion was detected on chromosome 6. However, we cannot excluded that there might be subtelomeric deletion of 6p, which was not detected by any SNP on the array platform due to highly repetitive region. We assume that the input of the 6p deletion might be insignificant comparing with 13q proximal deletion, which includes a number of reliable genes: for example GJB6 is associated with autosomal dominant deafness; FGF9 is associated with multiple synostoses syndrome, which also includes deafness; SGCG associated with muscular dystrophy limb girdle type 2C; and SACS is casing spastic ataxia Charlevoix Saguenay type. Thus, the child may be under a high risk of having deafness, muscular dystrophy and ataxia based on the deleted regions.

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P13.87

Dissecting the genotype in syndromic intellectual disability using whole exome sequencing in addition to genome-wide copy number analysis

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When a known microimbalance affecting multiple genes is detected in a patient with syndromic intellectual disability, it is usually presumed causative for all observed features. Whole exome sequencing (WES) allows questioning this assumption. In this study of three families with children affected by unexplained syndromic intellectual disability, genome-wide copy number and subsequent analyses revealed a *de novo* maternal 1.1Mb microdeletion in the 14q32 imprinted region causing a paternal UPD(14)-like phenotype, and two inherited 22q11.21 microduplications of 2.5Mb or 2.8Mb. In patient 1 carrying the 14q32 microdeletion, tall stature and renal malformation were unexplained by paternal UPD(14), and there was no altered *DLK1* expression or unexpected methylation status. By WES and filtering with a mining tool, a novel *FBN1* missense variant was found in patient 1 and his mother, who both showed clinical features of Marfan syndrome by thorough anthropometric assessment, and a novel *EYA1* missense variant as a probable cause of the renal malformation in the patient. In patient 2 with the 22q11.21 microduplication syndrome, skin hypo- and hyperpigmentation and two malignancies were only partially explained. By WES, compound heterozygous *BLM* stop founder mutations were detected causing Bloom syndrome. In male patient 3 carrying a 22q11.21 microduplication inherited from his unaffected father, WES identified a novel missense variant in the *OPHN1* X-linked intellectual disability gene inherited from the unaffected mother as a possible additional cause for developmental delay. Thus, WES seems warranted in patients carrying microdeletions or microduplications who have unexplained clinical features or microimbalances inherited from an unaffected parent.

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P13.88

Targeted Next Generation Sequencing of TAAD genes using DNA isolated from paraffin embedded tissue

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Thoracic Aortic Aneurysms and Dissections (TAAD) may occur isolated or as part of syndromes, like Marfan or Loeys-Dietz syndrome. Mutations in several different genes are known to cause TAAD. Diagnosis is often made during autopsy. If available, paraffin embedded tissue can be used for *post-mortem* genetic testing. However, DNA extracted from this tissue is often degraded. Since next generation sequencing (NGS) with the Illumina MiSeq platform requires DNA fragments around 300 base pairs, it should be possible to analyse DNA isolated from paraffin embedded tissue with this platform.
We isolated DNA from paraffin embedded tissue from six deceased TAAD. Library preparation was carried out using a customized TruSeq Custom Amplicon Kit from Illumina. Sequencing was performed with the Illumina MiSeq.
185 exons of the genes *ACTA2*, *COL3A1*, *FBN1*, *MYH11*, *SLC2A10*, *SMAD3*, *TGFBR1*, and *TGFBR2* have been analysed. The results showed that the quality of the sequences depended directly on the quality of the isolated DNA. When the fixation of the tissue was poor (e.g. unbuffered formalin, no homogenous fixation), the coverage was very low. With good fixation, the sequencing results resembled that of DNA isolated from blood. Between 5% (poor fixation of the tissue) and 91% (good fixation/DNA from blood) of the 185 exons had a coverage greater than the threshold that we set at 50x.
In the six analysed patients, we found some new sequence variations with yet unclear relevance.
In conclusion, DNA from paraffin embedded tissue can be analysed by NGS, when the fixation has been done properly.

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P13.89

Mutation analysis of primary immunodeficiency patients by target enrichment and next-generation sequencing

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Primary Immunodeficiencies (PID) are a heterogeneous group comprising over 150 diseases caused by congenital defects of the immune system. These diseases are mainly characterized by severe recurrent infections, and can often be life-threatening. Diagnosing patients with PID is however challenging, since more than 179 genes are known to cause these diseases.
In this study, we adopted a selector-based sequencing capture assay together with Illumina sequencing, to identify disease-causing mutations in all 179 PID genes simultaneously, and evaluated the usefulness of this targeted assay for molecular diagnosis of PID. The exons of the 179 genes were captured and sequenced in 34 patients, 15 of which had at least one known causal mutation at the onset of the experiment.
Sequencing reads were aligned against the targeted regions, and variants were detected using an in-house developed software tool.
In order to narrow down the number of potential disease-causing mutations per patient, variants were filtered according to read depth, variant allele ratio and variant consequence.
Using the applied criteria, 17 out of 21 (~81%) known single base or short indel variants were detected, resolving of the mutation status for 12 out of 15 individuals (80%).
Sequencing of the unknown samples resulted in the discovery of several new mutations that could be confirmed by Sanger sequencing.
We will present the outcome of our mutation analyses, and discuss the usefulness of the developed assay for first tier cost efficient molecular diagnosis of PID, reducing the number of patients subjected to more expensive exome and genome sequencing.

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P13.90

The Shared Variant Database

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DNA diagnostics is based on sharing information on gene variants found in patients with a certain phenotype. Yet, most variants are not shared and valuable information gets lost. This is especially true for exome/genome sequencing projects, where only the results of successful projects and of these only the causing variants identified get published.
We have developed the Shared Variant Database (SVD) to support easy and pre-publication sharing of all variants and phenotypes. Upon submission a report is returned summarizing, per variant, its frequency in the database. To address privacy concerns, data can be submitted as a pooled set. The SVD accepts input in VCF and BED formats for variant calls and coverage respectively. For variants that have a low frequency the submitter has the option to contact submitting colleagues.
The public part of the SVD shows data that were made public by their submitters and summary data from other sources. Variants can be shared with or without links to an individual. Submitters have the option to mark variants that are of special interest. These options should facilitate sharing data of unsolved cases with the intention to initiate a collaboration with others that have similar cases/findings.
An earlier version of the SVD, the Diagnostic Variant Database (DVD), was implemented to support the Dutch medical centers performing exome sequencing, facilitating effective filtering of variants frequently occurring in the Dutch population.

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P13.92

Next Generation Sequencing in molecular diagnostics of previously undiagnosed clinical conditions

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Our project is aimed at identifying the molecular bases of previously undiagnosed genetic diseases in Polish patients by Next Generation Sequencing (NGS), mainly whole exome sequencing and multiplex PCR-NGS.
Whole exome sequencing was performed for DNA samples of nine patients, representing undiagnosed clinical cases, and a healthy control. Exome-enriched libraries were prepared and sequenced using the TruSeq chemistry and GA II or HiSeq 2000 instruments (Illumina). The exome analysis was performed using specialised open access, commercial and company-developed bioinformatic tools. A pipeline was designed for data quality analysis, mapping and SNP/indel detection for diagnostic purposes.
As a result of our studies, a previously described heterozygous mutation in the COL1A2 gene (c.1009G>A) was identified as resulting in osteogenesis imperfecta symptomes in one patient, and its inheritance in the patient's family was analysed. Exome data analysis also allowed us to identify two mutations in the CAPN3 gene responsible for the muscular dystrophy symptoms, indicating the Limb-Girdle Muscular Dystrophy type 2A diagnosis. Both mutations are single nucleotide deletions, leading to frameshifts. The first mutation is one of two most common mutation in the CAPN3 gene (c.550delA), the second mutation (c.1642delC) has never been reported before. Polyphen 2 and SIFT analyses suggested its pathogenicity and possible targeting of improperly terminated mRNA for nonsense mediated decay. The biparental inheritence and carrier status of both mutations in the patient's family were analysed.
The results of our studies allow us to propose whole exome sequencing as a tool that can be applied to diagnose Mendelian disorders.

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P13.93

Whole-exome sequencing in Altaian families with autosomal recessive nonsyndromic hearing loss (the Republic of Altai, Southern Siberia)

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Mutations in more than 70 different genes were found as the causes of hereditary Non-Syndromic Hearing Loss (NSHL). Identification of NSHL-responsible genes is a significant challenge due to extreme genetic heterogeneity of the disorder. Here we explore the utility of whole-exome sequencing (WES) for identifying candidate causal variants in unrelated families from Altai with undiagnosed potentially autosomal recessive NSHL (ARNSHL). We performed WES on 6 siblings, two from each of three Altaian extended families (F#38, F#40, F#54), with proportion of affected to non-affected siblings as 4:5, 4:5, 5:3, correspondingly. Likelihood of mutations compromising *GJB2* gene has been ruled out in these families. Sequence processing and variant calling were performed using standard bioinformatics tools. A custom filtering system was used to prioritize novel variants of candidate genes. We identified a novel missence homozygous mutation (NM_194248.2:c.1111G>C) in gene *OTOF* known in association with ARNSHL (DFNB9) in both affected siblings (F#54) and missence homozygous mutation (NM_030665.3:c.5254G>A) in gene *RAI1* previously associated with Smith-Magenis syndrome (OMIM#182290) and Potocki-Lupski syndrome (OMIM#610883) in four affected siblings (F#38, F#40). Subsequent Sanger sequencing of extended families confirmed cosegregation of homozygous mutations c.1111G>C (*OTOF*) with hearing loss in all affected siblings of F#54, and c.5254G>A (*RAI1*) in all affected siblings in F#38, F#40. Non-affected siblings from all families were either wt/wt or heterozygous for c.1111G>C (*OTOF*) in F#54, and for c.5254G>A (*RAI1*) in F#38, F#40.
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P13.94

NGS diagnosis on Willebrand disease : about a highly homologous pseudogene ?

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Von Willebrand Disease (VWD) is a common autosomally inherited bleeding disorder which results from a quantitative or qualitative deficiency of von Willebrand factor (VWF), a glycoprotein that is essential to primary haemostasis. The VWF gene (VWF) is located on the chromosome 12, resides within 178 kb of genomic DNA and consists of 52 exons which gives rise to a 9kb mRNA. Systematic identification of the causal VWD sequence variations has been hampered by the large size of the VWF, but the main difficulty resides on a 97% homologous partial pseudogene from exon 23 to exon 35 (21kb of genomic DNA). Cost and time consuming of Sanger method sequencing remain a serious obstacle for most diagnostic laboratories. However, Next Generation Sequencing (NGS) have radically changed the technical perspectives over the last years. Then we have planed to test NGS for VWD diagnosis to evaluate the accuracy of this resequencing technique for this highly homologous region. We have selected the Agilent HaloPlex target enrichment system to prepare sequencing library samples running out on illumina MiSeq platform. The HaloPlex probes library design has been made on the Agilent web-base SureDesign , (free software) and comprised VWF exons, introns, 5' and 3' UTR and the known promoter. This design foresee a coverage rate of 95.84% of the whole gene. 48 patients have been tested and raw datas will be analysed and compared to capillary sequencing. Results will be discussed on the ESHG poster.

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P14.01

Rapid sequencing of clinical samples using the Ion Proton™ system

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We are evaluating the Ion Proton™ System (Life Technologies) as a platform for massively parallel sequencing of clinical samples (MPS). This technology has several potential advantages as compared to other platforms for MPS. A sequencing run only takes 3 hours and the mapping and variant calling analysis is completed within 24 hours. Sequencing of human exomes using the Ion PI™ chip typically results in the identification of 60-70,000 SNPs per sample, with ~98% of these overlapping variants reported in dbSNP. We successfully recover known insertions of lengths up to 25 bp and deletions of up to 37 bp. The bioinformatics analysis has been streamlined using in-house database system, based on R and MySQL, where all detected variants from all our exome sequencing runs are stored. This system allows for very efficient filtering of SNPs and indels between any group of samples in a matter a seconds. In addition to exome sequencing, Ion Proton™ is being used for clinical applications such as identification of fusion transcripts from cancer samples, mutation screening using panels of candidate genes and whole-genome sequencing of bacterial or viral human pathogens. The introduction of the Ion PII™ chip will enable whole-genome human sequencing in the time frame necessary to be useful for an increasing range of clinical applications.

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P14.02

A fast solution to NGS library preparation with low nanogram DNA input

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Next Generation Sequencing (NGS) has significantly impacted human genetics, enabling a comprehensive characterization of the human genome as well as a better understanding of many genomic abnormalities. By delivering massive DNA sequences at unprecedented speed and cost, NGS promises to make personalized medicine a reality in the foreseeable future. To date, library construction with clinical samples has been a challenge, primarily due to the limited quantities of sample DNA available. To overcome this challenge, we have developed NEBNext® Ultra DNA Library Prep Kit, a fast library preparation method using novel NEBNext reagents and adaptors, including a new DNA polymerase that has been optimized to minimize GC bias. This method enables library construction from an amount of DNA as low as 5 ng, and can be used for both intact and fragmented DNA. Moreover, the workflow is compatible with multiple NGS platforms.

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P14.03

Next Generation Sequencing application to challenged sequencing on homologous genes CLCNKA and CLCNKB, implicated in two forms of Bartter syndrome.

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CLCNKA and CLCNKB encode for renal chloride channels ClC-ka and ClC-kb involved in Bartter syndrome (BS), a rare recessive salt-losing tubulopathy. These genes are highly homologous, with a 95% identity and a similar genomic organisation. In order to improve CLCNKB analysis and to sequence CLCNKA, we have developed a NGS protocol for both genes in a same run.
The DNA library was prepared using nebulised specific LR-PCR following manufacturer recommendations. Each patient was tagged with two distinct MIDs, one for each gene. The library was clonally amplified using emulsion PCR, enriched and loaded on a picotiter plate on a GS junior (Roche). Data were analysed with Reference mapper (Roche) and Seqnext (JSI medical). Both genes were sequenced for 7 patients and 3 controls and only CLCNKB in 2 patients and 3 controls. We compared NGS results to Sanger sequencing to determine specificity and sensitivity.
The main obtained coverage varied from 40 to 200. All coding variants found with Sanger analysis were found with NGS. The sensitivity was near 100% for the common regions tested and the analytic specificity was verified by the right match between the reads and reference sequence on the entire chromosome 1.
In conclusion, this study allowed us to develop a whole specific sequencing of the homologous CLCNKA and CLCNKB genes in a same run. The use of NGS in diagnosis requires secure interpretation with two softwares, in order to avoid misinterpretation of contiguous variants and homopolymers regions, and a well defined threshold for variant interpretation.

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P14.04

Combination HRM and MLPA for identification of methylation status at the H19 and KCNQ1OT1 DMRs in patients with Beckwith-Wiedemann syndrome

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Methylation alterations in *H19* and *KCNQ1OT1* differentially methylated regions (DMRs) on chromosome 11p15.5 are relatively common in Beckwith-Wiedemann syndrome (BWS). Gain of methylation in *H19* DRM or loss of methylation in *KCNQ1OT1* DMR result in heterogeneous overgrowth which is also associated with high risk for tumor development. The goal of the study was to combine methylation-sensitive high-resolution melting analysis (MS-HRM) and methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) for the identification of *H19* and *KCNQ1OT1* DMRs methylation status in 43 patients with Beckwith-Wiedemann syndrome. The genotypes of these samples were compared to traditional methylation-specific PCR results. Of 43 BWS samples, 30.2% (13/43) were hypomethylation in *KCNQ1OT1* DMR, 2.3% (1/43) were hypermethylation in *H19* DMR, 2.3% (1/43) were paternal uniparental disomy (UPD), and 65.1% (28/43) were negative. The detection rate for BWS at two DMRs is 34.9%. These two methods produced consistent results regarding methylation status and yielded neither false positive nor false negative results. Moreover, this study provides a representative picture of the distribution of methylation status of *H19* and *KCNQ1OT1* DMRs in clinically ascertained BWS cases in the Taiwanese population. The MS-HRM and MS-MLPA clearly demonstrated that these are significant tools for quantification of methylation in molecular epigenetic diagnosis.

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P14.05

FAVR (Filtering and Annotation of Variants that are Rare): methods to facilitate the analysis of rare germline genetic variants from SOLiD and Illumina datasets

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Characterising genetic diversity through the analysis of massively parallel sequencing (MPS) data offers enormous potential to significantly improve our understanding of the genetic basis for observed phenotypes, including predisposition to and progression of complex human disease. Great challenges remain in resolving genetic variants that are genuine from the millions of artefactual signals.
FAVR is a suite of new methods designed to work with commonly used MPS analysis pipelines, with a focus on relatively rare genetic variants. The most important and novel aspect of FAVR is the use of signatures in comparator sequence alignment files during variant filtering, and annotation of variants potentially shared between individuals. The FAVR methods use these signatures to facilitate filtering of (i) platform and/or mapping-specific artefacts, (ii) common genetic variants, and, where relevant, (iii) artefacts derived from imbalanced paired-end sequencing, as well as annotation of genetic

variants based on evidence of co-occurrence in individuals. We applied conventional variant calling applied to whole-exome sequencing datasets, produced using both SOLiD and TruSeq chemistries, with or without downstream processing by FAVR methods. We demonstrate a 3-fold smaller rare single nucleotide variant shortlist with no detected reduction in sensitivity. The principles described herein were applied in our publication identifying XRCC2 as a new breast cancer risk gene and have been made publically available as a suite of software tools.
FAVR is a platform-agnostic suite of methods that significantly enhances the analysis of large volumes of sequencing data for the study of rare genetic variants and their influence on phenotypes.

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P14.06

Rapid Single Cell Targeted Sequencing: Applications in Tumorigenesis

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Current sequencing technologies have allowed the genomic community to delve deeper into complex cellular systems on a massively parallel scale. We now, for example, have a better understanding of cancer evolution, as well as the general genomic heterogeneity within a single tumor sample. Rare mutations and copy number variations (CNVs) are known to drive tumorigenesis and disease progression, but the amount of sample DNA required for deep sequencing is a limiting factor. This hindrance means that our understanding of cancer and other cellular systems, such as embryo development, is limited to the sub-population level. A detailed understanding of cellular genomic hierarchies, whether on a temporal or spatial scale, requires an improved capacity to perform quantitative analysis on a single-cell level. In this study, we have developed methodologies using as little as 1 picogram of input material to generate sequence information across 100's to 1000's of amplicons. Sequencing performance did not show any appreciable amplification bias as measured by strand bias and coverage uniformity. Using a custom AmpliSeqTM assay, individual cells from primary and metatstatic ovarian tumors were sequenced at ultra-high coverage (>1000x), allowing us to study low frequency mutations and changes in gene copy numbers, and hence the evolution of ovarian cancer at the cellular level.

T. Harkins: A. Employment (full or part-time); Significant; Life Technologies. **C. Lee:** A. Employment (full or part-time); Significant; Life Technologies.

P14.07

First experiences with the GS Junior 454 in molecular genetic analysis of patients with hereditary breast and ovarian cancer at the Center of Familial Breast and Ovarian Cancer, University of Cologne

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Mutations in *BRCA1* and *BRCA2* are linked to the development of hereditary breast and ovarian cancer. In 2012, 74.500 women and 600 men got breast cancer and 7.200 women got ovarian cancer in Germany, while a hereditary predisposition is suspected in about 10% of these cases. Family history and age of onset are the most important inclusion criteria for genetic testing. Since August 2012, we have analysed 237 index patients with hereditary breast and/or ovarian cancer using the GS Junior 454 (Roche) and the BRCA kit (BRCA Master™ Dx kit, Multiplicom). The *BRCA1/2* genes contain many homopolymer stretches of at least 6 bp in length. Since these stretches are difficult to detect with the GS Junior 454 platform, we additionally employed a homopolymer assay fragment analysis (BRCA HP, Multiplicom), which allows rapid detection of small insertions/deletions in approximately 30 of these coding homopolymer stretches in *BRCA1/2*. In this cohort, 36 out of 237 index patients were tested positive for pathogenic *BRCA1/2* mutations. Of those, 10 individuals show disease-causing alterations located in homopolymer stretches that were easily detected by homopolymer assay pre-screening. Thus, our results warrant homopolymer pre-screening prior to costly next generation sequencing analyses.

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P14.08

New splicing vector pSAD: Splicing functional analysis of a hybrid "maxi-minigene" with exons 19 to 27 of BRCA2

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At least 50% of all mutations identified in BRCA genes in breast/ovarian cancer patients are variants of unknown clinical significance (VUS). Several studies have shown the link between disease and splicing disruptions due to mutations, including predicted nonsense or frameshift mutations. We aimed to investigate the role of splicing aberrations of BRCA2 in breast/ovarian cancer.
We followed a simple strategy consisting of bioinformatics analysis with NNSplice and Human Splicing Finder of DNA variants and splicing functional assays of hybrid minigenes. Splicing reporter plasmids allow to perform functional analysis without the need of patient RNA. We designed a new splicing vector, pSAD, which enabled us the creation of a "maxi-minigene" that includes nine exons of BRCA2 (19 to 27, MGBR2_19-27), where exon 27 substituted the second vector exon. The wt minigene produced a mRNA of 2,174 nucleotides. We validated the new MGBR2_19-27 with four splicing variants of the exons 19, 20, 23 and 24, which were tested in a previous report. Thirty-six out of 166 variants reported in these 9 exons were bioinformatically selected and introduced by PCR-mutagenesis in the wt MGBR2_19-27. Fifteen variants (42%) of all types (synonymous, nonsense, frameshift or missense) altered splicing by different mechanisms. Altogether, most variants disrupted the canonical splice sites; one affected a splicing silencer and another the polypyrimidine tract.
Aberrant splicing represents a relevant pathogenic mechanism in hereditary breast/ovarian cancer. Splicing functional assays with the new vector pSAD are valuable tools to discriminate between benign and pathogenic DNA variants of any human disease genes.

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P14.09

A novel technique that distinguishes low-level somatic DNA variants from FFPE-induced artifacts in solid tumors by next-generation sequencing (NGS)

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Several applications, including somatic mutation detection in cancers, require methods that can efficiently detect low-frequency variants (< 10% minor allele frequency, MAF) in DNA extracted from formalin-fixed-paraffin-embedded (FFPE) tissue. We have developed a novel technique that can distinguish true variants from fixation artifacts with high sensitivity and specificity by investigating each of the two DNA strands independently. TruSeq Custom Amplicon technology was used to generate sequencing libraries and deep sequencing was carried out to an average depth of 20,000X with a minimum of 1000X. The targeted re-sequencing assay* investigates ~14 kb of exons in 26 genes commonly mutated in solid tumors. Testing of more than 200 samples with a MAF ≥5%threshold revealed the presence of a large number of potentially false positive calls when data from only one strand of DNA was analyzed, but this number was significantly reduced (e.g. >50% for G>A) when both strands were considered. Conclusion: This technique can distinguish FFPE artifacts from true variants and therefore provides increased accuracy for the detection of low-frequency variants by NGS.
*Research Use Only

N. Udar: A. Employment (full or part-time); Significant; Illumina. **R. Haigis:** A. Employment (full or part-time); Significant; Illumina. **T. Gros:** A. Employment (full or part-time); Significant; Illumina. **N. Kerry:** A. Employment (full or part-time); Significant; Illumina. **B. Barnes:** A. Employment (full or part-time); Significant; Illumina. **D. Pokholok:** A. Employment (full or part-time); Significant; Illumina. **M. Ross:** A. Employment (full or part-time); Significant; Illumina. **A. Lucio-Eterovic:** None. **Q. Zhang:** None. **M. Zenali:** None. **E. Jaeger:** A. Employment (full or part-time); Significant; Illumina.

P14.10
Characterisation of the RNU2 CNV, a bulky neighbour for BRCA1

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Structural variation in the human genome has gained considerable attention in the recent years as it accounts for much of the variation between human genomes. However, the question of the implication of multi-allelic CNVs in complex traits remains largely open as most of them cannot be genotyped by array technology. Among them, the *RNU2* CNV is missing from the latest human genome assembly. However, this locus has been previously shown to contain a variable number of tandem repeats and to reside close to the breast cancer susceptibility gene *BRCA1.*

We developed new tools to fully characterize it. By FISH analyses on combed DNA, we precised its location 124 kb telomeric of *BRCA1*, we determined for the first time the exact allelic number of repeats in 41 individuals and found a range of 6-82 and a level of heterozygosity of 98%. Analysis of the 1,000 Genome Project data sets confirmed the high degree of polymorphism and concerted evolution of this CNV. By taking advantage of its location within the *BRCA1* linkage disequilibrium block, we studied the *RNU2* array transmission through a large number of generations and found that the *RNU2* CNV displays an unexpected high degree of meiotic stability.

These new approaches extend our knowledge of a recently neglected CNV that could be valuable for evaluating the potential role of structural variations in disease due to its location next to a major cancer susceptibility gene.

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P14.11
A novel approach for multiplex ultrasensitive detection of somatic mutations in tumor samples

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Systematic characterization of somatic mutations in cancer genomes has improved our understanding of this multifaceted disease and has provided opportunities for targeted therapeutic development. Despite much progress several analytical challenges remain to be resolved, in particular the detection of low abundance somatic mutations. These genetic events are hard to identify due to the heterogeneous nature of most tumor tissue samples and where the mutation may differ from the highly abundant wild type sequence by only a single nucleotide. A method capable of accurate detection of these rare variants would allow identification of genetic determinants for the initiation and proliferation of tumors at an early stage of disease progression or would serve as a tool for monitoring residual disease.

We have developed a novel approach for ultrasensitive high-throughput multiplex mutation detection and used this technique to detect as low as 0.125% of a mutant sequence in a mixture model. The process consists of multiplex PCR followed by a mutation specific single base extension reaction. The extension reaction utilizes a single mutation specific chain terminator labeled with a moiety for solid phase capture. Captured, washed, and eluted products are interrogated for mass and mutational genotypes are identified and characterized using MALDI-TOF mass spectrometry. For further validation of this methodology, we successfully verified rare mutations in a set of characterized cell-lines with ultra-low fractions of defined somatic mutations. This new method, when compared to next generation sequencing platforms, offers a relatively simple workflow with quick turnaround time and minimal sample input requirements.

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P14.12
Comparative study for the evaluation of a new technology for cystic fibrosis screening

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Objectives

Cystic fibrosis (CF) is one of the most frequently diagnosed autosomal-recessive diseases in the Caucasian population. Screening for Cystic Fibrosis Transmembrane conductance Regulator (CFTR) gene mutations, including poly T, is strongly recommended in infertile couples planning a pregnancy by assisted reproductive technology (ART). This study evaluated the performance of the new Nanochip CF70 kit (Savyon Diagnostic, Israel), a microarray assay, and compared it with the Innolipa kits (Innogenetics, Belgium)

Methods

From January to July 2012 we analyzed 392 blood samples with Innolipa and Nanochip technologies that identify respectively 70 and 56 CFTR mutations. Both tests include the most common Italian mutations and the poly-T screening. Discordant results were analyzed with the Devyser CFTR Core Kit (Devyser, AB, Sweden), MLPA (MRC Holland), Direct Sequencing (DS) on the 3730 DNA Analyzer (AppliedBiosystems), and Sequenom's MassArray system (Diatechpharmacogenetics, Italy)

Conclusions

Innolipa and NanoChip were concordant for 371/392 samples. 21/392 (0.5%) discordant results were tested with the aforementioned technologies: DS confirmed Innolipa results in 18/21 samples and Nanochip results in 1/21, while Devyser and Sequenom did not recognize some mutations not included in their panels. DS was essential for the identification of two different homozygous deletions; although they were not present in Innolipa panels, in 2/21 samples Innolipa indicated a mutation with the warning *no interpretation possible*

In this study the Innolipa assay confirmed its reliability and Nanochip showed that it could become competitive with slight changes to the software

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P14.13
Induced pluripotent stem cells derived from human chorionic villi

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Trophoblast cells (TCs) are placenta fetal components that can be isolated from patients undergoing chorionic villi sampling for prenatal diagnosis. In consideration of some of the features of these cells we speculated that TCs could be used to produce induced pluripotent stem cells (iPS). To validate this hypothesis, we employed human chorionic villi and a polycistronic lentiviral vector (hSTEMCCA-loxP) encoding Oct4, Sox2, Klf4 and c-Myc genes necessary to cell reprogramming. Stem cells specific morphological, molecular and immunocytochemical markers (ALP; OCT4; SSEA3; SSEA4; TRA1-60) confirmed the successful reprogramming. Additionally, we evaluated their ability to differentiate into the three embryonic germ layers (ecto, endo and mesoderm) by immunocytochemical characterization and their ability to in vivo form teratomas.

This study was carried out on two different samples: a cystic fibrosis (CF) patient carrying E831X/F508 mutations and a wild type one. Following reprogramming, the presence of the mutations were confirmed in iPS-CFTR cells and then cells were induced to differentiate into alveolar type II cells (ATIICs; a lung cell lineage). Within the cell population generated, the presence of ATIICs was confirmed by molecular analyses.

To date, this represents the first example of iPS cells derived from a very early extra-embryonic fetal tissues like chorionic villi (hiPS-CVS). These data suggest that hiPS-CVS can be considered a valid cell model to accomplish pathogenesis studies and possibly represents a valid tool for future therapeutic applications.

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P14.14

Digital PCR modeling for maximal sensitivity, dynamic range and measurement precision

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A mathematical framework is developed for modeling a digital PCR system with factors impacting the measurement precision such as the number of available reaction chambers, sample volume reduction (due to a variety of causes), and false negative/false positive rates. This framework is used to develop graphics showing the relationship between precision and the supported dynamic range. The impact of total input sample volume on the lowest limit of detection or sensitivity is also illustrated.
The model predicts an increase in supported dynamic range for the same number of reaction chambers with the use of two dilution points (using half the number of reaction chambers for each dilution). The loss of half the number of reaction chambers to a second dilution point incurs a slight loss in the detectable concentration range. However, this loss is more than offset by the gain in concentrations now detectable because of an overlapping effect of the second dilution point. Beyond two dilutions, gains are not found to be as significant. The results also predict how to leverage available reaction chambers for precise detection of two targets present at largely different proportions. A large subset of the chambers is dedicated to detecting the rare type and the remaining chambers are dedicated to detecting the wild type at a different dilution. The curves showing precision versus concentration will be used to plan two digital PCR experiments for genetic quantification and rare allele detection respectively.

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P14.15

Single cell analysis with QX100 Droplet Digital PCR system

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Over the last decades, it has become increasingly evident that gene expression profiles can vary from cell to cell, even within an apparently homogenous population. The analysis of this heterogeneity has become a focus of interest in various fields of biology, especially in stem cell research. The main obstacle for analysis of gene expression at the single-cell level is the low amount of starting material. This requires a high level of confidence in results obtained from unique samples, making it nearly impossible to be accurately done by traditional quantification methods such as qPCR. Droplet digital PCR (ddPCR) provides absolute quantification of individual molecules with high precision, and without the requirement for standard curves or pre-amplification steps. Using Bio-Rad's QX100 ddPCR system, we developed a method that measures single-cell gene expression with a great accuracy, thus enabling us to simultaneously analyze expression of different targets in material from the same cell. In order to perform this, we carefully evaluated various cell lysis and cDNA synthesis methods and developed a protocol that is fully compatible with ddPCR, easy to use and capable of analyzing gene expression in single cell without pre-amplification. This method allows us to measure the expression of genes of interest in a single cell, minimizing the stochastic effect of sampling and empowering us to accurately and sensitively detect and quantify low-expressing genes in single cells.

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P14.17

Development of a next-generation sequencing approach for molecular diagnosis of dystrophinopathies

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Duchenne Muscular Dystrophy (DMD) and its milder allelic variant, Becker Muscular dystrophy (BMD) are both caused by mutations in the dystrophin gene (DMD) located on Xp21. The human DMD gene is the largest one spanning >2,200 kb of genomic DNA. It contains 79 exons involving various mutation types and regions. The mutational spectrum includes copy number mutations (CNMs) and point mutations that account respectively for approximately 65% and 35% of DMD mutations. Current diagnostic assays involve a variety of methodologies but theses methods are time-consuming. The main objective of our study was to implement Next Generation Sequencing (NGS) technology for diagnostic applications in DMD or BMD patients. For this purpose, we assisted the Multiplicom Company to develop a multiplex PCR assay of the 79 exons and splice sites (Multiplicom's MASTR™ - Multiplex Amplification of Specific Targets for Resequencing) for further sequencing on the Roche 454 GS-FLX sequencer. We compared data generated by this approach with those obtained by classic Sanger sequencing in a cohort of patients with known and unknown mutations, and tested its quality and reproducibility. The results showed complete consistency between this NGS strategy and Sanger sequencing, with a gain in term of length of experiments and data analyses. Another major interest of this technique is its potential ability to exploit the multiplexed PCR amplicons to determine exonic CNMs. A single technique should allow screening the majority of DMD mutations, reducing therefore costs and diagnostic delay in most cases.

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P14.18

Evaluation of methodologies for the analysis of human exomes using DNA purified from saliva.

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Saliva provides a non-invasive sample collection option which can greatly improve the success of a research study by ensuring donor compliance and enabling collections in difficult environments. Saliva collected using Oragene self-collection kits provide results equivalent to blood as demonstrated via published GWAS studies over the last 10 years. In this study we show that DNA from saliva collected using Oragene is a reliable sample for exome arrays and exome sequencing. Furthermore, we compare results between the two platforms. Saliva samples (2 mL) were collected using Oragene self-collection kits. DNA was extracted using the prepIT•L2P kit, an alcohol recipitation-based extraction kit that yields high quality, high molecular weight DNA. Samples were quantified using a Picogreen protocol, A260/A280 ratios were evaluated using a UV spectrophotometer and DNA integrity was assessed using agarose gel electrophoresis. All samples were of sufficient quantity and quality for analysis using the Illumina HumanExome v1.1 arrays and for targeted exome sequencing. Ten samples were processed on the exome arrays and all reported a call rate >99.8%. Three samples were exome sequenced, for which we obtained > 98.5% exome coverage at a mean depth >72x. We then evaluated the variants which are shared amongst the array and target capture platforms and evaluated the concordance between them. Saliva collected using Oragene provided DNA of sufficient quality and quantity for both exome array and targeted sequencing analysis. Data obtained demonstrates the samples perform equally well using either approach and that data is highly concordant across methodologies.

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P14.19

Haloplex, the next generation in exome sequencing

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Exome sequencing allows samples to be sequenced at a fraction of the costs of whole genome sequencing while retaining information on the important protein-coding portions of the genome. Haloplex technology offers a very short (3 day) sample prep time, that combined with the fast Illumina HiSeq2500 and MiSeq sequencers brings the turn-around time of exome sequencing to days instead of weeks. Here, we present the first exome sequencing results obtained with Haloplex capture technology within a week.
We processed 8 samples with the Haloplex exome capture including 1 sample from HapMap. The generated libraries were sequenced on the Illumina MiSeq, HiSeq 2000 and HiSeq 2500 systems. The generated data was com-

pared to SureSelect v4 data from the same samples. Haloplex requires only 200 ng of DNA input amount and provides a two day turn around time for sample preparation. 90% of the exome is covered at 10x using only 4 Gbp of sequencing. The datasets from Haloplex are highly concordant with SureSelect all exon V4 results (99.6% SNP concordance). Haloplex excels at calling insertions and deletions in and near repeats due to the structured nature of the resulting reads but both data sets have specific benefits and strengths which will be described. In conclusion, Haloplex exome capture is a solid platform for exome sequencing. Its short runtime and low input requirements make Haloplex exomes ideally suited for clinical genetics applications.

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P14.20

Determination of transgene copy number in stably-transfected mammalian cells by PCR-capillary electrophoresis assay

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The quantitative determination of transgene copy number in stably-transfected mammalian cells has been traditionally estimated by Southern blot analysis. Recently, other methods have been available for appraisal of gene copy number, such as real-time PCR.
We describe here a new method for quantitating the transgene copy number in stably-transfected mammalian cells. Method is based on the relative quantification of amplicons after multiplex PCR reaction in which we used fluorescently labelled primers.The visualisation of amplified fragments was performed by capillary electrophoresis. Sequence specific for our target gene (prothrombin) and internal control originating from genomic DNA (18S rRNA) were amplified in the same PCR tube. We calculated mean peak height ratio of target:control gene with standard deviation for every cell clone sample and used this method to identify stably-transfected mammalian cell clones bearing same copy number of vector containing target gene. The results of our assay were confirmed by real-time PCR. Our study showed that method is equally informative and less expensive compared to traditionally used assay. Apart from saving time and material, this method has the advantage of using a very small amount of DNA. Also, since PCR is carried out in a single tube, any factor affecting the PCR will affect both target and internal control and hence the ratio remains unchanged.
In conclusion, herein described method proves to be fast, reproducible and low-cost and can be used as a rapid screening tool for the determination of gene copy number in basic research of gene expression.

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P14.21

Using biotinylation for plasmid DNA binding to streptavidin-cell-penetrating peptide conjugate

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Gene therapy strategies based on plasmid DNA are more preferable than viral-based gene delivery. Plasmids are more stable in vivo and known to be less immunogenic. Retroviral-based vectors integrate in actively transcribed genes that can lead to disruption of tumor suppressor genes. Gene delivery methods based on plasmid DNA are not likely to cause mutagenesis and they do not integrate in chromosomes providing increased safety. Despite the potential advantages of using plasmid DNA, delivery issues with plasmid vectors exist.
One of the ways of plasmid delivery into a cell can be covalent bonding of the plasmid to a cell-penetrating peptide (CPP). However, common covalent linking methods are limited by the concern that the covalent bond between CPP and plasmid may alter the target gene expression. To avoid this, few preselected nucleotides must be linked with CPP. Besides the covalent bond, for this purpose may be used disulfide or biotin-streptavidin interaction.
In this work, we have created a biotinylated circular plasmid DNA to further its binding to the streptavidin-CPP. In one strain of the pEGFP-N3 plasmid we replaced 1-3 thymines by biotinylated uracils. This modification was carried out in previously inserted 30 b.p. sequence between SV40 polyadenylation signals downstream of the EGFP gene and f1 origin for single-stranded DNA production. The presence of biotinylated uracils in purified plasmid was verified in restriction analysis followed by dot blot procedure. Streptavidin-conjugated alkaline phosphatase was used to detect the biotin-uracils. The ability to express EGFP was tested in Hela cell culture.

D.S. Polyakov: None.

P14.22

Human Genome Structural Variation Discovery and de novo Assembly at the Single Molecule Level using Nanochannel Genome Mapping

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As a result of the remaining limitations of DNA sequencing and analysis technologies_even ten years after the completion of the human genome project_there remain about 400 gaps in the human reference sequence assembly, hundreds of millions of unassembled bases in those regions, and no effective tools to comprehensively characterize the structural variation in an individual's genome. Despite the ungapped reference sequence being of extremely high quality, it is not feasible to create similarly high quality assemblies of individuals to detect and interpret the many types of structural variation that are refractory to high throughput or short-read technologies. We present a single molecule genome analysis system (Irys) based on NanoChannel Array technology that linearizes extremely long DNA molecules for direct observation. This high-throughput platform automates the imaging of single molecules of genomic DNA hundreds of kilobases in size to measure sufficient sequence uniqueness for unambiguous assembly of complex genomes. High-resolution genome maps assembled de novo preserve long-range structural information necessary for structural variation detection and assembly applications. We have used Irys genome mapping for the assembly and characterization of two human genomes. From these assemblies, we have spanned many of the remaining gaps, identified known and novel structural variants and phase some haplotype blocks, including in the MHC region. We also resolve and measure long tandem repeat regions that are likely impossible to assemble by other methods.

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P14.23

Gold-Nanobeacons for gene silencing: evaluation of the effect and toxicity of a new nanotheranostics tool

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Antisense therapy is a powerful tool for post-transcriptional gene silencing suitable for downregulating target genes associated to disease. Gold nanoparticles (AuNPs) have been described as effective intracellular delivery vehicles for antisense oligonucleotides providing increased protection against nucleases and targeting capability via simple surface modification. We constructed an Antisense Gold-nanobeacon consisting of a stem-looped oligonucleotide double labelled with 3'-Cy3 and 5'-Thiol-C6 and tested for the effective blocking of gene expression in colorectal cancer cells. Due to the beacon conformation, gene silencing was directly detected as fluorescence increases with hybridisation to target. Vectorisation via the AuNPs circumvents the need of transfection agents but with comparable silencing efficiency to that of commercial RNAi strategies with the advantage that AuNPs provide additional protection against circulating RNases. An integrated toxicology evaluation was performed based on acute cell toxicity, genomic DNA damage and proteome profiling showing that the proposed nanotheranostics tool does not exhibit significant toxicity, which is extremely relevant when translating into in vivo systems.

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P14.24

Reprogramming through the pluripotent state as a new paragigm for human cell rejuvenation

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Direct reprogramming of somatic cells into induced pliripotent stem cells (iPSCs) provides a unique opportunity to derive patient-specific stem cells with potential application in autologous tissue replacement therapies and without the ethical concerns of embryonic stem cells (hESC). However, cellular senescence which contributes to aging and restricted longevity, has been described as a barrier to the derivation of iPSCs, suggesting that aging might be an important limitation for the derivation of iPSCs for therapeutic purposes from elderly individuals. Recently, we developped an optimized 6 factor based reprogramming protocol that may cause efficient reversing of cellular senescence and reprogramming into iPSCs. We demonstrated that iPSCs derived from senescent and centenarian fibroblasts have reset telomere size, gene expression profiles, oxidative stress and motochrondrial metabolism, and are undistinguishable from hESC. Finally, we further demonstrates that re-differentiation led to rejuvenated cells with a reset cellular physiology, defining a new paradigm for human cell rejuvenation. These results provide new insights into iPSC technology and pave the way for regenerative medicine for aged patients.

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P14.25

A rapid and simple method for NGS library normalization

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Library preparation is a major contributor to labor and reagent costs for Next Generation Sequencing (NGS). This is particularly true for higher throughput applications, such as targeted re-sequencing, where many samples are prepared in parallel. NGS platforms require careful quantification of libraries, and sets of barcoded libraries require normalization prior to pooling. We present a simple method for NGS library normalization that obviates the need for library quantitation and dilution. Five DNA samples (1 matched lung normal/tumor pair, 1 FFPE DNA, and 2 high molecular weight DNAs) were carried through a 207-plex amplicon library preparation workflow with 5, 10, and 20ng of DNA input. Libraries were prepared in triplicate for each sample at each input level. After library construction and normalization, 45/45 (100%) of libraries were within a two-fold concentration range (115 - 204 pM, average 156 pM, SD 16.5 pM), when measured by qPCR. Sequencing of these libraries showed no adverse effects on accuracy (>99.5%), uniformity of amplicon representation (>99%), or bases with no strand bias (>99%) compared to control libraries (non-normalized, individually diluted). The method has been shown to be highly tunable, making it applicable to a variety of NGS platforms.

G. Roma: A. Employment (full or part-time); Significant; Life Technologies/ Ion Torrent. **S. Roman:** A. Employment (full or part-time); Significant; Life Technologies/ Ion Torrent. **C. Van Loy:** A. Employment (full or part-time); Significant; Life Technologies/ Ion Torrent. **D. Topacio:** A. Employment (full or part-time); Significant; Life Technologies/ Ion Torrent. **K. Rhodes:** A. Employment (full or part-time); Significant; Life Technologies/ Ion Torrent. **M. Andersen:** A. Employment (full or part-time); Significant; Life Technologies/ Ion Torrent.

P14.26

Hyperspectral Imaging for Label Free High Resolution Characterization of Chromosomes

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In Cytogenetics the specific structure of chromosomes is routinely identified by expensive and time consuming staining techniques like GTG (G- banding by trypsin using Giemsa) banding or FISH (Fluorescence Insitu Hybridisation). In both cases a broad expert knowledge is necessary to understand and diagnose complex diseases and particularly to visualize small structural aberrations.
We have made efforts to substitute the state of the art karyotyping technique to a fast low-cost and automatic label-free karyotyping procedure using absorption and stray light spectroscopy in the UV-VIS range combined with classical light microscopy. Therefore we designed a hyperspectral imaging system with a scanning software for marker free karyotyping. This system allows visualizing the stray light interference pattern of unstained chromosomes resulting from small variations in size, thickness, refractive index and field of chromatin condensation of chromosomal substructures on a nanoscale. Up to 150 individual spectra along the chromosome are registered. The spatial pixel resolution can therefore be as small as 50nm. The spatial location is indeed limited to the diffraction limit of microscopy (5Mb), but the detected substructures remain in the spectra and even can be optically resolved using Scanning Near-Field Microscopy (SNOM).
The complex spectral signature can be analyzed by means of Multivariate Curve Resolution (MCR) or Principle Component Analysis (PCA) which is now integrated in our scanning software. The unique banding pattern of unstained chromosomes can be visualized in a novel high resolution karyogram resulting from the hyperspectral data analysis.

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P14.27

WildFire: an In-situ Isothermal PCR Method forming Billions Monoclonal Colonies for NGS

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We have developed a new massive parallel monoclonal technology, called "WildFire", where sequencing libraries are *in-situ* isothermal amplified directly on the surface of a 5500 flowchip. Sequencing libraries (~ 40 uL per lane) are added directly to the 5500-series Genetic Analyzer flowchip, whose surfaces have been coated with a special library-adaptor capture oligonucleotide. A DNA polymerase reaction mix is added, and in a single isothermal step lasting ~ 30 minutes, single templates are amplified *in-situ* on the flowchip. The net density of sequencing-colonies created in this manner far exceeds anything currently utilized in next-generation sequencing, reaching ~ 1.5 million colonies per mm^2 per flowchip surface. During *in-situ* amplification, the capture oligonucleotide is "consumed", and each individual nucleic-acid fragment "spreads" (like a WildFire) inside the flowchip until reaching an adjacent library fragment(s). When the individually-growing fragments "meet", the amplification step terminates, because all of the surface-bound primer was consumed. These "self-assembled", spatially resolved, monoclonal colonies, are then sequenced by SOLiD chemistry. The resulting colony-sequencing reads maintain the same high accuracy as our bead-based method. Full genomes (from bacterial to human), exomes (human), and transcriptomes (human) have now been sequenced using WildFire technology. WildFire technology greatly improves NGS workflow, increases throughput, and significantly decreases net cost-per-genome.

K.Q. Lao: A. Employment (full or part-time); Significant; Life Technologies.

P14.28

Non - destructive aDNA extraction from museum specimens

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Museum collections contains valuable genetic information. Most of them haven't been available, mainly because specimens should be intact. The possibility of obtaining DNA by non- destructive way from conservation fluid allows to explore these valuable specimens.
Analysis include exhibits, which were stored in two types of preservative fluids: ethanol/glycerol and ethanol/glycerol/formalin. Specimens are dated from first decade of XIX century to the second half of the XX century. Method starts from vacuum filtration of the conservation fluid. DNA were isolated from the filters, contained small fragments of tissue and whole and fragmented cells. DNA was isolated according Ivanow's method with our own modifications, then evaluated using agarose gel and Quantifiler real time - PCR test. Next, in view of the genetic material's age and the degradation stage, the samples were tested by multiplexing STR or SNP. In order to verify the results, two various tests which ranges overlaped, were used.
The obtained results allow to accept these method as reliable. This approach may be useful for researches as well as for museum curators because recovery of DNA may be connected with necessary periodical conservation and

complementation of conservation fluids. The conclusion: it is possible to obtain valuable researching genetic material without the necessity to damage or destroy the museum exhibits.

T.M. Dobosz: None.

P14.29

Ravine syndrome: a mutation in a degenerated LINE-1 element harbored by a long intragenic non-coding RNA

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Familial recurrence of an extreme phenotype of infantile anorexia in a Caucasian isolate in Reunion Island led us to suspect autosomal recessive inheritance of this rare progressive encephalopathy. Here, we report homozygosity mapping of the locus of this syndrome, called Ravine. Complete DNA sequencing of the 400-kb linkage locus reveals a point mutation in a primate-specific retrotransposon that is transcribed as part of a unique long non-coding RNA, SLC7A2-IT1A/B, which is expressed in the brain. Silencing this RNA whether by siRNA or antisense leads to increased neuronal apoptosis, consistent with the inappropriate dosage of this RNA in vivo and with the phenotype. Structural analysis of the sequence reveals homology to piRNA as well as a small RNA-like hairpin. We assess the global impact of the mutation by transcriptomic analysis of brain samples, and discuss the different mechanisms that could underlie the effect of the mutation. We believe that this work could contribute in the understanding of the significance of subtle variations in retrotransposons.

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P14.30

Towards a Theory of Science Policy for Genetics

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Many politically charged topics in Western liberal democracies have science at their core: embryonic stem cell research, climate change, GM crops, or alternative energy strategies. But science has always been politically charged -- from the early 17th century academies advising governments, to the Horizon 2020 budget debate in Europe and the "fiscal cliff" in the United States. We argue that science has become especially challenging for policy-making precisely because liberal democracies lack a coherent way to accommodate pluralistic views about scientific innovation. Indeed, given the importance of science in society, it is surprising that so little attention has been given to providing a coherent approach that would explain the way science policy works. Such an approach, if it could be developed as theory, might help systematize public decision making about science and more effectively contribute to desirable social goals. Human genetics is only the most recent of many science policy topics where comprehensive ethical analysis has been brought to bear (rightly) on key issues such as informed consent, privacy, and governance. This may be why regulation of direct-to-consumer genetic testing, establishing consent protocols for biobanks, or constructing privacy protections for GWAS studies does not admit of harmonized policy. It may also be that insufficient effort has been made to articulate the fundamental political values that underpin progress in science policy. To supplement the role that ethical theory has played in genetics, we sketch the basic features of a theory of science policy using political philosophy to engage these normative problems.

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P14.31

Breakpoint characterization of large deletions using PacBio and Illumina sequencing technologies

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The characterization of large deletions identified by multiplex ligation-dependant probe amplification (MLPA) or microarray analyses can be challenging. Here, we present the applicability of single-molecule (PacBio RS) and second-generation (Illumina) sequencing technologies to the characterization of large genomic deletions. The three DNA samples used in this study harbor hemizygous deletions previously characterized using a Sanger approach. Deletions in two samples of length 26,887 bp and 302,580 bp, respectively, affect the *FBN1* gene in patients with Marfan syndrome, whereas a deletion of 3,408,306 bp comprises the entire *COL3A1* gene in a patient with Ehlers-Danlos syndrome vascular type (EDS IV). By testing these DNA samples, our methods determined that both next-generation sequencing platforms were able to identify the position of deletion breakpoints. Our results point out various advantages of next-generation sequencing platforms when characterizing genomic deletions. However, special attention must be paid to identical sequences flanking the breakpoints, such as poly(N) motifs. Consequently, for the characterization of large deletions an alternative procedure is provided by our approach, which can be performed with reasonable DNA amount and is less work intensive and time consuming than a Sanger approach.

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P14.32

Next generation sequencing: aligning the futures of genomic medicine

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Genomic approaches to cancer treatment are already informing a number of clinical trials in oncology (e.g. microarray-based cancer signatures trials). This is animating powerful visions about the development of increasingly personalised and effective cancer therapies.
However, in this paper we show that next-generation high-throughput technologies, offering an unprecedented level of genomic resolution, are likely to steer the emergent sociotechnical phenomenon of cancer genomics even further into as yet uncharted territories.
Our paper will draw on the authors' direct engagement with an International Cancer Genome Consortium project (CAncer GEnome of the KIDney - CAGEKID) and a number of other European projects in the field of high-throughput genomics. We will provide first-hand ethnographic analyses of the anticipatory orientations of early actants in cancer post-genomics.
What are the traces of this imagined future in present day practices of cancer post-genomic research? What sociotechnical relationships are being reconfigured, and how? What material practices, biomedical platforms and actor-networks are being mobilized to design the future of post-genomic clinical oncology? Our inventory will reconstruct the assemblage of a sociotechnical infrastructure made of new actors, circulating samples, shared databases, collaborative projects, but also of restructured ordering tools such as new informed consent forms and feedback practices.

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P14.33

The challenges of quantifying the use of bioresources in genetic research

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Sharing data and bioresources is essential for optimising knowledge production. However, establishing a valuable bioresource that can be used for various genetic researches over time requires considerable time and efforts that often lack proper recognition. To address this issue, one approach is to develop adequate tools to assess bioresource uses and their specific contribution in the development of novel scientific knowledge. The concept of a Bioresource Research Impact Factor (BRIF) was introduced in 2003], and later further developed [1]. BRIF aimed to be a quantitative indicator filling a

gap in the complex environment of scientific production assessment. Rather than a fixed indicator we tend to propose a framework for measuring bioresource impact that could be used for valuing the activity of a bioresource over time.
The methodology of Requirement Engineering (RE) was used to formalise the steps towards the development of a BRIF instrument. The requirement analysis resulted in the following entities/ideas to be assessed:
- Defining obstacles to sharing samples and data
- Pointing to specific issues regarding genetic use
- Choosing adequate identifier scheme for bioresources
- Identifying and weighing parameters to be considered in various metrics
- Analysing the role of Journal guidelines and policies for resource citing and referencing
- Assessing policies for resource access and sharing and how they influence the bioresource use.
This work allows proposing a framework for the operational development of BRIF. It still requires input from stakeholders within the biomedical community. Nat Genet 2011, **43**(6):503-4.

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P14.34
Detection of mRNA knockdown in live cells using a novel nanoparticle technology

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Modulation of gene expression using techniques such as RNAi has become a fundamental tool in the study of gene function and biological pathways. To determine the efficiency of gene knockdown using most widely used detection methods, cell samples must be sacrificed by lysis or permeabilization and fixation. In addition to sample destruction, another disadvantage of these techniques is that they yield results that only reflect the average expression of the gene in the collected cell population. One nondestructive option is the use of transfected reporter constructs. However, although the cells remain alive, the technique cannot reveal endogenous gene expression and can have negative effects on cell health. An ideal RNA detection agent provides a noninvasive approach to interrogating gene expression while enabling sorting of live cells that can be separated and directly used for downstream studies.
Here we describe the detection of Survivin mRNA levels in LNCap and SCC12 with and without the presence of Survivin siRNA treatment. The changes in gene expression can be determined in the cells while they remain in cell culture in real-time, providing more physiologically relevant data in living cells. This technique utilizes a fluorescent reporter that can be detected using fluorescent microscopy for visualization or flow cytometry for single cell resolution and quantification. This allows for a more dynamic understanding of knockdown efficiency.
The ability to specifically detect RNA levels in live cells on a cell–by-cell basis provides new opportunities to link biological pathways and physiological processes to gene functions.

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P14.35
New simple method for TPMT alleles determination using multiplex HRMA

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The thiopurine methyltransferase (TPMT) is a crucial enzyme in metabolism of thiopurine drugs: azathioprine and 6-mercaptopurine which are used in the treatment of leukemia or inflammatory bowel diseases (IBD). Genetic polymorphism of the TPMT gene correlates with activity of this enzyme which determines an individual reaction and dosing of thiopurines. The three main TPMT alleles: TPMT*2 (c.238G>C), *3A (c.460 G>A, c.719A>G) and *3C (c.719A>G) accounting for 80% to 95% of inherited TPMT deficiency in different populations in the world. The aim of the study was to establish a rapid and highly sensitive method for analysis of these three major TPMT alleles. Here we present the molecular test based on multiplex high melting resolution analysis (HRMA) for genotyping of c.238G>C, c.460 G>A and c.719A>G.
We analyzed DNA samples from 100 clinically diagnosed IBD patients treated with thiopurine drugs and known genotypes at codons 238, 460 and 719 of the TPMT gene. One probe was TPMT *1/*2, one *3A/*3A, seven *1/*3A and other 91 wild type *1/*1. Our results obtained with multiplex HRMA indicated 100% accuracy compared to data from classical sequencing and RFLP. We conclude that multiplex HRMA can be used as a sensitive and efficient alternative diagnostic method compared to conventional techniques for determination of TPMT*2 and *3 alleles.

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P14.36
Whole Genome Sequencing of a Family Trio Using DNA Extracted from Saliva

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Saliva collected using Oragene is a non-invasive sample type and an alternative to blood that provides large amounts of high quality genomic DNA, as demonstrated through published GWAS studies over the last 10 years. In this study we show that DNA extracted from saliva is a reliable sample for whole genome sequencing and performs similarly to DNA from blood. Saliva samples were collected from a family trio consisting of a mother, father and male child. Saliva was collected using Oragene self-collection kits. The DNA was extracted using the prepIT•L2P kit, an alcohol precipitation-based extraction kit that yields high quality, high molecular weight DNA. Library preparation, sequencing and assembly were performed at Complete Genomics (CGI). Sequencing performance of DNA from saliva was compared against a publicly available trio data set sequenced from blood DNA, sequenced by CGI. There was no statistical difference between the number of known variants (p=0.064), novel variants (p=0.243), indels (p=0.069) and frameshifts (p=0.075) called among saliva samples compared to blood samples on average. In saliva, we observed minor differences in synonymous reference mismatch calls, missense variants and total sites called. These differences are likely due to differences in donors and sequencing coverage between the saliva and blood samples, work is ongoing to determine the cause. Data obtained from the family trio indicated excellent sequencing performance of all three samples confirming that saliva collected using Oragene is an excellent source of DNA for whole genome
sequencing.

M. Tayeb: A. Employment (full or part-time); Significant; DNA Genotek Inc. **S. Rabuka:** A. Employment (full or part-time); Significant; DNA Genotek Inc. **R.M. Iwasiow:** A. Employment (full or part-time); Significant; DNA Genotek Inc.

P14.37

Computer-aided facial recognition of individuals with X-linked hypohidrotic ectodermal dysplasia

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X-linked hypohidrotic ectodermal dysplasia (XLHED) is a genetic disorder characterized by hypohidrosis, hypotrichosis and hypodontia. Early diagnosis of XLHED is a key factor in reducing the risk for severe hyperthermia and pulmonary infections. Patients display a distinct craniofacial phenotype characterized by retruded appearance of the midface with maxillary hypoplasia and mandibular prognathism. In this study, a novel facial dysmorphology analysis system based on 2D photographs (FDNA®) was used to identify XLHED-affected individuals.

Following photograph acquisition from male patients with clinically and/or genetically-confirmed XLHED (n=27 newborns, n=64 age 1-10 yrs, n=42 age 11+ yrs, plus controls), 130 facial fiducial points were localized per photograph and their geometrical relationships determined. The final classification was based on these measurements as well as on a global „gestalt" detector. Cross validation of the recognition capability involved 20 rounds of analysis with the data split randomly to training and testing data.

In this proof-of-concept study, the statistical power of the test in identifying XLHED patients using average area under the ROC curve was 96% for individuals >11 years; 97% for children 1-10 years, and 98%-99% for newborns. Overall, the system had a high level of specificity (97-100%) at 90% sensitivity across all age groups.

These results demonstrate the strength and utility of computer-based facial analysis utilizing ordinary photographs in the identification of XLHED-affected patients. With further refinement, a universal newborn screening platform is plausible that will alert families to this clinical condition. Early diagnosis may also be critical for successful ectodysplasin therapy in this disorder (NCT01775462).

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P15.01

Investigation of intra individual gene expression variations in venous blood from healthy volunteers

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High-throughput sequencing techniques as RNA-Seq may soon be used to discover diseases with diagnostic tests at the earliest possible onset. RNA-Seq techniques create a high resolution readout of transcript levels. The Gene-expression profiles have a near digital character, which exceed sensitivity and dynamic range of the hybridization-based array technologies. In order to discover small changes in transcript abundance that may lead to a disease, within individual variability, but also technical and statistical biases of high-throughput sequencing need to be well described. Pipelines as the Tophat Cufflinks offer the user a number of custom settings to fine-tune the analyses result. We will investigate the impact of these settings on the prediction of transcript abundance systematically and describe variation with technical replicates and autologous day-to-day samples further. Consolidated guidelines will be generated and applied in subsequent analyses together with further samples from later-time points within the same individual.

Human venous blood samples will be collected from 3 healthy volunteers on a day to day, week to week and month to month basis over 2 years. Multiplexed polyadenylated RNA samples were run on the Illumina HiSeq system and analyzed with a Tophat Cufflinks pipeline.

Characterizing the dynamic range of intra-individual transcriptomes on the basis of statistical, chemical and biological variations is a crucial step in applying RNA-Seq in diagnostics. Here, we will analyze if current statistical methods can result in biological different answers within one individual and will provide recommendations for RNA-Seq studies.

S. Lehnert: None. **M. Jaspers:** None. **H. Cuppens:** None.

P15.02

Comparative genome-wide analysis using whole-genome sequencing and high-density SNP genotyping array data

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Next-generation sequencing and array-based high-density genotyping are both powerful approaches to study genetic variation that affect disease development, tumorigenesis, response to drugs etc.

In our study, the aim is to (i) develop a workflow to compare CNVs obtained from high-coverage whole-genome sequencing to the ones from high-density SNP genotyping array data; (ii) accurately detect rare CNVs with effect on health in a longevity cohort collected by Estonian Genome Center of Tartu University of Estonia.

We report a cohort of 49 unrelated individuals older than 83 years. Whole-genome sequencing experiment was performed using TruSeq DNA Sample Preparation Kit and HiSeq 2000. For genome-wide SNP genotyping we used HumanOmni2.5-8v1 BeadChips (Illumina, Inc).

Our study includes the estimation of efficiency and accuracy of calling structural variants from sequencing data and comparison of the concordance with CNV information obtained from SNP genotyping. To test if rare variants could affect ageing and age-related diseases, we will compare the state and frequency of genomic alterations between different age groups and geriatric subjects. By integrating both sequencing and SNP genotype data we will characterize some of the functional spectrum of genetic variation in our study cohort.

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P15.03

Exon-level aCGH in over 2000 patients from the Deciphering Developmental Disorders Project.

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The Deciphering Developmental Disorders (DDD) project is a family based study focused on rare genetic variants in patients with undiagnosed developmental delay. The majority of probands have been pre-screened by clinical microarray, which limits the enrollment of patients to those whose phenotypes cannot be easily explained by large copy number events. Exome sequencing and array comparative genomic hybridization (aCGH) are used to scan patient genomes for a wide range of genomic variation. The primary source for copy number (CNV) discovery in the DDD project is high-resolution (exon-level) aCGH. This platform comprises of 2x1Million probe Agilent arrays and is heavily targeted towards exons and conserved regions while retaining median backbone coverage of 3Kb. For the detection of CNV we have developed a high performance change point detection pakkage CNsolidate, which makes use of multiple weighted algorithms. Using this approach, we are able to rank detections based on differential weighting functions between component algorithms. To facilitate the direct reporting of patient variants back to the NHS via the DECIPHER database, we have implemented a dedicated clinical reporting pipeline. We compare patient CNVs to high quality CNV reference sets containing population frequency estimates (CNV Consensus) and a manually created list of developmental disorder genes (DDG2P). We applied CNV detection and clinical variant filtering to over 2000 patients from the DDD project. Here we assess the performance and detection rates using CNsolidate, describe a global CNV burden analysis

and present some examples of variants classified as pathogenic using the clinical reporting pipeline.

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P15.04

3DM: Data integration and next-generation variant effect predictions

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Many point mutations are located in protein-coding regions of the human genome. Determining their effects on the structure and function of a protein remains a very difficult problem, as is illustrated by the poor performance of tools such as PolyPhen and SIFT. In response to the growing demand for high-quality variant effect predictions we present 3DM; a data integration & mutation prediction platform for protein families. 3DM relieves many of the burdens that researchers face in dealing with the growing amounts and complexity of biomedical data. For each protein family a large amount of information that is extracted from protein structures, alignments and scientific literature, among others, is available. All this information is integrated and validated, and can be analysed via a number of different methods and tools.
By intelligently combining all this heterogeneous information 3DM is able to provide state-of-the-art predictions about the effects of genetic variations.
On a set of > 800 validated LongQT syndrome related variants (~200 neutral variants, ~600 pathogenic variants), 3DM was able to reach an accuracy of more than 95%. In contrast, PolyPhen was able to correcty predict only ~65% of the variants. The extremely accurate predictions made by 3DM shows that the availability of high-quality, integrated data is essential for assessing the effects of mutations.

B. Vroling: None. **T. van den Bergh:** None. **R. Kuipers:** None. **H. Joosten:** None.

P15.05

GS data online treatment : a 454 dedicated tool to facilitate quality assessment, variation selection and annotation

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Following the improvements of second generation sequencing technologies, benchtop sequencers are now available to a number of laboratories. 454 sequencing is the oldest of these technologies and its pros and cons are now well characterised. However, it does not really benefit from the burst of academic implementations of bioinformatic software. Identifying mutations in the clinical context of genetic heterogeneous disorders represents a promising approach. These conditions often require the sequencing of several genes before the identification of pathogenic genotypes, and therefore could benefit from medium-throughput methods. Usher syndrome is characterised by a congenital deafness associated with retinitis pigmentosa. We designed the liquid capture of the 9 responsible genes coupled with 454 sequencing. Given the lack of analysis tools and the weaknesses of the built-in software concerning the identification of some major disease causing alterations, we implemented GSdot, a web-based application available at https://neuro-2.iurc.montp.inserm.fr/454/, designed to assess, filter and annotate variants identified by the process. GSdot, launched right after base-calling and alignment, assesses some quality scores and computes the average depth of coverage for each analysed region. It also applies some simple rules to the candidate variations to eliminate a maximum of artefacts, and adds annotations (HGVS nomenclatures, gene and exons localisations) to the remaining candidates using the third-party software Annovar and Mutalyzer. A comparison of hundreds of variants previously identified by Sanger among the same cohort showed that GSdot treatment does not produce any false negative, and therefore can be used as an efficient tool to study genetic heterogeneous disorders.

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P15.06

Detection of allele-specific gene expression on next generation sequencing data

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Some studies observed that variation of gene expression between alleles is common, and this variation may contribute to human variability of several traits. The goal of our study is to develop a statistical framework aiming to measure and detect allele-specific gene expression (GE) differences from global GE experiments conducted using next generation sequencing (NGS) technology. Our method for identification of allele-specific differential expression (ASDE) is based on the likelihood estimation of the observed data depending on the parameter Θ (the relative amount of the reference allele with respect to the alternative allele). ASDE was estimated by a Likelihood Ratio Test (LRT). Only genes with at least two heterozygous sites were selected. Currently we tested the method on a total of 7 mantle cell lymphoma samples (MCL), 12 muscle cell samples (MC) and 15 lymphoblastoid cell lines samples (LCL). Using an arbitrary threshold of LRT > 250 we observed 15, 22 and 33 ASDE genes for MCL, MC and LCL, respectively, on a total of 10910, 8357 and 13328 expressed genes. For MCL samples we observed 3 and 1 ASDE genes in common with MC and LCL, respectively. No common ASDE genes were observed between MC and LCL. We plan to improve the method to identify haplotypes underlying ASDE, and to investigate the correlation between ASDE genes and their chromosomal location or specific classes of functionality. These bioinformatic results should be confirmed by wet lab experiments. In conclusion we developed a simple method to detect ASDE genes from NGS data.

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P15.07

Cafe Variome: Connecting Diagnostic and Disease Networks by Enabling 'Open Discovery' of Data, Without Sharing Of Data

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Diagnostics laboratories assess DNA from many patients affected by inherited disorders. To infer the likely pathogenic role of sequence variants identified in such settings, it would be useful to check whether the same changes had previously been seen by other laboratories/patients with related phenotypes. However, this option is precluded due to difficulties of data sharing between labs, or with others. Typically, any information exchange that does take place occurs on a one-to-one basis, via telephone or email contact.
To address this deficiency the novel ‚Cafe Variome' platform has been developed to enable ‚open discovery' of the existence (rather than actual substance) of relevant mutation data. By enabling networks of labs to easily query for the existence of variants, without necessarily revealing the underlying data, this overcomes issues of patient confidentiality.
Hence, Cafe Variome is not a database but is a searchable ‚menu'. The platform enables data owners/submitters to specify and update lists of who can search for records of interest (using various search parameters), and to determine how results are returned to those users: either as core data, or links to data at source, or via computationally facilitating data access requests.
The core ‚open discovery' concept underlying Cafe Variome could be deployed in many other settings - and the underlying technology is sufficiently generic and flexible to enable this. Therefore, as well as currently piloting the system with networks of diagnostic labs, we would welcome further collaborations in any and all potential areas of application.

O. Lancaster: None. **R. Dalgleish:** None. **D. Atlan:** None. **A.J. Brookes:** None.

P15.08

System and pathway analysis of variants associated with immune cell levels

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Bioinformatic analyses aiming to discern causal polymorphisms, underlying regulatory mechanisms, pathways and protein interaction networks, were performed for a set of SNPs found to be associated with levels of 95 immune cell types in the SardiNIA study. These immune traits, representing the ma-

jority of leukocyte cell populations (monocytes, T and B cells, Natural Killer cells, regulatory T cells, dendritic cells, and their subsets), were assessed by polychromatic flow cytometry in a sample of 1,629 individuals, and analyzed by genome-wide association scan.
The data, comprised of 81 variants, associated genes, traits and autoimmune diseases, were visualized in network setting in Cytoscape, allowing for better exploration of the multi-way relationships among them. The genes, to which the lead variants and those in linkage disequilibrium map, were inspected in a context of protein network and analyzed for the enrichment of pathways, Gene Ontology terms and semantic interconnections. The SNPs effects were assessed both in terms of an impact on the gene product and of potential regulatory function, taking advantage of genome-wide data on genomic and epigenetic features and 3D chromatin structure, made available through ENCODE project.
The results point to genes and processes with well established role in hematopoiesis and immune system, but also to involvement of an extensive part of signaling network. Regulatory function of DNA fragments containing several variants have been hypothesized, including looping to promoter regions of surrounding genes, enhancer or insulator function, or transcription factor binding.

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P15.09
Whole genome methylation profile of patients with Balkan endemic nephropathy

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Background: Balkan endemic nephropathy (BEN) represents a chronic progressive interstitial nephritis in striking correlation with uroepithelial tumors. The disease has endemic distribution in several Balkan countries and shows predominance of affected females.
DNA methylation is a primary epigenetic modification that is involved in cancerogenesis, genomic imprinting, gene silencing etc. The significance of CpG-island methylation in normal development, cell differentiation and gene expression is widely recognized.
Methods: We performed whole genome DNA methylation analysis on pool-DNA blood samples from 159 affected individuals and 170 healthy controls. We determined the methylation status of 27000 CpG-islands throughout the whole genome in healthy controls and BEN patients (Agilent DNA-methylation array 1x244k). We obtained the methylation profile of BEN patients from Serbian and Bulgarian endemic regions. Results were processed by Agilent Genomic Workbench Lite software v.6.5.0.18.
Results: Comparison of the methylation profiles of BEN patients and corresponding controls revealed the differentially methylated regions (DMRs). We then compared the DMRs between all patient-control pairs to determine common disease associated changes in the epigenetic profiles
SEC61G, IL17RA, HDAC11 were found to be differently methylated throughout all patient-control pairs. The CpG-islands of all 3 genes were hypomethylated in patients and hypermethylated in controls. Dysregulation of these genes could be common mechanism in BEN in both endemic regions and in both genders.
Conclusion: Our data strongly suggests that immunologic alterations have a significant role in BEN etio-pathogenesis. These results are in accordance with major BEN pathological characteristics.
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P15.10
Epigenetic alterations as a pathomechanism of genetic disease, example of Beckwith-Wiedemann Syndrome.

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Beckwith Wiedemann syndrome (BWS) is a model disorder for the study of epigenetic mechanisms.
BWS is a pediatric overgrowth disorder involving a predisposition to tumor development. The clinical presentation is highly variable, some cases lack the hallmark features of exomphalos, macroglossia and gigantism (EMG syndrome).
The molecular etiology of BWS syndrome is rather heterogenous. Dysregulation of imprinted genes found within a genomic region on human chromosome 11p15 results in the BWS phenotype through a number of different mechanisms leading to either primary epigenetic alterations or genetic alterations that change the relative contributions of parental alleles. Clinically, the BWS molecular subgroups are associated with different recurrence risks and mostly important with different predispositions to embryonal tumors, which necessite different current tumor surveillance.
We present the results of our around 200 examined patients with clinical suspicion of BWS syndrome and the different molecular subgroups. Up to 50% of BWS patients with positive molecular fiding have biallelic rather than monoallelic expression of the insulin-like growth factor 2 (IGF2) gene. Another 50% of patients have an epigenetic mutation resulting in loss of imprinting in this region.
Unique observations in this disorder point out to an important embryonic developmental window, relevant for example to the obsevations of monozygotic twinning which seems to predispose for the epigenetic dysregulations. Another actual topic is the higher rate of epigenetic dysfunction after assisted reproduction.
We discuss the insights of how the study of BWS has contributed to our understanding of the mechanisms of growth control, oncogenesis and genomic imprinting.

Z. Gencik-Guillier: None.

P15.11
Genome-wide association scanning for asymmetry of the human planum temporale

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The Planum Temporale (PT), a triangular shaped surface on the upper part of the temporal lobe, is for most people larger on the left hemisphere than the right. The degree and direction of this asymmetry has been linked to gender, handedness and language dominance. Although present to a degree in other primates, PT asymmetry is pronounced in the human brain and is already visible at 31 weeks of gestation. While this suggests a role for genetic mechanisms, nothing is known about the specific genes that determine this landmark brain asymmetry.
We performed a genome-wide association meta-analysis of PT asymmetry in three population cohorts: the Brain Imaging Genetics cohort from the Netherlands, and two Study of Health in Pomerania cohorts from Germany. A grey matter volume asymmetry measure was derived from T1-weighted MRI scans.
Our combined sample of 3100 subjects showed a genome-wide significant association on chromosome 2q32.2. Because of the sexual dimorphism of PT asymmetry, we subsequently used the meta-analyzed GWAS results to test for enrichment of association in sex-hormone related pathways. We found that, after correcting for the multiple pathways tested, the ´steroid hormone receptor activity´ pathway was significantly associated with PT asymmetry.
This study is the first genome-wide scan for genetic effects on PT asymmetry. Variants in the genes and pathways identified may affect the development of this structure and its role in cognition.

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P15.12

Fast detection of de novo copy number variants from case-parent trio SNP arrays.

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To infer de novo copy number variants from SNP arrays for case-parent trios, we exploit the trio design and define a statistic called „minimum distance" to capture differences in copy numbers between offspring and parents using genome wide SNP array data. We show that this approach reduces technical variation from probe effects and genomic waves, which is the major source of false positive identifications in copy number analyses. Following segmentation of the minimum distance by circular binary segmentation, final inference regarding de novo copy number events is based on a posterior calling step. We apply both the „MinimumDistance" approach and the joint HMM implemented in PennCNV to a study of oral clefts, validate several detected de novo regions by qRT-PCR, and assess the overall concordance of these two algorithms. Our analysis of the oral cleft trios reveals that genomic waves represent a substantial source of false positive identifications in the joint HMM, despite a wave-correction implementation in PennCNV. The minimum distance is an effective statistic for reducing technical variation contributing to such false de novo discoveries. Computationally, MinimumDistance provides a nearly 8-fold increase in speed relative to the joint HMM. Using trios not selected for phenotype as controls, we identify a region on chromosome 7p14.1 with a (genome-wide) significantly higher number of de-novo deletions in the case-parent trios ascertained through an oral cleft.

I. Ruczinski: None.

P15.13

Comparison of copy number variation (CNV) calling performance in large numbers of technical replicate SNP array data using three different, widely-used CNV calling algorithms

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Copy number variants (CNVs) have been shown to explain part of the heritability in various multifactorial diseases. Many of these findings are derived from SNP-array data generated in the course of large genome-wide association studies (GWAS). This is not without challenges, however: SNP-arrays contain an ever increasing density of probes which results in a decreased signal-to-noise ratio. The latter causes problems for automated CNV calling algorithms and is a major cause for the unambiguous calling of smaller (<350kbp) and/or low frequency CNVs. Recent studies have therefore focused on the much more reliable calling of larger CNVs (number of consecutive marker or length) and often considering the easier to detect deletion events only.
This study compares the performance and differences in CNV calling using three widely-used CNV calling algorithms: CNVPartition, QuantiSNP2 (v2.2), and PennCNV. As SNP array data a large number of technical replicates (n>500) all genotyped at the University of Bonn on Illumina's HumanOmni-Express- and HumanOmni1M-arrays were used.
We observed an unexpectedly high fluctuation in the prediction of cnv events throughout the three algorithms. All gave comparable findings for larger findings (> 1Mbp) but suffered to give consensus results for smaller variants. Since the replicates were typed on the same array type, this allowed to evaluate effects of the chemistry or the operator in the lab. Results will be shown and based on that parameters will be presented that allow for a better evaluation of the quality of CNV callings from SNP array data.

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P15.14

CNVs and de novo deletion identified in GSTM1 gene in Rheumatoid Arthritis

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Introduction: At least 12% of the human genome has been identified as copy number variable (CNV). We analysed CNVs of Glutathione S-Transferase Mu 1 class gene (GSTM1), a candidate gene in Rheumatoid Arthritis (RA). Three methods for identifying CNVs were compared in trio families with RA.
Patients and Methods: Nine trio families were genotyped using a multiplex standard PCR (mPCR), which lead to identify three genotypes (-/-, -/+, and +/+) of the gene. Two methods of CNVs quantification were used: a quantitative PCR (qPCR, Life Technologies) for a relative quantification and Droplet Digital PCR (DDPCR, BioRad) for an absolute quantification using up to 20,000 reactions for one sample.
Results: mPCR identified genotypes -/- and -/+. qPCR and DDPCR revealed zero to two copies of the gene. All samples with two copies were heterozygous for GSTM1 deletion (-/++). A de novo deletion of a GSTM1 copy was observed in a RA patient. qPCR analysis using a calibrator with a known CN or the Most Frequent CN (MFC) could have generated heterogeneous results. DDPCR did an absolute quantification of CNVs with a high number of replicates and no calibrator or MFC was needed.
Conclusion: Comparison of technologies identifying CNVs of this candidate gene lead us to conclude that DDPCR is the best method to use in this field of investigation. We highlighted then rare events such as de novo deletion in families of RA patients. Further analyses on larger samples are required to better understand the impact of GSTM1 CNVs in RA.

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P15.16

Variant discovery in BRCA1 and BRCA2: Comparison of the efficiency of NextGENe software and the Torrent Suite Variant Caller plugin

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Second-generation sequencing technologies are now widely used in a research setting to screen large numbers of samples for known variations, or as primary detection for new ones. Computational methods that rate the discovered variations and separate the sequencing errors from the real SNPs and mutations are an inseparable part of every NGS pipeline.
In our study the BRCA1 and BRCA2 genes were sequenced in 16 patients with breast cancer on a single Ion 316 chip, using the Ion Torrent PGM platform. The native Ion Torrent Variant Caller and the NextGENe software by SoftGenetics were used separately to analyze the results. The calls that appeared in both analyses were forwarded for confirmation with Sanger sequencing.
NextGENe reported a smaller total number of variations over all 16 patients (224) in the sequenced regions than the Variant Caller (312), which was due to the strict settings of the mutation filter. 106 of those variants were common for the two groups. The filtering was tuned down in two steps, yielding two more NextGENe result sets with 325 (123 common) and 1951 (201 common) variants, respectively. The 201 common variants had a higher frequency than the calls made by Variant Caller alone, and higher scores than those made by NextGENe only. This shows that the combined use of the two filters can increase the quality of the final results, even when they are set to register almost all variations in the data, as in the case of de novo variant discovery.

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P15.17

Evaluation of DNA methylation in breast cancer patients

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Epigenetic alterations, in addition to the highly characterized genetic changes, are key contributors to breast carcinogenesis. Altered DNA methylation is commonly observed in the tumorigenesis. The aberrant promoter hypermethylation of cancer-related genes in tumour-derived DNA from plasma samples was presented as a useful tool for detecting and monitoring cancer.
The purpose of our study is to investigate relationship between DNA methylation and breast cancer progression. Quantitative DNA methylation analysis of 11 genes (APC, ADAM23, CXCL12, ESR1, PGR B, CDH1, RASSF1A, SYK, TIMP3, BRMS1 and SOCS1) was performed by pyrosequencing. For determining of methylation levels we analysed DNA isolated from paraffin-

embedded tumour tissues, plasma and blood cells from 37 sporadic breast cancer patients and plasma and blood samples of 50 healthy controls.
We have observed DNA methylation in nearly half of investigated patients in RASSF1A, APC, ADAM23, CXCL12 and SYK genes in tumour samples. Cumulative methylation results showed different methylation levels in tumour and plasma samples where notable higher portion of methylation was found in tumour. Differences between DNA methylation in plasma samples of patients and healthy controls were not significant. This finding cast some doubts on the utility of DNA extracted from plasma samples to identify hypermethylation of specific gene promoters for diagnosis and prognosis of cancer. Preliminary results suggest that DNA methylation in the several studied genes in tumour tissues could be useful biomarkers for the identification of breast cancer with metastasizing potential.
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P15.18
The Association Between TWIST, RARβ2, ESR1 Gene Promotor Hypermethylation and the Histopathologic Type of Breast Cancer in Turkish Population

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Backround: Breast cancer is the most common cancer in women. Histopathology plays an important part in determining the treatment strategy for women with breast cancer.
TWIST expression in breast tumors correlate with increased disease recurrence, metastasis and poor disease-free survival. Steroid receptor gene family members such as RARβ2 and ESR1 genes are methylated and silenced in a fraction of breast cancer.
Method: In this study the promoter methylation levels of TWIST, RARβ2 and ESR1 gene which are associated with breast cancer were investigated by Quantitative Methylation Sensitive High Resolution Melting Analysis (QMS-HRM).We analysed primary tumor core biopsies from 80 high-risk primary breast cancer patients (tumors ≥2 cm and/or lenfatic metastase and/or distant metastases and/or under 40 years) and their histopathologic types were associated with the methylation levels.
Results: In our study the promoter hypermethylation status were observed at different rates; TWIST, RARβ2 and ESR1 methylation frequencies were 25%, 88.75 and 72.5%, respectively. The promoter hypermethylation levels of the genes found to be significant with lymph node positivity, ER positivity and HER2/neu negativity.
Conclusions: Our study is important as being the first study that analyzes the association between histopathologic type of breast cancer and TWIST, RARβ2, ESR1 gene promotor methylation status in Turkish population.
Key words: Breast cancer, Histopathologic Type, TWIST gene, RARβ2 gene, ESR1 gene, Methylation, MS-HRM Analysis

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P15.19
Analysis of Apoptosis regulatory pathway in sporadic breast cancer through Gene Regulatory Network

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In multicellular organisms, gene regulatory networks along with transcriptional factors (TF) control the global gene expression and the dynamics of protein output in living cells. An extensive analysis of networks facilitates to analyze coordinated gene expression changes to find altered molecular pathways and genes in cancer development. The aim of the study was to predict the innovative biological networks that describe transcriptional alteration (up or down regulation) in genes/pathways which could contribute to the pathogenesis of cancer and other associated disease. The expression of several candidate genes (MDR1, ATM, BCL2, CASP3, CASP8, CHK2, TR10C, TR10D, TR10B, CFLAR, H2AX, IFNG, IL10, IL4, IL6, P53, MDM2, TGFB1, TNFA, TNF10, BRCA1 and BRCA2), as a part of another study in sporadic breast cancer was used for constructing the biological network which comprised of both transcriptional regulatory relationships and integrated the protein interactome. We also developed a set of TF-gene regulatory relationships, using UCSC genome browser. The human cancer combinatory gene regulatory network was found to be a hierarchical scale-free network with PAX4-transcription factor as the most important regulators. It is known that PAX4 functions as a potent tumor suppressor and plays a critical role in Cancer growth as well as in the functional morbidity causes Diabetes, which also provides a logical link between cancer and diabetes. We believe our work provides a scaffold combinatorial gene regulatory network allowing systematic study of apoptotic gene regulation, and provides a pipeline which could be extended to reveal conditional combinatorial regulatory landscapes correlating with specific cellular contexts.

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P15.20
Methylation analysis of NFkB-related genes in celiac gut mucosa

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Celiac disease (CD) is a complex, immune-mediated enteropathy caused by ingested gluten with strong genetic and environmental risk determinants, and is an excellent target for the study of gene methylation. IL6 has been shown to induce nuclear translocation of DMT1 through NFkB and JAK/STAT pathways and to alter CpG island methylation. In this work, we studied the methylation pattern of the promoter regions of several NFkB-related genes.
Using pyrosequencing of bisulfite treated DNA, we analyzed 4-6 CpG islands in 8 NFkB-related gene promoters in 17 celiac biopsy pairs (at diagnosis and after >2 years on gluten free diet) and 13 non-celiac biopsies. We also studied IL6 expression. Additionally, samples from 8 patients at diagnosis and 8 treated patients were incubated for 12 hours with gliadin, with a modulator of NFkB pathway, with both compounds or without any of them.
Compared to controls, RELA presented lower methylation levels at diagnosis while MAP3K7 and TRADD showed the opposite pattern. Treated patients presented intermediate methylation percentages, suggesting a partial reversion of the aberrant methylation induced by active disease. Methylation levels of different genes showed significant linear correlation in patients but not in controls. Methylation percentages did not correlate with IL6 RNA levels, although IL6 was overexpressed in active CD. Preliminary results suggest that incubation of biopsies from treated patients alters methylation in several genes, and NFkB modulation seems to partially reverse gliadin effects. Correlation between different genes was not observed in these samples, pointing to cell subtype selection underlying the observed events.

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P15.21
Genome-scale DNA Methylation Analysis of Sporadic Colorectal Cancer by Infinium HumanMethylation 450 BeadChips.

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Infinium HumanMethylation 450K BeadChip arrays were used to examine genome-wide DNA methylation profiles in 22 sample pairs of colon cancer (CRC), adjacent tissues and 19 colon tissues samples from cancer-free donors. We show that tumor and both normal methylation profiles can be clearly distinguished from one another and that the main source of methylation variability is associated with disease status. Although we have identified a number of genes potentially involved in the field cancerization their total impact on methylation in normal tissue is rather low.
At CpG sites level we showed that common CRC-specific methylation patterns consist of at least 15,667 CpG sites significantly different from both versions of the norms. 10,342 of them were hypermethylated and 5,325 hypomethylated. Hypermethylated sites were common in maximum number of sample pairs, located mostly in CpG islands and significantly enriched for known cancer-specific differentially methylated regions, while hypomethylated were mostly located in CpG Shores and were generally sample-specific.
Despite of considerable variability in methylation data we selected a panel of 14 highly robust candidate methylation markers located in genes SND1, ADHFE1, OPLAH, TLX2, C1orf70, ZFP64, NR5A2 and COL4A. This set was

successfully cross-validated using methylation data of 247 CRC samples from The Cancer Genome Atlas consortium (AUC = 0,981(95% CI: 0.9677-0.9939), sensitivity = 100%, specifity = 82%).
In summary, our results demonstrate the significance of aberrant DNA methylation in CRC tumorigenesis and report a large number of novel differentially methylated loci some of which may serve as candidate markers for diagnostic purposes.

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P15.22
Facing the challenge of increased genetic and clinical heterogenities

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With next-generation sequencing techniques, geneticists are in great need of bioinformatic solutions to face the deluge of literature and gene identifications. A challenge is thus to provide both the medical and the scientific audiences with ready and easy access to high-quality synthesized information on rare human diseases in relation with the genes. Originally founded in 1986, GenAtlas is a world-reference database of genes and diseases that has ever since been constantly maintained and updated. It provides a flow of information from gene structure to expression, protein structure to expression and function, mutations to gain or loss-of-function consequences. Reciprocal links are established with different databases such as Ensembl, HGNC, Genecards, UniGene, NCBI Gene and Refseq, HGMD as well as OMIM, UniProt, OrphaNet, GeneReviews, confirming international recognition. The specificity of GenAtlas is that it records data manually extracted from scientific papers and annotated, making it a genuine compilation of genetic diseases with updated and curated links to relevant original articles. Through proof-of-concept cases, we will present how the new web-interface can help facing the challenge of clinical and genetic heterogeneity, as well as the characterization of non-coding RNA genes, i.e. microRNA and long non-coding RNA as bona fide causal genes.

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P15.23
Qualifying the role of genes in the determination of diseases: a new service of Orphanet

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The identification of genes related to diseases is growing exponentially. However, relationships between genes and diseases are not always explicit. There is no standardised, systematic annotation of gene-disease relationships in genetic databases that is easy to use for research purposes. Orphanet has maintained a database of genes linked to rare diseases since 2007. Genes are also entered in the database if tests are available in order to search for susceptibility factors for non-rare diseases. There are currently 2,498 genes linked to diseases in the database. Each gene-disease relationship is assigned one the following categories: germline or somatic causing mutations, part of a fusion gene, major susceptibility factor, modifying gene or gene playing a major role in the phenotype (of a chromosomal abnormality). The evidence is established from the literature, by cross-referencing with other genetic databases (i.e. OMIM, Geneatlas), by seeking expert advice or by collective decisions made by the scientific staff at Orphanet in very difficult cases. Whether the information has been assessed or not is indicated in the website by a visual pictogram as well as in the downloadable files.
The *gene-relationship-disease* triples can be visualised via the Orphanet website (www.orpha.net) by disease or gene search, or extracted from Orphadata (www.orphadata.org). They will be used in innovative bioinformatics projects (for instance, OpenPHACS) as nanopublications.

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P15.24
Automating the collection and quality control of clinical variant data.

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The diagnostic mutation database (DMuDB https://secure.dmudb.net) was established in 2005 to allow the secure sharing of diagnostic variant data. Currently the database contains 45117 variant reports from 78 genes. Since 2011 DMuDB has been available to diagnostic laboratories worldwide and is now used by 105 laboratories.
As DMuDB has grown, the need for ensuring data quality has become more apparent. To identify incorrect nomenclature we have developed a quality control pipeline using the Taverna Workflow Management System (http://www.taverna.org.uk/) and the Mutalyzer web service (https://mutalyzer.nl/). In addition, we have identified instances where the pathogenicity of a variant differs between submissions. This has required us to establish protocols for re-contacting users regarding their submissions.
Much of the data submitted to DMuDB is in the form of users spreadsheets that require reformatting prior to database submission. This is time consuming and inappropriate for next generation sequencing (NGS) data. We are developing mechanisms for incorporating submission to DMuDB into laboratories LIMS systems. We are enabling the input of patient phenotype data using standards such as Human Phenotype Ontology (HPO). Capturing phenotype data will increase the usefulness of variant data to the clinical community. This particularly applies to variants identified by NGS, which may have been identified by the sequencing of large gene panels.
Our experience of databasing clinical variants suggests that future development of large variant data repositories will require a "hands on" approach by database curators in order to maintain data quality.

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P15.25
Estimation of the timing and rate of *de novo* mutagenesis in a healthy monozygotic twin pair

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Characterizing *de novo* mutations regarding their origin, timing and rate has great importance in terms of providing insights into mutational processes, human diseases and evolution. Outstanding progress of the next generation sequencing technologies has facilitated the investigation of *de novo* mutations and led to the estimation of human *de novo* single nucleotide variant (SNV) rate at ~1.2 × 10−8 bp/generation. However, the timing of these *de novo* mutations is still unknown. Hence, we performed deep whole genome sequencing of a male monozygotic twin pair and their parents at (21-41x) and identified ~3.2 million SNVs for each individual. Through the comparative analysis of the genotypes of both twins and their parents for each of these SNVs in a synchronized manner, we discovered a total of 97 *de novo* SNVs. Among them, 46 were shared by the twin pair indicating a parental or pre-twining zygotic origin. We found 37 *de novo* SNVs to be specific for twin-I and 14 for twin-II, which indicates that these SNVs had occured in the post-twinning embryo, particularly in the mesoderm from which the blood cells derived. To date, we validated 12 pre-twinning and 2 post-twinning *de novo* SNVs using Sanger sequencing, while other candidates are currently being tested at the time of this abstract submission. Our findings provide knowledge about the rate and timing of *de novo* mutagenesis in healthy individuals; and further reiterate the importance of post-zygotic mutations leading to somatic mosaicism in what could be a wide spectrum of human diseases.

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P15.26
Locus Reference Genomic sequences: reference sequences for the reporting of clinically relevant sequence variants.

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Locus reference genomic (LRG) sequences are internationally recognized reference sequences designed specifically for the reporting of clinically relevant variants. An LRG provides a stable genomic DNA sequence for a region

of the human genome which will never be changed and so is not versioned. They are compiled and maintained by the NCBI and EBI, as part of the GEN2PHEN and RefSeqGene projects (www.ncbi.nlm.nih.gov/refseq/rsg), in collaboration with the community of diagnostic and research labs, locus specific database (LSDB) curators and mutation consortia. The LRG project aims to create an LRG for every clinically relevant genomic locus. Over 600 LRGs have been created so far.

All information relating to an LRG is contained within a single XML file, which comprises separate sections for the fixed and updatable annotation. The fixed (stable) section contains the genomic sequence and annotation for transcripts and exons used as reference standards. The updatable section contains mapping information for the LRG sequence, as well as annotation of additional transcripts, co-located genes, LSDB information, and alternative exon and amino-acid numbering systems.

The stable nature of these reference sequences enables unambiguous reporting of variants in LRG coordinates, as recommended by the Human Genome Variation Society (HGVS) and European Molecular Genetics Quality Network (EMQN). LRGs can be viewed in the Ensembl and NCBI genome browsers and databases. Tools such as Mutalyzer (https://mutalyzer.nl) and Variation Reporter (http://www.ncbi.nlm.nih.gov/variation/tools/reporter/) also support LRGs.

All LRG records are available on the LRG website (http://www.lrg-sequence.org), which also provides instructions on requesting LRGs and the complete LRG specification.

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P15.27
Rapid Identification of Clinically Relevant Variants from Human Sequencing Data

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Biological interpretation of thousands of potentially deleterious variants is a bottleneck in discovering valuable causal insights from DNA resequencing studies, often requiring months of effort after completion of the variant calling step. Ingenuity® Variant Analysis (www.ingenuity.com/variants) is an application that leverages an extensive knowledge base of millions of expert-curated mutation and biomedical findings from the literature and integrated reference information to enable real-time interactive filtering and rapid prioritization of variants. We have extended it to compute a synthesis of the current knowledge about variants to provide automated initial clinical assessments, identify variants implicated in diseases consistent with observed clinical features, and extensive coverage of pharmacogenetic impacts. Aside from empowering clinical researchers to immediately identify reportable variants in medical genomes, we have optimized gene-level burden tests on the order of 100x faster than conventional methods while delivering consistent results, and pathway level causally-consistent algorithms to find the few variants that are most compelling for follow-up in multi-sample studies. Using a combination of causal analytics, statistical and genetic analysis at the variant, gene, and pathway levels, and the ability to visualize how variants impact disease progression, we will demonstrate the application of a context-rich knowledge base to discover clinically relevant cancer driver variants and novel causal variants for human genetic disease.

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P15.28
High-throughput screening of animal venoms for isolation of novel drug leads

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Multiple studies have shown the potential of some animal venom compounds for the treatment of different diseases where medical needs are not properly addressed by the current available treatments. Animal venoms are small effective molecules with extraordinary target selectivity and low side effects with huge natural and chemical diversity. These properties make animal venoms especially suitable as sources for drug development. Due to technical and economical limitations in traditional peptide screening techniques little work has been done so far in venom-based research for drug therapy. With the advent of Next-Generation Sequencing platforms novel and more efficient strategies for drug discovery are now feasible to improve Human Health.

Here we describe the results of the first pilot project that was performed to reconstruct and characterize three venom transcriptomes that were sequenced on two different NGS platforms. We functionally compared the results of the different datasets to provide new insights into venom composition. Our results are part of a comprehensive international project aiming at full venom toxin identification for drug lead discovery and drug development. The VENOMICS project is a FP7 HEALTH initiative which aims to analyze 200 venoms using the most advanced proteomics and transcriptomics techniques to obtain a collection of 10,000 venom peptides that will be further used for drug development to target different metabolic diseases such as obesity and diabetes.

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P15.29
DNA-methylation changes at multiple loci in patients with imprinting disorders due to epimutations and in children born small for gestational age

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A subset of patients with imprinting disorders due to epimutations shows DNA methylation defects at multiple loci (MLMD). Within the German "Imprinting Network" we analyzed the methylation pattern at imprinted loci in 127 patients with imprinting disorders caused by epimutations and 98 children born small for gestational age (SGA). In a step-wise screening approach we first determined peripheral blood DNA methylation patterns of 10 imprinted loci in all patients by bisulfite pyrosequencing and compared them to those of 50 healthy controls. In addition to 6/127 patients previously diagnosed with MLMD, bisulfite pyrosequencing suggested aberrant DNA methylation at multiple loci in additional 15/127 patients with classical imprinting disorders. No SGA born child showed MLMD (Bens et al, EJHG, 2012).

All patients suggestive for MLMD in pyrosequencing with left over material (n=19), one "positive control" for each classical imprinting disorder (n=6) and all children born SGA were further analyzed using the HumanMethylation450k BeadChip (Illumina) interrogating genome-wide DNA-methylation patterns at 485,577 CpG loci. Results were compared to those of 20 healthy controls. MLMD was confirmed in all previously diagnosed cases, 3 patients suggestive for MLMD in pyrosequencing, one SGA born child and one "positive control". The extent of methylation changes varied with the *KCNQ1* locus being most frequently affected consistent with the clinical phenotype of BWS in 3/11 cases. This study also revealed that multiple imprinting defects are more frequent in growth disorders than in neurogenetic disorders caused by imprinting defects.

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P15.30
Association of IGFBP-5 exon-I methylation and gene expression in breast cancer

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Breast cancer is the most frequently diagnosed oncological disease. Insulin-like growth factor binding protein-5 (IGFBP-5) is one of the critical members of IGF system in both normal cell physiology and tumorigenesis. IGFBP5 expression shows diversity in breast cancer patient tissues. Aberrant CpG methylation in regulator sequences of this gene could be responsible for the difference of IGFBP-5 expression pattern in breast tumor.
The aim of our study was to evaluate the significance of IGFBP-5 exon-1 methylation status on expression level of IGFBP-5 mRNA in patients with breast cancer. Thirty pairs of breast carcinomas and their normal breast tissue counterparts from the same patients were randomly selected for methylation-specific PCR (MSP) experiment. Methylation status of first exon and its transcriptional effect were evaluated using a MSP and expression level by real time PCR, respectively.
According to our results, different methylation patterns of the region were detected between healthy and cancer tissues of 4/30 patients. Gene expression and methylation status were correlated in two of these patients, whereas in one there was no correlation. In the fourth patient tissues, in which methylation and unmethylation were detected in healthy and cancer tissue, respectively, the expression data is not available yet.
In conclusion, methylation level of IGFBP-5 exon-1 region has been shown to be rarely present in breast cancer tissue, but it may affect mRNA expression level of the gene. Further studies are needed to confirm these findings. Ongoing studies about promoter sequence methylation of IGFBP-5 gene are being conducted.

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P15.32
Exome Database

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SNVs and indels derived from exome sequencing are usually filtered by minor allele frequency and mode of inheritance in order to identify candidate variants for Mendelian disease. We developed a database that stores both pedigree information and variant data generated by an analysis pipeline. A corresponding web frontend allows to filter the data in an interactive way. Standard queries include searches for rare heterozygous, compound heterozygous, homozygous, de novo and somatic variants. Variants are connected with a number of annotations such as position in transcripts, amino acid change, functional predictions, and presence in dbSNP, HGMD and the 1000 Genomes data. Furthermore, quality scores, and read depth and links to external databases are provided. In addition to publically available frequency data, variants identified in in-house exomes of individuals with unrelated phenotypes are used as controls.
In addition to frequency based searches, one can also perform gene and disease-based searches, thus displaying the variation content of genes or focusing the search on known disease genes or HGMD mutations.
The database also contains data about the average read depth per exon and the proportion by which each exon is covered at least 20-times. These data can be used in a diagnostic context to define the regions of a gene which have been appropriately analyzed and to identify genes which possibly carry deletions of entire exons.
Lastly, the database is connected with a LIMS and provides basic quality statistics, such as sequence amount, read depth, sample contamination and discordance between specified and identified sex.

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P15.33
GeneTalk: a compound heterozygotes filter for exome data

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The identification of disease causing mutations in next-generation sequencing (NGS) data requires efficient filtering techniques. We developed a web-based compound heterozygous filter that is suited for data from NGS projects and that is easy to use for non-bioinformaticians. We analyzed the power of compound heterozygous mutation filtering for rare recessive disorders in single individuals, trios and more complex pedigree structures based on exome data of more than 10,000 individuals from 10 different population backgrounds. Depending on the ethnicity there are characteristic sets of highly variable genes that can be used to filter candidate genes. Compound heterozygous filtering rules in combination with population frequency data yield a highly effective prioritization in disease gene discovery.

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P15.34
Gene-centered viewing, storing and sharing of exome/genome variant and phenotype data

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The favourite view of sequence variant data in DNA diagnostic centers is gene-centered. We have developed a new version of the LOVD platform (Leiden Open-source Variation Database, http://www.LOVD.nl) facilitating the analysis of exome and genome sequence data. During installation, web services retrieve gene and transcript information on the fly. Imported variant data are stored using chromosomal nucleotide positions as a reference. Data can be stored and displayed in several ways: variant-by-variant or all connected to one individual. Using the existing LOVD functionality, users have the option to perform query per gene or individual, to link to other resources of interest, to get genome browser views of the data and to using web services to access variants stored in other gene variant databases. LOVD 3 has the unique option to independently store both the phenotypes screened and the variants detected. This gives submitters the chance to share inconclusive results, allowing collaborators with matching data to join the gene identification project and crack the case together. In addition, LOVD3 has a new access level, designated “collaborator”, allowing submitters to share otherwise non-public data with other submitters, e.g., to share detailed phenotype information with other diagnostic labs only.
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P15.35
Circadian clock in whole blood cells

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Circadian rhythm is approximately 24-hour cycle in behavioural, physiological and biochemical processes in diverse living organisms. One of underlying principles of successful rhythmicity is cycling gene activation, which leads to the changes in the transcriptome of different tissues. We investigated gene expression in the peripheral blood lymphocytes as a function of time of the day when the blood sample was taken. In total mRNA samples from1086 individuals were analysed using Illumina HumanHT-12 v3 BeadChip arrays. We found 181 statistically differentially expressed transcripts throughout the day (from 9 am to 18 pm), including previously known rhythmic genes as well as novel candidate genes that might be related to circadian rhythms of blood. These results were replicated in KORA cohort samples, although in shorter time-span. This finding demonstrates daytime-dependent effect on gene expression of whole blood cells, indicating potentially functional circadian clock in peripheral blood cells.

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P15.36

Gene expression profiling of non-injurious mechanical ventilation in an animal model of sepsis-induced ARDS

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The acute respiratory distress syndrome (ARDS) is a severe inflammatory disease characterized by pulmonary edema and hypoxemia. It commonly affects critically ill patients following insults such as sepsis. The only effective treatment is mechanical ventilation (MV) using low tidal volumes (LTV), although it is unclear how it exerts its protective effects. To identify the molecular mechanisms modulated by MV, we performed microarray gene expression analysis using a clinically relevant model of sepsis-induced ARDS by cecal ligation and puncture in male Sprague-Dawley rats. Following this first injury, rats were exposed to different MV regimes, using high tidal volume, LTV, or simply non-MV. Total lung RNA was extracted, DNase digested, integrity evaluated and hybridized to microarrays. Comparisons against the non-septic control rats revealed a total of 2038 deregulated genes (at 5% false discovery rate) in the overall experiment. Functional predictions in the 1013 deregulated genes of the LTV group supported the involvement of chemotaxis, proliferation and cellular differentiation, lung development, cell matrix adhesion, water transporter channels, neuronal development and axonogenesis, processes related to edema formation and pulmonary fibrosis commonly observed in the lungs of ARDS patients. We expect that further integrative analysis of this data along with existing human genomic information from this and other respiratory conditions will provide valuable candidate genes for downstream association studies of genetic variants with ARDS pathogenesis.

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P15.38

Genome-wide association study of DNA methylation and whole blood expression profiles in KORA

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DNA methylation is one of the main epigenetic mechanisms regulating the activation and inactivation of genes in a tissue specific manner. Although it is well-known that DNA methylation is essential for the regulation of gene expression, so far no epigenome-wide study is published that has analyzed the influence of the methylation state on transcript levels in whole blood leucocytes.

We analyzed 732 individuals from the population-based KORA F4 cohort with whole blood DNA methylation data measured on the Illumina HumanMethylation450 BeadChip and whole blood gene expression data on the Illumina Human HT-12 v3 Expression BeadChip. After quality control we applied a linear regression model using methylation intensity values (M-values) for 460.000 CpG sites and almost 30.000 expression probes. We obtained more than 12 million significant associations when using the Bonferroni threshold of 3.7E-12 including 76,720 CpG sites and 5,145 expression probes. To our surprise, more than 80% of the associations were between CpG sites and transcripts across different chromosomes. On the one hand, this might suggest a high degree of inter-chromosomal interactions; however, on the other hand, the extremely high proportion of non-cis interactions questions the biological significance of the identified associations and needs to be verified. Future strategies include replication, adjustment for leucocyte subtypes and genetic variants, stepwise exclusion of sites and transcripts with multiple hits, and functional annotation of the significant hits. This study suggests a complex relationship between the methylation state and transcript levels and demonstrates the challenges in interpreting results from multi-omics analyses.

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P15.39

Architecture of aging-regulated genome-wide expression profiles in humans

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Summary: During adulthood physiological ability declines with age and is associated with genome-wide changes in gene expression profiles. Genome-wide age-regulated expression trends could provide an unbiased description of temporal changes during aging. We developed a robust statistical methodology to identify age-regulated expression trends in human cross-sectional datasets, and we show that the pattern of aging-associated changes in expression profiles differ between tissues. We identified that in *Vastus lateralis* muscles and in brain cortex molecular aging starts already during early midlife and is progressed through two distinct checkpoints. In contrast in Kidney cortex and in blood changes starts only around the eighth decade. Our results suggest that in human molecular aging progresses through at least two checkpoints, which are temporally and spatially distinct between tissues. This open new hypothesis for how aging is initiates and progressed in humans.

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P15.40

SNP detection by RNA-seq

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The massively parallel sequencing of the transcriptome (RNA-seq) produces several millions sequences (reads) that are usually used to quantify digitally the genes expression over the trascriptome. Since RNA-seq is based on sequencing, it's possible to identify loci that are likely to be polimorphic using alignment mismatches between the tested sample and the reference genome sequence. The goal of this study is to compare the most common alignment programs (bowtie2, tophat2, bwa, gsnap) to verify the reliability of SNP detection in RNA-seq samples. For the analysis were used data of 5 RNA-seq samples from individuals (2 leukemia and 3 chronic myeloproliferative syndrome) previously genotyped with the Affymetrix Chip GenomeWideSNP 6. After alignment, genotypes of polimorfic loci were detected directly from the pileup computed by the program samtools. More than 10000 polymorphic loci were detected by RNA-seq and were in common with the corresponding loci on the Chip. In the first analysis an allele was considered a true variant if called by at least five reads. Using this threshold the average genotype error rate between RNA-seq and Chip is ~10%. The same computation performed with no threshold value (1 read was enough to call the alternative allele) shows an error rate of ~ 0,03%. The results suggest that SNP detection is possible using RNA-seq and that variants called by few reads have to be interpreted as true variant and not as background noise. A more detailed study on differential allele counts needs to be performed to ascertain possible biases of RNA-seq.

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P15.41

SH-imi-goldnanoparticles a possible drug target carrier: an evaluation using epigenetic histone dimethylation levels

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This study refers a 2-mercapto-1-methylimidazole (SH-imi) gold nanoparticles (AuNPs)-conjugated synthesis, their interaction within different cell lines and epigenetic effect after cell internalization. We describe SH-imi-AuNPs cell intake following H3K4 and H3K9 dimethylation analysis in three different cell lines: human neuroblastoma cells SH-SY5Y, androgen-responsive lymph node prostate cancer cell line LNCaP, and DU-145 cells, a non-androgen sensitive cell line. Specifically we study the level of dimethylation in H3K4 as epigenetic marker of euchromatin, and dimethylation in H3K9 as transcriptional repression marker.
After an hour of treatment with SH-imiAuNPs, histones were extracted and dimethyl-H3K4 and -H3K9 evaluated. The three cell lines all show decreased levels of H3K4me2 and increased levels of H3K9me2. Additionally we observe a general chromatin closing trend within the three cell lines with minor different effect.
To better understand the nanogold biological interaction we incubated SH-SY5Y for an hour using decreased concentration of SH-imiAuNPs, within the range of 100 ng/ml to1 ng/ml. We found that cell damage decrease with the concentration of gold nanoparticles. Fluorescent microscopy and binarization algorithm based on thresholding process with an RGB input image, still show the presence of SH-imiAuNPs into cells. In particular, our results underline that lower concentration of nanoparticles lead to a new characteristic nano-phenotype in which the cells reached a new steady-state healthy condition. Further, under these conditions SH-SY5Y cell lines are able to rescue cell phenotype making the system suitable for drugs nano-carrier studies.

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P15.42

High-resolution HLA Typing of HapMap NGS Samples From 1000 Genomes Paired Illumina Reads

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HLA typing from NGS data is gaining importance as the cost of sequencing and sample preparation is falling rapidly. Since the MHC is the most polymorphic region of the human genome with significant segmental duplication this makes the usual NGS analysis, which includes reference-alignment of the NGS reads highly unreliable.
Omixon has developed algorithms to solve HLA typing by directly mapping NGS reads to the IMGT/HLA database to determine the best matching HLA type from whole genome and whole exome data. We are presenting methods to determine HLA types up to four field if possible from public data using paired Illumina reads of the 1000 Genomes (KG) samples. Data from 218 samples of the HapMap set (137 Coriell IDs) with known HLA types (A,B,C) were used for validation. Criteria for typing confidence and accuracy is also presented. Automated filtering and typing of a single sample takes minutes on a commodity PC with our database-driven search algorithm. Results show that although the KG data was not intended to use for high-resolution HLA typing, read sets with proper read-length and quality can provide the correct types above 90% concordance.

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P15.43

Exome sequencing in a family with hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is a heart muscle disease with a prevalence of 1:500 in the general population. It is an inherited autosomal-dominant disease, characterized by left ventricular hypertrophy. Mutations in one of the known sarcomeric genes are identified in 50-60% of cases. However, in the remaining genetic cause leading to HCM is unknown.
The aim of the study was to identify the genetic cause of HCM in a family with no identified mutation. We sequenced the exome of three affected distant relatives. Exome libraries were prepared using the TruSeq Exome Enrichment Kit (Illumina) and sequenced on Illumina HiSeq2000. On average, 4.5 Gb, corresponding to 65,000,000 reads of sequence (12% duplicate reads) were generated per exome. Alignment was done using BWA v.0.5.9 against the human reference genome GRCh37/hg19. Approximately 70% of the bases were covered at least 20-fold. For variant calling, the GATK pipeline (Broad Institute) was used. For putative mutation identification, we selected only variants shared by the three sequenced individuals and which were novel (absent in dbSNP135) and heterozygous (dominant inheritance). After filtering, we identified 12 variants in 12 different genes. All were genotyped in the whole pedigree (8 affected, 3 unaffected individuals) to check for co-segregation. Two variants in genes not previously reported to be associated with HCM remain candidates. None of them is obviously related to cardiac dysfunction suggesting a new mechanism leading to HCM in this family. Current resequencing of the two genes in 200 unrelated HCM cases should definitely identify the causative gene.

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P15.44

An integrated 'imputation in a box' pipeline for GWAS studies

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Genetic imputation has been a major component of many bioinformatics analysis pipelines including GWAS, signal refinement and meta-analysis. Moreover, modern genomic research moves towards identification and discovery of novel markers that contribute to complex diseases. These markers often belong to the lower side of the frequency spectrum. In order to impute these sufficiently we need vast amounts of data both in the study and the reference panel. Hence imputation tend to be very laborious, complex and demand modern High Performance Computing (HPC) environments. Finally very limited work has been done to improve comparability, reproducibility and sharing of existing pipelines.
Here we propose a web application that contains analysis pipelines for almost all imputation software (mach/minimach, impute2 and beagle). The pipelines include protocols for format conversion, sample and chromosome chunking, quality check, accuracy metrics and visualization, all in simple CSV format and as editable elements in a database. It also includes the widely used reference panels of 1000 Genomes (GIANT release), HapMap2 and tools for creation of custom panels. The application supports various flavours of HPCs including local server, (pbs) cluster, grid and cloud infrastructure. The complete computation can be monitored and managed through a web interface or commandline interface.
The proposed approach can be conceptualized as an "imputation in a box" application that belongs to the family of MOLGENIS 'compute' ecosystem that also includes NGS and GWAS pipelines. All has been used successfully for imputation in the BBMRI-NL Genome of the Netherlands. Availability: http://www.molgenis.org/wiki/ComputeStart

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P15.45

Bioinformatic approaches for predicting the development and progression of inflammatory bowel disease

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So far, genome wide association studies identified 167 loci which contribute the IBD development. Scientists use different statistic methods for evaluating markers, however, no one have reached sufficient specificity and sensitivity to transfer genetic tests in clinical practice. To confirm previously identified SNPs in Slovenians, we genotyped 635 patients and 276 controls. A robust evaluation technique using repeated cross-validation was performed to identify the number of SNPs needed for optimal performance. Recur-

sive Feature Elimination (RFE) using Support Vector Machines (SVM) was used to assess the effectiveness of classification for different SNP combinations. SVM-RFE (evaluated using 20 runs of 10-fold cross-validation) identified a combination of approximately 19 SNPs that can be used for optimal performance in terms of area under the ROC curve (AUC). Using an optimal set of SNPs we were able to achieve AUC of 0.828, sensitivity of 0.515 and specificity of 0.900. By designing risk profiles, based on different number of SNPs, and deriving relative risk from odds ratio we could poorly predict who will develop IBD and who will not, however we reached predictive value up to 70 % to distinguish between two major sub-phenotypes and up to 96 % to predict which Chron's disease patients will require biological therapy. Results of our study hold a great promise and hope for application of SNPs as biomarkers in clinical practice. Predictive models, based on genetic characteristics may be used as diagnostic and prognostic tests of each individual, encourage positive lifestyle and guide clinical decisions in the near future.

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P15.46

Placing landMarks in the Knowledge Space: crowd-sourcing landmark publications for benchmarking text-mined predictions

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Data production and the volume of biomedical literature are growing faster than human comprehension. Keeping up to date with new discoveries even within one's own field of expertise is no longer possible. To make it easier for researchers to keep up with landmark biomedical discoveries, we introduce a simple, a web-based tool called LandMark, that allows researchers to log their newly discovered gene-disease associations, drug-disease associations, and protein-protein interactions. The interface is a minimal web-form that takes only minutes to complete, but then archives the discovery in a public database making it easy for the research community to monitor and cite new discoveries. Users who file a new discovery at LandMark will receive a brief report about their concepts based on our semantic analysis of MEDLINE abstracts. Furthermore, by registering at the web site, users will receive automatic updates for future discoveries involving the diseases, genes, proteins or drugs of their choice. LandMark allows individual researchers, institutions and publishers to publicly claim landmark discoveries and serves as a transparent service to help resolve conflicts about priority in landmark discovery. LandMark stores information using the novel semantically enabled nanopublication format, allowing automatic interoperability with other semantic data and proper attribution. Over time, LandMark will result in a gold-standard test set of discoveries for benchmarking text-mining and automated knowledge discovery systems. LandMark can be accessed at: http://nanopub.org/landmark/

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P15.47

Genome-wide analysis of macrosatellite repeat copy number variation in HapMap panels identifies differences and commonalities in repeat size variation

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Macrosatellite repeats (MSRs) belong to the largest repeat structures in the human genome and usually span hundreds of kilobases of genomic DNA. Because of their polymorphic nature, MSRs represent an extreme example of copy number variation. Their structure and function however, is poorly understood. We studied six autosomal and two X chromosomal MSRs among 270 HapMap individuals from Central Europe, Asia and Africa for copy number variation, stability and genetic heterogeneity.
Repeat array size distribution analysis indicates that all MSRs are highly polymorphic. The most genetic variation was observed among Africans and the least among Asians. A mitotic mutation rate of 0.4-2.2% was detected, which exceeds meiotic mutation rates of MSRs and possibly explains the large size variability typically observed for these MSRs. By means of a novel Bayesian approach, statistical support for a distinct multimodal rather than a uniform allele size distribution was detected in all but one MSRs, with evidence for equidistant intervals between the modes.
These results suggest that MSRs are restricted in their configurations and that deviations thereof may cause disease, as is the case for facioscapulohumeral muscular dystrophy, which is most often caused by contraction of the D4Z4 MSR below a threshold of 10 units. This study represents the first comprehensive study of MSRs in different human populations and identifies commonalities and differences in their organization and function of the human genome.

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P15.48

Inter-individual methylation variability in differentially methylated regions between maternal whole blood and first trimester CVS

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DNA methylation is the most studied form of epigenetic regulation, a process by which chromatin composition and transcription factor binding is altered to influence tissue specific gene expression and differentiation. Such tissue specific methylation patterns are investigated as biomarkers for cancer and cell-free fetal DNA using various methodologies. We have utilized methylDNA immunoprecipitation (MeDIP) and real-time quantitative PCR to investigate the inter-individual methylation variability of 22 differentially methylated regions (DMRs) in fifty 1st trimester Chorionic villus samplings (CVS) and fifty female peripheral whole-blood (WB) samples. qPCR results from MeDIP and genomic DNA (Input) assays were used to calculate fold enrichment values for each DMR. For all regions tested MeDIP enrichment was higher in CVS than in WB samples as expected, and mean average enrichment values across all regions tested was 2.9 and 0.2 respectively. The variability of the two datasets was assessed using their mean coefficients of variation, giving a score of 0.4 for CVS and 0.9 for the WB cohorts. Despite the inter-individual variability noted for these regions, mean enrichment values for CVS were significantly different than those for WB in all DMRs tested ($p<0.001$). This observation is reinforced by the absence of overlap in CVS and WB enrichment value distributions for 16 of 22 DMRs. Our data indicate that inter-individual variation in methylation is an important variable to consider during biomarker discovery, yet the difference in methylation status across tissues is large enough to allow for robust tissue specific methylation identification.

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P15.49

Menopausal effects on metabolic signatures of a Finnish population cohort

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Background: Recent studies have revealed general age- and sex-related patterns in metabolite profiles, but have not provided information on serum lipid markers or specifically addressed menopause as a factor affecting metabolite levels. After menopausal transition, women rapidly lose their advantage in cardiovascular disease risk compared to age-matched men, which has partly been explained with pro-atherogenic alterations in serum lipids. We aimed to analyze menopause- and age-related changes in the metabolite profiles of a Finnish population cohort.
Methods: Our study sample consisted of 7060 non-diabetic and non-lipid medicated individuals from FINRISK 1997. The levels of over 100 NMR-measured serum metabolites were individually treated as dependent variables in linear regression analysis with BMI and smoking taken into account. The transcriptomic changes in menopause were analyzed in a similar

linear analysis of 518 individuals of FINRISK 2007.
Results: Our results highlight a systematic, consistent shift towards a more atherogenic lipid profile by age, observed in all Apolipoprotein B-100 parameters. In women, the steepest slope of change strictly overlaps the time of menopausal transition, whereas in men the pattern shows a gradual change beginning already at early middle age. In transcriptomic analysis, the expression levels of several liver lipid-related genes were observed to vary with menopausal status.
Conclusions: Our results provide population-based knowledge on detailed lipid measures which may add valuable insights to CVD-risk assessment especially in women, emphasizing the need to stratify analysis by sex and the importance of menopausal status alongside age in all CVD- and lipid-related risk analysis.

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P15.50
Whole genome bisulfite sequencing of acute lymphoblastic leukemia cells

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Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer in the developed countries. DNA methylation of CpG sites plays an important role in cell differentiation and establishment of cell identity by regulation of gene expression. Aberrant patterns of methylation have been associated with a wide variety of diseases including cancer. We and others have previously documented large differences in the patterns of ALL epigenomes compared to control immune cells and common patterns have been identified in different subtypes of ALL. Because of its biological importance, abnormal methylation patterns could play a major roll in the establishment and malignancy of ALL. Hence, methylome analysis can shed light on the molecular mechanisms involved in ALL pathogenesis.
Bisulfite treatment of DNA coupled to high-though sequencing offers a unique possibility to unravel the genome-wide cytosine methylation landscape at base-pair resolution. In addition, bisulfite sequencing can give information relating to haplotype specific methylation. To investigate the distribution of methylation in ALL cancer cells, we have generated whole genome bisulfite sequencing data at high coverage to characterize the methylomes of four samples, belonging to different subtypes of ALL. We will present a detailed view of the methylation patterns in the four ALL patients together with analysis of RNA-Seq data that we will use to infer how methylation affects gene transcription. Furthermore, we will also investigate the occurrence of allele-specific methylation and how it is connected to allele-specific gene regulation.

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P15.51
DNA Methylation Patterns Associated with Smoking in Twins Discordant for Smoking Status

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It is well established that methylation changes in DNA at cytosine bases, constitutes an important part of epigenetic regulation of human gene expression. Although even monozygotic twin pairs havedifference in their methylation patterns which grow with aging, the specific factors associated with DNA methylation profiles are not well understood. Smoking is one of the most important health determinants associated with numerous diseases. While interactions between smoking habits and genetic susceptibilities are a priority health issues, epigenetic angles regarding such interaction mechanisms are not well understood. In this study we have selected 11 pairs of monozygotic twins in a Korean twin-family cohort study, The Healthy Twin Study. Monozygotic twin pairs consisting of "never smoker" and "current smoker" by self-administered questionnaire were further validated by urine cotinine level. 4 pairs with conflicting information between questionnaire and cotinine levels were excluded. Average age of twins were 43 (7 male and 4 female pairs), and the cumulative smoking of the smokers were 14.7 pack-years. Illumina 450K genome-wide methylation array was used for analysis, and 484,130 probes passed QC (detection p<0.05) to be used in this analysis. Beta value of methylation for each probe, and gene-based methylation score were used for analysis. Wilcoxon Signed Rank Test was used for screening reactive methylation regions. When we checked for the regions responsible for the smoking difference, 250 regions from 232 genes showed significant differences (p<0.001) between smokers and non-smokers. Several regions were suggested for possible candidate regions of "smoking methylation" hot spots.

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P15.52
DNA metylome comparison of various human tissues

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Introduction: DNA methylation is one of the basic molecular mechanisms involved in the spatial regulation of gene activity and maintaining the genomic integrity. To complement the current knowledge of normal human DNA methylation, we have analyzed DNA methylation in 17 somatic tissues from four individuals using Infinium HumanMethylation 450 BeadChips (Illumina, San Diego, USA) that covers 486,428 CpG sites all over the genome.
Results: The related tissues (e.g. spleen artery, coronary artery and aorta) successfully co-clustered in the hierarchical clustering analysis. Of the 17 tissues studied, only a small number of CpGs (2%; 10 707 CpGs) were fully methylated in all the analysed tissues and these genes have mostly functions in the reproductive system. The always-methylated CpGs were mostly located in gene body regions. On contrary, the mostly unmethylated CpGs (15%; 72 444 CpGs) were located in the proximity of transcription start sites of the genes that are involved in different cellular processes. We have correlated the DNA methylation patterns with available gene expression data and discovered a number of tissue-specific differentially methylated DNA regions. We have used Sanger sequencing to confirm the differential DNA methylation at selected regions of the genome.
Conclusions: Our results show that human somatic tissues have distinct DNA methylation patterns. There are also general similarities in the location of methylated and unmethylated CpG-s, independent of tissues and genes. Our data may also help in understanding the role of gene-body methylation and methylation of CpG island shores.

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P15.53
Genome-wide DNA methylation profiling in myocardial infarction

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Methylation of CpG islands is an important epigenetic regulation mechanism in organ development and differentiation, aging and several diseases. To investigate the role of differential methylation on myocardial infarction (MI) risk, we examined the methylation levels of more than 450K CpG sites in 206 cases and 206 matched controls belonging to the Italian section of the EPIC cohort. EPIC healthy volunteers were recruited between 1994-98 and followed up for MI and other diseases. For the CpG methylation level assessment on blood DNA we used the Illumina HumanMethylation450 BeadChip. Data were analyzed according to standard procedures (MethyLumi, Bioconductor). To account for sex specific methylation and risk profiles, logistic regression analyses were conducted separately for males and females. All analyses were corrected for matching variables (age, season, center of recruitment) and cardiovascular risk factors when significantly different between cases and controls (smoke, BMI, waist/hip ratio). No significant association of single CpG methylation change has been found at the genome–wide significant threshold (p<10-7) with MI risk. However, in a

genome-wide "regional" association analysis, we found multiple significant signals (p<10-7) of differential methylation between cases and controls in 2 genomic regions for females (Chr5, Chr1) and in 4 regions for males with borderline significance (Chr6, Chr7, Chr11, Chr17). QTLs associated to MI, blood pressure regulation and metabolic disorders have been described in these regions. These results suggest that different methylation profiles between cases and controls can be involved in the regulation of these regions and in the modulation of MI risk.

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P15.54

Contribution of methylomic and proteomic approaches to deciphering the pathophysiology of nasal polyposis, a model of chronic inflammatory diseases

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Nasal polyposis (NP), a chronic inflammatory disease affecting the upper airways, represents an accessible model to investigate the mechanisms underlying chronic inflammation. To test a possible implication of DNA methylation in NP, we combined proteomic (iTRAQ/nano-LC-MALDI-TOF-TOF) and methylomic (Illumina-450K-methylation array) approaches in human nasal epithelial cells from patients with NP (n=3) compared to control (n=3). Considering methylomic and proteomic data for which we found a +/- 30% difference between NP patients and controls, we identified 640 genes with differential CpG methylations; for 20 of them, we quantified the corresponding protein by proteomic analysis. Twelve of these genes displayed a methylation status that correlated perfectly with the expression level of the corresponding proteins: (i) nine genes encoding proteins overexpressed in NP contained one or several undermethylated CpG (DUOX1, POSTN, PRDX3, TXNDC17, HSPG2, LASP1, TAP1, LGALS7 and HLA-DRB1) and (ii) three genes encoding underexpressed proteins contained one or several overmethylated CpG (RASA3, HLA-DQA1 and HLA-C). Interestingly, it turned out that these 12 genes were known to play a role in inflammation, oxidative stress or tissue remodeling, four of which having already been implicated in NP (DUOX1, POSTN, HLA-DRB1 and HLA-DQA1). Moreover, 39 additional genes with differential methylation status would also play a role in chronic inflammation. These first results, which are currently validated by the use of demethylating agents, underline the interest of this promising strategy based on the combination of global methods for studying the possible role of DNA methylation in chronic inflammatory diseases.

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P15.55

The development of bioinformatics pipelines for the analysis of genetic variant data in a cloud environment.

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Next generation sequencing technologies have been rapidly assimilated into genetic diagnostics, enabling the development of a new range of diagnostic tests encompassing large numbers of genes. Inevitably, such tests identify many more variants than "traditional" testing. Only one of these may be disease causing but all must be evaluated to identify this mutation, greatly increasing the workload on the clinical scientists.

To address this problem, we are developing reusable bioinformatics workflows using the Taverna Workflow Management System (http://www.taverna.org.uk/). These workflows group variants according to their positions within genes (i.e. exonic, intronic and near intron/exon boundaries), compare variants to data from 1000 Genomes and NHLBI exome sequencing projects and web services such as Mutalyzer, Pubmed, Ensembl and WAVe are used to evaluate variant pathogenicity and provide a report for clinical scientists.

Developing workflows using Taverna enables sharing with other bioinformaticians via myExperiment (http://www.myexperiment.org/). As well allowing workflows to be reused, this will improve the quality of shared data by allowing the analysis methods to be shared along with variants.

Deploying the workflow in a cloud infrastructure (Amazon Web Services) ensures the solution can scale up to the increasing workload. Software consumes IT resources on-demand and executes the workflow concurrently for any number of tests with a minimal overhead. The development of a cloud infrastructure for NGS will enable the sharing of data, bioinformatics and computing resources between researchers and should promote the standardisation of analysis across the molecular diagnostics community.

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P15.57

Statistics and observations on 200 exomes: the de Duve Institute (UCL) NGS platform

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The field of human genetics is being revolutionized by exome and genome sequencing. A massive amount of data is being produced, always faster. For example, targeted exome sequencing can be completed in a few days using NGS, allowing new variant discovery in a few weeks. The *Université catholique de Louvain* acquired such a next generation sequencing platform, installed in the Laboratory of Human Molecular Genetics at de Duve Institute. The platform is composed of a Solid 5500XL (Life technologies), a Personal Gene Machine (Ion Torrent, Life technologies) and a cluster for bioinformatics processing. The platform offers services such as exome sequencing and RNA Seq, accessible to the Belgian research community.

Here we present statistics and observations on the 200 exomes that have been analysed since September 2011 (150 exomes generated by our Solid 5500XL and 50 exomes generated by an Illumina HiSeq 2000). We present statistics on runs (effects of run parameters on coverage, proportion of duplicates, ...), variants (number of variants called, number of stops gained according to the calling software) and gene coverage (number and efficiency depending on the enrichment kit). Observations include comparison between pair-end and ECC runs, description of the reasons why we lose coverage compared to the expectations, effects of running the same experiment multiple times on coverage, proportion of variants that can be eliminated using paired blood when searching for somatic variants in tissues, and how some false positive variants (due to systematic errors or homologous regions) can be automatically detected.

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P15.58

VariantDB : A flexible annotation and filtering portal for NGS data

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Background:

Causes of many hereditary disorders can be identified through detailed inspection of the patients' genome using Next Generation Sequencing technologies. However, interpretation of the vast amount of data, typically over 20.000 variants per patient, became a bottleneck for routine implementation. To help streamlining NGS data analysis through various trimming, mapping, annotation and filtering tools, centralised web-based interfaces, such as the Galaxy platform, have been developed. Although Galaxy provides many bioinformatic tools, it lacks flexibility in downstream annotation and filtering of the resulting variants.

Methods & Results:

We built VariantDB, a versatile annotation and filtering database implemented in MySQL, with a PHP/CGI frontend. Data added from Galaxy or FTP-upload are private, but can be shared with collaborators. Automatic annotation by ANNOVAR and snpEff, includes allele frequencies (1000g/ESP6500/dbSNPv135), functional impact (RefSeq/Ensembl/UCSC) and pathogenicity predictions (SIFT/MutationTaster/PhyloP/Polyphen2/gerp++/LRT). Custom annotation sources can be added to the xml-based configuration. Family relations allow filtering for de novo or segregating variants. Results are

presented in a tabular overview, with selected annotations and hyperlinks to IGV, or exported as text files.
Conclusions:
VariantDB allows on-demand variant filtering based on the implemented annotations. The intuitive web-interface is a very powerful tool for the interpretation of NGS data.
URL: http://www.biomina.be/app/variantdb/

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P15.59
Store, align and explore your genome outside the Cloud, at home, on your PC

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Cost of whole genome sequencing is rapidly approaching 1000 euro, putting it within reach of amateur geneticists, scientifically curious consumers as well as teachers developing hands-on *omics training courses. Current direct-to-consumer offers do not give consumers the possibility to analyse the actual data, try various alignment algorithms and their parameters nor with the thresholds for calling variants. The raw sequence data remain at the provider and the algorithms used are a black box.
Consumers like amateur geneticists and training course developers prefer to have control of the analysis pipeline for didactic or other purposes and thus need easy to use, flexible software that runs on standard PC's. We present GensearchNGS which we developed to analyse a whole genome on standard PC/Mac, from read alignment to variant detection and annotation. It includes an easy to use graphical user interface, integrating various public and proprietary alignment algorithms through plugins. Open file format standards offer the freedom to exchange data, with for example LOVDs gene variant database to view/query the database with its own associated functionality.
Using only 2 machines we aligned a ~30x coverage whole genome dataset in 40 hours, detected SNPs and short indels, measured coverage and annotated variants with information from Ensembl. To further annotate variants, we connect to the Variant Database archive in the Netherlands, which allows collecting frequency information on the detected SNPs.
This system will allow private exploration and analysis of whole genome raw read data, requiring nothing more than one or two desktops or laptops.

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P15.60
Estimating exome genotyping accuracy by comparing to data from large scale sequencing projects

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Next-generation sequencing (NGS) based methods, such as exome analysis, are currently being introduced as a tool for mutation detection into routine diagnostics. This increases the need for platform-independent methods of quality control. To date, quality scores have been used on a base- or genotype-level to indicate the reliability of single base or variant calls. However, the great variety of NGS platforms and analysis pipelines hinders the direct comparison of genotype-specific quality scores between platforms or for entire data sets and currently no criteria exist for assessing the overall quality of an exome. We present a genotype-weighted metric to compare all exome variants identified in a single sample together to an appropriate high-quality reference data set, with which we estimate the exome-wide genotyping accuracy based simply on the reported variants and without any further knowledge about the data generation. Our method represents a new way to evaluate the quality of entire whole-exome sequencing data in addition to current recommendations for sequencing depth and genotype likelihoods. The distance value of our metric corresponds to a quality parameter for an entire exome and allows comparing the quality of multiple exome datasets from the same or different NGS platforms.

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P15.61
Single Base Resolution DNA Methylomes from Circulating Cell Free DNA as a Basis for Comparative Analyses

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Circulating cell-free (ccf) DNA is present in the plasma of healthy individuals; however, distinct physiological and disease conditions, including pregnancy and cancer, contribute additional nucleic acids into the ccf DNA pool. Differential DNA methylation is a method of differentiating the DNA added from the inherent ccf DNA. Whole genome bisulfite sequencing (WGBS) was performed on a set of unmatched samples including ccf DNA from 9 non-pregnant (NP) female donors, genomic DNA from 7 buffy coat and 5 placenta samples, and ccf DNA from 7 pregnant females. We first created a methylome map of ccf DNA from non-pregnant donors at single base resolution. We found CpG cytosines within longer fragments were more likely to be methylated, linking DNA methylation and fragment size in ccf DNA. Next, we performed a series of pairwise comparative analyses to identify differentially methylated regions (DMRs). Comparison of the methylomes of placenta and NP ccf DNA enabled the detection of greater than 50000 DMRs, the majority resulting from of placenta hypomethylation. Further investigation revealed the presence of domains exhibiting consistent hypomethylation in placenta samples relative to NP ccf DNA spanning megabases. DMRs identified when comparing placenta to NP ccf DNA were recapitulated when comparing methylation patterns in pregnant ccf DNA to NP ccf DNA, confirming the ability to detect differential methylation in ccf DNA mixtures. Collectively, these data advance the biological understanding of ccf DNA and detail the ccf DNA methylome at single base resolution, serving as the foundation for future testing based upon ccf DNA.

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P15.62
Omics Connect Toolbox: A management, validation and visualisation toolkit for complex omics data

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Data generated from the panoply of modern omics technologies are large and complex, making it difficult for researchers to integrate and explore such information. Here we present the 'omics connect toolbox': a set of user-friendly software tools that can be deployed locally in research and biobank environments to assist with omics data validation, management, viewing and sharing.
The 'Automated Bio-Curator' (ABC) tool is designed to help researchers validate and curate their data. This software is built upon a pre-existing data validation engine (cutplace), enhanced to process biological datasets against a set of user-defined rules. The output of ABC highlights data rows and fields that are defective in some way, and suggests and automates corrections.
For core Omics Connect databasing, we have implemented the Observ-OMX data model via the MOLGENIS platform. This allows highly flexible but powerful multi-omics data management, browsing and filtering including mo-

dules for xQTLs, GWAS, NGS, big data pipelines, and sharing. A straightforward tabular data format allows researchers to exchange data without the need for technical know-how on top of ABC.
Most critically, Omics Connect enables direct co-visualisation of various types and scales of omics data via a customised dalliance genome browser. Users can add, delete, edit, or disable tracks to suit their needs. The system employs DAS to serve data sourced from diverse local or online sources to view in the context of other private or publically available data using the Omics Connect browser or other DAS enabled browsers, to facilitate complex data integration and interpretation.

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P15.64
Phenotype terminologies in use for genotype-phenotype databases: need for a standardisation

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The community requires stable standardised terminologies in order to achieve interoperability between databases intended for clinical research that include phenotype descriptions. This is crucial in interpreting genomic rearrangements as well as future high-throughput sequence data. Relevant terminologies used by different communities to describe phenomes were cross–referenced: PhenoDB (2846 terms), London Dysmorphology Database (LDDB; 1318 terms), Orphanet (1243 terms), Human Phenotype Ontology (9895 terms, 22/08/2102), Elements of Morphology (AJMG; 423 terms), ICD10 (1230 terms), as well as medical terminologies in use: UMLS (7,957,179 distinct concept terms), SNOMED CT (>311,000 concepts), MeSH (26,853 concepts) and MedDRA (69,389 concepts). We established a strategy to compare them in order to find commonalities and differences, using ONAGUI as a tool to detect exact matches. The non-exact matches were verified manually by an expert. This exercise showed that, given the multitude of needs and applications in the field of rare diseases, it is not currently realistic or even desirable to have one terminology for all applications. Prominent terminologies have different focuses and user bases. We propose: either to build a consensual terminology using the concepts shared by existing terminologies to describe phenotypes, or to publish the cross-referencing between the existing terminologies, as it has been done for disease nomenclatures. This would ease the interoperability of databases without disturbing the habits of different user groups. The best option would be to implement both.

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P15.65
KD4v: Comprehensible Knowledge Discovery System for Missense Variant

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Understanding the effects of genetic variation on the phenotype of an individual is a major goal of biomedical research, especially for the development of diagnostics and effective therapeutic solutions. The KD4v (Comprehensible Knowledge Discovery System For Missense Variant) server allows to characterize and predict the phenotypic effects (deleterious/neutral) of missense variants. In this work, we describe the use of a recent knowledge discovery from database (KDD) approach using Inductive Logic Programming (ILP) to automatically extract knowledge about human diseases. We extracted background knowledge from MSV3d, a database of all human missense variants mapped to 3D protein structure. We identified 9000 mutations with known three-dimensional structure that were known to be involved in human disease. After using ILP for learning, we obtained a set of rules that classify a mutation as 'deleterious' or 'neutral' and that can be interpreted by both computer and humans. We then performed a clustering analysis to reduce the number of rules, by combining rules that covered similar mutation sets. The results of this analysis help to improve our understanding of the relationships between structural, functional or evolutionary features and deleterious mutations. Inferred rules can also be applied to predict the impact of any single amino acid replacement on the function of a protein. The web server is available at http://decrypthon.igbmc.fr/kd4v.

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P15.66
Cis- and *trans*- Gene Regulation Patterns over Obesity-associated Proteins

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Understanding how genes regulate complex traits is still elusive. The architecture of genetic control over gene-expression levels was dissected by e-QTL previously. We attempted to extend the e-QTL study to proteomics level, by selecting proteins related to obesity. At 1st stage, 5 obesity-discordant monozygotic twin pairs (MZ) were selected, and sera of lean and obese cotwins were pooled; after removing 6 major proteins, 128 proteins were identified showing significant differences in both groups by liquid chromatography (LC) tandem mass spectrometry (MS/MS) . At 2nd stage, 261 individuals from 35 families (28 MZ) with varying degree of obesity were screened for 128 proteins using a quantitative LC- multiple reaction monitoring (MRM) mass spectrometry (MS) as a tool for the quantification of proteins. Finally, GWA analysis on 38 proteins identifiable in individual MRM proteomics assay, were conducted (Affymetrix Genechip v6). Heritability (h2) estimates of most proteins ranged between 0.37 and 0.59, showing moderate to high level of genetic control. GWA analysis (GenABEL) revealed that most proteins are under multiple genetic control (p<10E-6), while some proteins do show evidence of *cis*-regulation (p<10E-3, *Hemoglobin subunit epsilon; C4b-binding protein; Apoprotein A-I*). *Ecto-NOX disulfide-thiol exchanger 2* (*X* chromosome) showed the strongest association with ectopic genes, *RSU1* on chromosome 10 (P<10E-26). Our findings suggest that network from genes to proteins are already extremely complex indicated by ubiquitous multiple trans-regulations; and that multi-omics analysis would be able to make up "missing heritability" considering evidence of stronger genetic controls over protein levels than on obesity phenotypes.

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P15.67
Epigenetic analysis in the BMPR2 gene promoter region in patients with pulmonary arterial hypertension

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Pulmonary arterial hypertension (PAH; OMIM 178600) is a rare and progressive vascular disorder characterized by increased pulmonary vascular resistance, vascular remodelling and right heart failure. The gene mainly related to PAH is the bone morphogenetic protein receptor type II (*BMPR2*). DNA methylation is an epigenetic mechanism characterized by the presence of CpG islands mainly on the promoter regions. These modifications are associated with transcriptional silencing of associated genes. Given the complex and multifactorial nature of PAH, it seems likely that epigenetic events could influence the establishment or progression of PAH.
Forty one patients with familial, idiopathic and secondary PAH and 21 controls were included in this study. Genomic DNA, obtained from peripheral blood, was modified with sodium bisulfite. We searched for CpG islands in 5000 bp upstream the 3´UTR region of *BMPR2* using the Methyl Primer Express v1.0 software.
We found one CpG island of 750 bp containing 57 CpG sites, proximate to the 3´UTR region. To analyse the methylation status within this region we designed 3 pairs of methylation- and unmethylation-specific primers. No differences in the methylation status were observed between patients and controls.
In conclusion, methylation does not seem to be related to PAH, at least in the region of *BMPR2* studied.

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P15.68
Determining the quality and complexity of next-generation sequencing data

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Current methodologies for determining the quality and complexity of next-generation sequencing (NGS) data are rather limited as they heavily rely on reference-based quality measures and screening for a set of potential artefacts. To address these limitations, we have developed a new methodology (kLib) that is independent of a reference sequence in determining the complexity and quality of NGS data and allows for pairwise comparison across a series of samples. We have applied kLib on four cohorts of NGS data that consist of 59 targeted resequencing, 43 whole-exomes, 49 full-genomes, and 465 RNA-Seq. In each cohort, kLib could precisely detect and separate various technical variations that were introduced during sample prep or sequencing such as high duplication rate, high number of off-target reads, low capture performance, differing capture protocols, bimodal insert-size distribution, high amount of library chimaeras, and sample mix-up. Notably, some of the aforementioned artefacts could only be characterised after vigorous quality assessments and would have otherwise been missed. These often hidden artefacts undermine the potential application of NGS in clinical diagnostics as they may result in obscured downstream analysis. In addition, we show that kLib allows for estimating the complexity of NGS data, a vital property of NGS in diverse studies ranging from de novo assembly applications to detection of a shift in bacterial abundance in a series of metagenomes. The various techniques that constitute this new paradigm in robust evaluation of the quality and complexity of NGS data allows for a transition from basic research to clinical diagnosis.

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P15.69

ADAR Regulates RNA Editing, Splicing and Transcript Stability As Revealed by Genomic and Molecular Analyses

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ADAR adenosine deaminases mediate A-to-G RNA editing. We carried out DNA and RNA deep sequencing, RNA interference, RNA-immunoprecipitation and mass spectrometry in human B-cells to examine the functions of ADAR proteins. We uncovered over 50,000 previously unknown A-to-G editing sites. These editing sites reside in mRNA and long non-coding RNAs (lncRNAs). ADAR gene knockdown abolished editing at most sites, thus validated A-to-G editing at these sites. In addition, ADAR knockdown affects expression levels of several thousand genes, in an editing-independent manner, suggesting ADAR plays roles in processes other than editing. We subsequently identified more than 100 proteins in the ADAR protein complex, including splicing factors, heterogeneous ribonucleoproteins and other proteins involved in RNA processing. We showed that ADAR and RNA-binding proteins, such as HuR, bind to common transcripts and co-regulate RNA stability. Moreover, ADAR plays a role in modulating alternative splicing. Our findings underscore the complexity of RNA editing and processing and interactions between different steps.
In this presentation, I will describe results from genomic, transcriptomic and proteomic analyses which allowed us to identify tens of thousands of unknown A-to-G editing sites and to characterize ADAR's roles in gene regulation. The presentation will focus on findings that underscore the complexity of RNA processing and suggest that there are co- or post-transcriptional mechanisms yet to be determined.

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P15.70

Transcriptome sequence genomic analysis in three tissues of a twin cohort reveals complex causes of allelic expression

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In this study we used RNA-seq data in three tissues: fat, LCLs and skin in a sample of ~400 female twin pairs (2330 RNA-seq samples in total) to address the question of tissue specificity of genetic regulation and to dissect the underlying causes of allelic expression. We found 9861 genes with at least one significant cis eQTLs for fat, 10015 for LCL and 9243 for skin (FDR=10%). We also found that between 75% and 80% of the eQTLs are shared among two tissues and that 50% are shared by the three tissues. RNA-seq data allows the quantification of allele specific expression (ASE). At a 10% FDR we observed that 9.5%, 9.3% and 9.1% of the heterozygous sites had a significant ASE effect in fat, LCL and skin. The observed ASE can be caused by genetic or epigenetic /environmental factors. To quantify how much variation in allelic expression is due to genetic factors we estimated the heritability of ASE ratios. We found a heritability of 42% for fat, 45% for LCL and 66% for skin, suggesting that epigenetic / environmental factors have a substantial effect on allelic expression. We also observed that cis genetic effects explain just a fraction (68% in fat, 37% in lcl, and 44% in skin) of the total genetic effects, suggesting potential epistatic effects between trans and cis genetic variants. These results demonstrate a complex genetic architecture for allelic expression. We are exploring further to find putative GxG and GxE interactions affecting allelic expression.

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P15.71

Shift and night work may shape the DNA methylome in human

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Due to modern life style people feel increasingly compelled to work at times which are incompatible with their biological day-night rhythms. Numerous studies reported a link between night and shift work and diseases like sleep problems, the metabolic syndrome and an increased risk for several cancer entities.
Since DNA methylation allows adaption of genetic information according to environmental conditions, we wondered whether night and shift work might be reflected by specific changes in the DNA methylome of those workers.
To avoid genetic contributions we applied DNA methylation analysis using the Infinium HumanMethylation450k BeadChip preferentially on twin pairs discordant for working times, one twin working at shift or night and the other twin working regularly at daytime. The participating twins were selected from the GEMINAKAR twin follow-up study recruited from the Danish Twin Registry. For the majority of the subjects DNA was available from a previous study conducted during 1997-2000 allowing analysis of long-term effects of different working times on the DNA methylome.
By now, we have hybridized the DNA of 32 monozygotic and 39 dizygotic twins to the microarray, allowing the parallel interrogation of more than 450,000 CpG loci.
An interims analysis based on 17 samples revealed 407 CpG loci corresponding to 262 genes differentially methylated between individuals working at day time or with night shift ($p<0.001$).
Hence, shift work may shape the DNA methylome.
As part of the SAME project this study is co-financed by INTERREG 4 A programme Syddanmark-Schleswig-K.E.R.N. by EU funds from the European Regional Development Fund.

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P15.72

Comparison and validation of loss of function variants called by different GATK methods

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The choice of an appropriate variant calling pipeline for exome sequencing data is becoming increasingly more important in translational medicine projects and clinical contexts. Within GOSgene, which facilitates genetic analysis as part of a joint effort of the University College London and the Great Ormond Street Hospital, we performed a comparison between the variant calls generated by the classic GATK caller UnifiedGenotyper and the new variant discovery tool HaplotypeCaller. We performed an experimental validation of the loss-of-function (LoF) variants called by the two methods using Sequenom technology. UnifiedGenotyper showed a total validation rate of 97.6% for LoF SNPs and 92.0% for insertions or deletions (INDELs) while HaplotypeCaller was 91.7% for SNPs and 55.9% for INDELs. We conclude

that in our working environment, UnifiedGenotyper remains the caller of choice, being an accurate method, with a high validation rate of error-prone calls like loss-of-function variants.

Table 1: Validation of variants by caller comparison

	Outcome	Inter-section	Unified Genotyper only	Haplotype Caller only
SNPs	Validated	97 (98.0%)	27 (96.4%)	2 (22.2%)
	Not validated	2 (2.0%)	1 (3.6%)	7 (77.8%)
	Fail	3	0	1
	Total number of assays	102	28	10
	Total number of working assays	99	28	9
INDELs	Validated	35 (92.1%)	11 (91.7%)	3 (10.0%)
	Not validated	3 (7.9%)	1 (8.3%)	27 (90.0%)
	Fail	4	0	12
	Total number of assays	42	12	42
	Total number of working assays	38	12	30

The validation rates of LoF SNPs and INDEL calls from both methods (Intersection) or uniquely called by UnifiedGenotyper or HaplotypeCaller. The failure rate of validation assays (fail) on the genotyping chip is given.

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P15.73

Whole genome TRIO analysis using CLC Genomics Workbench

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The increasing use of next generation sequencing in medical genomics settings uncovers the fact that bioinformatics analysis is still a bottleneck. This especially holds true if whole genome sequencing is applied. CLC bio's Genomics Workbench provides efficient and accurate algorithms capable of analyzing such large data sets. In the presented work we describe how the "Trio Analysis" tool of CLC bio's Genomics Workbench can be used to analyze and compare an Illumina Platinum CEO trio data set composed of mother, father and son.

Our strategy for re-sequencing approaches is based on CLC bio's optimized resources and time efficient mapper, its algorithms to uncover variants and annotate them with data from public resources and its further downstream tools to compare and filter variants. The analysis results can be visualized by built-in solutions.

Here, we present the full workflow performed in CLC bio's Genomics Workbench to identify *de novo* variants in a trio dataset. This strategy can be applied to other families to provide insights into inheritance mechanisms, such as the inheritance of recessive variants, and detect possible health consequences.

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P15.74

Characterising the genetic architecture of the miRNA response to Mycobacterium tuberculosis

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Tuberculosis is a major public health problem, with 1.7 million deaths annually worldwide. Despite evidence to suggest there is a heritable component to susceptibility to Tuberculosis, little is known about the underlying genetic and epigenetic factors. In recent years, expression quantitative trait loci (eQTL) studies have provided abundant information about both the genetic basis of variation in gene expression and inter-individual regulatory variation, offering new insight into the aetiology of susceptibility to complex disease. However, despite their critical role in the immune response, the characterisation of the genetic architecture of miRNAs remains comparatively unexplored. Here we aimed to understand variation in miRNA expression in the presence/absence of Mycobacterium tuberculosis (MTB), the aetiological agent of TB, and to dissect the genetic basis of such variation. To this end, we profiled miRNA expression, by microarray, in dendritic cells before and after MTB infection for 18 hours in 65 individuals. We observed a significant change in expression for 40% of miRNAs upon infection, as well as a marked shift in the distribution of correlations between miRNA and, previously characterised, mRNA profiles, implying extensive remodelling of regulatory networks. To understand the genetic architecture of this response we mapped miR-eQTLs before and after infection, by combining our expression data with whole-genome genotyping of the same individuals. We found over 3% of miRNAs to be associated with a cis-eQTL, including 2 infection-dependent associations. One of these miRNAs, miR-326, was also significantly dysregulated upon infection, providing a novel candidate for influencing variability in the response to MTB.

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P15.75

PON-P, accurate predictor for variation pathogenicity

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High throughput sequencing data generation demands the development of methods for interpreting the effects of genomic variants. Numerous computational methods have been developed to assess the impact of variations because experimental methods are unable to cope with both the speed and volume of data generation. We have developed Pathogenic-Or-Not-Pipeline (PON-P), which provides higly accurate predictions for the effects of missense variants. The accuracy is 0.90 when the prediction reliability is 0.95. The method is based on machine learning and utilizes random forest classfier. It has been trained and tested with a dataset of some 32 000 experimentally verified disease-causing or benign cases available in VariBench, database for variation benchmarks. The original version (Olatubosun et al., 2012) has been further developed. A related tool is available for mismatch repair (MMR) repair gene variations (Ali et al., 2012). PON-MMR has accuracy of 0.87. When applied to 758 unclassified variants in InSiGHT database, it could classify 248 cases (40.3 %) as pathogenic or benign. The tools are available at http://bioinf.uta.fi/PON-P/ and http://bioinf.uta.fi/PON-MMR/. The tools are freely available and can handle large numbers of cases. PON-P can be used for screening and prioritizing variants in order to determine deleterious ones and for further experimentation.

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Olatubosun, A., Väliaho, J., Härkönen, J., Thusberg, J. and Vihinen, M. (2012) PON-P: Integrated predictor for pathogenicity of missense variants. Hum. Mutat. 33, 1166-1174.

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P15.76

Investigation of genome-wide DNA methylation marks associated with FV Leiden mutation in patients with venous thrombosis

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Several lines of evidence are emerging to suggest that DNA methylation marks are involved in the susceptibility of various complex human diseases including thrombosis-related disorders. As part of a general project aimed at identifying DNA methylation-sensitive regulatory mechanisms associated with biomarkers of the fibrinolysis/coagulation cascade, we measured genome-wide DNA methylation levels in peripheral blood samples of 349 patients with venous thrombosis (VT) using the dedicated Illumina HumanMethylation450 bead array. In the current study, we undertook a genome-wide analysis of 485,577 CpG sites to assess whether patients carrying the FV Leiden mutation might be associated with specific DNA methylation profiles. We identified a locus on chromosome 1 characterized by two CpG sites whose DNA methylation levels differed significantly ($p = 6.12\ 10\text{-}12$ and $7.24\ 10\text{-}11$) between carriers (N = 98) and non-carriers (N = 251). Of note, the smallest p-value observed at the F5 gene, also on chromosome 1, was $p = 0.571$. We further tested whether these two CpG sites were associated

with a biomarker of VT risk known to be under the strong influence of FV Leiden mutation, the agkistrodon contortrix venom (ACV) test that explores the Protein C anticoagulant pathway. Strong associations were observed (p = 9.47 10-10 and p = 3.02 10-7) which subsequently vanished (p > 0.10) after adjusting for FV Leiden mutation. These results, if confirmed in independent samples, would add novel insights into the epigenetic regulation of the coagulation cascade.

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P16.001

Correlation between Apolipoprotein E gene polymorphisms and the dose for acenocoumarol maintenance in Bulgarian patients

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Acenocoumarol is an anticoagulant which acts through interference with the recycling of vitamin K in the liver, leading to reduced activation of several clotting factors. Apolipoprotein E plays a central role in the uptake of the lipid-soluble vitamin K.
The aim of this study was to evaluate whether variations in the ApoE gene influence acenocoumarol maintenance dose in Bulgarian patients on anticoagulant therapy.
The genotypes of 64 patients with acenocoumarol treatment in maintenance doses were determined by High Resolution Melting assay. The doses for acenocoumarol maintenance were compared among patients with different genotypes.
In the group of patients, the frequencies of ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, ε4/ε4 genotypes were 9.4%, 1.6%, 1.5%, 67.2%, 20.3%, 1.6%, respectively. The acenocoumarol maintenance dose showed a genotype related effect. The acenocoumarol dose of group ε2 (ε2/ε3) was 5.78 ± 0.37 mg/d, higher than that of group ε3 (ε3/ε3, 2.87 ± 0.41 mg/d, P=0.013) or group ε4 (ε4/ε4, ε3/ε4, 2.43 ± 0.58 mg/d, P=0.002). ApoE genotype explains 9.9% of acenocoumarol dose variance (analysis of variance, P=0.01). However, the regression analysis of ApoE together with data on VKORC1*2, CYP2C9*2 and CYP2C9*3 polymorphisms show that only VKORC1*2 and CYP2C9*2 are significantly associated with the acenocoumarol maintenance dose.
In conclusion, our study results show that ApoE polymorphisms have a minor influence on the individual dose for acenocoumarol maintenance in Bulgarian patients on anticoagulant therapy.

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P16.002

Association of LOXL1 and HTRA1 genes polymorphisms with both forms of advanced age-related macular degeneration in Turkish patients

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Age related macular degeneration (AMD) is clinically heterogenous disorder and the most frequent cause of irreversible blindness in the elderly population. Several single nucleotide polymorphisms (SNPs) have been linked to the risk of developing AMD. Complement Factor H (CFH) and ARMS2/HTRA1 genes were shown to be associated with a distinct component of the AMD phenotype. Recently, lysyl oxidase-like 1 (LOXL1) gene was shown to be involved in a significant fraction of exudative AMD in Japenese populations. Y402H variant in CFH and A69S polymorphism in ARMS2 genes were shown to be associated for both types of end stage AMD in Turkish patients in our previous studies. The purpose of this study is to determine whether polymorphisms in the HTRA1 and LOXL1 gene are associated with advanced AMD in Turkish patients.
Genotyping of 87 age-matched healthy individuals and 95 unrelated late AMD patients were performed by direct sequence analysis. Both genes were analyzed for deviations from Hardy-Weinberg equilibrium and no significant deviations were detected (HTRA1 controls p=0.92 HTRA1 cases p=0.15; LOXL1 controls p=0.05 LOXL1 cases p=0.14). The association between AMD and genotypes were evaluted and analyzed by logistic regression method. According to the statistical analysis, HTRA1 AA genotype is found to be highly associated with AMD (AA genotype OR=5.33,1.95-14.58%CI;p= 0.001) when compared to GG genotype. However, LOXL1 genotypes did not show any significant association with AMD (P=0,847). This study suggest that HTRA1 rs11200638 polymorphism is highly associated for both types of end-stage AMD in Turkish populations whereas LOXL1 is not.

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P16.003

Effect of Gas6 c.834+7G>A polymorphism and the interaction of known risk factors on age-related macular degeneration pathogenesis in Hungarian patients

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Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly in the developed world. Numerous genetic factors contribute to the development of the multifactorial disease. We performed a case-control study to assess the risk conferred by known and candidate genetic polymorphisms on the development of AMD. We searched for genetic interactions and for differences in dry and wet AMD etiology.
213 patients with exudative, 67 patients with dry AMD and 106 controls were tested for 12 polymorphisms in Apolipoprotein E, complement factor H, complement factor I, complement component 3, blood coagulation factor XIII, HTRA1, LOC387715, Gas6 and MerTK genes. Gas6 c.834+7G>A polymorphism was found to be significantly protective against wet type AMD (OR=0.50, 95%CI:0.26-0.97, p=0.04). Multiple regression models revealed a genetic interaction in the dry AMD subgroup. In the absence of C3 risk allele, mutant genotypes of both CFH and HTRA1 behaved as strong risk factors (OR=7.96, 95%CI:2.39-26.50, p=0.0007, and OR=36.02, 95%CI:3.30-393.02, p=0.0033, respectively), but reduced to neutrality otherwise. The risk allele of C3 was observed to carry a significant risk in the simultaneous absence of homozygous CFH and HTRA1 polymorphisms only, in which case it was associated with a near-five-fold increase in the odds of dry AMD (OR=4.93, 95%CI:1.98-12.25, p=0.0006). Our results suggest a protective role of Gas6 c.834+7G>A polymorphism in exudative AMD development. In addition, novel genetic interactions were revealed between CFH, HTRA1 and C3 polymorphisms that might contribute to the pathogenesis of dry AMD.

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P16.005

Variant Association Tools (VAT) for association analysis of large scale sequence and exome genotyping array data

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Currently there is great interest in detecting rare variant associations with complex traits. The vast majority of methods developed to test for rare variant associations aggregate variants across a region, which is usually a gene. We developed variant association tools (VAT), a tool-set that implements a quality control and association analysis pipeline for rare variant association studies using sequence or genotyping array data. Highlights of VAT include variant site/call level quality control, summary statistics (HWE, Ti/Tv, etc), phenotype/genotype based sample selections, variant annotation, selection of loci for analysis and a number of rare variant association methods for analysis of qualitative and quantitative traits. The association testing framework implemented in VAT is regression based which readily allows for flexible construction of association models with multiple covariates, weighting (based on allele frequencies or predicted functionality), interactions terms and models for pathway analysis. VAT is capable of rapidly scanning through data using multi-processes computation, adaptive permutation and conducting multiple association tests simultaneously. Additionally a programming interface is provided to readily facilitate user implementation of novel association methods. The VAT pipeline can be applied to sequence data, imputed data and genotyping array data, e.g. exome chip. VAT is highly beneficial in performing association analysis on small to large scale studies of complex traits making use of the latest genotyping and sequencing technologies.

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P16.006

Expression of CTLA4 isoforms is altered in patients with childhood asthma and is changing during treatment with inhaled corticosteroids and leukotriene receptor antagonist

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Cytotoxic T lymphocyte antigen 4 (CTLA4), an important regulatory molecule in the process of antigen presentation, was previously associated with pathogenesis of autoimmune diseases and asthma. To further evaluate CTLA4 as a potential biomarker, we
have measured the expression of CTLA4 gene isoforms with qPCR in previously untreated children with mild to moderate asthma before and 4-6 weeks after the therapy with inhaled corticosteroids (ICS) or leukotriene receptor antagonist (LTRA).Median relative
expression of full length CTLA4 (flCTLA4) isoform in asthmatics was 0.440 ± 0.425, compared to 1.000 ± 0.738 in healthy controls (p<0.0001), and of soluble CTLA4 (sCTLA4) isoform in asthmatics was 0.580 ± 0.468 compared to 1.040 ± 1.080 in healthy controls (p<0.0001). After ICS treatment, the
sCTLA4 expression in asthmatics increased from 0.435 ± 0.295 to 0.645 ± 0.573 (p=0.049), while after LTRA treatment decreased from 0.480 ± 0.520 to 0.310 ± 0.355 (p=0.008). Our study shows that CTLA4 expression is dysregulated in asthma patients. ICS and LTRA treatments showed opposite effect on sCTLA4 expression which may be a reflection of thedifferent mechanism of action of both drugs in asthma.

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P16.007

Clinical and Genetic Investigation of Bardet-Biedl Syndrome in Tunisia: A 30 years prospective population based, cohort study

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Bardet-Biedl syndrome (BBS) is a ciliopathy and pleitropic autosomal recessive disorder characterized by a wide spectrum of clinical signs including progressive retinal degeneration, polydactyly, obesity, learning difficulties, renal tract and genital anomalies as well as other less frequent features such as for instance anosmia, or Hirschrpung disease. Clinical diagnosis was established if at least four major features are present in the patient. The phenotype spectrum of BBS is variable; some manifestations could appear during childhood. BBS is considered as a rare disorder. The aims of this study were to analyze the genetic epidemiology of BBS in Tunisia, further define the genotype- phenotype correlation. **Materials and methods:** Today 50 unrelated families including 75 patients were collected. Molecular analysis was performed using two successive methods, first genome wide scans with microsatellites markers and then exon capture coupled with high-throughput sequencing of thirteen ciliopathies genes. **Results and discussions**: The current prevalence of BBS in Tunisia has been estimated at 1:156 000. Mutations were identified in the 28 analyzed families. Most frequent mutations were described in *BBS1* and *BBS2* genes. This data expands the mutations profile of *BBS* genes in Tunisia and suggests a divergence of the genetic spectrum comparing Tunisian and other populations. Two founder mutations were identified in *BBS2* and *BBS8* genes. **Conclusion**: This study showed the genetic heterogeneity of BBS in Tunisia. Molecular diagnosis of BBS has to be established in the country in order to offer the genetic counseling and prenatal diagnosis for affected families.

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P16.008

Confirmation of a founder effect in Northern European population of a new beta-globin mutation (codons 8/9 (+AGAA))

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Beta-thalassemia is a genetic disease caused by a defect in the production of the beta-like globin chain. More than 200 different mutations are described that can lead to the disease and are mainly found in populations that have been exposed to malaria parasites. We recently described a duplication of 4 nucleotides in the first exon of beta-globin gene in several families of patients originating from Nord-Pas-de-Calais (France). In order to determine if this mutation has arisen there or has been introduced by migrants from regions of the world where thalassemia is endemic, we genotyped 4 unrelated mutation carriers and 32 controls from Nord-Pas-de-Calais for 97 european ancestry informative markers (EAIMs). Using these EAIMs and comparing with population reference panels, we showed that the patients were very similar to the controls and were closer to North European populations than to South European or Middle-East populations. Using the genotypes at 12 microsatellite markers surrounding the beta-globin gene of the 4 mutation carriers plus an additional one recently discovered, we found that they shared a common haplotype that signs a founder effect that was estimated to have taken place 225 years ago (9 generations).
Rare beta-thalassemia mutations have already been described in patients sampled in non-endemic regions but it is the first proof of a founder effect in Northern Europe. How this finding could challenge the hypothesis of a selective advantage of mutation carriers when exposed to malaria would be discussed.

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P16.009

Whole population-based study of a Polynesian genetic isolate underscores gene environment interaction in biliary atresia

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Despites a century-old characterization of biliary atresia (BA), nothing is commonly assumed about heritability of this disease, its onset, its susceptibility factors nor its physiopathology. In this study, we provide the clues to thoroughly revisit BA as a multifactorial disease, related to genetic ancestry. Among the 152,866 children born in French Polynesia between 1979 and 2009, 40 had biliary atresia accounting for an incidence of 26.1/100,000 live births, which is the highest incidence worldwide. Epidemiologic data and follow-up study over 30 years period reveal statistically significant influence of environment with seasonality of births. In all 40 patients, we also studied genealogic and clinical data. Genome-wide analyses of 250,000 SNPs in 24 patients and their parents were performed. Genetic isolate was evidenced by genealogy, inbreeding coefficient calculation (F=0.045) and principal component analysis. Contribution of environmental factors was integrated into an original statistical modeling. While homozygosity mapping revealed no significantly shared region, suggestive linkage to chromosome 2q13.1 was identified in patients with environmental constraint. Transmission disequilibrium test analysis further identified suggestive association with SNPs from 14 loci. Using bioinformatics, we could identify that the majority of genes that were encompassed in those loci were involved in the same highly significant pathway, specific to liver. Our findings provide a novel view of a complex genetic make-up in an isolate population underlying BA. Our study design unveils an approach to study the contribution of both ethnic and environmental factors in a rare disease.

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P16.010

Appraising the clinical validity for pharmacogenetic markers of fluoropyrimidine response in the treatment of colorectal cancer

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Pharmacogenetic tests are not routinely used for fluoropyrimidine treatment stratification despite a high frequency of adverse events leading to significant morbidity and mortality. We have conducted a pharmacogenetic study of early adverse events in a cohort study of 254 colorectal cancer patients treated with 5-fluorouracil or capecitabine. Our objectives were to test the clinical validity of nine previously identified markers of toxicity in a broad clinical setting; and to identify any associations between severe adverse events and six additional candidate variants of proximal enzymes in the pharmacodynamic and pharmacokinetic pathways.
We did not identify any significant associations for variants of the folate metabolizing enzymes and toxicity. However, two pharmacokinetic enzymes were associated with this phenotype. We found a significant association with *TYMP* L471S (adjusted OR = 2.70 [1.23; 5.92], p = 0.01) and a signature of rarer *DPYD* mutations (adjusted OR = 6.76 [1.99; 22.96], p = 0.002).
If a prognostic test for early adverse events analysed the *TYMP* and *DPYD* variants as a signature, the sensitivity would be 45.5 %, with a positive predictive value of just 33.9 %. The poor clinical validity reflects the modest effect size or low allele frequencies of the individual predictive markers considered in this study. The combined effect of risk alleles for a given phenotype may be much larger or indeed smaller than implied by their individual effect sizes but meta-analyses of individualized data sets such as this will be required to identify genetic interactions and prognostic genetic signatures with promising clinical validity.

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P16.011

Genetic analysis of BRCA1 and -2 haplotypes in patients with breast- and/or ovarian cancer in South Tyrol

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Introduction:
The purpose of this study was to determine haplotypes of BRCA1 and BRCA2 for patients with breast and/or ovarian cancer using common single nucleotide polymorphisms (SNPs).
Patients and Methods:
Patients with breast and/or ovarian cancer, unselected for family history, were identified through the local cancer registry. After written informed consent, participants provided a blood sample for genetic analysis. Genomic DNA was extracted by standard technique. Direct sequencing of the complete coding region of BRCA1 and 2 was performed. A set of 12 SNPs in BRCA1 and 16 SNPs in BRCA2 that were repeatedly present in our patient group were selected for genotyping. Haplotypes were designated as in Frosk et al (2007). Rare haplotypes were confirmed by LightCycler PCR and melting curve analysis.
Results:
Blood was obtained of 183 patients. Ten patients (5.5%) with a deleterious BRCA mutation were excluded from haplotype analysis. In the remaining 173 patients, 11 different BRCA1 haplotypes and 12 different BRCA2 haplotypes were found. Of these, 4 BRCA1 haplotypes and 6 BRCA2 haplotypes reached a frequency greater than 5%, representing >93% of all BRCA1 and >84% of all BRCA2 haplotypes, respectively. Four BRCA1 haplotypes were detected only once.
Conclusions:
Common haplotypes in BRCA1/2 can be reliably determined using a limited number of SNPs. Using haplotypes, instead of single polymorphisms, might help to elucidate the impact of genetic variation in BRCA1/2 on cancer risk, since contradictory results in the literature could be due to the use of single polymorphisms only.

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P16.012

Frequency analysis of the CCR5-Delta32 allele in medieval and modern Romanian population

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The CCR5-delta 32 mutation in human chemokine receptor gene can be considered a rare example of a beneficial mutation. The mutation gives its homozygous carriers complete resistance against HIV infection and has been proposed to provide protection against different lethal epidemics. Although the origin of the mutation indicates prehistoric times, repeated waves of lethal epidemics over medieval centuries strongly modified the European gene pool via bottleneck effect and seem to increase the frequency of CCR5-delta 32 up to 20% as a result of selective advantage.
In order to investigate if the lethal epidemics of the medieval times selected the allele we analyzed the presence of this mutation in aDNA isolated from the skeletons discovered in Piața Universității archeological site and compared them to the frequency in a control group from local modern population. This archaeological site is one of the biggest medieval cemeteries from Romania providing the opportunity to sample the same population at different points during the medieval period and beyond.
The comparisons couldn't reveal strikingly different frequencies of the CCR5-delta 32 allele. Our preliminary results based on 18 aDNA of 150 samples and 74 of 2500 modern DNA indicate that the frequency of the allele in medieval Romanian population is not statistically significant when compared to contemporary one suggesting that lethal epidemics had little effect on its present day frequency. We can conclude based on preliminary data that increase in the allelic frequency of the mutant CCR5-delta 32 allele has been positive selected before the medieval centuries.

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P16.013

Genetic polymorphisms involved in the up regulation of CD40L, in blood donors

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Introduction: CD40-CD40 ligand (C40L) signaling pathway is a key way in the cooperation between immune and inflammatory cells. The CD40/CD40L is also involved in a variety of autoimmune and inflammatory disorders caused by a deficiency or abundance of one/both of the receptor-ligand molecules. Some genetic polymorphisms were shown as regulators of protein expression. This study was conducted to investigate the polymorphisms responsible for the CD40L up regulation in blood donors.
Donors and Methods: We genotyped 450 blood donors (200 collected at the French Blood Establishment Auvergne Loire, France and 250 collected at the Blood Banks of Monastir and Sousse, Tunisia).
The studied CD40L polymorphisms are located in the 5'UTR regulatory region, successively rs3092952 SNP A> G and rs201992677 [-/CAAA], an InDel SNP modifying the number of repeats of a CAAA microsatellite.
We performed Denaturing High Performance Liquid Chromatography method and automated sequencing.
Results and discussion: The two studied SNPs are in linkage disequilibrium so they should constitute a haplotype. Surprisingly, all the male donors presenting the G allele rs3092952 were also found to carry seven CAAA repeats (rs201992677) and even 8 CAAA repeats in 2 cases.
A significant difference was observed between the two populations (Tunisian and French).
The next step will be to study whether the CD40L protein up expression is related to the G allele (previously known) or/and to the variable number of tandem CAAA repeats. This could induce a consequence on hyper activation of blood recipients in transfusion.

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Functional analysis of Immunochip candidate genes in Celiac Disease

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Celiac disease (CD) is an immune mediated multigenic disorder caused by intolerance to ingested gluten that develops in genetically susceptible individuals. The major genetic risk factor is HLA DQ2/DQ8 but it is not sufficient to explain all genetic susceptibility to CD. GWAS and IMMUNOCHIP performed in CD identified 39 non-HLA regions associated with CD, and a long list of candidate genes mapping these regions has been proposed.
The aim of this work was to analyze the expression of those genes in the intestinal mucosa of active and treated CD patients and controls, to question their implication in CD development, as well as the influence of the associated SNPs in their expression.
Gene expression analysis was carried out using the Fluidigm BioMark™ HD Real-Time PCR System, in intestinal biopsies of 15 CD children at diagnosis and after >2 years on GFD, and 15 non-celiac controls. Statistical analyses to assess differential expression, genotype effect and correlation were performed.
Ten out of 46 genes were differentially expressed when comparing active and treated patient mucosa, 15 genes showed differences between active disease and controls and 3 of them were constitutively downregulated in patient mucosa, independent of disease status. Correlation was found in the expression of some of the analyzed genes, and in depth studies may be able to clarify these relationships.
Several genes under association peaks seem to have a functional implication in the disease, but other genomic elements, such as miRNA or lncRNAs in the region should not be ruled out.

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P16.015

Survival in autosomal dominant cerebellar ataxias differs according to the mutational mechanism: prognostic implications

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Autosomal dominant cerebellar ataxias are a group of diseases affecting the cerebellum and its afferent/efferent tracts, characterized by genetic heterogeneity (SCA1 - 34). We studied 1013 index cases and estimated survival in 461 index cases and 224 affected relatives with known mutations. Median age at death (n=461) was 68 years [28-97]. Comparison of 409 subjects with SCA due to CAG expansions (SCA1, 2, 3, 6, 7, 17, DRPLA) and 52 subjects with non-polyglutamine SCAs (SCA 11, 13, 14, 15, 23, 25, 28, 31, 32, 36) showed that survival was significantly shorter in the former (67 years [28-97] versus 83 years [45-95], p< .0001). SCA with polyglutamine expansions died 16 years earlier and have a significantly reduced survival. Among polyglutamine SCA median age at death in SCA1 was 63 years [58-65], significantly earlier than SCA 2, 3, 6 and 7 (Log Rank 34.0, p= .0001). Death occurred 8.1 years earlier in the offspring of polyglutamine SCA (p< .001) indicating anticipation. Despite significantly earlier age at onset in non-polyglutamine SCA (37.8 years ± 13.3 versus 29.0 years ±17.7, p< .001), the disease progression was faster in polyglutamine SCA (duration between age at onset and first examination 21.9± 14.6 years and 8.9± 6.9 years respectively, p< .0001). Duration between age at onset and death was 18 years in index cases with polyglutamine SCA.
Survival and severity among SCAs was significantly different according to the underlying mutational mechanism.

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Extensive genetics of Cerebral Cavernous Malformation in Italy

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Cerebral Cavernous Malformations (CCM) (OMIM 116860; Orpha code: ORPHA 164) are a diffuse cerebrovascular disease characterized by abnormally enlarged and leaky capillaries arranged in mulberry-like structures with no clear flow pattern. The lesion might predispose to seizures, focal neurological deficits or fatal intracerebral hemorrhage. However a CCM can also remain neurologically silent. It might either occur sporadically or as an inherited disorder with incomplete penetrance and variable expressivity.
The Familial form of this disease (FCCM), is defined as the occurrence of CCMs in at least two family members and/or the presence of multiple CCMs and/or the presence of a disease-causing mutation in one of the three genes in which mutations are known to cause familial CCM. Diagnosis is made by clinical history and family history; physical examination including neurological, cutaneous and retinal; brain and/or spinal cord MRI; and when available, histopathologic examination of tissue specimens. The diagnosis of FCCM can be confirmed by molecular genetic testing of the following three genes in which mutations are known to cause FCCM: KRIT1/CCM1, MGC4607/CCM2, and PDCD10/CCM3. Sequencing analysis of the three genes should be integrated by Multiple Ligation Probe Assay (MLPA) to search for genomic rearrangements.
We present here data from 87 Italian FCCM analyzed both by sequence and MLPA analysis. We identified KRIT1/CCM1 mutations in 65.52%, MGC4607/CCM2 mutations in 17.24%, PDCD10/CCM3 in 12.64% and no mutations in 2.3% of the analysed cases.
According to our results, more than 90% of the familial form of the disease present a genetic mutation.

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Pulmonary and extrapulmonary parameters are significantly associated with *IL8* polymorphisms in Chronic Obstructive Pulmonary Disease.

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RATIONALE: COPD is characterized by airflow limitation and tobacco smoking is its major risk factor. IL8 is a proinflammatory chemokine mainly involved in the initiation and amplification of acute inflammatory reaction. Here we aimed to test the association of *IL-8, CXCR1* and *CXCR2* polymorphisms and susceptibility to disease.
METHODS: 9 tagSNPs were genotyped in 744 caucasian individuals (202 COPD patients, 90 smokers with normal lung function and 452 healthy controls). Clinical variables were measured at baseline and annually over a 2 years period. Pulmonary compromise was measured using lung function, and the extrapulmonary manifestations were determined using the BODE index. We also determined plasma levels of IL-6, IL-8, IL-16, TNF-α, MCP-1, MMP-9, PARC and VEGF in COPD patients.
RESULTS: No significant association was found between gene variants or haplotypes with predisposition to disease when comparing COPD patients with control groups. No relationship was observed between tagSNPs and cytokines levels. Significant associations were found between the rs4073 (T/A), rs2227306 (T/C) and rs2227307 (T/G) in the *IL8* gene and FEV1%, FEV1/FVC and GOLD status (p<0.05) at baseline in COPD patients. The rs4073A, rs2227306C and rs2227307T alleles were significantly associated with patients who scored higher in the BODE index and showed an important decrease in their FVC during the 2 years follow-up period.
CONCLUSIONS: Despite no significant association was found between gene variants and COPD susceptibility, three polymorphisms in the IL8 gene appear to be involved in a worse prognosis of disease, by a reduction in lung capacity and an increased BODE index.

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Type and frequency of CNVs in a group of Bulgarian patients with congenital malformations and intellectual disability

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Introduction: The development of microarray technology revealed an unexpected large number of deletions and duplications in the human genome whose clinical significance is unknown in some cases. This requires a thorough population study of CNVs in healthy individuals and patients.
Methods: Oligo array-CGH (BlueGnome CytoChip oligo 2X105K, v1.1, 35kbp backbone resolution) was applied in 52 patients with developmental delay and multiple congenital anomalies.
Results: A total of 247 CNVs were detected, of which 15 pathogenic, 108 with unknown clinical significance and 124 benign - mean number of CNVs per patient – 4,5.
Unknown CNVs in chromosomal loci 2q37.3, 10q11.22, Xp22.33 (19 variations) were found in over 5% of patients, which were probably not pathogenic.
The most common benign variations were in 15q11.2, 8p11.23, 6p21.32, 3q26.1, 14q11.1 and 12p13.31 loci, occurring in over 10% of the patients.
Conclusion: We determined benign and probably not pathogenic variants in Bulgarian patients. There is an obvious need for large population studies. This would facilitate the interpretation of unknown genomic imbalances in clinical aspect. Besides, it would help the widespread introduction of CGH microarray in diagnostic practices - postnatal and prenatal genetic diagnosis.
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Gene-gene interaction between APOE and USF1 for coronary heart disease in TARF study

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Background: Epistatic gene-gene interactions could contribute to the heritability of coronary heart disease, but limited investigations have been reported. USF1 is a key regulator of several genes involved in lipid and glucose metabolism as a transcription factor. APOE has been identified as a target gene of USF1. APOE gene polymorphisms (SNP) have been shown to associate with plasma apoE concentrations, lipid levels and cardiovascular disease. In this study, we investigated a possible interaction between these two genes on coronary heart disease risk in Turkish Adult Risk Factor (TARF) Study.
Methods: A population-based cross-sectional survey conducted from 1998-2011 included 1874 randomly selected adults (mean age 54.4±11.9 years, 49% men) participating TARF study. 111 of these individuals were diagnosed as coronary heart disease. Genotyping of -219G/T (rs405509), +113G/C (rs440446) and ε2/ε3/ε4 (rs429358 and rs7412) polymorphisms in *APOE* gene and also ex11G/A (rs3737787) and int7C/T (rs2073658) polymorphisms in *USF1* gene was performed using the Taqman technology (ABI 7900HT, Applied Biosystems, CA). Gene-gene interaction effects were analyzed using traditional multiple regression models.
Results: The logistic regression model showed there were significant interactions effect between *APOE* -219G/T and +113G/C polymorphisms and *USF1* int7C/T on coronary heart disease of the Turkish adults (P for interaction =0.009 and =0.01, respectively). After adjustment for age, gender, body mass index and diabetes mellitus, these interactions were also showed (P for interaction =0.004 and =0.02, respectively).
Conclusion: Gene-gene interaction between *APOE* and *USF1* plays an important role in coronary heart disease risk in Turkish population.

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Polimorphisms of CYP2B6 and CYP2D6 genes associated with drug metabolism in Roma population samples

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The human cytochrome P450 B6 and D6 (CYP2B6 and CYP2D6) enzymes, members of the cytochrome superfamily, play major role in the metabolism of several substrates such as drugs and other xenobiotics. These phase I enzymes are known to commonly contribute the biotransformation of clinically important pharmaceuticals, including anticancers (tamoxifen), antidepressants (anandamide), anxiolytics (sertraline), opioid analgesics (methadone). The purpose of this study was to investigate the allelic frequencies of three major functional gene variants, CYP2B6 G516T, CYP2D6 C100T and CYP2D6 G1846A in Hungarian Roma population compared with Hungarian Caucasian controls. A total of 426 Roma cases (151 males, 275 females; mean age 43.3±0.87 years) and 431 Hungarian controls (248 male, 173 females; mean age 37.6±0.61 years) were genotyped for three SNPs using RT-PCR assay and direct sequencing. We found significant differences in the presence of rare variant in case of CYP2B6 G516T ($p \leq 0.001$) and CYP2D6 C100T ($p=0.003$) and G1846T ($p=0.022$) between the Hungarian Roma and Hungarian general population. The 516T allele frequency was 67.1% in the Roma group, 42.7% in controls, whereas the minor CYP2D6 100T allele was present in 53.3% in Romas, while 41.1% in healthy Hungarians. The allele frequency of 1864A was 45.1% in Roma samples and 36.2% in controls. Our results suggest, that both pharmacogenetical and clinical factors including ethnicity can influence the efficacy of individual drug therapy.

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CFTR gene rearrangements in Argentinean cystic fibrosis patients

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Introduction: Cystic Fibrosis (CF) is an autosomal recessive disease mainly caused by point mutations and small deletions in the CFTR gene. Besides, more than 30 large deletions that remain undetected when using conventional PCR techniques have been described in CFTR gene. Some of these deletions can reach frequencies up to 6% in certain populations. In Argentina, the study of 32 mutations prevalent in Caucasian population detects about 76% of cystic fibrosis alleles. To date, it is unknown the contribution of CFTR rearrangements in the development of CF in this population.
Objective: To determine the frequency of large deletions in the CFTR gene in Argentinean cystic fibrosis patients with at least one unidentified mutation.
Materials and Methods: The number of copies from all 27 exons in the CFTR gene was studied by Multiplex Ligation-dependent Probe Amplification (MLPA) in 74 CF patients with one or none identified mutation.
Results: CFTR rearrangements were detected in 11 of the 91 alleles studied (12%), 7 as a second mutation and 2 in homozygote form. Six different rearrangements were identified: CFTRdele2, CFTRdele2, 3, Del17a-17b-18, CFTRdele19, Del22-24 and CFTRdele1-24. CFTRdele2, Del17a-17b-18 and CFTRdele19 were found in more than one allele.
Conclusions: The analysis of the CFTR gene deletions allowed increasing the sensitivity of the molecular study. The frequency of CFTR deletions found in this group of CF patients suggests that this methodology should be incorporated into the diagnostic algorithm in Argentinean population.

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Graphically testing bi-allelic markers for Hardy-Weinberg equilibrium

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In genetic association studies markers are usually tested for Hardy-Weinberg proportions (HWP). Such tests are relevant, as deviations from HWP may be the result of genotyping error, can result from population stratification or can be indicative of marker-disease association. The classical test for HWP is the the chi-square test, though nowadays exact test procedures have become increasingly popular. Testing becomes unwieldy if large numbers of markers are tested simultaneously, leading to large amounts of statistics and p-values. In this talk we show that all HWP testing can be done gra-

phically, by simultaneously representing all markers in a de Finetti diagram or ternary plot. Hardy-Weinberg equilibrium traces a parabola inside the ternary plot. Curves that delimit the acceptance region of the HWP test can be traced in the plot as well. For chi-square tests (with or without continuity correction) the limits of the acceptance region are smooth curves. The acceptance region of exact tests can be represented by zigzag lines. In this way the significance status of large databases of markers can be glanced from a single diagram. Systematic heterozygote excess or dearth for the markers under study is then easily detected. Alternative graphical presentations for representing marker data using log-ratio transformations of the genotype counts are also considered. In log-ratio coordinates, the Hardy-Weinberg law is a straight line. Examples of graphical tests with HapMap data and markers involved in colon cancer will be shown. Software for making all diagrams (the R-package HardyWeinberg) will be discussed.

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P16.024

Whole exome sequencing to find de novo causal variants: sample size and significance

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Whole exome sequencing technology enables the detection in patients of de novo variants that might be responsible for their condition. Sometimes, a single trio is investigated in a diagnostics-like setting, while in other studies, large series of patients with the same disorder and their parents are sequenced to find genes with multiple hits. Questions arise about whether particular results can be considered significant and about how many samples are needed to find a significant result. We formulated equations to help answering these questions for specific experiments.
For example, the equations show that in the case of a single average-sized gene and a follow-up cohort, a second missense de novo hit in the gene would be significant with p<0.01, when at least one hit is found in a follow-up sample of up to 300 patients. When a series of patients is sequenced, a more heterogeneous disease will require a larger cohort to find multiple hits in the same gene. At the same time, in a larger cohort, more hits per gene must be found to be considered significant. This trade-off will be demonstrated. Also, the size of the gene matters. For example, finding a double hit in an average-sized gene in a sample of 50 patients occurs with p=0.02 by accident, while finding a double hit in one of the 5% largest genes occurs with p=0.09 by accident, and will therefore not be considered significant.
We will present some guidelines and rule-of-thumb-numbers.

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P16.025

Paternal age explains a major portion of de novo germline mutation rate variability in healthy individuals

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De novo mutations (DNM) are an important source of rare variants and are increasingly being linked to the development of many diseases. Recently, two studies using individuals with common psychiatric disorders, autism spectrum disorders (ASD) or schizophrenia, have shown a strong link between the fathers' age at time of conception and identified DNM hotspots across the genome1,2. Though they were both revealing, these studies were conducted on cohorts of patients with psychiatric disorders known to result in part from DNM. Using disease-free familial quartets we show for the first time that the overall ratio of somatic:germline DNM is 1:9 and that there is a positive correlation between paternal age and germline DNM in healthy subjects. Interestingly somatic and all CNV DNM did not vary with paternal age. We also found that DNM were not evenly distributed across the genome, which adds support to the possibility of DNM hotspots.

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Genetic epidemiology of hearing loss in the Republic of Tuva (Southern Siberia)

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Hearing loss is a heterogeneous disorder due to genetic or environmental causes, or both. Congenital (or early onset) sensorineural profound hearing loss is most likely caused by genetic factors. We created the uniform register of 1407 persons with different hearing impairments and conducted genetic epidemiological analysis of hearing loss in the Republic of Tuva located in Southern Siberia and bordered on the south with Mongolia. The total population of the Republic of Tuva is ~310 000 and mainly represented by indigenous people, Turkic-speaking Tuvinians. We revealed 982 patients with severe-profound hearing impairment that is ~ 1 per 300 inhabitants of the Tuva have socially significant hearing loss. Moreover, congenital aural atresia and different ear deformities was found in 55 persons. Then, we analyzed the types of hearing impairments and excluded the patients with conductive and mixed forms of hearing loss as well as patients with presbycusis or sensorineural deafness apparently caused by environmental factors. As a result, a group of patients with congenital (or early onset) sensorineural severe-profound hearing loss included 542 individuals. Mutation screening of the GJB2 gene entire coding region in 173 patients (mostly Tuvinians, and a few Russians) from this group revealed mutations p.W172C, c.235delC, p.V37I, c.299_300delAT, and c.35delG (only in Russian patients) in 24.3% of all examined patients. The p.W172C mutation makes the largest contribution to deafness in Tuvinian patients. Carrier frequency of p.W172C in normal hearing Tuvinians (N=117) was found to be 4.3%.
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Prevalence of Mutations in the GJB2 and GJB6 Genes in patients with Non-syndromic Deafness in UAE population

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Hearing loss, affecting millions of people, is the most common form of sensory impairment. It is a genetically heterogeneous disorder. The various deafness forms so far studied appear as monogenic disorders. They are all rare with the exception of one, caused by mutations in the gene encoding the gap junction protein connexin26, which accounts for about 1/3 to 1/2 of the cases of prelingual inherited deafness in Caucasian populations. This study focuses on two of the most common genes which cause autosomal recessive deafness, GJB2 and GJB6, to determine the frequency for common mutations in these genes. We specifically aim to estimate the prevalence of 35delG, 167delT, and 235delC in the GJB2 gene and D13S1830 deletion mutation in the GJB6 gene in individuals with nonsyndromic hearing loss and in healthy individuals in the population of United Arab Emirates. Common mutations were screened for in extracted genomic DNA using PCR-RFLP and multiplex PCR techniques. Eighty-eight patients with non-syndromic hearing loss participated in the study from Sharjah, Abu Dhabi, and Al-Ain centers. The 35delG mutation was the most common in our patients, and was the only one detected in 8 (homozygous for 35delG) out of 88 patients (9%). None of our tested patients were positive for the 167delT or 235delC mutations. Using multiplex PCR assay, the results showed a homozygous wild type genotype for the del (GJB6-D13S1830) mutation in both deaf and normal subjects alike. In addition, none of the seventy-four tested healthy individuals carried a mutation in 35delG, 167delT, or 235delC.

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Effect modification of SNPs in predicted milk rich miRNA target sites on the association between breastfeeding and diarrhea

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Breastfeeding is a well known protection factor for diarrhea. Recently, several studies have shown that immune related miRNAs are present in mammal milk and colostrums. To indirectly test the function of milk miRNAs on diarrhea susceptibility, we explored the effect modification of SNPs in predicted milk rich miRNA target sites on the association between breastfeeding and diarrhea.

SNPs that might modify the binding of the 32 most abundant milk miRNA based on *in silico* predictions were retrieved from the PolymiRTS v2.0 database. After filtering for SNP functionality, around 600 SNPs were available in INMA (Infancia y Medio

Ambiente) birth cohort (N=719). The effect modification of these SNPs on the association between breastfeeding (any and predominant >=16 weeks) and diarrhea at age 1 year, was tested using PLINK. The four top SNPs (rs7467, rs940774, rs216463 and rs8053471) based on arbitrary p interaction values were selected for replication in two European cohorts: Generation R (N=2457) and COPSAC (N=345).

The most consistent result was for rs8053471. In particular, any breastfeeding and predominant breastfeeding decreased the risk of diarrhea in children with the rs8053471 minor allele [combined OR (95%CI) for any breastfeeding >=16 weeks: AA 1.10 (0.85;1.42), AG 0.62 (0.44;0.90), GG 0.45 (0.22;0,93)]. Rs7467 and rs940774 showed some trend of replication for predominant and any breastfeeding, respectively.

In summary, these preliminary results suggest that the relationship between breastfeeding practices and diarrhea protection might be, in part, mediated by milk miRNAs. Further investigations are needed to validate these results.

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P16.029

Polymorphism in maternal gene *RFC-1* A80G can increase the risk of Down syndrome pregnancies: a systematic review and meta-analysis

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First described in 1866, Down syndrome (DS) is resulting from a failure of chromosome 21 segregation during maternal meiosis in about 95% of cases. Maternal polymorphisms linked to folate metabolism have been studied as a risk factor for DS. A80G is a common polymorphism related to the folate transporting protein (RFC-1). It has been associated with DS, but inconsistent results have emerged from studies that investigated such association. Therefore, we performed this meta-analysis to derive a more precise estimation of this association. Studies were identified by searching the PubMed database for relevant articles in English published before january 2013 using the following criterion: (reduced-folate carrier or RFC-1 or A80G) and (Down syndrome or Trisomy 21). Case reports, editorials and review articles were excluded. The meta-analysis examined the association between gene polymorphism and maternal risk of DS for the dominant and recessive models of the mutant allele. The associations were indicated as a pooled odds ratio (OR) with the corresponding 95% confidence interval (CI) and significance level was considered for $p < 0.05$ values. Data from 11 case-control studies were included in the meta-analysis. The meta-analysis of dominant and recessive models showed a significant association between *RFC-1* A80G polymorphism and the birth of children with DS [random effects pooled OR = 1.28 (CI: 1.002-1.645) P= 0,0475] and [random effects pooled OR = 1.24 (CI: 1.029-1.512) P= 0,0241], respectively. Our study has demonstrated that there is a significant association between *RFC-1* A80G polymorphism and the risk for a DS pregnancy.

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P16.030

Polymorphism g.37190613G>A of the ELMO1 gene related with diabetic nephropathy in Mexican Mestizos.

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Introduction. ELMO1 gene encodes a protein involved in cytoskeleton organization. Variations in this gene have been associated with diabetic nephropathy, as the case of the polymorphism g.37190613G>A. In Mexican population there is no previous report on this issue Aim: To analyze the distribution of alleles and genotypes frequency from polymorphism g.37190613G>A in Mexican population. Methods: Genomic DNA of 103 mestizos from Northwest and 50 Half Breeds (with ancestry Yoruba and Zapoteca) of the Southern Lowlands, of Oaxaca, was extracted of peripheral blood leukocytes by the Miller method. The polymorphism was determined by PCR-PASA. The Hardy Weinberg test was validated by a X2. Results: This study population shows the presence of ancestral (G) and the reference (A) allele. The MAF rate, as well as heterozygosity index was estimated. The allele and genotype distribution is in Hardy Weinberg equilibrium, beging similar to the European and Asian populations. The populations here studied differs of native Africans, which ancestral allele is the most frequent. No carriers were found with homozygote G genotype. However northwestern mestizos show the expected distribution, having more Spanish ancestry. Conclusions. This study suggests that the ancestral allele from g.37190613G>A polymorphism, was lost during miscegenation of the different ethnic groups after the conquest of México. The allele A is now cosidered as a wild-type, transmitted by Negroid Asians and Spanish whose arrived in the Costa Chica and Southern Lowlands, in the 16th and 17th century as well as its a relationship with an increased risk of diebetic nephropathy in Mexican population.

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P16.031

Genetics of sphingolipids in hypertension

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Several attempts to decipher the genetics of essential hypertension have been done, but only a few genes have been identified. Unsolved population heterogeneity and the insufficiency of the prevailing monogenic approach to capture genetic effects in a polygenic condition are the main reasons for the modest results obtained. The physiologically heterogeneity of diastolic and systolic blood pressures was resolved by partition of the study population by combined latent class analysis and structural equation modelling into an ensemble of 14 physiological more homogeneous subpopulations. Two-gene interactions were evaluated for the sphingolipid metabolic network, and he phosphatidate and redox metabolic networks by variance decomposition and by a information theory approach. On average more than 5,000 highly significant interactions were detected by variance decomposition in each of the subpopulations including 160 single nucleotide polymorphisms (SNP) in 82 genes. The number of interactions were reduced to less than 0.5% by only including interactions with significant mutual information. The analysis suggests that acid ceramidase and sphingosine kinase-1 to be functional hubs in blood pressure regulation.

Of the 675 interactions with significant weighted mutual information 38 increased the prevalence of hypertension (diastolic and/or systolic), while 27 decreased the prevalence of hypertension in the study population. These interactions included genes from the networks mentioned above in a complex pattern of specific genotypes. Thus, the sphingolipid metabolic network was established to be of significance in regulating the blood pressure by itself and by integrated interaction with the phosphatidate and redox genetic networks.

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P16.032

Comparison of multi- and single-sample variant calling in high-coverage exome data

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Genetic variants are typically ascertained on a 'per sample' basis following high-coverage exome sequencing,. However, calling variants simultaneously across all sequenced individuals better captures shared genetic variants and yields more accurate genotype calls at these sites. As a result, this approach has recently been proposed for calling genotypes following exome sequencing. However, when studying rare phenotypes the focus is typically not on shared genetic variation but on very rare or private mutations. The utility of multi-sample calling for the ascertainment of such variants remains unclear.

Using data from the UK10K project, we make direct comparisons across almost 1,000 exomes called using both single-sample (SAMtools and GATK) and multi-sample calling (SAMtools). We compare how well each method identifies variants across the minor allele frequency spectrum (including singletons) in terms of the number of called variants (sensitivity), and the quality of these calls (specificity) (using the Ti/Tv ratio as a proxy for quality). Comparisons between the two approaches are performed across a range of variant quality score recalibration (VQSR) filtering thresholds

Preliminary analyses suggest that while multi-sample calling provides three to four times more variants unique to this method (depending on the VQSR threshold), the quality of these variants is considerably lower (Ti/Tv ~1.6) than those variants unique to single-sample calling (Ti/Tv ~2). In addition, single-sample calling provides around 5% more singleton variants per sample. Analyses are still ongoing with aim of ascertaining the best approach for accurately calling very rare or private mutations following exome sequencing.

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P16.033

Heterozygosity in familial Mediterranean fever is not causal but represents a susceptibility factor for clinically-similar complex disorders

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Background. Familial Mediterranean fever (FMF) is an autosomal recessive autoinflammatory disorder due to *MEFV* mutations and one of the most frequent Mediterranean genetic diseases. The observation of many heterozygous patients in whom a second mutated allele was excluded led to propose that heterozygosity could be causal; however, this might often be coincidental due to high rate of mutations in Mediterranean populations.

Objective. To better delineate the pathogenicity of heterozygosity in order to help genetic counselling and better manage the disease.

Methods. Complementary statistical approaches were used: genotype comparison in siblings from 24 familial forms, estimation of FMF prevalence at population levels, genotype study in 557 patients from four Mediterranean populations.

Results. Nearly all affected siblings of probands carrying two *MEFV* mutations also carry two mutated alleles. At population level, we did not observe any contribution of heterozygosity to the disease prevalence. However, patients are more prone to be heterozygous as shown by the higher ratio heterozygous carriers/non carriers in patients than in healthy individuals ($p<10^{-7}$-$p<0.003$). The risk for heterozygotes to develop FMF was estimated between 5.10^{-3} and 2.10^{-2} and the relative risk between 4.6 and 8.4. Finally, heterozygous patients were frequently part of families displaying a complex mode of disease inheritance.

Conclusions. This is the first statistical demonstration that heterozygosity is not responsible for classical Mendelian FMF, but constitutes a susceptibility factor for clinically-similar complex conditions. We also provide a first estimate of the risk for heterozygotes to develop FMF.

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P16.034

The Mutation Analysis of the *MEFV* Gene in Patients With Familial Mediterranean Fever in Turkey

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Purpose: Familial Mediterranean Fever (FMF) is a chronic inflammatory disease with otosomal recessive inheritance characterized by recurrent acute periods of fever, abdominal pain, rash and polyserositis. The mutations of the gene encoding pyrin protein play an important role in the etiology of the disease. In this study, we analyzed the MEFV mutations the patients referred to our center with a diagnosis of FMF according to the criteria of Tel- Hashomer.

Materials and Methods: We screened the mutations of the most frequently affected exons (2, 3, 5, 10) of the MEFV gene with DNA sequence analysis in 217 patients referred in Diskapi Yıldırım Beyazit Training and Research Hospital ,The Center of Genetic Diagnosis, Ankara, Turkey. The demographic and clinical data with pedigree analyses of the patients were performed with chi-square and fisher exact tests.

Results: Total 31 different mutations were found in the screened exons of 207 patients. The most frequent mutations were M694V, V726A, E148Q, and M680I. The most common clinical symptoms were abdominal pain, fever, and joint pain. Conclusion: FMF, occurring most commonly in Jewish, Turkish, Arabic and Armenian people, is a disease with a difficult differential diagnosis. The disease has an increasing frequency in Turkish people and is a candidate for important health problem in near future. The settling of diagnostic algorithms, the correct use of molecular methods and the exact definition of genotype-phenotype correlations will all help to reduce the number of patients with FMF waiting for the correct diagnosis.

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P16.035

Origin of the Manouche gypsies in France - how did it all begin: lesson learnt from a founder mutation associated with Glanzmann thrombasthenia

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The c.1544+1G>A substitution at the 5′ splice donor site of intron 15 of the ITGA2B gene, called the French Gypsy mutation, causes Glanzmann thrombasthenia, an inherited hemorrhagic disorder transmitted as an autosomal recessive trait and characterized by an altered synthesis of the platelet αIIbβ3 integrin. So far, this mutation has only been found in affected individuals originating from French Manouche families, strongly suggesting a founder effect. Our goal was to investigate the origin of the French Gypsy mutation. We estimated the age of the mutation by a likelihood-based method that uses the length of the shared haplotypes among a set of patients. For this, we genotyped 23 individuals of Manouche origin; consisting of 9 Glanzmann thrombasthenia patients homozygous for the French Gypsy mutation, 6 heterozygous carriers and 8 homozygous wild-type individuals. They were genotyped for four single-nucleotide polymorphisms using high-resolution melting curve analysis, and for two CA repeats in the BRCA1 and THRA genes at chromosome 17, using fragment analysis gels. We found that a haplotype of five polymorphic loci covering a 4-cM region was strongly associated with the French Gypsy mutation, suggesting a founder effect. The estimated age of this founder mutation was 300-400 years (range 255-552 years). Thus, all carriers of the French Gypsy mutation c.1544+1G>A at intron 15 descended from a common ancestor 300-400 years ago. Finally, on the basis of our results, we will also detail in this work the possible migration history of the Manouche gypsies in France.

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P16.036

Phospholamban p.Arg14del mutation causes arrhythmogenic cardiomyopathy

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Background. Recently, we showed that the c.40_42delAGA (p.Arg14del) mutation in the phospholamban (PLN) gene can be identified in 10-15% of Dutch patients with dilated cardiomyopathy or arrhythmogenic cardiomyopathy. The arrhythmogenic burden of the p.Arg14del mutation was illustrated by the high rate of appropriate ICD discharges and a positive family history for sudden cardiac death.
Methods. Our goal was to evaluate the geographical distribution and the origin of this mutation and to estimate the prevalence in a Dutch population cohort. We investigated the postal codes of the places of residence of p.Arg14del mutation carriers and places of birth of their ancestors. In addition, a large population-based cohort (PREVEND) was screened for this mutation.
Results. By April 2012, we had identified 101 probands carrying the PLN p.Arg14del mutation. A total of 358 family members were also identified, resulting in a total of 459 mutation carriers. The majority of mutation carriers live in the northern part of the Netherlands and analysing their grandparents' places of birth indicated that the mutation likely originated in the eastern part of the province of Friesland. In the PREVEND cohort we identified six heterozygous PLN p.Arg14del mutation carriers out of 8,267 subjects (0.07%).
Conclusion. The p.Arg14del mutation in the PLN gene is the most frequently identified mutation in Dutch cardiomyopathy patients. The mutation that arose 575-825 years ago is likely to have originated from the northeastern part of the province of Friesland and is highly prevalent in the general population in the northern part of the Netherlands.

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P16.037

Characterization of CCG repeats in *FMR1* gene and risk for Fragile X syndrome (FXS) among Ashkenazi population.

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Introduction: FXS is the most common cause of inherited intellectual disability. FXS is usually caused by CGG trinucleotide expansion in *FMR1* gene. Normal alleles have < 55 CGG repeats. Premutation alleles (55-200 repeats) may expand to full mutations (>200 repeats) in female meiosis. In addition, interspersed AGG repeats decrease the risk while un-interrupted CGG repeats are associated with allele instability and expansion. FXS carrier rate varies in different populations. This study describes the carrier rate and stability of *FMR1* alleles in a large cohort of Ashkenazi women.
Methods: 4344 Ashkenazi and 4985 non-Ashkenazi females without a family history of FXS or intellectual disability were analyzed for CGG repeats using Southern-blotting and PCR. AGG interruptions were evaluated in 326 Ashkenazi and 298 non-Ashkenazi using Amplidex® kit.
Results: Both populations showed two major peaks of 30 and 29 repeats. Ashkenazim had higher frequency of 30 repeats, and lower frequency of other peaks (P <0.0001). Significant higher rate of premutation allels was detected among Ashkenazi, only in 55-59 repeats' range (1:114 versus 1:277 in non-Ashkenazi). Loss of AGG interruptions (<2) was less common in Ashkenazi with normal alleles compared to non-Ashkenazim (9% versus 19% , p= 0.0002).
Discussion: While Ashkenazi females have high carrier rate of FXS, most Ashkenazi carriers have a low-range premutation allele, that carries a low risk of expansion to a full mutation. In addition, normal size allele have increased stability in Ashkenazim compare with non-Ashkenazim. Thus the risk for offspring with FXS may be lower than expected in Ashkenazim.

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P16.038

The FTO gene polymorphisms are associated with body composition and bone mineral content in the Chinese postmenopausal women

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Genome-wide association studies have identified *FTO*, *ACACB*, *SEC16B*, *MC4R*, and *CDKAL1* genes that can contribute specifically to the risk of abdominal adiposity. However, it is unknown whether these genetic factors affect relative body fat distribution in the abdominal visceral and subcutaneous compartments. To investigate the association between *FTO*, *ACACB*, *SEC16B*, *MC4R*, and *CDKAL1* gene polymorphisms and body composition in the Chinese postmenopausal women, five hundred and fifteen post-menopausal women were recruited and 99 of them were followed-up within duration of 2 years. Body composition and fat distribution were measured by dual-energy x-ray absorptiometry (DEXA). Three SNPs of the *FTO* gene and five SNPs from *ACACB*, *SEC16B*, *MC4R*, and *CDKAL1* genes were selected for genotyping on these post-menopausal women. We found that post-menopausal women carrying TT genotype of FTO rs14211085 increased their BMI by 0.67, while those carrying CC+CT genotype decreased BMI by 0.59 during a period of two years (p=0.017). We also found that the waist circumference of women carrying CT+TT genotype of *ACACB* rs2239607 was significantly increased by 3.48 cm compared with their counterparts (0.30 cm) during a period of two years (p=0.012). We further observed that post- menopausal women carrying GA+GG genotype of *FTO* rs9930506 had significantly higher total lean tissue as well as bone mineral content compared with their counterpart (p<0.05). Moreover, women bearing CC+CT genotype of *FTO* rs14211085 had significantly higher bone mineral content compared with their counterpart. Our findings provide initial evidence that the FTO may contribute to body composition and bone mineral content.

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P16.039

Use of genetic data in Europe: balancing informed consent and research freedom

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The EU Commission proposal for a Regulation revising the protection of individuals with regards to the processing of personal data (Com (2012) 11 final) (the "Regulation"), was launched in early January 2012 in order to remedy the legal uncertainty stemming from fragmentation in the way personal data protection was implemented across Member States. Another key issue was to increase the Regulation's ambit to better cover emerging fields resulting from rapid technological developments. Since January 2012, the Council of the EU and the European Parliament (December 2012) have proposed amendments that will have important consequences for medical research, especially since they concern data processing "for historical, statistical and scientific research purposes" (art.83) and notably in the context of health (art.81). Also, the Regulation now separately defines and regulates "genetic data" by including it as sensitive data having new legal ramifications for the way researchers can conduct their studies. Moreover, informed consent ("IC") procedures have been reinforced as they are seen as the only way to protect the autonomy of research participants. Consequently, IC has to be "freely given specific, informed and explicit"; which may nullify most of the current exceptions designed for research, ignoring the specific, unique nature of long term genetic research. An emphasis on IC to protect autonomy ignores many other available governance mechanisms (e.g. information disclosure, transparency, etc.). Indeed, transparent information exchange better balances informed consent and research freedom and should therefore be considered the best option for promoting and protecting fundamental human rights.

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P16.041

Frequencies of the GJB2 gene mutations in genetic non syndromic hearing loss patients from Eastern Sicily .

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Genetic nonsyndromic hearing loss (NSHL) is a pathological condition present in 1-2/1000 neonates. The inheritance pattern is autosomal recessive (80%) (DFNB), autosomal dominant (17%) (DFNA), X-linked (2-3%) (DFN) and mitochondrial (<1%). In Caucasian and European populations, NSHL are attributable to mutations in the *GJB2* gene that encodes connexin 26 protein (CX26). In the cochlea, CX26-containing gap-junctions are proposed to maintain K^+ homeostasis between outer hair cells and endolymphatic space during the auditory transduction.
The aim of our study was to determine the prevalence and the spectrum of the *GJB2* mutations in NSHL patients from Eastern Sicily.
In this study, a group of 196 unrelated NSHL patients (age range, 4-40 years) (43 females and 110 males) was enrolled and was age-sex matched with 196 healthy subjects. All patients underwent age-specific audiological evaluation. DNA samples were analyzed for allelic variants in the coding exon of the *GJB2* gene by PCR amplification and direct sequencing. The sequencing samples were compared with a wild type sequence obtained from the site http://www.ncbi.nlm.nih.gov/gene/2706 (NG_008358.1 RefSeqGene).
In our patients, the molecular analysis of the *GJB2* gene showed different mutations: the 35delG deletion (16%), the R184W (6%) and 167delT (4%) genetic variants. Moreover, V153I, W24X, E47X and M163V mutations encountered with a frequency of 2%. The frequency of the 35delG was significantly different from the control group (p<0.001), as well as the other mutations (p<0.01). We also found the M163V mutation, a very rare variant that we here described for the third time in the world literature.

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P16.042

Interaction of a genetic risk score of triglyceride risk variants and measures of insulin sensitivity on fasting serum triglyceride levels in a Danish population

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Background: Thirty-two susceptibility loci have in meta-analyses been shown to associate with serum triglyceride at genome-wide significance levels. To test the hypothesis that the impact of a genetic risk-score (GRS) of validated triglyceride risk variants may be modulated by the level of whole-body insulin-sensitivity or specific environmental factors we constructed an unweighted (uw-GRS) and a weighted GRS (w-GRS).
Methods: A total of 31 variants were genotyped in 6,784 individuals from the Inter99 study-population. Individuals receiving lipid-lowering medication were removed (n=80). In a cross-sectional design, we investigated whether BMI, measures of insulin sensitivity (HOMA-IR, $ISI_{matsuda}$ and BIGG-SI) or lifestyle factors (alcohol consumption, physical activity, diet and smoking) interact with the effect of the GRS on fasting serum triglyceride levels.
Results: Fourteen risk variants associated with increased triglyceride levels (p<0.05), having per allele effect sizes ranging from 1.8%-14.9%. The uw-GRS and w-GRS associated with triglyceride levels with a per allele increase of (OR[95%CI]) 2.6%(2.2-3.0), $p=2.1\times10^{-42}$ and 0.7%(0.6-0.8), $p=3.9\times10^{-78}$, respectively. We demonstrated an interaction between the w-GRS and BMI ($p=6.5\times10^{-3}$), HOMA-IR ($p=2.6\times10^{-4}$), $ISI_{matsuda}$ ($p=2.0\times10^{-4}$), and BIGG-SI ($p=3.3\times10^{-3}$) in relation to triglyceride levels. Hence, the GRS had a stronger effect among individuals who were insulin resistant. We found no interaction with any of the lifestyle factors. Similar results were obtained for the uw-GRS.
Conclusion: Our findings suggest that individuals who are insulin resistant may be more susceptible to the cumulative genetic burden of the triglyceride variants. Therefore, these genetically-destined at-risk individuals may benefit more from targeted interventions that aim at increasing insulin sensitivity.

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P16.043

Impact of a genetic risk score on incidence of coronary artery disease and myocardial infarction and fasting serum lipids in a prospective population-based cohort of 6,127 Danish individuals

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Abstract
Background: Genome-wide association studies have identified genetic risk-variants of coronary artery disease (CAD). We aimed to estimate the effect of a genetic risk score on 1) incidence of CAD and myocardial infarction (MI), and 2) baseline fasting serum lipid traits.
Methods: Data on genotype and quantitative traits was available in the population-based Inter99 study (n_{total}=6,127). Information on CAD (n_{cases}=374) and MI (n_{cases}=124) was obtained from national registries (mean follow-up=11.6 years). Participants were array-genotyped with the HumanCardio-Metabo- or Humanexome-chip for 34 CAD risk-variants. A genetic risk score (GRS) was calculated as sum of risk-variants. Risk of MI and CAD were calculated using a Cox regression-model automatically adjusted for age.
Results: The sex-adjusted GRS associated with incidence of MI and CAD (effect per GRS tertile HR[95%CI]= 1.27[1.01 - 1.59], p=0.04, and 1.19[1.05 - 1.36], p=0.007, respectively). Adjustments for sex, BMI and smoking-status showed association (HR[95%CI])= 1.28 [1.02 - 1.60], p=0.03 and 1.20[1.06 - 1.37], p=0.005, respectively). For the same model, the GRS associated with total cholesterol (β[95%CI]: 11.6%(8.3 - 14.9), $p=7.58\times10^{-13}$), serum triglycerides (2.7%(1.2 - 4.2), $p=4.2\times10^{-4}$), LDL-cholesterol (9.2%(5.0 - 13.5), $p=2.25\times10^{-5}$), triglyceride/HDL ratio (3.6%(1.7 - 5.6), $p=2.1\times10^{-4}$) and remnant cholesterol (3.2%(1.2-5.2), p=0.002). The GRS showed a nominal association with HDL-cholesterol (-1.1%(-2.24 -0.01), p=0.053). Finally, association of GRS on MI- and CAD-incidence was adjusted for total cholesterol and systolic blood-pressure (1.26[1.01-1.59], p=0.04 and 1.19[1.05-1.36], p=0.008, respectively).
Conclusion: A CAD GRS influences classical MI-risk factors suggesting that observed increased risk of CAD and MI could partly be mediated through altered lipid levels.

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P16.044

Higher risk of death among MEN1 patients with mutations in the JunD interacting domain. A GTE cohort study (Groupe d'étude des Tumeurs Endocrines).

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Multiple Endocrine Neoplasia syndrome type 1 (MEN1) is a rare disease secondary to mutations in MEN1 and predisposes to endocrine tumours. Although genotype-phenotype studies failed to identify a statistical correlation, some families harbour recurrent tumoral patterns. This is the first genotype-phenotype correlation study in MEN1 disease considering a time dependent statistical analysis, testing intra-familial correlation and evaluating the impact of the mutation localization in MENIN interacting domains in a large cohort. We report on the MEN1 cohort from the GTE network. Patients with MEN1 mutation and clinical follow up were included, i.e. 262 families, 806 patients. Mutations type, localization and 9 interacting factors of the MENIN protein were compared to occurring tumor types and death ages. The genotype-phenotype study was performed using a frailty Cox model

with gender adjustment and FDR correction procedure. The intra-familial correlation was assessed using a dedicated statistical method. There was no significant correlation between mutation type or localization and the different phenotypes. Overall survival was significantly reduced when mutations affected the JunD interacting domain (HR=2.13 (CI-95%[1.26; 3.60]). Patients had a higher risk of death from cancer of the MEN1 spectrum (HR=2.44 (CI-95%[1.25; 4.77]). Intra-familial correlation was demonstrated and completed with estimates of the heritability coefficients for tumor types. This result suggests that loss of interaction between JunD and MENIN ought to be a modifying factor of the disease expressivity and might result in the development of more aggressive tumors. This study highlighted the intrafamilial correlation, and helps follow up and care for MEN1 families.

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P16.045
How diverse is GJB2 in Portugal?

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To anyone acquainted with the genetics of deafness, GJB2 is the most familiar and well known gene, in which novel mutations are still being found. From the beginning it became clear that some GJB2 mutations are more prevalent in certain world regions or ethnicities and nowadays, with more than 200 different mutations, polymorphisms and novel variants described, population specificity is even more evident. Thus, when faced with probands requiring GJB2 diagnosis or genetic counseling, it is very helpful to have full knowledge of mutation prevalence in their region of origin.
Previous studies concerning deafness genetics in Portugal, and focusing on GJB2, have suggested that mutation spectrum of this gene in our population it's not exactly like other Mediterranean or western European countries. Portuguese history may explain part of this specificity.
In this study we set out to extend the investigation on GJB2 mutation prevalence in the general population by screening about 900 random neonates from different Portuguese regions in a thorough representation of our country's population. Screening of GJB2 gene was performed by sequencing the entire coding region. GJB6 deletions del(GJB6-D13S1830) and del(GJB6-D13S1854) were tested by multiplex PCR.
A great variability of GJB2 variants was observed and some interesting regional differences were found, supporting our previous studies. The prevalence and distribution of those variants will be discussed in detail, and compared with the rest of Europe and other world populations.

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P16.046
The Malta BioBank and the Maltese Genome Project

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The Malta BioBank is the first national archive of biological samples. It houses a population bank and a clinical bank with specific collections. The population bank has a very large collection of random Maltese neonates, pooled neonatal samples, multiple births and senior citizens. The clinical bank has specific collections such as the Globin Bank, the Coeliac collection, the Parkinson's collection, the Diabetes collection and a small collection of rare diseases e.g. cystic fibrosis.
The two main projects of the biobank are: 1) the Globin Bank which includes abnormal haemoglobins (Hb) and thalassaemia in the Mediterranean and genetic control of globin gene expression; and 2) the Maltese Genome Project which includes origins, mobility and genetic epidemiology of the Maltese population for gene discovery research.
The Globin Bank currently holds more than 250 Hb F Malta I ($p.\alpha_2G\gamma_2117Arg$) carrier samples and other samples with haemoglobin variants such as Hb F Sardegna ($p.\alpha_2A\gamma_275Thr$). The F Malta I variation has been found to be in tight linkage disequilibrium with the adult Hb variant Hb Valletta ($p.\alpha_2\beta_287Pro$).
A unique family with HPFH was found to be haplo-insufficient in the transcription factor KLF1 (p.K288X) on chromosome 19.

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P16.047
From GWAS to WGS: a whole-genome association study in 250 trios of the Genome of the Netherlands Project.

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Introduction: The Genome of the Netherlands Project (GoNL) was set up to characterize genomic variation in 250 pedigrees of Dutch ancestry. 231 trios and 19 quartets were sequenced at medium depth of coverage (>12x) by BGI (Shenzhen, China). We explored how this whole-genome sequencing data set could be used for an association analysis with height, weight, body mass index (BMI), and lipid traits (LDL-C, HDL-C, triglycerides, and total cholesterol).
Methods: We included 748 samples in the association analysis and called 19,763,454 bi-allelic SNPs. We excluded SNPs based on Hardy-Weinberg equilibrium ($P < 1 \times 10^{-6}$) and minor allele frequency < 1%, leaving 8,337,919 SNPs for further analysis. We used FaST-LMM to incorporate family structure in the association testing.
Results: No SNPs reached genome-wide significance ($P \leq 5 \times 10^{-8}$) for any trait. There was no evidence of population stratification or inflated type 1 error rate. Testing loci previously associated with the traits studied, we observed a clear excess of significant associations with the expected direction of effect. We also tested various gene-based tests, and explored how to correct effectively for the correlated test statistics.
Discussion: Power to test for association is limited with a small sample size. Nevertheless, we were able to replicate a bulk association signal for known loci of all phenotypes studied. We expect that the primary value of the GoNL whole-genome sequencing panel will be for imputation in much larger samples already genotyped in the GWAS era.

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P16.048
TWB1802, the first release of GWA control samples from Taiwan BioBank

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A population-specific reference is essential for hunting disease genes and developing personalized medicine. About 2% of the 23 million Taiwan residents population are the aboriginal peoples, belonging to the Austronesian family, while the majority is of Han-Chinese ancestry. Taiwan BioBank aims to build a national-wide biomedical research database that integrates genomic profiles, life styles and environment information of 300,000 Taiwan residents. Currently, Taiwan BioBank targets on disease gene-mapping using a genome-wide association (GWA) study approach. This study is set to conduct a population-specific reference that may serve as a global control sample for future GWA studies of diseases in the Taiwan population. A total of 2090 DNA samples, including 158 replicates, are randomly selected from a cohort study of the Taiwan BioBank and genotyped using the TWB array, which is designed for the Taiwan population by Taiwan BioBank. After removing replicates and samples with cryptic kinships, 1802 un-related samples are compiled as the first TWB release (TWB1802), including 899 males and 903 females. A total of 623,190 SNPs pass QC filters. Population structure analysis shows that all TWB1802 samples are clustered with the HapMap Asian groups (CHB, CHD, and JPT). Admixture analysis shows that the majority of TWB1802 may have southern or northern Han Chinese origins while some individuals show signals of admixture of Han Chinese and Austronesian origins. A publically-accessible web-based calculation platform, Taiwan View (http://taiwanview.twbiobank.org.tw/taiwanview/twbinfo.do) is built to perform on-line GWA analysis using TWB1802 as a control group. Further expansion with more than 20,000 Taiwanese samples is undergoing.

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P16.049

Genetic and environmental factors involved in normal hearing function and age-related hearing loss

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The vast majority of genetic and environmental/lifestyle factors underlying normal hearing function and age-related hearing loss (ARHL) remain to be identified. To reach this goal we decided to run 1) a series of Genome Wide Association Studies (GWAS) on hearing traits and ARHL, 2) expression studies by immunohistochemistry and confocal microscopy in wildtype mice on a list of 24 genes identified by GWAS, 3) a search for environmental/lifestyle factors (i.e. degree of education, smoking and drinking habits) by epidemiological studies.
Up to now, using a series of isolated populations/communities coming from Europe, Caucasus and Central Asia for an overall number of 3815 individuals, two GWAS for hearing quantitative traits (one sex separated), and one GWAS for ARHL have been performed. By meta-analyzing data some significant and suggestive loci (p≈10-8,10-7) have been found. As regards expression studies, 5 genes (Arsg, Slc16a6, Dclk1, Gabrg3, Csmd1) out of the 24 selected ones show strikingly specific expression in the cochlea, while additional 8 (Ptprd, Grm8, Kiaa056/GlyBP, Evi5, Irg1, Rimbp2, Ank2, Cdh13) display expression in multiple cell types of the cochlea. As regards environmental/lifestyle factors, the epidemiological analyses revealed that among all the investigated variables, coffee consumption was associated at low and high frequencies (p=0.006) while the intake only at high frequency (p=0.003) in 4 out of 10 countries investigated. Moreover, a statistical significant association between ARHL and level of education was detected (p=0.0003) confirming previously reported data. Additional studies are in progress to further confirm and expand present results.

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P16.050

Thresholds for significance in GWAS used in practice: a systematic analysis

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Background: In genome-wide association studies (GWAS), an association is considered as significant if its p-value is below the genome-wide threshold of significance. The choice of a threshold requires a fine balance between power and the risk of false positives and the current practice for this choice has not been systematically described. We conducted a systematic analysis to describe genome-wide significance thresholds in published GWA studies.
Materials and methods: We identified all GWASs published during the first half of 2011 and retrieved the genome-wide threshold of significance as stated by the authors. Most GWAS were followed by a replication stage in an independent sample, and we also systematically extracted the threshold for a SNP to be included in the replication stage.
Results: We identified 167 published GWASs over the study period. One third of studies (56) did not cite any significance threshold for their results. Sixty-seven studies (40%) considered $5x10^{-8}$ as genome-wide significant threshold. For the remaining 44 studies, the thresholds were more liberal (median 10^{-7}). A replication stage was performed in 122 GWASs. Thresholds to include SNPs in a replication stage were liberal (median 10^{-5}), varied considerably between studies, and were justified in only 14 studies (11%).
Conclusion: The most commonly used genome-wide threshold was 5x10-8, although reported in less than half of the manuscripts. No consensus emerged for the choice of the threshold to include SNPs in a replication stage. Establishing recommendations for thresholds would increase the interpretability of positive signals reported in the literature.

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P16.051

A meta-analysis of genome-wide association studies identifies a novel locus associated with thrombin generation potential

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High thrombin generation levels have been associated with the risk of venous thrombosis and ischemic stroke. In order to investigate the genetic architecture underlying the inter-individual variability of thrombin generation, we measured three quantitative biomarkers for thrombin generation, the Endogeneous Thrombin Potential (ETP), the Peak height and the Lag-time in two independent samples, the Three City Study and MARTHA, with genome-wide genotype data. By applying an imputation strategy based on the 1000 Genomes project to these two samples totalling 2,100 individuals inferred for ~6,6M markers, genome-wide significant associations were observed for ETP and Peak at the F2 gene ($p = 4.62\ 10^{-22}$ and $p = 1.05\ 10^{-8}$, respectively), a well-established susceptibility locus for thrombin generation. Further conditional analysis on the F2 signal revealed suggestive evidence ($p < 10^{-6}$) at 6 additional independent single nucleotide polymorphisms (SNPs) for at least one of the three phenotypes. These 6 SNPs were tested for replication in a third independent sample of 800 individuals part of the MARTHA12 study. For one SNP located on chromosome 9, the association with Lag-time was highly significant ($p = 3.26\ 10^{-7}$). When the results of the three data sets were combined, the overall evidence for association of the chr. 9 locus with Lag-time reached $p = 1.35\ 10^{-11}$. This locus has never been reported associated to thrombin generation, paving the way for novel mechanistic pathways in the aetiology of thrombotic disorders.

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P16.052

Replication of GWAS identification of Kawasaki disease susceptibility genes and a functional study affirm a role for BLK in disease susceptibility

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SNPs in *BLK* gene, which encodes B-lymphoid tyrosine kinase, showed the most significant associations with Kawasaki disease in two recent genome-wide association studies (GWAS) of Han Chinese and Japanese populations. In this reports, we conducted a meta-analysis to combine the evidence regarding *BLK* from these two previous GWAS and further replicated the associations in Korean and European populations. A top SNP identified in Han Chinese, rs2736340 in BLK, provided compelling evidence for associations in our meta-analysess of studies in Han Chinese and Japanese ($P = 3.97\times10^{-25}$) populations, all Asian populations ($P = 4.741\times10^{-31}$), and overall population samples ($P = 1.032\times10^{-24}$). Moreover, we showed that *BLK* expression was significantly induced in white blood cells in Kawasaki disease patients at the acute stage, and the risk allele of rs2736340 expressed lower levels of BLK in lymphoblastoid cell lines. These results provide clues about the involvement of BLK in Kawasaki disease pathogenesis.

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P16.053

Power comparison study of association tests with a general regression model

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Introduction: In most GWAS in the absence of information on the underlying model, the additive model is used for association studies. This may result in significant lack of power in case of strong departure from additive effect. In this case, a General regressive Model (GM), allowing to test for both additive effect and dominance deviation from additive effect, may be more appropriate for association tests.
Aims: to compare the power of GM to other classical association tests under a large panel of models.
Method: Powers were estimated by simulating 1 million replicates of 300 cases and 300 controls for 3 disease prevalence (1%, 5% and 10%), various SNP frequencies (between 0.1 and 0.5), various risk allele's genetic effect (Odds-Ratio between 1.2 and 4) and different transmission modes (recessive, dominant, additive). Four association tests: general, recessive, dominant

and additive models were applied to all replicates to estimate their respective powers to detect association.
Results: Although GM has one supplementary degree of freedom, its power to detect association is close to the power obtained by analysis under the true (simulated) model. Furthermore, GM is much more powerful than the additive model when the true genetic model is recessive. The test on dominance deviation parameter was able to estimate the correct model in more than 95% of replicates.
Conclusion: We recommended applying GM model instead of the additive model for GWAS. Reanalysis of GWAS data of multifactorial diseases using GM is likely to detect new variants that have been missed by classical methods.

M. Dizier: None. **A. Philippi:** None. **C. Julier:** None. **F. Mathieu:** None.

P16.054
Region-based association analysis of human quantitative traits in related individuals

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Regional-based association analysis instead of individual testing of each SNP was introduced in genome-wide association studies to increase the power of gene mapping, especially for rare genetic variants. For regional association tests, the kernel-machine-based regression (KMBR) approach was recently proposed as more powerful alternative to collapsing-based methods. However, the vast majority of existing methods for KMBR are applicable only to unrelated samples. We propose a new method for KMBR association analysis of quantitative traits in samples of relatives. The method is based on the GRAMMAR+ transformation of phenotypes followed by using existing KMBR software for unrelated samples (for example, SKAT). GRAMMAR+ transformation can be calculated with the 'polygenic' procedure in the GenABEL package v 1.7-2 (http://www.genabel.org/). We compared the performance of kernel-based association analysis on the material of the GAW17 by using our transformation, the original untransformed trait, and environmental residuals. We demonstrated that the distribution of the test statistic under the null hypothesis does not correspond to the declared distribution when original traits or environmental residuals are analyzed by SKAT. Only the GRAMMAR+ transformation produced type I errors close to the nominal value and had the highest empirical power. Therefore, the new method can be applied to the analysis of related samples by using existing software for KMBR association analysis developed for unrelated samples. GRAMMAR+ transformation is a computationally efficient alternative to other methods, including correction on relative structure in KMBR analysis via covariance matrix, because our trait transformation allows the use of KMBR methods with smaller computational complexity.

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P16.055
Searching for Hashimoto's missing heritability using Canine Lymphocytic Thyroiditis: a mission "possible"?

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Dogs are not only man's best friends in our daily life, but also in the long-term challenge of unravelling the genetics behind complex diseases. Canine lymphocytic thyroiditis (CLT), shares similarities in etiology, clinical signs and disease progression with Hashimoto's thyroiditis (HT), one of the most common autoimmune diseases in humans. The aim of our study is to identify genetic risk factors associated with canine autoimmune hypothyroidism, and to investigate these in human HT patients.
We performed a genome-wide association study using 115 Swedish Giant Schnauzer dogs (71 cases and 44 controls), considering presence of autoantibodies against thyroglobulin, increased levels of thyroid stimulating hormone (≥40 mU/l) and decreased levels of thyroxine (≤5 pmol/l) in the definition of the disease phenotype. We used kinship matrix and manual pedigree check in order to control for relatedness and applied a mixed model in the association analysis. An association on CFA11 was consistently confirmed ($p<10^{-5}$), spanning a 7 Megabase region of extensive linkage disequilibrium. An additional association, with a potentially really intriguing biological meaning, was found on CFA 18 ($p<10^{-4}$).
Once we have confirmed the associations by including more samples and identified haplotypes and genetic variants associated with the disease, we will be able to evaluate them in Hashimoto´s patients as well. Studying CLT may help solve a piece of the intricate puzzle behind the development of Hashimoto's disease, and possibly unravel a fraction of its missing heritability.

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P16.056
Evaluation of SNP's in promoter region of Gγ-globin gene and their relation with fetal hemoglobin in healthy individuals and sickle cell anemia patiens in Colombia.

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The sickle cell anemia (SCA) is a genetic disease (autosome recessive) caused by a mutation in β-globin gene that provoke a structural change in hemoglobin. There are modulators of SCA, once is fetal hemoglobin (HbF). The HbF is a complex character, but have been identified several mutations in promotor region of Gγ-globin associated to increasing of HbF levels. The objective of this survey was evaluate SNP's in proximal promotor of Gγ-globina gene to establish markers associated to HbF expression both in sickle cell patients and healthy people in Colombia. 163 Blood samples were taken of individuals of Atlantic and Pacific colombian coasts: 49 blood samples from sickle cell patients and 114 from healthy people. 15 SNP's were evaluated, neither showed relation with modulation of HbF expression in sickle cell anemia patients, but two SNP's, -309 (A→G) and -16 (C→G) were associated to HbF in healthy people. Is possible that regulation factors in cis could be different between sickle cell patients and healthy people and therefore the SNP's evaluated here were not present in important transcriptions elements in these patients meanwhile in healthy people do.

Y. Mendoza: None. **C.J. Fong:** None. **G. Barreto:** None.

P16.057
Assessing the contribution of chromosome X to complex traits: Two new loci for height and evidence for incomplete dosage compensation

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Most association studies omit chromosome X. Here we show the benefit of testing for the associations of a dense set of chromosome X variants with metabolic and anthropometric traits.
We imputed 1,250,369 non-pseudoautosomal X chromosome markers in 19,563 individuals in seven Northern European cohorts and investigated the contribution of these variants to the levels of twelve quantitative traits (BMI, height, WHR, insulin, glucose, CRP, HDL-C, LDL-C, TC, TG, systolic and diastolic blood pressure) for which multiple associated autosomal loci have been previously identified. Using a linear mixed model we estimate that the genotyped and well-imputed chromosome X markers (MAF>1%) together account for up to 2% (for height, CRP and systolic BP) of variance in these traits. In a chromosome X-wide association analysis we identified two associations ($p<5\times10-8$): Common SNPs in two independent ($r2<0.02$) loci in Xq21.1 were associated with height. Given the potentially incomplete dosage compensation in some loci in X we evaluated gender differences in the associations: in the other height locus the effect was considerably larger in women (explained variance 0.55% and 1.09 cm difference between homozygotes in 31-year-old women) in a manner consistent with both X chromosomes being active in this locus. The associated variants have a who-

le-blood-cis-eQTL with ITM2A, a gene implicated in early cartilage development. Supporting our observations, ITM2A has been previously shown to variably escape from X-inactivation.
Our results show that chromosome X contributes to the variability of many complex traits with potential sexually dimorphic associations and hence support including it in future GWAS.

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P16.058
Spectrum of mutations in a large cohort of unrelated haemophilia B patients

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Hemophilia B is 6 times less frequent than hemophilia A and characterization of deleterious mutation is less reported. We present here the result of molecular studies performed in 400 hemophilia B unrelated patients.
The functional region of *F9* gene, including the 8 exons with the flanking splice junction, the promoter and part of the 3'untranslated region surrounding polyadenylation site were studied by DNA sequencing. In all patients negative after complete gene sequencing, search for duplication was performed using quantitative fluorescent multiplex-PCR (QFM-PCR). A causative mutation was identified in 368/400 (95%) patients, mainly point mutations, a whole *F9* gene deletion in 6 patients, a large *F9* gene deletion encompassing one or several exons in 12 patients, a LINE sequence insertion in one patient and a mutation located in the Leyden region in 15 patients. For the first time a *F9* gene duplication was identified in 3 unrelated families. Thirty-eight (11%) of the identified mutations have not been reported so far. The strategy used to predict the deleterious consequences of these new variations will be presented. Collection on the international database of the mutations responsible for all hemophilia B cases, whatever the severity is particularly helpful to improve genetic counselling, especially for pregnant women with low FIX:C level, no family history of hemophilia and discovery of unknown mutation.
To conclude, only 5% of the patients had no mutation identified after extensive gene analysis. Next generation sequencing will probably help us to solve these cases.

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P16.059
‚Estimation of genetic diversity in NS3-protease region of hepatitis C virus using 454 pyrosequencing'

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Viruses are highly diverse in their genetic constitution, both within and between infected hosts. They adapt to the host's environment with a high mutation rate and can make efficient treatment very difficult. Hepatitis C virus (HCV), which causes nearly 200 million chronic infections worldwide is curable with treatments which comprise immunoregulator and direct antiviral agents. Our analysis is a case study in which viral samples from 40 patients with chronic HCV are being sequenced using a 454 Roche GS Junior at different stages of treatment. Using variant callers, we obtain the frequency of the variants that are found in the viral population during the course of the treatment. We study the viral diversity during the treatment to assess whether the treatment actually decreases the viral diversity. Our main objective is to test if the viral diversity at the onset of the treatment is an important determinant of the treatment outcome. This would ultimately help in understanding the evolution of the virus and provide further insights into drug design and personalized treatment of HCV.

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P16.060
GWAS Identifies Novel Susceptibility Loci on 6p21.32 and 21q21.3 for Hepatocellular Carcinoma in Chronic Hepatitis B Virus Carriers

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Genome-wide association studies (GWAS) have recently identified KIF1B as susceptibility locus for hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC). To further identify novel susceptibility loci associated with HBV-related HCC and replicate the previously reported association, we performed a large three-stage GWAS in the Han Chinese population. 523,663 autosomal SNPs in 1,538 HBV-positive HCC patients and 1,465 chronic HBV carriers were genotyped for the discovery stage. Top candidate SNPs were genotyped in the initial validation samples of 2,112 HBV-positive HCC cases and 2,208 HBV carriers and then in the second validation samples of 1,021 cases and 1,491 HBV carriers. We discovered two novel associations at rs9272105 (HLA-DQA1/DRB1) on 6p21.32 (OR = 1.30, P = 1.13×10^{-19}) and rs455804 (GRIK1) on 21q21.3 (OR = 0.84, P = 1.86×10^{-8}), which were further replicated in the fourth independent sample of 1,298 cases and 1,026 controls (rs9272105: OR = 1.25, P = 1.71×10^{-4}; rs455804: OR = 0.84, P = 6.92×10^{-3}). We also revealed the associations of HLADRB1*0405 and 0901*0602, which could partially account for the association at rs9272105. The association at rs455804 implicates GRIK1 as a novel susceptibility gene for HBV-related HCC, suggesting the involvement of glutamate signaling in the development of HBV-related HCC.

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P16.061
Association of hypoxia inducible factor-1 alpha gene polymorphism with physical performance in Lithuanian athletes

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A nonsynonymous coding single-nucleotide polymorphism (Pro582Ser, c.C1772T, rs11549465) of the hypoxia inducible factor-1 alpha (*HIF1A*) gene has been shown to be associated with changes of maximal oxygen consumption (VO_2max) during training. Given this evidence and the impact of *HIF1A* on oxygen metabolism in muscle activities, we hypothesized that variant of the *HIF1A* gene might be associated with Lithuanian elite athletes status and their VO_2max level. A total of 249 Lithuanian elite athletes (112 endurance-oriented, 50 power-oriented, 87 "mixed group") and 266 healthy unrelated individuals (controls) were genotyped(PCR-RFLP). Anthropometric measurements, anaerobic muscle strength (short-term explosive muscle power, anaerobic alactic maximum power) and aerobic VO_2max test were evaluated. The results revealed that the frequencies of the *HIF1A* genotypes were different in the athletes compared to controls (Pro/Pro 74.7%; Pro/Ser 24.9%; Ser/Ser 0.4% *vs.* Pro/Pro 75.2%; Pro/Ser 21.4%; Ser/Ser 3.4%; P=0.038). The frequency of the *HIF1A* Ser allele was higher in the power-orientated (18%) athletes compared to endurance-oriented (12%), "mixed group" (10.9%) and controls (14.1%). Muscle strength phenotypic indexes did not differ between athletes depending on their *HIF1A* genotype ($P>0.05$). Interestingly, we found that athletes carrying the Pro/Ser genotype exhibited significantly higher VO_2max (69.5*vs*.60.3 ml/kg/min, $P<0.05$) than those carrying the Pro/Pro genotype. Based on these findings, we conclude that *HIF1A* variation may be associated with Lithuanian elite athletes' status and athletes carrying the *HIF1A* Pro/Ser genotype with higher aerobic capacity. Of course, further studies will be needed to fully understand the mechanisms underlying the putative association between the *HIF1A* polymorphism and endurance performance.

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P16.062

Tolerance versus immunity: evidence of balancing selection at the HLA-G promoter region

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Human leucocyte antigen G (HLA-G) is a tolerogenic molecule which plays a crucial role in protecting the fetus from maternal immune attack. Besides, HLA-G expression can be induced in numerous pathological conditions where its immunosuppressive properties render it an efficient means of escape from immune recognition for pathogens and tumors. In contrast to classical class I HLA molecules which display extensive variability in the coding region, *HLA-G* rather exhibits a higher degree of variation in regulatory regions. In this study, we aimed to characterize the sequence variation and haplotype structure of the *HLA-G* promoter region to investigate the evolutionary history of the *HLA-G* promoter and shed some light into the mechanisms that may underlie *HLA-G* expression control. The 1.4-kb upstream regulatory region of *HLA-G*, which includes all of the known promoter elements, was sequenced in three new African populations (Serere from Senegal, Tori from Benin, Yansi from Congo) and these data were combined with the available sequence data from the 1000 Genomes project and the literature. Patterns of sequence variation, frequency spectrum and genetic differentiation of *HLA-G* promoter strongly supported a history of balancing selection at this region. An extended analysis of a 300-kb region surrounding *HLA-G* revealed that this region is the direct target of selection and is not involved in a hitchhiking effect. These results suggests that there might be a fine balance between optimal levels of expression from fetal to adult life, allowing both fetal tolerance and appropriate intrauterine defense against pathogens.

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P16.063

Founder Mutation for Huntington Disease in Caucasus Jews

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Huntington disease (HD) is an autosomal dominant disorder characterized by chorea, psychiatric illness and cognitive decline caused by expansion of the trinucleotide CAG repeat within the *HTT* gene. The prevalence of HD is well known among Europeans, but not in Jewish communities. We analyzed the clinical and demographic data of our HD patients and carried out haplotype analysis in order to look for and date a founder mutation. Between 2006 and 2011, 1,400 patients were evaluated in our adult genetics clinic. Eleven patients from 10 different families were diagnosed with HD; nine families are Caucasus Jewish (CJ) and one is Ashkenazi. Of the nine CJ, eight shared a 3.74 Mb haplotype, which is compatible with the HD-susceptible A1 haplogroup. We calculated the coalescence age of the mutation using the DMLE+2.0 software program to be between 4 and 10 generations, which correspond to around 80-200 years ago. In our clinic 90% of HD patients are CJ, although the CJ comprise only 1.4% of the Israeli population. These findings suggest a higher prevalence of HD among CJ compared to the general Israeli population and are consistent with a recent founder mutation in this group.

This should raise the index of suspicion for HD in CJ, thereby allowing for earlier diagnosis so that prenatal testing can be offered when desired, and treatment once it is available.

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P16.064

Risk scores derived from known lipid variants improve identification of individuals at increased risk of hypercholesterolemia

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Background Hypercholesterolemia (HC; total cholesterol > 6.5 mmol/l and/or use of lipid lowering medication) is a well-established modifiable cardiovascular disease risk factor. We determined the ability of genotypic risk scores to identify individuals at increased risk of hypercholesterolemia.

Methods and Results We calculated TC, LDL-C, HDL-C and TG risk scores in the Rotterdam Study (RS, n=10,072) and Erasmus Rucphen Family Study (ERF, n=2,715), using 157 SNPs discovered in recent meta-analyses of ≥100,000 individuals. Adding the TC score to a clinical model based on age, sex and BMI improved the prediction of HC (c-index in RS increased from 0.64 to 0.68 (P=2.1x10^{-31}) and in ERF from 0.71 to 0.75 (P=2.9x10^{-9})). The LDL-C, HDL-C and TG scores combined improved prediction similarly. In RS, Kaplan-Meier curves showed an approximately doubled cumulative incidence of HC in the highest TC risk score quartile compared to the lowest. In both cohorts, overall and in age strata, prevalence of HC increased with TC risk score quartile (P_{trend}=1.3x10^{-96} to 0.002). Odds ratios also increased with quartile, from 1.62 for the second to 3.5 for the highest quartile compared to the lowest in the total sample. Maximum OR was 3.9 for the highest quartile in the 65-74 years stratum (P=2.4x10^{-32}).

Conclusion Our results show that genetic risk scores can be useful in identifying individuals at increased risk of hypercholesterolemia. Our findings also suggest that a genetic risk score can help identify individuals that would benefit from earlier and more intensive monitoring of cholesterol levels.

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P16.065

Genetic Predisposition to Left Ventricular Dysfunction: A Multigenic and Multi-analytical Approach

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Background: Left ventricular dysfunction (LVD) is a complex, multifactorial condition, caused by mechanical, neurohormonal, and genetic factors. The present study was undertaken to identify the combination of genetic variants of renin-angiotensin-aldosterone system (RAAS), matrix metalloproteinases (MMPs) and inflammatory pathway genes and their possible interactions contributing towards genetic susceptibility to LVD in the background of coronary artery disease (CAD).

Methods and Results: The study included 510 consecutive patients with angiographically confirmed CAD. Among them, 162 with reduced left ventricle ejection fraction (LVEF≤45%) were categorized as having LVD. We analysed 11 polymorphisms in 9 genes of RAAS, MMPs and inflammatory pathways. Single locus analysis showed that AT1 A1166C (p value<0.001; OR=3.67), MMP9 R668Q (p value=0.007; OR=3.48) and NFKB1 -94 ATTG ins/del (p value=0.013; OR=2.01) polymorphisms were independently associated with LVD. A combined G-score consisting of risk alleles of associated polymorphisms showed much higher significance as compared to individual variations (p-value<0.001). High-order gene-gene interaction using Classification and Regression Tree analysis (CART) and Multifactor dimensionality reduction (MDR) approaches revealed AT1 A1166C and NFKB1 -94 ATTG ins/del polymorphisms jointly increased the risk of LVD to great extent (p-value=0.001; OR=8.55) and best four-factor interaction model consisted of AT1 A1166C, MMP7 A-181G, MMP9 R668Q and NFKB1 ATTG ins/del polymorphisms with an improved testing accuracy of 0.566 and CVC=9/10 with permutation p<0.001) respectively.

Conclusion: The AT1 A1166C independently and in combination with MMP9 R668Q and NFKB1 -94 ATTG ins/del polymorphisms plays important role in conferring genetic susceptibility to LVD in CAD patients.

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P16.066

Association study of TOLL and CARD with leprosy susceptibility in Chinese population

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Background: Previous genome-wide association studies (GWAS) identified multiple susceptibility loci that have highlighted the important role of *TLR* (Toll-like Receptor) and *CARD* (caspase recruitment domain) genes in leprosy.
Methods: A large three-stage candidate gene-based association study of 69 *TLR* and *CARD* genes was performed in the leprosy samples of Chinese Han.
Results: Of 4,363 SNPs investigated, eight SNPs showed suggestive association (P<0.01) in our previously published GWAS datasets (Stage 1). Of the eight SNPs, rs2735591 and rs4889841 showed significant association (P<0.001) in an independent series of 1,504 cases and 1,502 controls (Stage II), but only rs2735591 (next to *BCL10*) showed significant association in the second independent series of 938 cases and 5,827 controls (Stage III). Rs2735591 showed consistent association across the three stages (P>0.05 for heterogeneity test), significant association in the combined validation samples ($P_{corrected}=5.54\times10^{-4}$ after correction for 4,363 SNPs tested), and genome-wide significance in the whole GWAS and validation samples ($P=1.03\times10^{-9}$, OR=1.24). In addition, we demonstrated the lower expression of *BCL10* in leprosy lesions than normal skins and the formation of a highly significant gene network by *BCL10* and the eight previously identified genes ($P=1\times10^{-19}$) that regulates the activity of NFκB, a major regulator of downstream inflammatory responses, which provides further biological evidence for the association.
Conclusion: We have discovered a novel susceptibility locus on 1p22, which implicates *BCL10* as a new susceptibility gene for leprosy. Our finding highlights the important role of both innate and adaptive immune responses in leprosy.

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P16.067

LITGEN - revealing genetic structure of the population of Lithuania

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LITGEN project has been launched in 2011. The aim is to determine genetic diversity and structure of the population of Lithuania using informative variable genetic markers such as SNPs, CNVs, STRs of Y chromosome, mtDNA, autosomes, exome and whole genome for future extensive wide scale human genome studies in Lithuania.
First stage of LITGEN project was to collect samples from different ethno-linguistic groups of Lithuanians. Questionnaires and informed consent forms were obtained and signed by all participants. Phenotypic data such as diet, physical activity, genealogy and anamnesis were collected by questionnaires. About 1000 samples were collected and current database contains 259 triads. 150 triads were assigned to particular ethno-linguistic group as all members originated from the same region. Analysis of biochemical phenotype associated with cardiovascular diseases has been performed for all participants. Attention is paid to particular groups of genes or pathways involved in cardiovascular diseases, nutrigenomics, pharmacogenetic response, autosomal recessive diseases identifying genomic variants specific to the population of Lithuania. Data from questionnaires and biochemical phenotype are managed using BC|GENE data management platform. Y chromosome haplotype consisting of 17 STR markers was determined for male participants using commercial AmpFlSTR® Yfiler™ kit. Large scale genotyping was performed using HumanOmniExpress-12 v1.0. Further bioinformatical analysis is performed from the obtained data.
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P16.068

Local interactions capture both known and novel loci and provide new insights into eight human metabolic traits

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Local interactions between neighbouring SNPs are hypothesized to be able to capture variants missing from genome-wide association studies (GWAS) via haplotype effects but have not been thoroughly explored. Aided by recent improvements in software we were able to perform full pair-wise genome scans and conventional GWAS in eight metabolic traits in the Northern Finland Birth Cohort 1966 (NFBC1966) and the Atherosclerosis Risk in Communities study cohort (ARIC). Genome-wide significant interactions were detected in ARIC for systolic blood pressure between *PLEKHA7* (a known GWAS locus for blood pressure) and *GPR180* (which plays a role in vascular remodelling), and also for triglycerides as local interactions within the 11q23.3 region (replicated significantly in NFBC1966), which notably harbours several loci (*BUD13*, *ZNF259* and *APOA5*) contributing to triglyceride levels. Tests of the local interactions within the 11q23.3 region conditional on the top GWAS signal suggested the presence of two independent functional variants, each with supportive evidence for their roles in gene regulation. Local interactions captured 9 additional GWAS loci identified in this study (3 significantly replicated) and 73 from previous GWAS (24 in the eight traits and 49 in related traits). We conclude that the detection of local interactions requires adequate SNP coverage of the genome and that such interactions are only likely to be found between weakly linked SNPs. Analysing local interactions is a potentially valuable complement to GWAS and can provide new insights into the biology underlying variation in complex traits.

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P16.069

A frequency based approach for discriminating between causative and rare genetic variations in LQTS

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Long QT syndrome (LQTs) is a Mendelian disease characterized by delayed ventricular repolarization manifest with prolongation of the corrected QT (QTc) interval on the electrocardiogram. Genetic testing on LQT syndrome led to the worldwide identification of hundreds of genetic variations in 13 different genes. Interpreting and validating the effects of these mutations is still challenging especially in absence of functional studies that can confirm their deleterious effect. In this context, using NHLBI genetic frequency regarding 23 LQTS mutations supported by functional studies we estimated a Minor Allele Frequency (MAF) threshold to discriminate between a causative rare genetic and a low frequency variation.To assess the validity of our model we applied the estimated threshold 0.04% to a population of 337 LQTS probands carrying 204 unique single-nucleotide variations.Results showed that the median value of the QTc distribution was significantly higher for patients with rare mutations (MAF < 0.04%) comparing with those having common variants (p < 0.05). In addition, using data regarding 79 functionally validated variations, we tested the performances of the two bioinformatic tools Polyphen2 and SIFT in predicting the deleterious effects of the genetic variation.
Our data provided a validated frequency cut-off value to discriminate between causative mutations and low frequency variations in absence of in vitro or in vivo studies.

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P16.070

Methylenetetrahydrofolate reductase MTHFR C677T polymorphism and susceptibility to lymphoproliferative disorders in the Malaysian population

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Methylenetetrahydrofolate reductase (MTHFR) is a critical enzyme in folate metabolism. This enzyme is involved in DNA synthesis, DNA repair and DNA methylation. Several studies have shown that MTHFR C677T gene polymorphism is associated with susceptibility to lymphoproliferative disorders (LPD). The objective of this case-control study was to determine the association between MTHFR C677T gene polymorphism and risk of LPD in the Malaysian population. The C677T polymorphism was genotyped in 1,246 participants (524 lymphoid malignancy patients and 722 healthy individuals) using the Sequenom iPLEX MassARRAY platform. A total of 524 (42%) LPD patients and 722 (58%) controls participated in this study. Of the LPD patients, 32%, 9%, 42%, and 17% were diagnosed with diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), other non-Hodgkin lymphoma (NHL), and Hodgkin's lymphoma (HL) subgroups respectively. No statistically significant differences were observed for different genotypes between patients and controls in the pooled LPD group (p>0.05). Overall, there was significant association between C677T polymorphism and risk of FL (p =0.006). Subgroup analysis by ethnicity in patients with FL as compared with controls showed significant association in Malaysian Malays (p=0.016). However, sample size in this subgroup was small. This study suggests that C677T polymorphism might be a risk factor for susceptibility to FL in the Malaysian Malays. However, due to the small sample size of patients with FL, false positive finding cannot be ruled out.

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P16.071
Prevalence and diversity of monogenic hereditary pathology among the children of Rostov region (Russia)

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Results of prevalence and nosologycal forms of a monogenic hereditary pathology (MHP) among the children's of twelve investigated Districts of Rostov Region (Russia) was submitted. The total size of investigated populations was made 497460 people, including 97345 (19.57%) children. The ethnic structure of considered sample on more that 90 % is presented by Russian population. The study was conducted on the original protocol examined by standard protocol of medical genetic research elaborated in laboratory of genetic epidemiology, Research Centre for Medical Genetics. About 3000 MHP and syndromes of OMIM could be identified by this research. Clinical investigations were performed by pediatricians, clinical geneticists, neurologists, ophthalmologists, orthopedists, audiologist, and dermatologists, focused on diagnostic of MHP. The spectrum of MHP detected in the twelve Districts comprises 166 nosologycal forms - 95- autosomal dominant (AD), 57- autosomal recessive (AR) and 14- X-linked. The prevalence rate (for 1000 children) of children's population by all types MHP - AD, AR, and X-linked, separately for urban (1.86±0.21, 1.82±0.21, 0.53±0.16, respectively) and rural (4.38±0.28, 3.39±0.25, 1.30±0.22, respectively) populations was calculated. In rural populations, the prevalence is higher in 2 times higher than in urban. The total prevalence of MHP was 6.49±0.28/ 1000 children. Given that this study can detect only half of hereditary diseases, the total prevalence of hereditary disorders in children more than 1%. In this study, we studied the prevalence of hereditary diseases among children Republics Bashkortotan, Udmurtia and Chuvashia, in which the burden of hereditary diseases was 1.4%, 1.2%, and 1.1%, respectively

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P16.072
Mitochondrial DNA diversity in medieval and modern Romanian population

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Ancient DNA population studies may yield interesting results in cases where are indications from archaeology and history that a population demographic modification has taken place and significantly reduce the genetic diversity via bottleneck effect.

We analyzed mtDNA variation using aDNA samples from Piata Universității archaeological site and modern DNA to study nature and extent of temporal changes in genetic variation in Bucharest region during 16th-19th centuries. The archaeological site of Piata Universității cemetery unearthed about 676 graves with 900 skeletons. We have analyzed by now 18 aDNA samples out of a total of 150 dental pieces recovered from the skeletons excavated from the cemetery and 74 out of 250 modern DNA samples collected. Standard contamination precautions and authentication criteria were applied. Hypervariable regions I and II of ancient mtDNA were amplified and sequenced using twelve overlapping fragments, each with a length of approximately 100 base pairs. The majority of the ancient and modern mtDNA samples analyzed by now falls into the common West Eurasian mitochondrial haplogroups.

In conclusion, to fully assess the dynamics of the historical population composition by comparing genotypes in a temporal context we have to complete the comparative analysis of all aDNA and modern DNA samples. Moreover, in order to reveal possible genetic data changes caused by a possible population bottleneck corresponding to the waves of lethal epidemics, in which an almost one-third of the population was lost, we will also investigate a set of 17 fast evolving short tandem repeat loci (STR).

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P16.073
Multi-phenotype meta-analysis in up to 167,984 individuals evaluates pleiotropic effects on cardiometabolic traits at *FTO*, *FADS1* and *GIPR* loci

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Cardiometabolic risk factors are correlated epidemiologically and share common genetic predisposition, as confirmed by single-trait genome-wide association studies. A large-scale multi-phenotype analysis can be a valuable tool to dissect independence of effects of a genetic variant on metabolic traits and disease risk, e.g. pleiotropy. We aimed to a) evaluate the multi-phenotype analysis method using an *FTO* variant known to exert its action via body mass index (BMI) and b) explore multi-phenotype effects at *FADS1* and *GIPR*, both associated with a range of metabolic traits. We implemented a method that utilizes multiple logistic regression with SNP genotypes as the outcome and phenotypes as explanatory variables. Model selection was based on Bayesian Information Criterion scores summed across studies from derived multi-phenotype likelihoods. Analysis was performed in up to 167,984 individuals of European ancestry from 26 studies. Consistent with previous reports, the multi-phenotype meta-analysis confirmed that BMI drives associations with other traits at *FTO*, suggesting absence of pleiotropy. Variants at *FADS1* have an effect on lipids, fasting glucose and resting heart rate among others. At *FADS1*, the best models within multi-phenotype sets underscored independent effects for triglycerides and total cholesterol (P_{LRT}=1.3x10^{-102}). Variants at *GIPR* are associated with BMI, type 2 diabetes and 2-hour post load glucose and insulin. We were not able to detect pleiotropy at *GIPR*, possibly due to a smaller number of individuals with 2-hour glucose measurements in this study. The multi-phenotype method confirmed mediation effects for a known *FTO* variant and shows promise for dissecting pleiotropic associations.

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P16.074

A two-stage genome-wide association study identifies germline predisposition loci for myeloproliferative neoplasms

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Myeloproliferative neoplasms (MPN) are chronic hematological malignancies characterized by excessive production of terminally differentiated blood cells. Genome-wide association (GWA) studies in recent years have become the main tool for identification of common polymorphisms predisposing to diseases. So far a strong susceptibility haplotype spanning JAK2 gene has been identified for MPN. We aimed to find more loci conferring risk to develop MPN by performing a GWA study comparing MPN samples with healthy controls. Genotype data obtained by Affymetrix Genome-Wide Human SNP Array 6.0 for all samples was used for the analysis.
We used 372 Austrian MPN cases and 1757 German controls (KORA-gen) for initial GWA. The strongest association was detected for JAK2 locus (allelic association P=1.4x10-28, OR=2.59). To overcome the limitation of small sample size we designed a second stage of association validation for which we used four smaller independent cohorts which were also genotyped by the same SNP array platform: three cohorts from Italy (n=98, n=76, n=56) and one cohort from Czech Republic (n=37). All the SNPs that showed P value <10-3 in initial GWA were selected and the allele frequencies were compared to those of the four validation cohorts and their respective control groups. The analysis revealed three haplotypes on chromosomes 7p12.1 (P=9.06x10-6, OR=1.53), 13q12.11 (P=2.42x10-5, OR=0.69) and 15q26.2 (P=1.33x10-4, OR=1.44) that had similar frequencies in all validation cohorts and are thus candidates for common MPN predisposition. The current study also demonstrates the usefulness of multi-stage GWA analysis using SNP array data for validation.

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P16.075

Resolution of the paradoxical excess of duplications over deletions at the DPY19L2 locus in the general population

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We demonstrated previously that 75% of infertile men with round acrosomeless spermatozoa (globozoospermia) had a homozygous 200 Kb deletion removing the totality of DPY19L2. We showed that this deletion occurred by Non Allelic Homologous Recombination (NAHR) between two homologous 28 Kb Low Copy Repeats (LCRs) located on each side of the gene. The accepted NAHR model predicts that inter-chromatid and inter-chromosome NAHR create a deleted and a duplicated recombined allele, while intra-chromatid events only generate deletions. Therefore more deletions are expected to be produced de novo. Surprisingly, array CGH data show that in the general population, DPY19L2 duplicated alleles are approximately three times as frequent as deleted alleles. In order to shed light on this paradox, we measured the rates of de novo deletions and duplications at this locus on the sperm of three control donors. We achieved this by developing a digital PCR assay amplifying specifically the deleted and duplicated alleles. We measured that the average rate of de novo deletion was 8.9x10-6 (95% CI: 7.1x10-6; 1.1x10-5) whereas that of duplication was 3.9x10-6 (95% CI: 3.1x10-6; 4.9x10-6) showing an approximate two fold excess of de novo deletions over duplications, concordant with the NAHR theoretical model. We calculated that the excess of de novo deletions was compensated by evolutionary loss whereas duplications, not subjected to selection, increased gradually. We conclude that the purifying selection against sterile, homozygous deleted men, accounts partly for this compensation, but heterozygously deleted men also likely suffer a small fitness penalty.

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P16.076

Whole-genome sequencing of the Val Borbera cohort highlights low frequency variants associated with several complex traits

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Next-generation sequencing approaches allow the identification of population-specific low-frequency variants. A random sample of 110 individuals from the genetic isolate Val Borbera cohort (VBI) from Italy were whole-genome sequenced at low-coverage (6.5X).
After genome refinement, quality control and filtering steps, a total of 11,446,954 single nucleotide variants (SNV) and 812,109 INDELs were detected on autosomal chromosomes, and 372,230 SNVs and 29,671 INDELs were detected on the X-chromosome. Of these, 4,695,640 SNVs (about 39%) were at low frequency (MAF<=5%), whereas 2,603,184 (about 22%) were private to VBI when compared to two large-scale sequencing projects (UK10K and 1000 Genomes Project).
Prior to genotype imputation, the whole set of 1664 participants having genome-wide SNP array data were pre-phased using SHAPEIT2[1] . Imputation into the GWAS sample was based on a combined reference panel including the set of variants discovered in the pilot phase of the 1000 Genomes Project and the VBI WGS dataset. The final dataset includes 36,648,992 imputed SNVs and 1,380,736 INDELs.
We tested associations of individual SNPs at a large set of hematological, lipid and anthropometric quantitative traits [2], identifying several several suggestive signals driven by low-frequency variants. Replication experiments in other European sequenced populations are ongoing.
1. O. Delaneau, JF. Zagury, J. Marchini (2013) Improved whole chromosome phasing for disease and population genetic studies. Nature Methods [in press]
2. Xiang Zhou and Matthew Stephens (2012). Genome-wide efficient mixed-model analysis for association studies. Nature Genetics. 44: 821-824

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P16.077

Inferring negative selection from mutation genealogies

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Mendelian diseases are rare because natural selection prevents the dissemination of the disease-causing allele. Inferences about negative selection can provide important information about the probability of a given variant being the cause of a genetic disease. This is particularly important for non-coding variants, for which it is difficult to carry out functional tests.
The reconstruction of mutation genealogies from present-day chromosomes can provide us with information about past events. It is difficult to define the signature of negative selection from inferred genealogies and to quantify the probability of a given allele being a disease-causing variant, because the history of a variant reflects demographic history, the random transmission of alleles over generations and natural selection.
The relationship between branch length and ancestral haplotype size is purely statistical and the challenge is to develop simplifications with a minimal loss of information, because the precision of the reconstruction depends on the dimensionality of the graph. Most pathological variants are the product of recent mutations, and this assumption underlies allele frequency-based methods. Negative selection is therefore taken into account, albeit indirectly, through allele frequency, for the evaluation of variants of unknown significance. The analysis of graphs representing the genealogy of a variant may be viewed as an extension of this widely used method.
As haplotype decay is essentially stochastic, simple geometric structures that can be described unambiguously in mathematical terms can provide the algebraic framework for untangling the forces shaping the genealogy of a single allele.

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P16.078

Preliminary results of a genetic association study between candidate genes and nonsyndromic cleft lip and palate in the Brazilian population

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Nonsyndromic cleft lip with cleft palate (NSCLP) is a congenital malformation with a prevalence of 0.2% to 0.04% among live births. Environmental factors and pathogenic mutations in several genes have been described, as well as association studies between polymorphisms in candidate genes and NSCLP. Considering this, the aim of our study was to investigate the association between NSCLP and 39 candidate genes, which were selected based on NSCLP mutations and association studies and expression patterns during facial development. We evaluated 140 patients with NSCLP and 350 control individuals without NSCLP from Brazilian sample. A total of 251 tagging SNPs selected by Haploview software were used in the analysis, which screened the candidate genes. These SNPs were genotyped using the OpenArray™ TaqMan system. Logistic regression analysis was performed to analyze allele and genotype association. Seven SNPs in six different genes were statistically associated with NSCLP, even after correction for multiple testing [*MSX1* rs12532*GG (p=0.037); *BMP4* rs17563*AA (p=0.046); *JAG2* rs9972231*TT (p=0.030) and rs11621316*AA (p=0.027); *WNT9B* rs1443311*GG (p=0.025); *AXIN2* rs11655966*AA (p=0.010); *LHX8* rs12409145*AA (p=0,034)]. In addition, four SNPs showed an increased risk for NSCLP [*MSX1* rs12532*GG (OR=1.84, 95%CI=1.11-3.03); *BMP4* rs17563*AA (OR=1.71, 95%CI=1.11-2.63); *JAG2* rs11621316*AA (OR=1.88 95%CI=1.22-2.88); *LHX8* rs12409145*AA (OR=6.13, 95%CI=1.59-23.56)]. Interestedly, three SNPs presented a decreased risk for NSCLP [*WNT9B* rs1443311*GG (OR=0.20 95%CI=0.05-0.75); *AXIN2* rs11655966*AA (OR=0.50, 95%CI=0.33-0.76); *DVL2* rs2074222*GG (OR=0.52, 95%CI=0.34-0.81); *JAG2* rs9972231*TT (OR=0.34, 95%CI=0.14-0.80)]. This data suggests that these genes could be associated with formation and/or fusion of the facial processes and could predispose or protect to NSCLP.

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P16.079

Does Haplotype Tests Gain Power than Collapsing Tests in General Pedigree-based Association Studies for Detecting Rare Variants

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Recently, rare variants have received more attention with the advent of next-generation sequence technology. Some rare variants have been found to play a causal role in human disorders including psychiatric disorders. The established strategy is to collapse the rare variants as in the burden tests while analyzing the rare variants. In this study, we focus on the detection of the rare variants using pedigree data. A weighted haplotype test was considered in case-control design by Li et al which showed greater power than the collapsing tests through simulations. The haplotype-based approach has become attractive because haplotypes may carry some information of ungenotyped rare variants. Therefore, we aimed to investigate the performance of haplotype collapsing tests in family data. As the Pedigree-Disequilibrium-Test (PDT), the proposed haplotype based PDT test (hPDT) evaluates the difference in the counts of the transmitted and un-transmitted haplotypes from parents to affected siblings and the difference in the counts of haplotypes between affected and unaffected sibs.
In this study, ForSim is used to simulate sequence data in pedigrees. 200 families are randomly selected. To investigate the power, we randomly select 50% of the rare variants in the causal region to be causal. We further assume that r% (r% ranges from 5%, to 100%) of rare variants increase disease risk, whereas the remaining (100 - r)% decrease disease risk. As a comparison, the rare variants based PDT test (rvPDT) is also considered. Results indicated that when r=0.8, the power is 0.85 and 0.75 for hPDT and rvPDT, respectively.

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P16.080

Frequency of mutations in the *PAH* gene in PKU patients in Rostov Region

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Phenylketonuria (PKU) is an autosomal recessive inherited disorder arising from the deficiency of phenylalanine hydroxylase (PAH). In the majority of cases, PKU is caused by mutations in the *PAH* gene. More than 500 mutations have been described world-wide and the PAH enzyme has been fully characterized. Frequency and occurrence of mutations characterized by significant between-population differences. The most common mutation for Europeans is a missense mutation in exon 12 of the gene PAH - R408W.
DNA was extracted and examined by standard procedures from 77 patients with PKU. Were determined 8 mutations (R408W, P281L, IVS10nt546, R261Q, IVS12nt1, R158Q, R252W, I65T).
There are 3 mutations are the most frequently then others: R408W, IVS10nt546 and IVS12nt1. The frequency of these mutations is 72.73% in all patients and 77.60 % in Russian patients. R408W mutation makes up 62.34% in all patients (72.42% in Russian patients). About 25% of patients with R408W are heterozygous. 7.14% of patients had IVS10nt546 mutation. 90% of these patients were Turkish, Azerbaijani, Georgian and Armenian. IVS12nt1 mutation was detected in 4.31% Russian patients and 3.25 % in all PKU-patients. P281L, R252W, R261 and R158Q mutation frequencies in the PKU patients were 2.60% 1.95%, 1.95% and 1.29% respectively. There is no patient with I65T mutation.
All PKU patients in Rostov Region have a classic form, caused by the mutation in the *PAH* gene. There is no patient with BH4-deficient hyperphenylalaninemia in Rostov Region. The most common mutation for PKU patient in Rostov Region is R408W.

N. Vetrova: None. **M. Amelina:** None. **S. Amelina:** None. **O. Senyuta:** None. **R. Zinchenko:** None.

P16.081

Comparison of local and long-range phasing approaches in a Cilento isolate

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Population isolates have well-documented characteristics that can aid to identify rare variants associated with complex traits, namely reduced phenotypic, environmental and genetic heterogeneity. Besides, alleles that are rare in general populations may have become more frequent in those isolated populations, which could facilitate their identification. However, the cost of sequencing technology is still too high to allow the study of rare variants through the sequencing of the whole genome or exome for all individuals from an isolated population. To overcome this technical bottleneck, a cost-effective strategy consists in the sequencing of a limited number of previously genotyped individuals from the population followed by the imputation of the discovered variants onto the rest of the population.
Imputation involves a phasing process which can be performed using approaches that can be divided into two groups: local phasing and long-range phasing. The former family of approaches is based on linkage disequilibrium (LD) while the latter is based on Identity-By-Descent (IBD) and was designed to take advantage of relatedness between samples from isolated population. Here, we compare the performance of two recently published phasing approaches – SHAPEIT2 (Delaneau et al, 2013), a local phasing approach, and SLRP (Palin et al, 2011), a model-based long-range phasing approach - on samples from an isolated population from the Cilento region in Italy. This population is composed of three villages totaling about 2,000 genotyped individuals. Comparison is performed through simulations based on the genealogical structure of this isolated population.

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P16.082
Worldwide population differentiation of genes involved in drug response

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Human adaptation to spatially and temporally varying chemical environments has greatly influenced the present-day distribution of variants involved in xenobiotic response, including drug response. Documenting the general population differentiation patterns at pharmacogenetic genes and studying them in relation with natural selection can help us to better understand the genetic basis of drug response. In this study, we systematically investigated worldwide population differentiation and potential signals of natural selection in a comprehensive list of 45 genes with a known role in the pharmacokinetics or pharmacodynamics of drugs, identified as Very Important Pharmacogenes (VIP) by investigators of the Pharmacogenetics Research Network. To this end, we used frequency data from the 1000 Genomes Project, involving 14 worldwide populations, and estimated the degree of population differentiation, as measured by the fixation index FST, for all SNPs identified in VIP genes. Using genome-wide SNP data, we constructed several empirical distributions according to SNP physical position and function (non genic, genic, 5'-UTR, 3'-UTR, intronic, splice site, coding synonymous and coding non synonymous). An outlier approach based on these distributions allowed to identify highly differentiated variants in ADH1A, ADH1B, CYP3A4, CYP3A5, DRD2, P2RY1 and VKORC1 genes, reflecting the action of diversifying selection, while low levels of genetic differentiation were observed for the P2RY12 and SULT1A1 genes, reflecting homogenizing selection. Our results provide insights into the interethnic genetic differences in VIP genes and provide new candidates for putative functional variants that may contribute to the current variability in drug response within and among human populations.

B. Patillon: None. **P. Luisi:** None. **E. Génin:** None. **A. Sabbagh:** None.

P16.083
Genomewide linkage scan for principal components of phospho- and sphingolipids level in blood

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Phospho- and sphingolipids are a complex class of molecules involved in a number of important cellular functions. They influence membrane structure, intracellular signaling and interaction with the extracellular environment. The purpose of this study was to identify quantitative trait loci underlying variations in the lipids level in plasma.
One hundred and twenty eight species of phospho- and sphingolipid were measured in 821 individuals from an extended pedigree from the Erasmus Rucphen Family study. All individuals were genotyped using Illumina 6k linkage chip. Because many lipid levels were highly intercorrelated we redefined phenotypes in terms of principal components composed of linear combinations of the original traits. Linkage analysis was performed using MERLIN.
We identified two significant loci: 11q12.3 (LOD = 5.26) and 19q13.3-q13.4 (LOD = 4.14). The first peak included well known genes *FADS1* and *FADS2*. The second region has not been identified before although the potential candidate genes *TOMM40/APOE* were located on the boundary of the linkage interval. Our linkage peak was not explained by *TOMM40/APOE*, because the linkage signal was not decreased after conditioning on genotypes of rs2075650 and rs157580 in this locus. The next local association analysis of the linkage region demonstrated 33 SNPs with FDR-based q-value <0.05. Three of them (rs1167351, rs4802810, rs736574) demonstrated q-value 0.000135 – 0.000797. GWAS for these SNPs showed p-values 2.78×10^{-8} – 4.93×10^{-7}.
These results indicate the presence of new genes involved in the control of the lipids level in 19q13.3-q13.4 region. Identification of these genes may become possible after exome sequencing.

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P16.084
Next generation association studies in isolated populations

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Population isolates can enhance the power to detect association at low-frequency and rare sequence variation, because of potentially increased allele frequency and extended linkage disequilibrium. We have collected samples from two isolated populations in Greece (HELlenic Isolated Cohorts study, www.helic.org). All samples (n~3000) have information on a wide array of anthropometric, cardiometabolic, biochemical, haematological and diet-related traits. All individuals have been typed on the Illumina OmniExpress and exome-chip platforms, and 250 individuals have been whole-genome sequenced (WGS) at 4x depth. We are in the process of whole-genome sequencing all individuals at 1x depth. We have assessed the degree of relatedness compared to the general Greek population by calculating IBS. We find that 80% of subjects have at least one "surrogate parent" in the isolates, compared to 1% in the outbred Greek population. Using the GWAS and exome-chip data, we find that all of the 14 established fasting glucose level-associated variants (binomial $p=6.1\times10^{-5}$) and 23 of 30 BMI loci (p=0.002611) have the same direction of effect as published studies. Next generation association studies using the HELIC resource have the potential to address study design/methodological questions relating to very low depth WGS, characterise for the first time the population genetics of these populations, and link phenotype to genotype for traits of medical relevance.

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P16.085
Population diversity and history of the Indian subcontinent: Uncovering the deeper mosaic of sub-structuring and the intricate network of dispersals

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Past genomic studies have provided a broad picture of the peopling of the India: proposing a southern exit route through western coastline and migration from west and central Asia, through north-western corridor. We undertook a genome-wide study (Illumina 1M SNP chip) comprising 367 individuals, drawn from 20 ethnic groups of India representing all geographic regions, linguistic groups and social hierarchies, including hunter-gatherers from Andaman&Nicobar(A&N) Islands.
Our analysis corroborated with previous studies on the existence of deep rooted population structure in India, as evidenced by a high average pairwise FST ~0.04. The average pairwise FST was extremely high (>0.1) between A&N and the mainland Indian populations, indicating the high differentiation.
Unlike Europe, geography alone is a poor predictor of genetic diversity and/or ancestry, as indicated by PCA, ADMIXTURE and MANTEL test indicating complex local histories. There was poor correlation between pairwise genetic distance and geographical distance (0.33).
Our results of admixture estimation and hierarchical clustering reveal that genetically the populations of mainland India form four ancestry clusters (cross validation error minimum (0.52) compared to other competing models).
- TibetoBurman speaking populations of the northeast region
- Upper Caste groups of both northern(IE) and southern(DR) regions
- Austro-Asiatic(AA) speakers of central and eastern region
- DR speaking lower caste and tribal groups of southern region.

On inclusion of the Human Genome Diversity Panel (HGDP) data, we show that the A&N are close to the Polynasian populations, indicating early dispersal routes to Polynasia, Melanasia and Australia.

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P16.086

Ascertaining the effect of common and rare copy number variation in primary biliary cirrhosis risk

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Primary biliary cirrhosis (PBC) is an autoimmune disease of the liver, characterized by immune-mediated destruction of intrahepatic bile ducts. Recent SNP-based association studies have identified 25 independent risk loci, but the role of copy number variants (CNVs) in disease susceptibility has, thus far, not been investigated. Here, we present the first large-scale CNV-based association study of PBC using 2,981 cases from the UK PBC Consortium and 8,970 UK population controls, genotyped using the Immunochip. To accurately call CNV genotypes at both common (minimum aneuploid haplotype frequency (MAF)>1%) and rare CNVs (MAF≤1%) we used two different algorithms. PlatinumCNV, which performs 'per SNP' across sample CNV calling was used to ascertain common CNVs. In contrast, PennCNV, which takes a 'per individual' across SNP approach was used to call rare CNVs. We devised an extensive quality control (QC) pipeline to remove artefacts associated with potential confounding factors such as genotyping centre and DNA source, and performed a number of post-calling QC steps to reduce false-positive CNV calls. In our preliminary analyses to date we have identified one genome-wide significant common CNV (rs9611142: $P=1.30\times10^{-9}$, MAF=0.23) in high linkage disequilibrium (LD) with a known GWAS hit (rs2267407, $r^2=0.76$). Four common CNVs of suggestive significance ($10^{-8}<P<10^{-6}$) were also identified, one of which is not tagged by a known SNP association (maximum $r^2<0.01$). Further analyses of both common and rare CNVs are currently underway and validation and replication analyses are planned. Both CNV and SNP genotypes will be incorporated into our fine-mapping analyses.

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P16.087

Genetic and epigenetic determinants of human adaptation to different living environments.

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Over the past years, studies have suggested that epigenetic patterns, on top of playing an important role in the fine-tune regulation of gene expression, can also result from both inherited profiles and complex interactions with environmental factors. Epigenetic modifications could then allow fast and transient adaptation to new environments. We hypothesize that variations in epigenetic patterns may exist between populations, depending on their genetic background, their lifestyle and the environment to which they are exposed. To test these hypotheses, we will study 191 individuals from four populations: two African Pygmy populations historically relying on hunter-gathering: the Baka of Western Africa and the Batwa of Eastern Africa; and their respective neighbouring agriculturalist populations: the Nzime and Nzebi of Western Africa, and the Bakiga of Eastern Africa. Pygmy and non-Pygmy populations differ in their genetic background, lifestyle, environment, exposure to pathogens and cultural practices. We will focus on the study of their DNA methylation profile, as being the most accessible and characterized component of the epigenome. To this end, for each sample, we have obtained the genome-wide DNA methylation profile of >450,000 sites, and we genotyped one million SNPs genome-wide. These data will allow us to study the population variation in genetic and epigenetic profiles, and to map common and population-specific methylation quantitative trait loci. By integrating all these results with functional information from existing databases and natural selection information obtained by analyzing genotyping data, we hope to better understand the interaction between genetic diversity, environment, lifestyle and epigenetic modifications in humans.

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P16.088

Detection of population stratification on rare variant association studies for nicotine dependence

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To date, rare variants (MAF < 1 % or 5 %) have a proven role in complex diseases. The discovery of these genetic variants and their contribution to the development of diseases lead to several research issues in genetic studies. One of the research issues is that the prevalence of risk alleles varies across populations. Therefore, we considered population stratification based on principal components analysis to explicitly model ancestry differences between cases and controls and applied it into two rare variant detecting methods, i.e., combined multivariate and collapsing (CMC) and weighted sum statistic (WSS) methods. We used the latest publicly available dataset of the Genetic Architecture of Smoking and Smoking Cessation downloaded from the database of Genotypes and Phenotypes. This study includes 3,564 samples across populations (White and Black) genotyped on the Illumina HumanOmni2.5. We identified the specific ethnic association between nicotine dependence and CHRNA7 (p-value =4.25×10-10) in Black and THSD4 (p-value =3.45×10-11) in White which were originally implicated by bioinformatics studies using other nicotine dependence related data sets. The CHRNA7 gene included 113 SNPs (31 were rare) and THSD4 included 775 SNPs (133 were rare). Although these SNPs were not significant (p-value >10-5) by using a Trend test, they were detected by CMC and WSS methods while considering population stratification. In this study, we showed that considering population stratification in rare variant analysis could help gain more insight into identifying genetic risk factors for nicotine dependence.

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P16.089

Proteins network involved in Familial Hypercholesterolaemia

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Familial Hypercholesterolemia (FH) is an autossomal dominant disorder characterized by high levels of LDLc and premature cardiovascular disease. In the Portuguese FH Study 60% of clinical FH patients do not present any mutation in FH associated genes (LDLR, APOB, PCSK9, LDLRAP1), so the cause of hypercholesterolemia in these families should be in another gene of lipid metabolism.
The main aim of this project was the analysis of alterations, found by exome sequencing, in genes present in the protein networks of FH associated genes/proteins in order to identify the genetic cause of the hypercholesterolemia in these patients.
The exome sequencing of 5 index patients with clinical diagnosis of FH was performed in an Illumina sequencer and data analysis was performed by annovar software.
The networks of proteins associated with FH have identified 26 genes previously described in several studies as interacting with these genes. A total of 27 variants were found; a large number are synonymous alterations and the vast majority have been previously described in dbSNP. Eleven nonsynonymous alterations have been identified in 5 genes of lipid metabolism (LRPAP1, SORT1, LPA, LRP2 and EGFR) and one has not been described before in dbSNP. Family studies are being conducted to observe co-segregation of the alteration with the phenotype and determine if these variants can be alterations causing disease.
Identification of novel genes causing FH will improve patient identification and prognosis and can lead to the identification of novel lipid lowering drugs.

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P16.090

EURO-WABB: An EU Rare Diseases Registry for Wolfram Syndrome, Alström Syndrome, Bardet-Biedl Syndrome and other rare diabetes.

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Background. Wolfram, Alström and Bardet-Biedl (WABB) syndromes are rare diseases, which are characterized by presence of diabetes and visual involvement with significantly shorten life expectancy. There are no treatments available, and no access to well characterized cohorts of patients. The lack of specific health policies and the scarcity of expertise, translate into delayed diagnosis and difficult access to care. Genetic testing centres are concentrated in a few member states with unequal access to testing across the EU.

Methods/Design. EURO-WABB is an international multicentre large-scale observational study capturing longitudinal clinical and outcome data for patients with WABB diagnoses. Comprehensive clinical, genetic and patient experience data are collated into an anonymised and web-based disease registry. 700 participants will be recruited over 3 years from sites throughout Europe.

Discussion. The registry data will be used to increase the understanding of the natural history of WABB diseases, to serve as an evidence base for clinical management, and to aid the identification of opportunities for intervention to stop or delay the progress of the disease processes. The detailed clinical characterization will allow inclusion of patients into studies of novel treatment interventions or multi-national clinical trials. The registry will also support wider access to genetic testing, provide information for affected families and health professionals; and encourage international collaborations for patient benefit. It is hoped that the registry will create a more supportive environment and improved quality of life for WABB patients achieved through earlier diagnosis, prompt identification and management of complications and increased healthy life years.

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P16.091

A novel SVM-based approach for detecting rare variants of complex diseases

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Several strategies for the identification of rare variants (RVs) contributing to disease susceptibility have been proposed thus far. The most important feature of several recent proposed new statistical methods is pooling or collapsing multiple rare single nucleotide variants (SNVs) together such that collectively they would have a reasonable high frequency and effect. However, if the pooled RVs are associated with the trait in different directions or if some RVs are non-causal (i.e. they are not associated with the traits), pooling may weaken the signal in associated RVs and thus reduce statistical power. It is therefore helpful to determine whether a RV should be pooled by considering its association effect (including the association direction). Here, we propose a novel support vector machine (SVM) based approach to identify RVs associated with the disease based on the RVs' association effects. The disease may involve both common and rare variants with protective, deleterious or neutral effects. Environmental or gene-gene interactions can be accounted for in this approach. Simulation studies under a wide variety of scenarios and a data example showed the proposed approach outperformed other available approaches.

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P16.092

Pain pathways are associated with pain reduction in rheumatoid arthritis patients treated with anti-TNF medication

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Background Rheumatoid arthritis (RA) patients rate pain relief as the highest priority in treatment. Interestingly, recent research indicates that inflammation and pain pathways are important, but partly independent, targets of anti-tumour necrosis factor (TNF-inhibitor) therapy. Therefore, identification of genetic factors predicting pain relief is important in efforts to personalize treatment.

Objective We performed a genome-wide association study (GWAS) to identify genetic factors predicting pain reduction during anti-TNF treatment in RA patients.

Methods We conducted a GWAS including 919 RA patients treated with TNF-inhibitor. Association analysis using the relative pain change on a visual analogue scale (VASpain) 14 weeks after treatment initiation as outcome was performed under additive genetic model with adjustment for baseline VASpain. Pathway analysis was performed in Ingenuity.

Results 2,557,253 single nucleotide polymorphisms (SNPs) and 868 patients passed quality control. No findings reached the threshold for genome-wide significance (p-value $\leq 1x10^{-8}$) and lowest p-value identified was $7.27x10^{-7}$. Pathway analysis using all SNPs with a p-value<0.001 identified three canonical pathways to be overrepresented in our dataset. All three show a direct link to pain (processing): neuropathic pain signaling in dorsal horn neurons ($p=1.68x10^{-7}$), synaptic long term depression ($p=4.83x10^{-7}$) and glutamate receptor signaling ($p=5.97x10^{-7}$).

Conclusions We show that pain change after TNF-inhibitor treatment is associated with pain related genes. These genes do not show overlap with previous studies focusing on TNF-inhibitor treatment outcome based on the disease activity score. Identified pathways will be replicated in an independent cohort. Confirmed biomarkers can be used to personalize medication for the individual patient.

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P16.093

Genetic variation underlying common hereditary hyperbilirubinemia (Gilbert's syndrome) and the relationship with respiratory function in the UK 1946 birth cohort.

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Background: Bilirubin has potent antioxidant properties and raised serum levels have been linked with lower rates of respiratory disease. It is currently unknown whether the genetic variation underlying common hereditary hyperbilirubinemia (Gilbert's syndrome) is associated with differences in respiratory function.

Objectives: To examine whether the genetic variation of uridine diphosphate glucuronosyltransferase (*UGT1A1*), which has a major influence on serum bilirubin levels, also influences respiratory function.

Methods: Two functional variants of the *UGT1A1* regulatory region; rs8175347 (TA)n and rs4124874 c.-3279T>G were typed in 2,132 members of 1946 British birth cohort and regression models were fitted to examine the relationship with respiratory function.

Results: The frequency of the low activity variants of rs8175347 and rs4124874 associated with raised bilirubin levels were 0.30 and 0.44 respectively. There was a statistically significant association between *UGT1A1* (TA)n genotypes and lung function at age 43, and an interaction with smoking status at age 53. Mean FEV1 and FVC at age 53 for heavy smokers (≥20 cigarettes per day) with *UGT1A1* (TA)n alleles underlying Gilbert's syndrome was 472 ml (95%CI: 228 to 715) and 580 ml (95%CI: 280 to 881) higher respectively compared with heavy smokers without these alleles. The odds of moderate to severe COPD by age 53 were less than half in those with the *UGT1A1* (TA)n alleles underlying Gilbert's syndrome (odds ratio 0.48 [95%CI: 0.26 to 0.88])

Conclusions: Alleles of *UGT1A1* that cause raised serum bilirubin are associated with increased lung function in adults.

L.J. Horsfall: None. **R. Hardy:** None. **A. Wong:** None. **D. Kuh:** None. **D. Swallow:** None.

P16.094

REL and PADI4 polymorphisms and rheumatoid arthritis in the Tunisian population

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Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by autoimmunisation against citrulline, potentially leading to severe disability and premature mortality. Citrulline is generated by enzymes called peptidylarginine deiminase (PADs). Both genetic and environmental factors contribute to RA pathogenesis. PADI4 gene (1p36) has been mapped by linkage analysis in RA families. In addition, genome wide association studies (GWAS) had incriminated lately REL as a RA susceptibility locus. Considering these data, we investigated in the Tunisian population four single nucleotide polymorphisms (SNPs) mapping to PADI4 gene (PADI4 94 (rs2240340), PADI4 96 (rs1748032)) and REL gene (rs13031237, and rs6727504).
Two hundred and nineteen RA patients and 300 matched controls were enrolled. Genotyping was determined by Restriction Fragment Length Polymorphism-Polymerase Chain Reaction (PCR-RFLP) and confirmed by Mutagenically Separated PCR (MS-PCR). Frequencies of alleles and genotypes were analyzed using the khi-square test. Statistical significance was assumed at $P < 0.05$.
GT genotype (Rs13031237, REL gene) was significantly associated with the disease (OR = 1.71 (IC95%: 1.26-2.32), P = 0.018). Although, no evident association with anti-CCP or erosion was found. Rs6727504 was not polymorphic in the Tunisian population. In addition, we found no association between PADI4-94 and PADI4-96 SNPs and RA occurrence.
Our study is the first to examine the potential role of REL and PADI4 gene polymorphisms in the Tunisian and the North African populations. Our preliminary results should be confirmed by a larger cohort to validate our conclusion that SNP rs13031237 is in linkage disequilibrium with another polymorphism to identify.

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P16.095

No major impact of insertion/deletion polymorphism of the angiotensin-converting enzyme gene on susceptibility to rheumatoid arthritis in Serbian patients

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Angiotensin-converting enzyme (ACE) is upregulated in synovial stroma in rheumatoid arthritis (RA). Increased tissue ACE may potentiate synovial hypoxia and proliferation in RA. ACE gene insertion/deletion (I/D) polymorphism has consistently been associated with the variance of serum ACE concentrations.
The aim of this study is to determine genotypes of ACE gene I/D polymorphism in rheumatoid arthritis (RA) patients and to study the correlation between gotten genotypes and susceptibility to RA. A group of 260 RA patients and 101 healthy subjects were included in this study. Patients and controls were gender- and age-matched. DNA was extracted from peripheral blood leukocytes, and I/D polymorphism genotypes of the ACE gene was determined using polymerase chain reaction.
The distribution of ACE I/D genotypes in RA patients (DD 51.9%, ID 31.2 % and II 16.9%) were not significantly different from healthy controls (DD 45.6%, ID 33.6%; and II 20.8%). We have found a higher frequency of D allele in RA patients compared to the controls (67.5 % versus 62.3%) ($\chi 2=1.704$, p=0.191, OR=1, 25, and 95%: 0,892-1, 757) but without significant association. Also, comparison between ACE genotypes in subgroups of patients with RA made by gender, disease duration, values DAS28 and rheumatoid factor positive significant association was not observed.
Our data suggest that ACE gene I/D polymorphism is not a risk factor for RA in Serbian population.

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P16.096

Increased autoimmune diseases prevalence in Sardinian population explained by runs of homozygosity and genomic regions under positive selection

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Sardinia population is one of the European genetic outliers because of its historical and geographical isolation, characterized by an increased prevalence of certain disorders like type 1 diabetes or multiple sclerosis.
The objective of this study was to provide a plausible explanation of the increased autoimmune diseases prevalence in the Island, analyzing Runs of Homozygosity (ROH) and genetic regions showing signatures of positive selection.
About 1.2M Single Nucleotide Polymorphism (SNP) genotyped in 1077 Sardinian individuals were used to investigate the genetic population structure, as well as to estimate ROH and Extended Haplotype Homozygosity (EHH) regions.
Fixation index (F_{st}) values, λ inflation factors and Multidimensional Scaling analyses supported a high level of homogeneity in the Sardinian population.
Several short ROH (0.5Mb-2Mb), characteristic of the island population were identified. These regions contain genes already described as involved in the onset of several autoimmune diseases. Moreover, by means of EHH analysis, we identified various genomic regions showing footprint of positive selection and, once again, harbor genes associated with autoimmune diseases.
In conclusion, we confirmed the high genetic homogeneity of Sardinia, making this population very useful for association studies. Moreover, we showed that the high prevalence of certain diseases may be explained by the shared ancestry combined with the action of evolutionary forces. Therefore, estimation of ROH and EHH are efficient techniques to explain disease variability in isolated population.

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P16.097

Genetic structure of Romani populations in Croatia - analysis of autosomal STR loci

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Roma population is the largest transnational minority in Europe, made of numerous endogamous groups which share common Indian origin. Based on the history of early migrations to Europe, they might be classified into the Balkan Roma, the Vlax Roma, and the Northern/Western European Roma. In order to capture genetic variation of Roma population in Croatia we analysed 15 autosomal STR loci (AmpFlSTR Identifiler) on 536 Roma belonging to two major migration categories: Balkan and Vlax. Vlax Roma samples comprise individuals from two different linguistic groups (Munteni and Ardeleni). Diversity indices pointed to the lowest diversity among Ardeleni Vlax Roma. Moreover, Fst and Rst genetic distances show closer relationship between Balkan and Munteni Vlax populations than between two Vlax Roma populations. STRUCTURE program analysis revealed the highest deltaK for K=2, assigning Ardeleni Vlax individuals separately. Although it would be expected that Vlax Roma groups cluster together according to migration data, the clustering of Munteni Vlax population closer to Balkan Roma suggests that despite of common migration history the gene pool of Vlax Roma population shows pronounced differentiation, probably due to strict socio-cultural rules of the endogamous groups within migration category. High level of endogamy in Ardeleni Vlax group is also confirmed with our socio-cultural data collected in regard to this population. Due to extremely polymorphic nature of analysed loci further analyses are needed to entirely resolve genetic relations among endogamous Roma groups.

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P16.098

Investigating the Effect of Sequencing Depth on Low Coverage Next Generation Sequencing Association Studies

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Whole-genome and exome sequencing will allow researchers to probe the role of rare genetic variation in disease susceptibility. Low coverage sequencing with multi-sample calling has made large-scale case-control sequencing association studies feasible. However, cases and controls will often be sequenced at different depths, possibly as a result of using common controls. In this preliminary work we investigate how changes in depth affect variant discovery, genotype accuracy and power to detect association. Initial analysis was performed on whole-genome sequence data from 1,754 Crohn's disease cases sequenced at a mean coverage of 4X. In a series of simulations, sequencing reads were randomly downsampled from 4X to a mean depth of 2X. SNP genotypes were called for downsampled and original read depths using a combination of Samtools, GATK and Beagle. 37% of variants detected in the 4X data were missing after downsampling. The majority of these missing variants were rare (mean MAF 0.027 in 4X data), around half being either singletons or doubletons. For SNPs appearing in both 2X and 4X sets, genotype comparisons between depths showed rare (MAF < 0.005) and low-frequency (MAF between 0.005 and 0.05) SNPs had lower mean R-squareds (0.811 and 0.837 respectively) than common ones (SNPs with MAF > 0.05 had mean R-squared 0.954).
To extend our analysis we will investigate a wider range of sequencing depths. We will report how differences in sequencing coverage between cases and controls affect power to detect association compared to balanced designs where cases and controls are sequenced at identical depths.

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P16.099

Developing the Polygenic Model for Schizophrenia

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Genetic risk for schizophrenia is thought to comprise of thousands of variants including common single nucleotide polymorphisms (SNPs) of small effect. This is known as a polygenic effect and has been demonstrated through the polygenic model. Based on genome-wide association study results, the total number of associated risk alleles, weighted by their odds ratios, is counted for each individual in an independent sample and shown to be significantly different between cases and controls. Here we present three developments of this effective yet simple model; allowing for multiple populations, inclusion of linkage disequilibrium (LD) and inclusion of interactions.
In the original application, population stratification was controlled for in the association test to produce a single odds ratio for each SNP. The model was reformulated to include multiple odds ratios for the different populations, which were then weighted for each individual by the likelihood of them coming from that population. In the current polygenic framework stringent pruning is applied to ensure SNPs are independent, thus losing information from the SNPs that are removed. An alternative method is proposed that allows for LD at the association analysis stage, meaning more SNPs can be retained. Finally the model was extended to include interactions between independently associated SNPs. For each model odds ratios were calculated in the International Schizophrenia Consortium study, and fitted with data from the Molecular Genetics of Schizophrenia study. Each adaptation was compared to the original formulation.

E. Hannon: None. **P. Holmans:** None. **A. Pocklington:** None.

P16.100

Looking for a fine-scale genetic structure in Western France human populations

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The Common Variant Common Disease hypothesis was only partly verified through effective discovery of statistical associations. This empirical observation led the research community to reconsider the importance of rare variants.
The rare alleles of recent origin are likely to cluster geographically in communities with limited migration rates, like French rural populations before 20th century. Where these rare alleles strongly increase the risk of disease, we'd expect outbreaks of disease prevalence and enhanced power to establish the variant-phenotype relationship.
This work aims to assess whether the demographical history of some regions of Western France lends itself to a fine-scale population structure. This analysis was performed considering patients enrolled at Western France hospitals as having a Calcific Aortic Valve Stenosis (CAVS) diagnosis. Geographical location of individuals was defined according to the declared place of birth. This group of patients was genotyped genome-wide using Affymetrix Axiom chip.
In the preliminary Principal Components Analysis, the first two principal components revealed an important correlation with geography ($p < 1e-04$). There is a clear clustering of people from Brittany and people from Vendée, two regions geographically close.
These preliminary results show that population stratification can be observed even at a very low level in populations usually considered as open and panmictic. This strongly suggests that recent rare alleles are likely to cluster geographically even in these populations. Therefore, identification of rare variants inducing disease susceptibility can benefit from a strategy focusing on small geographic units.

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P16.101

Statistical Prioritization of Sequence Variants

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Association studies with sequence data require prioritizing variants for follow-up experiments. Statistical information contributes to this prioritization. Standard statistical methods rank sequence variants using exact p-values. However, one-sided and two-sided p-values can rank the same set of variants differently, suggesting problems in ranking sequence variants using exact p-values. Summarizing the disease-variant distribution into 2x2 tables, here we propose to rank sequence variants using the maxLRc, defined as the ratio of the conditional likelihoods evaluated at the maximum conditional likelihood estimate of the odds ratio versus at an odds ratio of 1. We have shown analytically that, under the best-case scenario when the disease-variant distribution is in quasi-complete (one empty cell) or complete separation (two diagonal empty cells), the maxLRc always results in the same rankings as one-sided exact p-values when the alternative hypothesis is specified in the "correct" direction (directional p-values). Under the more common case of overlap (no empty cell), there is no general agreement among two-sided p-values, directional p-values and maxLRc. However, using simulated data, we show that when the number of controls is greater than the number of cases, rankings assigned by maxLRc correlate better with the true rankings than p-values. We also illustrate our approach in a study of Rolandic Epilepsy. The maxLRc ranks variants using only information obtained from the observed data, whereas p-values incorporate the probability of more extreme observations that could have been observed. A more extreme observation is defined by the investigator's a priori belief in the direction of the association.

W. Li: None. **L.J. Strug:** None.

P16.103

A "population-based approach" to study the link between TAS2R genes, taste perception and food liking

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Variations at the TAS2R38 gene account for the major portion of differences in PROP (6-n-propylthiouracil) taste perception, which have been shown to influence food preferences and dietary behaviour. We examined the link between PROP taste responses, food preferences and TAS2R genes in six different populations of the Caucasus and Central Asia, located along the Silk Road. We reported, for the first time, genotypic frequencies of the TAS2R38 gene and PROP phenotype distribution in these populations. We found a strong relationship between PROP tasting and food preferences (r=0.67, p-value=0.009) using a "population-based approach", in which we exploit phenotypic differences between populations comparing a distance matrix based on PROP taste responses and a matrix based on food preferences. No

evidence of correlation was found between the distance matrix of food preference and the matrix of genetic distance based on TAS2R38 or the matrix based on the whole genome. Preliminary results of candidate gene analysis allowed us to identify others TAS2R genes that could cooperate with TAS2R38 in the modulation of PROP perception and as consequence food liking.
Besides increasing the knowledge of worldwide TAS2R38 prevalences and bitter taste, our results show that differences in food preferences among populations correlate with PROP status but not with the TAS2R38 gene, suggesting that PROP status is probably a marker for general taste sensitivity and as such is a major driver of food preferences. In addition, our work represent a starting point to study the involvement of multiple genes in bitter perception and food liking.

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P16.104

Molecular screening of δ- globin chain gene in a population from Eastern Sicily (Italy).

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In β-thalassemia carriers, a deficit of δ-globinic chain could mitigate the haematological phenotype with an increase of HbA2 (α2δ2) milder compared to the expected values. Therefore, a β-thalassemia carrier could not be identified. In Eastern Sicily (Italy), the Yalousa (δcd27) mutation is the most frequent variant (81%) of the δ- globin chain gene. It is localized at codon 27 and causes a δ+ thalassemia.
In our study, we selected 50 subjects from the Eastern Sicily (Italy), with values of HbA2 between 2.1% and 1.6%, associated with a normal haematological phenotype. Moreover, we recruited 4 **α**-thalassemia carriers, and 5 β-thalassemia carriers. DNA samples were analyzed by ARMS-PCR to detect the δcd27 mutation. To identify other mutations, we performed an automatic sequencing of the δ- globinic chain gene and GAP-PCR to detect the δ-Corfù hemoglobin.
We found the δcd27 mutation in 31/50 (62.0%) subjects and in the 5 β-thalassemia carriers. In the
4 α-thalassemic carriers, we found 2 subjects with δcd12 (Asn>Lys) (Hb Nyu) mutation at the heterozygous state (δ+ thalassemia), and 1 heterozygote subject for the δcd4 (Thr>Ile) genetic variant (δ+thalassemia).
Our study showed that there is a considerable heterogeneity of δ- globinic chain gene mutations.
Therefore, we conclude that the screening of δ- globinic chain gene is important for a complete typing of the β-thalassemia carriers. Indeed, the coheredity of several molecular defects of the globin genes may modify the phenotypic paintings so as to make them silent and hide conditions of great importance in genetic counseling of couples.

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P16.105

Telomere length in circulating leukocytes is associated with lung function and disease

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Rationale: Telomere length is recognized as a marker of biological age. Previous studies reported decreased telomere length in patients with chronic obstructive pulmonary disease (COPD), suggesting premature aging due to environmental exposure and/or chronic inflammation. Since the lungs are continuously exposed to environmental hazards, lung function per se may be a surrogate marker for biological age in light of the large inter-individual variability observed.
Objectives: We investigated the association of telomere length with respiratory disease (COPD and asthma), and spirometric indices: forced expiratory volume in one second (FEV1), forced vital capacity (FVC) and FEV1/FVC.
Methods: Our meta-analysis of 14 studies included 1,189 COPD cases with 16,115 controls, 2,834 asthma cases with 28,195 controls and spirometric parameters of 13,100 individuals. Associations were tested by linear regression, adjusting for age, sex, and smoking status.
Measurements and Main Results: We observed negative associations between telomere length and COPD (β=-0.0676, p=0.018) as well as asthma (β=-0.0452, p=0.024) with stronger effects in women compared to men. The investigation of spirometric indices showed positive associations between telomere length and FEV1 (p=1.62x10-7), FVC (p=2.38x10-4), and their ratio FEV1/FVC (p=6.13x10-3). The associations were weaker in apparently healthy subjects compared to COPD or asthma patients.
Conclusions: Our results indicate that lung function may reflect biological aging primarily due to intrinsic processes which are likely to be aggravated in lung diseases. Shortened telomeres in lung disease suggest that aging processes are involved in the pathogenesis of COPD and asthma.

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P16.106

Rare Variant Extensions of the Transmission Disequilibrium Test Detects Associations with Autism Exome Sequence Data

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Many population-based rare variant association tests have been developed to analyze sequence data. A drawback of these methods is that it is difficult to adequately control for population substructure and/or admixture and spurious associations can occur. For rare variants this problem can be substantial, because the spectrum of rare variation can differ greatly between populations. A solution is to perform analysis using the transmission disequilibrium test (TDT), which was developed to analyze trio data and is robust to population substructure/admixture. Sequence data is being generated on trios to detect de Novo events, and is also useful to detect association with transmitted variants. We extended the TDT to test for rare variant (RV) associations using four commonly used rare variant association methods. We demonstrate that for all RV-TDT tests type I error is well controlled even when there are high levels of population substructure or admixture. The power of the RV-TDT tests was evaluated using a number of population genetic and disease models. The RV-TDT was used to analyze exome data from 199 Simons Simplex Collection (SSC) autism trios. Using the RV-TDT an association was found between autism and rare variants in the *ABCA7* gene. Given the problem of adequately controlling for population heterogeneity in rare variant association studies and the growing number of sequenced based trio studies the RV-TDT is extremely beneficial to elucidate the involvement of rare variants in the etiology of complex traits.

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P16.107

Asymmetry of parental origin in Long QT syndrome

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Aims: We had noted preferential transmission of mutant alleles in Long QT syndrome (LQTS) families, which suggested a survival advantage for affected gametes. To determine whether this phenomenon depends on LQTS mutations or gene loci (LQT1, *KCNQ1* in 11p15.5; LQT2, *KCNH2* in 7q36.1; LQT3, *SCN5A* in 3p22.2), we studied parental and grandparental origins of alleles transmitted in LQTS and control families and the role of channel dysfunction in the transmission distortion.

Methods and Results: We studied 3782 genotyped members from 679 European and Japanese LQTS families (2748 carriers). We determined grandparental and parental origins of mutant alleles in 1892 children and 619 grandchildren, and grandparental origin of normal alleles in healthy children from 44 three-generation control CEPH families. In families from Europe and Japan, mutant alleles were significantly more frequently maternal than paternal in origin (61%, 1155 vs. 737 alleles,p<0.001). The ratio of maternal alleles in LQT1 (66%) was significantly higher than in LQT2 (56%, p<0.001) and LQT3 (57%, p=0.01). Unlike control families' Mendelian distribution of grandparental alleles, mutant grandparental LQT1 and LQT2 alleles in grandchildren showed excess maternal grandmother alleles. For LQT1, maternal origin mutant alleles bore a significant relationship to the level of dysfunction (dominant negative 67%, non-dominant 58%, p<0.03), however for LQT2 or LQT3 this association was not significant.

Conclusion: An excess of mutant alleles of maternal origin, most pronounced in LQT1, is homogeneous across ethnic groups. This is not linked to a locus-specific grandparental origin allele transmission distortion, but to potassium channel dysfunction.

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P16.108

Age-Dependent Association between Pulmonary Tuberculosis and Common TOX Variants in the 8q12-13 Linkage Region

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Only a small fraction of individuals infected with *Mycobacterium tuberculosis* develop clinical tuberculosis (TB) in their lifetime. Genetic epidemiological evidence suggests a genetic determinism of pulmonary TB (PTB), but the molecular basis of genetic predisposition to PTB remains largely unknown. We used a positional-cloning approach to carry out ultrafine linkage-disequilibrium mapping of a previously identified susceptibility locus in chromosomal region 8q12-13 by genotyping 3,216 SNPs in a family-based Moroccan sample including 286 offspring with PTB. We observed 44 PTB-associated SNPs (p < 0.01), which were genotyped in an independent set of 317 cases and 650 controls from Morocco. A single signal, consisting of two correlated SNPs close to *TOX*, rs1568952 and rs2726600 (combined p = $1.1x10^{-5}$ and $9.2x10^{-5}$, respectively), was replicated. Stronger evidence of association was found in individuals who developed PTB before the age of 25 years (combined p for rs1568952 = $4.4x10^{-8}$; odds ratio of PTB for AA versus AG/GG = 3.09 [1.99-4.78]). The association with rs2726600 (p=0.04) was subsequently replicated in PTB-affected subjects under 25 years in a study of 243 nuclear families from Madagascar. Stronger evidence of replication in Madagascar was obtained for additional SNPs in strong linkage disequilibrium with the two initial SNPs (p=0.003 for rs2726597), further confirming the signal. We thus identified around rs1568952 and rs2726600 a cluster of SNPs strongly associated with early-onset PTB in Morocco and Madagascar. SNP rs2726600 is located in a transcription-factor binding site in the 3' region of *TOX*, and further functional explorations will focus on CD4 T lymphocytes.

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P16.109

Distribution of eight SNPs of lipid level modifier genes in healthy Roma and Hungarian population samples

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The role of triglyceride metabolism in various diseases, such as cardiovascular or cerebrovascular diseases is still in the focus of numerous extensive investigations. Several polymorphisms associated with plasma lipid level changes are published in genome-wide association studies. Here we report the results of the genetic analysis of eight variants, rs12130333 at the ANGPTL3, rs16996148 at the CILP2, rs17321515 at the TRIB1, rs17145738 and rs3812316 of the MLXIPL, rs4846914 at GALNT2, rs1260326 and rs780094 residing at the GCKR loci. We genotyped 399 Roma and 404 Hungarian samples with PCR-RFLP method. Significant differences were found between Roma and Hungarian population samples in the allele frequency of ANGPTL3 variant (T allele frequency of rs1213033: 12.2% vs. 18.5% in Romas vs. Hungarians, p<0.025), of both MLXIPL variants (C allele frequency of rs17145738: 94.1% vs. 85.6%, C allele frequency of rs3812316: 94.2% vs. 86.8% in Romas vs. in Hungarians, p<0.025), and of GALNT2 variant (G allele frequency of rs4846914: 46.6% vs. 54.5% Romas vs. in Hungarians, p<0.025), while no differences could be verified in the remaining SNPs and the known minor alleles showed no correlation with triglyceride levels in any population samples. Our results suggest that these SNPs may be considered as risk factors for metabolic or cardio-cerebrovascular diseases in different population samples. The current study revealed fundamental differences of triglyceride modifying SNPs in Roma populations, although here we cannot associate the changes with triglyceride levels, the minor alleles still can have clinical implication as disease susceptibility.

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P16.110

Type 2 diabetes risk alleles near BCAR1 and in ANK1 associate with decreased β-cell function whereas risk alleles near ANKRD55 and GRB14 associate with decreased insulin sensitivity in the Danish Inter99 cohort

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Context: Recently, ten novel type 2 diabetes (T2D) susceptibility single nucleotide polymorphisms (SNPs) in *ZMIZ1*, *ANK1*, *KLHDC5*, *TLE1*, *ANKRD55*, *CILP2*, *MC4R*, *BCAR1*, *HMG20A* and *GRB14* loci were discovered in Metabochip-genotyped populations of European ancestry.

Objective: The present study aimed to characterize pre-diabetic quantitative traits underlying these SNP associations and to calculate the amount of inter-individual variation in glycemic traits explained by these and previous T2D susceptibility variants.

Design and Participants: 5,739 Danish individuals naïve to glucose-lowering medication were included in quantitative trait studies, while case-control

analyses were performed in 1,892 T2D patients and 6,603 normoglycemic controls. Participants without known T2D underwent an oral glucose tolerance test (OGTT), and measures of insulin release and sensitivity were estimated from insulinogenic, disposition, BIGTT and Matsuda indexes.
Results: We confirmed associations of *ZMIZ1*, *KLHDC5*, *CILP2*, *HMG20A*, *ANK1*, *ANKRD55* and *BCAR1* with T2D. The risk T-allele of *BCAR1* rs7202877 associated with decreased disposition index (*P*=0.02). The C-allele of *ANK1* rs516946 associated with decreased insulinogenic (*P*=0.005) and disposition (*P*=0.002) indexes. The G-allele of *ANKRD55* rs459193 associated with decreased Matsuda index (*P*=0.02) adjusted for waist circumference. The C-allele of *GRB14* rs13389219 associated with both increased insulinogenic (*P*=0.04) and decreased Matsuda (*P*=0.05) indexes. All validated European T2D variants still only explained a few percentages of glycemic trait variation.
Conclusion: If replicated in independent samples *BCAR1* rs7202877 may mediate its diabetogenic impact through impaired β-cell function. In addition, we substantiate previous evidence that *ANK1* rs516946 confers impaired insulin release and *ANKRD55* rs459193 and *GRB14* rs13389219 associate with insulin resistance.

M.N. Harder: None.

P16.111
Mediation of Genetic Effects

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We apply a genetic modeling via simulation to twin data from Nordic Twin Registry for obesity and cancer related measurements. For obesity, we use both BMI and other quantities derived from BMI to measure the weight growth. More specifically, the log(BMI) at baseline is regressed over other factors: ln(BMI_ij)= beta_i + alpha_i *j +gamma*age_i+epsilon_ij, for individual i and timepoint j (time since the baseline, in years). The alpha_i is then the log-weight growth rate (Hjelmborg et al. Obesity, 16(4), 2008). For cancer data, we use phenotypes available in the registry.
We follow the genetic modeling of twin data proposed by (Dite et al. and Stone et al; Cancer Epidemiol Biomarkers Prev; 17(12), 2008 and 21(7), 2012, respectively). In this modeling, the phenotype of a twin in a twin pair is regressed over both twins' co-variates. If the two twins in a twin pair are labeled as 1 and 2, Y denotes phenotype and X co-variate, then E(Y)= alpha + beta1 X1 +beta2 X2. It was shown that by varying a covariate experimentally, the expected value of the phenotype measure would change.
In our analysis, we assume a bivariate normal distribution for both (Y1,Y2) and (X1,X2). We treat phenotype measurements such as BMI, growth rate, at the baseline time as X, and that at the later time as Y. This approach would incorporate measurements at two points along a time course, thus enhance the power to detect the genetic component. We also introduce a random effects model for the stratification effects.

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P16.112
Association between *USF1* and type II diabetes in Turkish adults.

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Background: Upstream stimulatory factor 1 (*USF1*) belongs to the basic helix-loop-helix leucine zipper family. *USF1* controls expression of genes involved in lipid and glucose homeostasis and the gene has been linked with familial combined hyperlipidemia (FCHL). USF1 haplotypes have shown to be associated with glucose and lipid parameters in the EARSII study. We therefore evaluated the association of two *USF1* SNPs (rs3737787 and rs2073658) with type 2 diabetes (T2DM) in the adult Turkish population.
Methods: We genotyped 1831 subjects (895 males of mean age 52.91+/-11.80 and 936 females of mean age 52.5+/-11.82) and analyzed clinical data.
Results: The genotype (rs2073658; CC: 0.54, CT: 0.39, TT: 0.068; rs3737787; GG: 0.54, GA: 0.38, AA: 0.74) and haplotype (CG: 0.7, CA: 0.038, TG: 0.034, TA: 0.228 at D'=0.824) frequencies were determined. We observed an excess of TA haplotype and the two variants were in LD with each other. T2DM was more prevalent in males carrying the T (11.2% vs. 15.9% (p=0.036)) allele of rs2073658 compared to homozygotes for the C allele. After adjustment for confounding factors including age, physical activity, BMI, smoking and alcohol consumption logistic regression models revealed that male carriers of the T allele of the this polymorphism had higher risk of having T2DM (OR=1.81 [95%CI 1.20-2.74], p=0.005).
Conclusion: In this study we detected an association between the rare allele of the *USF1* gene and T2DM risk in Turkish adults for the first time.

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P16.113
Contribution of rs7756992 of CDKAL1 and rs4402960 of IGF2BP2 to the risk of type 2 diabetes in the Tunisian population.

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Aims: The insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2) and the cyckin-dependent kinase 5 regulatory subunit-associated protein 1-like 1 (CDKAL1) identified through GWAS studies have been shown to be associated with type 2 diabetes in various ethnic groups. The study aim was to investigate the association of the rs7756992 of CDKAL1 and rs4402960 of IGF2BP2 with T2D and diabetic complications (nephropathy and retinopathy).
Methods: We performed a case-control association study including 200 T2DM Tunisian patients (WHO criteria) with diabetic nephropathy (33%), diabetic retinopathy (41.5%) and 208 controls (age ≥40; fasting plasma glucose <6.1 mmol/l; without first degree family history of diabetes). Genotyping was performed using TaqMan technology. Statistical analyses were carried out using Stata 11 software (StataCorp, College Station, TX, USA).
Results: We found a significant association between the rs4402960 of IGF2BP2 and T2DM (p < 10-4). Furthermore, the rs7756992 of CDKAL1 was associated with the reduced risk of diabetic nephropathy in patients with diabetes (p = 0.001).
Conclusion: The present study confirms that the rs4402960 of IGF2BP2 gene is a strong candidate for type 2 diabetes susceptibility across different ethnicities. Interestingly, our study is the second one that described the rs7756992 of CDKAL1 gene as associated with diabetic nephropathy.

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P16.114
Cysteinyl leukotriene 1 and cysteinyl leukotriene 2 receptors are associated with atopic asthma in a founder population

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The atopy phenotype underlies many cases of asthma. The cysteinyl leukotriene receptor 1 (cysLT1) and 2 (cysLT2) genes have been important subjects of study because they are functionally and pharmacologically implicated in the atopy phenotype affecting many asthma subjects. In a founder population, we reported that the G300S variant of the cysLT1 receptor gene and the M201V variant of the
CysLT2 receptor gene are implicated in atopic asthma. In this report, we discuss the statistical association of both variants with the atopy phenotype - a phenomenon that may suggest that the interaction of
cysLT1 and cyLT2 gene variants gives rise to atopy in some populations. In vitro analysis has shown that cysLT1 and cysLT2 proteins directly interact within the
cell: representing a putative mechanism for the etiology of atopy in certain asthmatic populations.

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P17.1

Segmenting the human genome based on states of neutral genetic divergence

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Many studies have shown that levels of divergence generated by different mutation types vary and co-vary across the human genome. However, this phenomenon has been neither characterized simultaneously for multiple mutation types nor anchored to specific regions of the genome. A complete understanding of its mechanistic basis has also been lacking. Here we fit Multivariate Gaussian Hidden Markov Models to insertion, deletion, nucleotide substitution and microsatellite human-orangutan divergence estimates inferred in neutrally evolving regions of the human genome. We segment the genome into contiguous segments forming six divergence states, each characterized by a specific divergence profile, and associate these states with 35 genomic landscape features, parsing the contributions of different biochemical processes to mutagenesis. High divergence states inhabit GC-rich, highly recombining sub-telomeric regions, while low divergence states cover inner parts of autosomes. Chromosome X forms its own, lowest divergence state. The state of elevated microsatellite mutability is interspersed across the genome. This characterization is echoed in a segmentation based on human diversity data (from the 1,000 Genomes Project). Deviations from these general trends highlight the evolutionary history of primate chromosomes. We further demonstrate that genes and non-coding functional marks (ENCODE annotations) localize in the genome according to the underlying divergence states. In particular, functional elements are enriched in states with elevated divergence. These segmentations allow screening personal genome variants, including those associated with cancer and other diseases, and provide a framework for accurate computational predictions of non-coding functional elements. Therefore, our results provide a powerful resource for biomedical data analysis.

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P17.2

High impact of genetic drift on the isolated population of Rab Island - evidence from mitochondrial DNA diversity

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The Croatian Island of Rab is situated in the Northern Adriatic Sea. Evidence of human presence are seen from the Neolithic period and ever since this Island has been inhabitated by many ethnically different populations. After Illyrians and Romans, one of the most genetically and historically important periods was arrivals of Slavic tribes from 8th until 10th century. Here we present the evidence of high impact of the genetic drift on the genetic diversity and structure of the contemporary Rab Islanders. Analysis of mitochondrial DNA haplogroups of 163 autochthonous inhabitants from 5 settlements revealed relatively high level of haplogroup and haplotype diversity in the overall sample due to dynamic gene flow throughout history. On the other hand, only four haplogroups (H6, HV, J1c and U4) encompass more than one half of total maternal gene pool of this Island. Even so, every fourth contemporary Islander has one particular haplotype of U4d2 haplogroup. This is so far the most frequent finding of this haplogroup ever reported in any population (24.5 %). One possible explanation of such high deviation from average European frequency could be due to several epidemics of plague in 15th and 16th century when a vast majority of population died, in some cases almost 90 % of some settlements were devastated. The results of this study gave insight into microevolutionary processes that shaped the current maternal gene pool of this northern Adriatic Island.

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P17.3

Geographical Substructuring on Eastern Adriatic Islands

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This poster shortly portrays an analysis of the matrilineal legacy of two Eastern Adriatic islands, Hvar and Korčula. Both islands fit into a southern group of Eastern Adriatic islands, belonging to the cities of Split and Dubrovnik municipalities, respectively. We have analysed a total of 250 mtDNAs based on high resolution analysis of SNPs from the control and coding region. Results show a relatively high diversity of haplogroups: 7 haplogroups and 37 subhaplogroups, altogether. Despite a relatively small population (11103 on Hvar, 16182 on Korčula) and sample size (161 from Hvar, 89 from Korčula) of both Islands, the analysis showed a tendency of population substructuring depending on the geographical region of the island, when mitochondrial DNA haplogroup distribution is concerned. While Hvar islanders are divided into an Eastern and Western group, the Korčula islanders show a three-way distribution into Northern, Eastern and Western group. Also, two unexpected and rare haplogroups were discovered, L2a3 in a single individual on the island of Korčula, and F1b haplogroup in 10 individuals on the island of Hvar. Both lineages have been completely sequenced, and presented in a form of phylogenetic tree. We wanted to emphasize events such as founder effect, bottleneck, isolation and various historical, geographical and sociocultural factors that acted upon the maternal genetic landscape of contemporary populations on Eastern Adriatic islands.

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P17.4

Mitochondrial DNA Analysis of the Southeast European Genetic Variation Reveals a New, Local Subbranching in Hg X2

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High mtDNA variation in Southeastern Europe is a reflection of the turbulent and complex demographic history of this area, influenced by gene flow from various parts of Eurasia and a long history of intermixing. Our results of 1035 samples from four SEE populations (Croatians, Slovenians, Bosnians, Herzegovinians) show that their maternal genetic diversity fits within a wider European maternal genetic landscape, but in spite of the geographical proximity of sampled populations, certain differences can be observed in mtDNA haplogroup composition and variation. Also, we observed five new samples in the Bosnian population and one new Herzegovinian sample designated as X2* individuals, that could not be asigned to any of its sublineages (X2a'k) according to the existing X2 phylogeny. Subhaplogroup X2 is highly diversificated and spread over a wide geographic area, but usually at low frequencies (<5%). A detailed picture of X2 migrations across Europe, Asia and North America is still unrevealed and therefore intriguing. In attempt to clarify the phylogeny of our X2 samples, their mitochondrial DNA has been completely sequenced and no matching sequences have been found among over 18 000 mtDNAs in the updated mtDNA phylogenetic tree. We suppose that these lineages present SEE variants of the X2 clade and that they are signs of one or several local micro-differentiation processes that occured in a relatively recent demographic past. Additional sequencing of X2* individuals in this area is in progress and we expect our new insights will contribute to further resolution of the X2 phylogeny.

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P17.5

Y chromosome haplogroup analysis to estimate genetic origin of Balts

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Background. To obtain a more accurate portrait of ancestry of Balts Y chromosome haplogroupe (Y-hg) structure was determined in Russian individuals that inhabit historical regions of Baltic tribes and compared with incidence of Y-hg in Latvian population.

Material and methods. A study comprised 192 men - representatives of four

Northern-Western Russian regions (Mezen, Ustyuzhna, Sychevka, Starista) and 153 men representing Latvian subpopulations. To establish Y haplogroups DNA samples were hierarchically genotyped (using appropriate PCR followed by RFLP or sequencing) by 13 Y chromosomal binary markers (M9, SRY-1532, M17, Tat, M178, M170, P37, M253, M223, M172, YAP, M35, M201).
Results and discussion. It was found that distribution of major Y-hg's was relatively homogenous among analysed Russian and Latvian subpopulations. Only Russian subpopulation Mezen (Archangelsk district) showed the lowest frequency of the Slavic component
representing R1a haplogroup (22.5%) in comparison to other Russian (~55%) and Latvian (~40%) subpopulations. On the other hand the Fino-Ugric speaking population representing haplogroup N1c was the most common in Mezen (51%) in comparison to other Russian (~15%) and Latvian (~45%) subpopulations. Principal component analysis revealed that haplogroup N1c links Mezen with Eastern Latvian subpopulations Semigalians and Letigalians in one cluster, representing the hypothesis that the genetic history of N1c Y chromosomes in Baltic-speaking populations is distinct from that of the Uralic speakers.
Conclusion: Findings indicate that there are no significant differences in common Y-hg distribution among analysed Russian and Latvian subpopulations; however the analysis of Y-Hg genofund in Mezen indicates possible Fino-Ugric or Baltic ancestry.

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P17.6
Diverse functions of titin in non-muscle cells

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Introduction. Previously we have reported on the development of a new mouse anti-titin-A- band monoclonal antibody, named MAb Titl 5H1.1, using the synthetic peptide N- AVNKYGIGEPLESDSVVAK-C. In the human skeletal muscles, this antibody reacts specifically with titin in the A-band of the sarcomere and in different non-muscle cell types with nucleus and cytoplasm, including centrioles (Mikelsaar et al, 2010). We have specified the epitope area of MAb Tit1 5H1.1 to the hexapeptide N-AVNKYG-C which is highly conserved in the evolution and is related to Fn3 domains of the titin molecule. Our immunohisto- and cytochemical studies with MAb Tit1 5H1.1 in human, mouse and zebrafish tissues and cell cultures showed a striated staining pattern in muscle cells and also staining of centrioles, cytoplasm and nuclei in non-muscle cells (Mikelsaar et al, 2012). We have further studied the expression of titin in non-muscle cells and tissues of different organisms.
Results. Our immunohisto- and cytochemical studies with MAb Tit1 5H1.1 performed so far have shown the expression of titin in some cell types of nervous system of human and mouse organism. The study is in progress.
Conclusions. Our data show that titin may have in addition to the known roles in muscle cells and in non-muscle cells as a centriole associated protein also other roles in non-muscle cells to be specified. Acknowledgements. This work was partly supported by target financing SF0188096s08 of the Estonian Ministry of Science.

A. Mikelsaar: None.

P18.01
Hereditary diseases with specific mutations in Yakuts

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Several forms of pathologies, referred to as Yakut hereditary diseases, have been distinguished on the basis of the results of genetic epidemiological studies and molecular genetical analysis of Mendelian diseases in the population of the Republic of Sakha (Yakutia): spinocerebellar ataxia type I, myotonic dystrophy, oculopharyngeal muscular dystrophy, hereditary enzymopenic methemoglobinemia, and 3-M syndrome, SOPH syndrome, hereditary loss deafness 1A type. These diseases are characterized by a high prevalence among Yakuts as compared to their global incidence in the world and some of them have a specific mutations in Yakut population (oculopharyngeal muscular dystrophy, hereditary enzymopenic methemoglobinemia, and 3-M syndrome, SOPH syndrome, hereditary loss deafness 1A type). That results show that Yakut population is a genetical isolate and can be used in wide genetical studies for identify new genes.

N.R. Maximova: None.

P18.02
Difficulties in genetic counselling and prenatal diagnosis: An Omani consanguineous couple at risk for two distinct genetic syndromes

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We report on a female infant with multiple congenital anomalies including cardiac defects, dysplastic multicystic kidney, hypotonia, developmental delay and dysmorphic features. Parents are first cousins and of Arab descent with a history of primary infertility and one second trimester miscarriage. Karyotyping and FISH for 4p deletion revealed a normal result. On performing array-CGH non-contiguous interstitial deletions within the long arm of chromosome 6 [6q14.1 to 6q14.3 (deletion ~1.9Mb) and 6q15 (~1.6Mb)] were identified. Heterozygous deletions of this interval have been reported to cause developmental delay, dysmorphism, cardiac anomalies and hypotonia. Follow-up array-CGH studies did not identify the presence of the deletion in either parent confirming the abnormality to be of de novo origin. Cytogenetic testing was undertaken to rule out the possibility of a balanced translocation. The mother's result revealed a normal karyotype while the father showed a whole short arm translocation of chromosome 3 onto chromosome 13. It was explained to the parents that the translocation was not the cause of the 6q deletion and although the recurrence risk for the microdeletion syndrome would be low they were still at risk for unbalanced products of conception arising from the translocation. The couple, of Islamic belief, was five months pregnant at the time of counselling and declined amniocentesis (termination of pregnancy has to occur prior to ensoulment of the fetus at 19 weeks). This report highlights the counselling difficulties experienced and the challenges faced with the time constraints for prenatal diagnosis and consideration of termination in the Arab population.

Z. Bruwer: None. **U. Achandira:** None. **K. Al Kharusi:** None. **A. Al-Kindy:** None.

P18.03
Three peculiar families with alpha thalassaemia

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Molecular characterization of alpha globin genes is of increasing interest. Due to the massive immigration phenomenon, to diagnose alpha thalassaemia is essential, especially in people from Asia (high frequency of large deletions, resulting in a procreative risk of Hb Bart's Hydrops Foetalis and HbH disease). Also in the Italian autochthonous population the definition of alpha globin genes structure is necessary in order to clarify many cases of thalassaemia intermedia.
We report here three peculiar Italian cases.
1)Two siblings from Veneto Region with Thalassaemia Intermedia. The clinical phenotype is mild with intrafamilial variability. In the family we identified heterozygous Beta°Cod.39
and two different triplicate alpha with different extension.
2)A family from Toscana Region, in which father and son present a severe form of Thalassaemia Intermedia (with apparent dominant transmission). In both we found heterozygous Beta°Cod. 39 mutation and a large duplication, involving the entire alpha globin gene cluster and including the regulatory region HS40
3)A young girl from the Veneto Region with a haemoglobin variant (50% of total haemoglobin; the haematological parameters are abnormal and the percentage of HbA2 is below the normal range.
The girl is a compound heterozygote for the deletion -alpha3.7 and the point mutation alpha2IVS1[-5nt]. By Direct Sequencing of fusion gene -alpha3.7 we identified Hb Hasharon (50% of total haemoglobin, one variant gene of two active alpha genes).
The given examples highlight that the alpha globin gene cluster analysis can help to clarify different scenarios in thalassaemia: intrafamilial clinical variability, unexpected inheritance pattern and misleading haematological results.

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P18.04

Screening relatives for familial abdominal aortic aneurysm: don't forget the women

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Targeted screening of women and men with a genetic risk for abdominal aorta aneurysm (AAA) may add to population screening in reducing mortality from rupture. In order to identify individuals with a genetic risk for AAA, we performed a family history study of AAA patients and calculated the risk for individual relatives of having an aneurysm. Furthermore, we estimated the prevalence of familial AAA, and we investigated if specific clinical characteristics could help to distinguish familial AAA.
Family histories of 568 AAA patients were obtained by semi-structured questionnaires. Patients were classified as familial AAA when at least one first-degree relative was reported with an aortic aneurysm. Multivariable logistic regression analysis was performed to identify discriminative characteristics between familial and sporadic AAA.
Aneurysms were reported in 5% of all first-degree relatives (parents, siblings and offspring). Affected relatives occurred in 128 families, indicating a prevalence of 23% of familial AAA. Male and female patients had a similar prevalence of familial AAA. In patients with familial AAA, 17% of the first-degree relatives were affected, 26% of male relatives and 11% of female relatives. Index patients with familial AAA were younger at diagnosis and fewer had diabetes mellitus and hypertension.
The increased risk in male and female relatives in familial AAA showed that family screening - in contrast to population screening- should include women. Family history taking is important to recognise familial AAA and identify and inform relatives about their risk and offer relatives screening for AAA.

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P18.05

French Agence de la biomédecine annual report on genetic testing: a useful tool for public health

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The French Agence de la biomédecine is a public organization under the supervision of the Health Ministry created by the law on Bioethics in 2004. Its missions aim to guarantee quality, safety and transparency and anticipate technological developments regarding activities under its responsibility among which genetic diagnoses.
Evaluation of the activities at a national level is a key element of the expertise and regulations that must be managed by the Agency. In order to collect and analyze genetic laboratory activities the Agency has established a partnership with INSERM (Orphanet) to develop an online access to Orphanet's thesaurus.
This enabled us to harmonize the disease nomenclature for which genetic testing was performed by the laboratories. With over than 240 laboratories and more than 1,000 various disorders, this partnership was the best way to analyze the different tests performed by French laboratories.
This report communicates only diagnostic activities related to routine patient's results excluding research genetic testing. (Summary of the 2012 postnatal diagnostic genetic activities results will be available in March).
This annual report is public and available on the Biomedicine Agency website: www.agence-biomedecine.fr
The report provides critical information to support rare diseases plan decision process at national level. It gives a complete overview of genetic labs practice and technological evolutions and to some extent encourages genetic labs networking.
In conclusion, the French Agence de la biomédecine annual national report is a useful tool for public health.

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P18.06

Managing extreme anxiety in a mother preoccupied with a perceived physical defect in her normal infant

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Anxiety is a normal emotion experienced by parents facing potential diagnosis of a genetic condition in their child. Genetic counsellors are trained in assisting patients manage their anxieties and anxiety usually diminishes following reassurance from clinicians and receipt of normal results. It is important however, that we are aware of our limitations and able to recognise when further support beyond genetic counselling is required. A mother presented in a highly anxious state following the suggestion that her baby had features of a skeletal dysplasia. Several clinical examinations and investigations deemed the baby normal. During these months, we engaged with the mother regularly as her anxiety escalated following each normal result. We were challenged by her persistence that her child was abnormal. Her anxiety appeared extreme and we feared for her, and consequently the baby's, well-being. Referral to a psychologist and later to a psychiatrist ensued. The mother's anxiety proved intractable and ultimately resulted in a devastating outcome. Many challenging issues were presented, including managing excessive concern and preoccupation with a perceived physical defect in a normal individual. This is in contrast to the more common scenario of assisting families adapt to a new diagnosis in their sometimes perceived perfect child. We have reflected on our roles as genetic counselling professionals and questioned our practice, as standard counselling strategies appeared ineffectual. This case has emphasised the breadth of human emotions and reactions and has allowed us to consider the nature of such extreme anxiety in this unique counselling situation.

C.A. Shalhoub: None. **G. Betts:** None. **A.M. Turner:** None.

P18.07

CNVs involving the dystrophin gene detected incidentally in female patients referred for routine array-CGH

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Molecular karyotyping has progressively replaced conventional karyotype for investigating intellectual deficiency (ID). This technology has significantly improved the rate of diagnoses in ID but raises the possibility of unexpected but clinically significant findings. These variants cannot explain intellectual disability but may predict adult-onset disorders or have an impact on relatives. The problems raised by the incidental finding of CNV affecting cancer predisposing genes have been debated and some authors have proposed that the possibility of such a finding should be discussed during the pre-test counselling session and explicitly mentioned on the informed consent form. We raise the question of finding CNVs in X-linked genes in girl and consequences on genetic counseling. Dystrophinopathies are among the most frequent X-linked disorders. Intragenic deletions or duplications account for approximately 60% of mutations and are thus accessible to routine array-CGH. We report four cases of CNV involving the dystrophin gene detected on CGH-array in female patients without any family history of dystrophinopathy who were referred for developmental delay. There were three deletions and one duplication. The rearrangement was de novo in two and inherited in two. A deletion identified in a girl was found in her asymptomatic father. In case of maternal inheritance, prenatal diagnosis for dystrophinopathy was proposed, whereas the cause of developmental delay in the index case remained unsolved. Furthermore, information of at-risk relatives proved difficult in the absence of precise knowledge of the condition.

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P18.08

Clarifying assent

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Assent is a relatively young term in research ethics, but became an often mentioned ethical requirement in current pediatric research guidelines. Also the European Society of Human Genetics considers assent an important condition for the inclusion of children in biobanks. Although many emphasize the importance of assent, few explain how they understand the concept. In this paper we will discuss the concept of assent and its different

underlying ethical principles.
In the first category, assent appears to be a substitute for informed consent, grounded in respect for autonomy and protection against harm. We conclude that this interpretation of assent is not of added value as a majority of children cannot be considered competent to make autonomous decisions. In addition, other safeguards are more appropriate to protect children against harm. The grounds from the second category can be classified as engagement grounds. These grounds do justice to the specifics of childhood and are of added value.
Furthermore, we argue that it follows that both the content and the process of assent should be adjusted to the individual child and the study at hand. This can be referred to as personalized assent. Personalized assent is an appeal to the moral responsibility and integrity of the researcher. We will illustrate our interpretation of assent by means of case studies we conducted on pediatric biobanks.

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P18.09
Inheritance of autoinflammatory diseases: shifting paradigms and nomenclature

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Over 15 years have passed since the discovery of the first autoinflammatory gene, MEFV, responsible for familial Mediterranean fever. The identification of another gene, TNFRSF1A, in 1999 led to the concept of autoinflammation which characterizes rheumatologic conditions triggered by a defective innate immunity. Substantive progress has been made since then, with the identification of 18 autoinflammatory genes accounting for up to 24 disease entities showing overlapping symptoms. The accumulation of studies reporting patients with missing or excess mutations as compared to expected numbers, favors the hypothesis that these diseases are distributed along a continuum ranging from monogenic to multifactorial conditions, rather than featuring only classical modes of inheritance. Moreover, the probable interactions of environmental and epigenetic factors further obscure our understanding of the mechanisms underlying the phenotypic expression of patients.
This review explores the history of autoinflammatory gene discovery, discusses the nosological disparities stemming from the clinical vs pathophysiological definition of autoinflammatory diseases, and summarizes various inheritance patterns. This review calls for a consistent disease nomenclature and presents a reconciling hypothesis which places different sequence variants within the autoinflammatory disease continuum. Integrating these new concepts should help to facilitate communication between health professionals and promote personalized patient care.

I. Touitou: None.

P18.10
Targeted preconception carrier screening aimed at high-risk populations

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Autosomal recessive disorders cause serious morbidity and mortality in at least 25/10,000 children. Most affected children (>80%) are born unexpectedly without recognized family history of the condition. Preconception carrier screening aims to identify carrier couples with 1-in-4 risk of having an affected child, enabling informed reproductive choices before pregnancy. Many countries do not have population-based carrier screening programmes, and even for subpopulations at higher risk, testing is often not available.
In the Netherlands, in the absence of a nation-wide screening programme, we have taken the initiative to start offering carrier testing to high-risk populations: 1) An isolated community having high prevalent disorders due to founder effects; 2) Ashkenazi Jewish population (e.g. Tay Sachs disease); 3) Consanguineous couples; 4) People with African, Asian or Mediterranean ancestry (haemoglobinopathies); and 5) Caucasian people (cystic fibrosis). Each population requires a different approach, preferably embedded in a broad preconception care setting. A multidisciplinary approach is required, including, eg. primary care providers, clinical/molecular geneticists, and patients organizations. Possibilities and barriers for the implementation of screening will be studied. The psychological impact, stigmatisation/discrimination and informed choice will be monitored.
A great advantage is that services can be developed based on community needs, and can thus be delivered more effectively. New technologies allow simultaneous analyses of carrier status of multiple recessive disorders, potentially changing the screening landscape, but obviously raises new ethical and societal issues. By using a step-wise integrated approach targeting specific high-risk groups, an infrastructure for responsible genetic screening services (potentially even genome-wide) can be developed.

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P18.11
First case of Hb Randwick and beta-thalassemia in an Italian family

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When two or more globin gene defects are co-inherithed the results is quite often a production of complex hemoglobin asset not always easy to diagnose. In particular the laboratory evaluation at the protein level, in these cases, the bichemical assay could be very tricky and the diagnosis can be missed.
Very indicative is the case presented in this report: a girl, 10yo, presenting frequent episodes of anemia. One episode occurred during an infection by a parvovirus at 5yo. The child and her mother showed a very similar chromatographic profile (Variant II - Beta Thal Dual Kit, Bio-Rad) with an increased Hb A2 and moderate microcytosis in the child. The first level screening by biochemical test was in favour of a beta thalassemia trait in the child, but with the possibility to investigate additional defects.
The molecular analysis of beta genes identified in the proband the presence of beta thalassemia (beta° IVSI-1 at heterozigous state) and one additional T>G mutation at codon 15 on the second allele, corresponding to the rare variant "Hb Randwick" already described in 1988 in an Italian family living in Australia. This same defect was present also in the mother. Hb Randwick has been described as an unstable variant oh Hb because "in vitro" shows fenomeni di precipitation events by oxidative agents, tested in our laboratoty. Hb Randwick was never described before associated with beta thalassemia; the case presented is a new combination of two beta globin genes and indicate the possibility to better evaluate Hb instability.

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P18.12
Biobanking for genetic research: ethical, legal and social issues

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Biobanks are fundamental resources for biomedical research. They are particularly useful for research requiring large sample sizes, such as genetic studies. This paper addresses core ethical, legal and social (ESLI) issues that arise in biobanking for genetic research. Delving more deeply into the usual – and still necessary – considerations of consent, withdrawal, privacy, feedback, benefit sharing and governance, this paper addresses the particular issues raised by the very intersection of biobanking and genetics within the regulatory oversight of medical research. While not claiming exceptional status for either, it argues that both biobanks and genetic research represent specific instances where the established ethical norms and practice of medical research may not be fit for their purposes. From the fiction of anonymity to the conflation of requirements for banking with those for invasive or interventional research, this paper reassesses the ethical, legal and social requirements – and possibilities – of biobanking for genetic research.

K.A. Melham: None.

P18.13

Genetic discrimination in life insurance could be ethically acceptable.

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Genetic discrimination (GD) is a complex phenomenon that may have social, personal, familial and economic implications for individuals and populations. In Canada, there is currently a legal vacuum regarding the protection of individuals against GD. Concerns regarding GD by insurance companies are currently being expressed by both patients and professionals. These concerns can create barriers to genetic counselling and testing. They may also encourage individuals to resort to direct-to-consumer (DTC) companies for genetic testing outside the healthcare system.
This study explored ethical arguments regarding justifications of GD in the context of life insurance. Our analysis concluded that GD would be ethically acceptable if allowed only for amounts that exceed a minimal coverage (based on actuarial data) necessary for the promotion of equality of opportunity, as in the case of property and business start-up insurance. For example, in England and Wales, there is a moratorium prohibiting GD regarding minimal coverage (in life insurance the threshold is currently 500 000 £). Beyond this amount, differential treatment is permitted only if actuarial data exists to justify such treatment on the basis of customers' genetics. In Canada, similar protection against DG could promote access to genetic services and prevent the use of DTC testing, which currently does not ensure informed consent nor provides appropriate counselling. Appropriate protection can thus promote access to proper medical care and allow healthcare professionals to be less reluctant when referring their patients to genetic testing.

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P18.14

Cardiac conditions as an example of a new public health approach to genetic testing

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Public health genetics is facing a new, technology-driven challenge. Massive parallel sequencing (MPS) offers the opportunity of detecting mutations throughout the genome, thereby potentially allowing population screening for a wide range of conditions characterized by serious morbidity and mortality. Cardiac diseases offer an important opportunity to explore this challenge due to extensive knowledge about genes underlying serious but preventable genetic cardiac conditions. The first public health endeavor in the US, newborn screening, began in the 1960's to ascertain treatable conditions not evident in newborns. The criteria necessary for screening included preventable or treatable conditions with sufficiently high population frequency; significant morbidity and/or mortality and the availability of inexpensive and effective screening.Advances in technology such as tandem mass spectroscopy eventually allowed detection of many metabolic conditions from the same sample with little increased cost. That advance has generated debate regarding whether the classic criteria above are too restrictive and whether newborn screening should be expanded. Now our field faces the possibility of further expanding screening by using MPS to screen adults for high risk from undetected, highly penetrant mutations. If such a screening program were to be implemented, what criteria should be fulfilled? We propose to consider the following criteria: clinical severity; penetrance; and efficacy and acceptability of intervention. Using the example of cardiac conditions such as LQTS, HCM, DCM and others, we discuss these potential criteria and develop a scoring system to guide a new public health approach to genetic testing.

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P18.15

A nurse-led cardiogenetic follow-up clinic is highly appreciated by the family members at risk for DCM/HCM.

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Purpose: A growing number of first degree family members at risk for developing dilated/hypertrophic cardiomyopathy (DCM/HCM) are advised to be routinely followed up, but available health care resources are increasingly scarce. We compared a novel nurse-led cardiogenetic follow-up clinic with conventional follow-up by cardiologists.
Methods: First degree family members eligible for DCM/HCM follow-up were invited at the nurse-led FU clinic or attended the cardiologist's FU clinic randomly. Care consisted of ECG, echocardiography, assessment of his/her - and family's health and provision of information on DCM/HCM. Uptake and resource use were recorded. Patient satisfaction was collected using a validated questionnaire.
Results: 164 of 225 eligible family members (73%) participated (range 16-78 years, 47% men). 50% were mutation carriers and 50% had a positive family history of DCM/HCM. The uptake of the nurse-led clinic was higher than the cardiologist's clinic (72% vs. 55%, p=0.02). A follow-up consult took equal amounts of time (mean 15min, range 13-17min). The maximum patient satisfaction score was more often reached at the nurse-led clinic (85% vs. 48%, p<0.01). Family members at the nurse-led clinic more frequently reported that the caregiver appreciated his/her opinion (91% vs. 61%, p<0.01) and that they appreciated the caregiver's time highly (94% vs. 72%, p<0.01). Personal perceived control was equal.
Conclusions: Follow-up at a nurse-led cardiogenetic clinic results in larger uptake and more patient satisfaction whereas caregiver's time and perceived personal control are equal and costs are lower. A nurse-led cardiogenetic clinic can be considered an optimal alternative for conventional follow-up by cardiologists.

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P18.16

CCM3 mutations are associated with multiple meningiomas

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Cavernous angiomas affect roughly 0.5% of the population and are characterized by abnormal enlarged capillary cavities without intervening brain parenchyma. Clinical CCM symptoms include recurrent headaches, focal neurological deficits, hemorrhagic stroke, and seizures Three CCM genes are known: *CCM1/KRIT1*, *CCM2/ MGC4607* and *PDCD10/CCM3,* implicated respectively in about 65%, 20% and 15% of the CCM cases. The phenotypic characterization of *CCM3* mutated patients has been limited by the small number of patients harboring a mutation in this gene.
We report here molecular and clinical data of a large cohort of *CCM3* patients. Molecular screening for point mutations and deletions allowed to identify a mutation in 54 *CCM3* mutated index patients and 22 mutated relatives. Multiple extra-axial, dural based, lesions were detected in 7 unrelated patients and were proved to be meningiomas in 3 patients on pathological examination. Sequencing of the gDNA extracted from a meningioma for one of the patients showed a mutation occurring de novo on the wild type allele within the lesion. This "multiple meningiomas" phenotype is not associated with a specific *CCM3* mutation, and the association of these multiple extra dural lesions and multiple meningiomas was not detected in 189 patients with a *CCM1* mutation or 57 patients with a *CCM2* mutation.
CCM3 gene is as a novel gene involved in multiple meningiomas.

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P18.17

Nursing and genetics: A Brazilian multicenter study about factors of risk for cleft lip and palate

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The nurse participates in consultation family planning and prenatal care and develops health education activities. The cleft lip and palate (CLP) occurs in 1: 600-1000 newborns. The objectives of this study are to describe demographic and clinical characteristics and risk factors in FLP cases registred in the Brazilian Database on Orofacial Clefts (BDOC). It is descriptive and transversal study; used Fisher test and chi-square test, with a significance level of 5% (p <0.05). Of the 640 cases, 384 (60.0%) had at least one recognized risk factor; higher maternal education was associated to lower exposure to environmental risks (p = 0.000) and positive familial history was related to for non-syndromic cleft (p = 0.001). The identification of these and other factors associated with FLP and community profile allow the increase in guidance for family planning and prenatal care. Still, enables health education campaigns for health professionals and community activities related skills of nurses.

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P18.18

EuroGentest Clinical Utility Gene Cards - progress and perspectives 2013

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EuroGentest unit 2, addressing "Genetic testing as part of health care", holds the initiative for the creation of disease-specific, expert-authored genetic testing guidelines, the "Clinical Utility Gene Cards" (CUGCs). CUGCs are dealing with the pros and cons of the usage of genetic tests in a clinical setting. CUGCs give a balanced summary of the analytical and clinical validity, the clinical utility and cost-benefit issues of genetic testing in a disease-specific context. Due to their concise and standardized format, CUGCs offer quick guidance to all stakeholders, including clinicians, clinical geneticists, service providers and payers.
Each CUGC is authored by a multinational expert team. The manuscripts are peer-reviewed and published in the European Journal of Human Genetics (EJHG). All CUGCs are freely accessible on www.eurogentest.org, www.nature.com/ejhg and www.orpha.net. EuroGentest commissions the establishment as well as the annual update of the guidelines. Until now 83 CUGCs have become citable publications and 105 manuscripts are in progress. By the end of 2013, 300 CUGCs are intended to be published.
The CUGCs are well received by the scientific and clinical communities: the average number of downloads per gene card and year is over 1000. CUGCs represent either alternatives or complements to other guideline collections such as GeneReviews (http://www.ncbi.nlm.nih.gov/books/NBK1116/). An analysis of the CUGC commissioning procedure corroborates the experience of all actors in the field of rare diseases: the existence of a limited number of experts, their willingness to contribute, their individual overloading, and the ensuing long process from author invitation to completed review.

A. Dierking: None. **J. Schmidtke:** None.

P18.19

Elaboration of a tool for assessment and improvement of quality of decision-making at the multidisciplinary oncogenetic committee for colorectal cancer predisposition: a french experience

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The most common diseases that predispose for colorectal cancers are Lynch Syndrome and Familial Adenomatous Polyposis. MMR and APC (and MUTYH) genes are respectively responsible. A genetic counselling is imperative for an optimal care making for patients and families. Multidisciplinary committees (MDC) are usually organized, helping professionals to optimize a decision for gene analysis and patient/families follow-up, taking into account relevant experts advices. Our aim is to examine the evaluation and the improvement of quality decision-making for a family suspected for colorectal cancer predisposition. A disparate distribution of decisions has been suspected. In Lyon region we created a database to verify that and harmonize the different participants' work in MDC. Results: the 33 French oncogenetic main consultation centers have been contacted in order to describe the organization of their MDC. Answering rate reached 100%. Among these centers, 76% developed a specific MDC, whereas 24% used standard consultation. About 3.75 different medical specialities are gathered by MDC. Among them, there are oncogeneticists (100%), gastroenterologists (76%), genetic counsellors (84%), surgeons (32%), and biologists (36%). Twenty percent of centers having a specific MDC evaluate all their patient cases, whereas 80% select them. In Lyon region, a computerized tool is elaborated and will be widely disseminated to every collaborating partners of our MDC. It will enable us to standardize our decision-making and, by comparing decisions through quality criteria, to differentiate and categorize some patients/families groups. A better rationalization of care management, families' follow-up and prevention is thus targeted.

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P18.20

Complete Androgen Insensitivity Syndrome (CAIS) in a large East African Family

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A 27 year old female, from east Africa, was referred to us for infertility, primary amenorrhea and decreased pubic and axillary hair. She was the second of 9 sibs, 4 other females and 4 males. She had been married for 2 years and she never had a medical consultation for this reason. Testosterone was high. Absence of inguinal masses. There was absence of epididymides and vas deferens. Abdominal testes were seen by MRI; breasts and female adiposity were of normal development. Her karyotype was 46, XY. Her 15 year old sister has the same clinical features, and karyotype 46, XY. We did karyotyping for her 3 other sisters, two of them were 46, XX, and they are clinically normal. The last sister, age 9, was also found to be 46, XY and clinically normal. We offered gonadectomy to the 27 year old female. But it has been rejected. We extracted DNA samples from the mother and all the phenotypical females to provide proper genetic counseling in the carriers. These results will be presented at the meeting.
We haven't disclosed the diagnosis of CAIS to the affected individuals. However, the circumstance in which the diagnosis was done, due to the sociocultural and educational background and after 2 years of marriage, makes this decision extremely difficult. The ethical dilemma faced in revealing a diagnosis with such a devastating social impact for the patient, and the obligation to other carriers in the family, creates quite a unique source of debate.

M.M. Alwasiyah: None. **C. Trujillo:** None.

P18.21

Dynamic Consent - A Tool for Translational Research

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We have built a patient IT interface which uses a 'dynamic consent' approach. In this model, consent is not a mere communication exercise but a bidirectional, ongoing, interactive process between patients and researchers. Through the interface individuals can make and express preferences about the choices they are given about the use of their data and samples for research. The benefit of this interface is that it enables individuals to exercise their autonomy by giving informed consent for new types of research in real time rather than being asked to give a broad consent at the beginning of the research process when they are recruited into a biobank. The benefits for the research process are that recruitment is easier, less costly and more efficient; the legal and ethical requirements of consent can be met with ease; there is greater transparency and accountability in the research process and research findings can be returned to research participants as part of a personalised medicine approach. Dynamic consent has the potential to enhance patient confidence and enable long term patient-researcher collaborations in research.
This interface moves away from manual, paper-based processes to an e-governance system. We anticipate that the 'dynamic consent' interface will become an essential and sustainable component of research infrastructure and will further advance translational research initiatives. In this paper we present the dynamic consent model and show how it is being used as a tool for translational research and personalised medicine.

J. Kaye: None. **E. Whitley:** None. **D. Lund:** None.

P18.22

SIGN - Slovenian-Italian Genetic Network

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The "European Directive on the application of patients' rights in cross-border healthcare" allows patients residing in EU countries to receive medical treatments in another Member State and have their expenses reimbursed. Patients affected by rare diseases and in need of specialized care will greatly benefit from this. The directive will have to be enforced by European countries by October 25th 2013. It also aims to ensure quality and safety in health care.
The SIGN project is part of the Cross-border Cooperation Operational Programme between Italy and Slovenia and covers a three year period (2011-2014). Its objective is to improve both the accessibility and the quality of genetic services in the Italy-Slovenia macro-region and to provide patients affected by genetic diseases with equal opportunities of diagnosis and care.
The Institute of Medical Genetics of the University of Ljubljana is the programme leader and seven Slovenian and North-Eastern Italian institutions have joined the project.
The first stage of the project is ongoing and it involves mapping the specific clinical and diagnostic expertises of each participating institution and drafting guidelines to assess patients with genetic diseases.
Further to the EU Directive on cross-border healthcare, the SIGN project aims to develop telemedicine systems in order to provide remote genetics counselling performed by specialized personnel in areas where proper clinical services are lacking.
The project also fosters information spread to raise awareness among the general population and clinical specialists on the cutting edge diagnostic options that can be delivered to patients.

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P18.23

Direct-to-consumer genetic testing: development of a decision tool to support patients considering testing and the health professionals involved in their care

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Direct-to-consumer genetic tests are available via the Internet. One deliverable for the EuroGentest 2 project was the production of guidelines for 1) individuals who might be considering purchasing such a test and 2) health professionals from whom they might seek advice before or after using such tests. We addressed this task by first conducting three systematic reviews to ascertain the evidence on views and experiences of users and health professionals and the policies of professional and bioethics organisations. From the analysis, it appears that consumers' motivations include: general curiosity, to improve their general health, to ascertain the risk of a particular condition or to plan for future children. However, health professionals and bioethics organisations expressed concerns about potential harms resulting from use of these tests. Using the evidence obtained, we constructed a list of topics that should be included in any guidelines. We used a multidisciplinary expert workshop to produce a clinically relevant and pragmatic set of guidelines in the form of a decision tool. This is based on the health professional asking the initial question 'Why do you wish to have a test?'. According to the patient's response, the health professional and patient are guided through a pathway that includes relevant actions and information on the appropriateness of the test for a number of specific situations. This tool will be made freely accessible to health professionals and patients via the Internet. The rationale for each part of the decision pathway and the information provided will be discussed.

H. Skirton: None. **L. Goldsmith:** None. **L. Jackson:** None.

P18.24

Are you my father? How consumer ancestry DNA testing could undermine the anonymity of sperm donors

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Direct-to-consumer genetic testing for genealogical or ancestry purposes is a new development that could contribute to undermining privacy and confidentiality of consumers who purchase these services and of individuals who have never even participated in such testing. Recently, various companies have published testimonials on their websites describing how customers have found distant or close (parents, siblings) relatives. The advent of such services and the fact that a number of donor-sperm-conceived (as well as adopted children) have identified their biological father partly with the help of DNA testing raise important questions about whether promises of anonymity, which are still common practice in various countries, can be honored by clinics recruiting sperm donors. Healthcare professionals working in a context where anonymity in gamete donation is still an option for donors enter a new era where promises of absolute privacy become impossible to make. For policy makers, healthcare systems and gamete banks, it questions to what extent anonymity in gamete donations will remain a viable option in the future. It questions whether a few privacy breaches cases might provide sufficient evidence to completely change policies regarding anonymity and whether this might have an impact on the explicit framing of existing privacy risks in the recruitment process of potential gamete donors.

O. Rusu: None. **H.C. Howard:** None. **P. Borry:** None.

P18.25

A thirty-one years retrospective prenatal study of 47,XXX and 47,XYY dysgonosomies.

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Background: 47,XXX and 47,XYY dysgonosomies are two of the most common chromosomal abnormalities with a 1/1000 frequency. Their prenatal diagnosis (PD) is often fortuitous. We aimed to evaluate the different prenatal diagnosis parameters which enabled these PD and pregnancies outcomes to improve genetic counselling .
Methods: a retrospective collaborative study using data from 21 french laboratories over a period of 31 years from 1981 to 2011 was conducted by collecting the following data for each case: indication of the collection, nature of sample, maternal age, fetal karyotype and pregnancy outcome. All parameters were analyzed according to the implementation of multidisciplinary centers for prenatal diagnosis (MCPD) created since 1997
Results: 291 cases of 47,XXX and 167 cases of 47,XYY syndroms were collected. Karyotype was homogenous in 87.6% of cases. The main indication was advanced maternal age for 47,XXX (53.3%) and ultrasound findings (UF) for 47,XYY (35.9%). Outcome was similar regardless of the karyotype: 78.9% of delivery, 17.9% of termination of pregnancy (TOP), 3.2% pregnancy loss. There is a statistical difference for without UF pregnancies' outcomes in both groups before and after 1997, with a decrease of TOP from 28,6% to 5,4% ($p<0.0001$).
Discussion: As supposed, due to both maternal and paternal inheritance, 47,XXX dysgonosomy is twice more frequent than 47,XYY one. Difference in PD indication was mainly due to maternal age incidence for 47,XXX. Furthermore, we clearly showed that establishment of MCDP permitted to change the outcome of these pregnancies.

E. blondeel: None.

P18.26

RDDR: a Dysmorphology Diagnostic Network for newborn in the central part of Italy

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***Background*:** Dysmorphology is the study of birth defects or malformations arising from abnormal embryogenesis. A dysmorphology assessment of a newborn focuses on history examination and investigations that may lead to a diagnosis which is essential for patient management and for genetic counselling. In 2011 we developed RDDR network, aiming at improving the diagnosis of dysmorphic conditions in newborn in the central part of Italy.
***RDDR Workflow*:** RDDR (www.rddr.it) is developed on an online electronic software that, to date, links about 20 Centres of Neonatology which re-

present the Submitting Nodes. RDDR Network comprises clinicians from various dysmorphology centres in Lazio region with varying levels of expertise. Submitting Nodes transmit patients' clinical information and photographs. Accepted cases, appropriate for RDDR, are reviewed by RDDR dysmorphology experts throughout a forum section. Summary clinical reports are prepared an average a week after acceptance, depending on the timing and number of reviews, and are sent to the Submitting Nodes, and includes diagnostic suggestions and recommendations for further investigations or management of the patient.

Conclusions: RDDR Network enables clinicians of the submitting cases to access to a range of expert opinions and management advice, to increase individual and collective knowledge about dysmorphic rare conditions, and to raise current standards for the diagnosis, management, and increasing neonatologist skills in recognizing rare dysmorphic syndromes. RDDR was developed based on the experience of related European Dyscerne, A European Network of Centres of Expertise for Dysmorphology.

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P18.27

The psychosocial impact of risk-management for hereditary diffuse gastric cancer

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Between 30%-50% of cases of Hereditary Diffuse Gastric Cancer (HDGC) are caused by mutations in the E-cadeherin gene. *CDH1* mutation carriers have an earlier than average age of disease onset, and greatly increased risks of developing cancer. Individuals identified as at-risk of HDGC, either because of their family history or as a result of DNA testing, need to make decisions about risk management, whether they will have risk-reducing surgery (total gastrectomy) or continue screening. This retrospective qualitative interview project was undertaken to explore i) decisions about risk management, ii) the psychosocial implications of undergoing risk-reducing gastrectomy (RRG), and iii) individuals' information and support needs. The investigation included 42 patients; 27 had undergone RRG and 15 were having endoscopic screening. Factors influencing surgical decisions include: experiences of disease, RR surgery and screening within the family, risk perception, social factors (e.g familial obligations, life course issues) and tolerance of screening. Surgery was reported as having a number of psychological (e.g. impact on body image), social (e.g. impact upon family and earning potential) and physical costs and benefits. A number of information (e.g. impact on social life and reproductive options) and support (e.g. around eating practices) needs were identified.

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P18.28

Approaching education in the genomic age - widest possible audience or narrow focus

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Education has been proposed for some time as the solution to managing the impact of emerging genetic and genomic technologies. Programs targeting the widest possible audience including the general public, individuals and families affected by genetic conditions, those at risk, schools and the professionals who care for them have been conducted with success by the Centre for Genetics Education NSW Health(CGE) in Australia for over 20 years. However health professionals report great concern for the lack of preparedness for new technologies. Given the enormity and rapid increase in patient and support information now available online, difficulties associated with measuring the impact of community awareness campaigns on health outcomes, the density of information for health professionals in this changing field and the limited resources allocated to education, the best way forward has recently been unclear. In addition approaches to education have significantly changed with the accessibility and popularity of social media, online programs and tools. Following a review in 2012, CGE has adopted a new model to meet this challenge narrowing its focus in order to deliver wider benefits. The approach targets non-genetic trained health professionals to develop skills and knowledge to manage the impact of genetic and genomic technologies and in doing so strengthening and delivering wide patient benefits through their clinical practice including informed decision making, risk assessment, early appropriate referral, testing, detection, prevention, treatment and support. The model and strategies in place for 2013- 2015 are presented.

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P18.29

Identification of a Single-Nucleotide Polymorphism of TAS2R38 gene (Bitter receptor gene). Genotype and phenotype characterization of a sample of Ligurian students

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In Liguria region an educational project for teachers and students of last two years of high school has been going on in collaboration with University of Genoa since many years involving lab activities, lectures and training sessions. During the last two meetings of Italian Society of Human Genetics a special session was dedicated to school to disseminate in Italy the Genoa experience. One of the most successful activities was the study of bitter-tasting ability by the analysis of the TAS2R38 gene polymorphisms, a didactic model of genotype-phenotype correlation. Three SNP of this gene - that codes for a transmembrane receptor - cause three amino acids substitutions in P49A, A262Ve I296V, resulting in two common haplotypes, PAV (taster) and AVI (non taster). Individuals who have two dominant alleles (PAV/PAV or PAV/AVI) are sensitives to bitter substances, while those not sensitives (AVI/AVI) are recessive for this trait. The project involves thirty teachers and six hundred students and comprises a first part developed in schools: DNA extraction by mucose buccal cells, Bitter Taste Test by PTC paper, Questionnaire of food preferences, Bioinformatics. The second part takes place in a didactic laboratory of University: amplification of DNA trait including the SNP rs 713598, polymorphism identification by enzyme restriction and gel electrophoresis.
The activity allowed students:
- to evaluate genotype/phenotype correlation
- to calculate the frequences of PAV and AVI genotypes
- to know that a number of SNPs are inherited as a haplotype
- to consider the role of genetic variability of taste perception

B. Zanini: None. **V. Marini:** None. **M. Borriello:** None. **R. Ravazzolo:** None.

P18.30

The Clinical Laboratory Genetics profession in Portugal

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Portugal has several public and private laboratories dedicated to human genetic diagnosis, following high quality standards and professionals with qualifications in accordance with the standards of other European countries. The Clinical Laboratory Genetics profession is included in the career designated *Técnico Superior de Saúde, ramo de Genética (TSS-genética*). To integrate this career a biomedical sciences background is needed (e.g. degree in Biology, Biochemistry, Pharmaceutical Sciences) followed by a 3 year internship that takes place in authorized genetic laboratories. The Portuguese health system is facing significant challenges due to reforms that are under implementation. Alongside the rationalization of expenses, it is very important to regulate the creation of several professions, an issue that has to be addressed by the Portuguese health services authorities.
The Workgroup for the Clinical Laboratory Genetics Specialty (GT-EGCL) designated by the Board of the Portuguese Society of Human Genetics (SPGH) has created a database/registry of the Clinical Laboratory Genetics Specialists working in public and private areas in Portugal. The results obtained by the GT-EGCL that will be presented, allowed the characterization of the Clinical Laboratory Genetics Profession in Portugal.The GT-EGCL fully endorses the inclusion of the Clinical Laboratory Genetics into Directive 2005/EC/36, towards the recognition of this specialty at the European level. This Europe-wide recognition is of tremendous importance not only to maintain the high-standards in clinical practice in human and medical genetics areas, but also for the cross-border mobility/recognition of these Portuguese professionals.

L.C. Ramos: None. **P. Jorge:** None. **B. Marques:** None. **M. Avila:** None. **P. Rendeiro:** None. **M.D. Quelhas:** None. **J.B. Melo:** None. **I.M. Carreira:** None.

P18.31
Points to consider for prioritizing genetic tests

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Given the need of many European health care systems to contain costs of public health care, decisions have to be made which tests to cover from public budgets if resources are not sufficient to fund them all. This study reports results from EuroGentest activities on fair and reasonable prioritization of genetic tests. It is based on literature searches, interviews and a stakeholder workshop oriented at the ethical principles of accountability for reasonableness. Implicit prioritization of genetic services appears to be prevalent throughout European health care systems. For explicit, fair and reasonable prioritization of genetic tests, a range of criteria need to be considered. These include, in particular, medical benefit of testing, a priori risk of developing the condition, and costs of testing as well as further criteria such as time window for treatment to be effective, severity of the condition or whether the test is diagnostic or predictive. Quantitative methods like discrete-choice experiments can be used to attach relative weights to these criteria. Furthermore, data sources such as the Clinical Utility Gene Cards could be used for operationalizing the criteria and estimating priority scores. However, their validity is limited by the evidence regarding the criteria for most genetic tests. Also, weights attached to the criteria can vary depending on decision context. Theory and practice of explicit, fair and reasonable priority setting of health care resources in Europe are still at an early stage. The results from this study provide important points to consider for their further development.

F. Severin: None. **W. Rogowski:** None.

P18.32
External Quality Assessment of Genetic Counselling: results of a European survey and proposal for a pilot assessment

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on behalf of the, Genetic Services Quality Committee of the ESHG, Austria.

One objective of the ESHG Genetic Services Quality Committee (GSQC) is to explore the existing Quality Frameworks and the needs for a European Quality Assessment (EQA) scheme for genetic counselling. A GSQC working group surveyed all European national societies of human genetics by a web-based questionnaire. The working group aimed to obtain an overview of which quality assessments were already in place for genetic counselling, and where they were used. If applicable, national societies were asked to give information on quality assurance for the training of professional counsellors and the counselling process itself. National representatives were asked whether the genetic centres in their country participated in national external quality assessments and whether there was a need for European/international quality assessment (comparable to the EQA schemes for clinical genetic laboratory services). It was not the task of this working group to comment on guidelines, minimal quality criteria, or national quality assessment schemes.
Representatives of 15 out of 32 countries completed the questionnaire. The main outcome was that quality assessment ranged from none (the situation in most countries) to well-organised on a national level (the minority). It is clear that there are few quality assessment activities for genetic counselling and all countries expressed the need for an EQA.
There will be a symposium directly preceding the Paris ESHG 2013 conference, where the challenges and opportunities an EQA scheme may encounter will be discussed. The working group will present a proposal as to how an EQA for genetic counselling could be achieved.

C.M.A. van Ravenswaaij-Arts: None. **C. van Asperen:** None. **E. Dequeker:** None. **L. Tranebjaerg:** None. **L. Garavelli:** None. **B. Peterlin:** None. **B. Cope:** None. **R. Hastings:** None. **H. Skirton:** None.

P18.33
Patients' experiences of the FH cascade testing programme in Wales

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Familial hypercholesterolaemia (FH) is an inherited condition giving rise to high blood cholesterol. The heterozygous form is common, affecting 1:500 in the UK, of whom it is estimated that 75% are unaware of the disorder. If left untreated, 50% of males will develop coronary heart disease by the age of 55. It can be effectively treated through the use of statins. The UK department of health 2003 white paper 'Our Inheritance, Our Future' stated that FH cascade testing was a 'paradigm for how to use genetics to improve healthcare outcomes for a complex disorder.'
In 2010, the Welsh Assembly government in conjunction with the British Heart Foundation, funded an all-Wales genetic cascade testing service with lipid clinics, FH specialist nurses, genetic counselors and cascade testing of relatives. Semi-structured interviews were conducted in 2010/2011 with 12 patients (aged 18-70) that were involved in the FH programme.
Many interviewees recounted very strong family stories regarding FH, with several people having had a diagnosis for many years. However there were several examples of problems in receiving appropriate treatment or testing previously and poor understanding of the disorder by GPs. The experience of the all-Wales FH service was generally positive among both the previously and the newly diagnosed. The majority of participants were happy to provide details of their family for cascade testing.
This cascade testing programme is the first to be funded in the UK. It has identified new cases of FH and was also appreciated by participants who had an existing diagnosis.

J.L. miller: None.

P18.34
Families' Genetic Counseling Experiences Following a Positive FMR1 Newborn Screen

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Newborn screening for *FMR1* expansions presents unique genetic counseling challenges distinct from those encountered after standard newborn screening. These include a complex inheritance pattern; the variable phenotypes associated with age, gender, and repeat size; the potential for adult-onset disorders in infants and relatives; and the unavailability of medical treatment. We describe the genetic counseling experiences of families with screen positive infants in the Fragile X Newborn Screening Study. Using a PCR-based screening test that detects full mutations (200 repeats or greater) and premutations (55-199 repeats) 3795 infants born at UNC Hospitals were screened. Informed consent from both parents, when the father was reasonably available, was obtained. Twenty-one babies from twenty families screened positive; all had premutations. All twenty families were contacted by phone three months after the birth and invited for a genetic counseling visit for diagnostic and carrier testing. Fourteen families were scheduled, four could not be re-contacted, one withdrew and one is pending. Eleven samples were obtained for diagnostic testing. There were four falsely elevated screening results but only one remained in the carrier range after re-testing. Parents could decide about placing the diagnostic results into the infant's medical record. Before parental testing, we discussed implications for relatives, risks for adult-onset symptoms, and the potential for discrimination. Twelve parents in eleven families requested carrier testing; two parents declined. We will summarize parents' initial reactions to learning the result, factors influencing their subsequent decisions about testing, and their responses to the wider implications of their newborn's results.

M. Roche: None. **C. Powell:** None. **D. Skinner:** None. **A. Wheeler:** None. **D. Bailey:** None.

P18.35
Translation and adaptation of the Genetic Counselling Outcome Scale (GCOS-24) to Danish

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Background and aim: The ability to measure patient outcomes from genetic counselling is a prerequisite for evidencebased development of practice. The Genetic Counselling Outcome Scale (GCOS-24) is a recently developed patient reported outcome measure. The aim of this project was to develop a Danish version: GCOS-24-Danish.
Methods: GCOS-24 was translated from English to Danish by two independent translators. The translations were combined and then back-translated by two native English-speakers. All versions were reviewed by an expert committee. Alternative wordings and disagreements between back-translations and the original version were examined. The test developers were consulted regarding ambiguous wording. Forty candidates among patients seen earlier and in our waiting room were asked to fill out the preliminary GCOS-24-Danish. Willing respondents were interviewed about their experience. Distribution of responses and results of interviews were examined and the

GCOS-24-Danish adjusted accordingly. Results: There were differences between the original version and the back translations. Problems in the translation of semantic, conceptual and idiomatic equivalence were identified and resolved. Twenty-one questionnaires were returned and fifteen interviews conducted. Informants' responses and analysis of their understanding as displayed linguistically led to adjustment of two items to ensure cultural adaptation. Discussion: The process was more difficult than anticipated. Each step provided new insight regarding perception of genetic counseling and genetic conditions and led to adjustments of the original translation, leading to development of a tool better-suited to the target population. We would recommend the described approach when attempting translation of patient reported outcome measures.

B.R. Diness: None. **G. Overbeck:** None. **T. Duelund:** None. **T.B. Hammer:** None. **S. Timshel:** None. **M. McAllister:** None.

P18.36

An attempt to evaluate the efficiency of the genetic counseling for families with affected children or relatives

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The genetic counseling was essentially directive until recently, its efficiency being often assessed by changes in reproductive plans according to the geneticist's advice. The directive genetic counseling at present tends to be replaced with non-directive one. The efficiency of genetic counseling in Russia is poorly investigated. The current review is dedicated to these problem. The presented qualitative study is aimed to estimate the genetic counseling efficiency by a number of parameters, including satisfaction, reduction of disquietude (stress), the guilt feeling relieving for the birth of affected child. The study was conducted in the outpatient department of the Research Centre of Medical Genetics (RCMG) of the Russian Academy of Medical Sciences (RAMS) from 2007 to 2011. 226 respondents 17 to 67 years old with an affected child/children or affected relatives were interviewed before and after genetic consulting. The interviews were analyzed using Statistica 10.0 software. Most respondents (n=213, 94%) indicated that genetic counseling is important or very important to them. We identified a high level of anxiety and guilt feelings among respondents (60% respondents with affected children experienced guilt feelings and 40% were very concerned about the possibility of the disease recurrence in their family). The level of anxiety is significantly reduced after counseling ($p<0,05$). We are pleased to record a high level of satisfaction with genetic counseling among respondents: 197 (87%) of respondents considered it useful or very useful, 212 (93%) indicated the investment of time and money to be entirely or substantially justified, 181 (80%) reported satisfaction with advice.

E.E. Baranova: None. **E.K. Ginter:** None. **V.L. Izhevskaya:** None. **A.S. Sergeev:** None.

P18.37

Palestinian Physicians knowledge of genetics, and their attitudes towards genetic testing and genetic counseling.

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The Genetic and Metabolic Diseases Center at Al Quds University, provides valuable genetic services for the Palestinian community. As part of the center's mission to improve the knowledge of genetics and genetic testing among health care workers, a survey assessing the knowledge of basic genetics information, practices and genetic counseling was conducted by the center in the fall of 2012.
A cross-sectional survey was used whereby data was gathered from the target population at a single point of time. The target population for this project was physicians in the fields of pediatrics, gynecology, primary care and neurology.
The aim of this survey was to assess multiple criteria regarding physician's knowledge of genetic diseases and genetic testing. Additional aspects included their basic knowledge of genetics and hereditary concepts, awareness of the availability of genetic tests in Palestine, awareness of the importance of genetic counseling and its availability, and the management of patients with genetic diseases.
Surveys were distributed in twelve districts in the West Bank to physicians working in government hospitals, private clinics, and non government health centers. Our sample was about 150 surveys from all the fields mentioned above. The primary results showed a variation in experiences dealing with genetic diseases, and the appropriate response to it. Continuous education about the common genetic diseases is essential. There is a general consensus among the participants on the importance of developing a comprehensive national policy dealing with early genetic screening and genetic diseases management.

E. Tinah: None. **A. Ayyad:** None. **S. Khatib:** None.

P18.38

French professionals in Genetic Counselor careers

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The profession of genetic counselor in France was recognized in 2004, based on the recommendations of a mandate commissioned by the Health Minister to explore the medical demographics of France. The report predicted a shortage of health professionals in the field of genetics, particularly in light of the rapid development of molecular testing. Development of the profession was supported by a legal framework, and today 107 genetic counselors have graduated from the specific educational program which awards the Professional Master's Degree of Human Pathology, entitled Master in Genetic Counseling and Predictive Medicine. Here we will trace the development of the profession in France and review the demographic characteristics of the students and genetic counselors practicing the profession today.

C. Cordier: None. **N. Taris:** None. **N. Philip:** None. **M. Voelckel:** None.

P18.39

Genetic counselling data and patient's clinical records: Adapting practice to legislation, in Spain.

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According to legislature data derived from gene testing, genetic consultations, and specially genetic counselling; represents a set apart from the rest of the patient's clinical record (CR); for it contains information that can reveal the person's health, ethnicity, and indirectly (i.e. genetic pedigree) about others. That is why under Spanish law it's considered "especially protected personal information" and any media containing such records is subject to what has been termed "reinforced protection."
Unfortunately the Spanish legislation as of today hasn't establish well defined protocols to ensure the protection of said data, therefore the responsibility of designing and implementing strategies towards this end has by default fallen upon health-care providers.
We've compiled the strategies used at both our centre and national institutions with which we maintain communications. These strategies are the following:
• Not including the data into the electronic clinical record so that it is accessible only as hard copy
• Anonymizing the data on the service of origin's terminal
• Having the data in the electronic CR but encrypted
• Restricting access to selected consultants.
• Requiring patient's authorization
• Unrestricted access within electronic CR but with a pop-up style reminder of it's privileged status
• Unrestricted access with user's tracking.
What we advise and have applied to some extent at our institution is a combination of the above namely tracking users, maintaining reminders of its nature and having the patient waive if so wishes the ban on restricting the data from his or her current healthcare providers

M. Rodríguez Pedreira: None. **B.P. dos Santos:** None. **A. Mosquera Rey:** None.

P18.40

Communication of information on genetic studies in paediatric research: what are the present ethical challenges in large scale genomics and longitudinal studies?

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The vulnerability of children requires special protection in any type of research, particularly genetic research using large scale sequencing. While genetic data are considered as sensitive data in law, ethical issues raised in genetic studies involving minors are emphasized. Their participation im-

plies ipso facto, the involvement of a third person legally responsible in the decision making process: the legal representative. This is especially relevant in the manner of communicating the information to the legal representative and to the minor in large scale genomics and longitudinal studies because the maturity of the children will increase along with the advancement of the research. Therefore it is necessary to adapt the language in order to gradually involve the children. Such communication raises ethical challenges, in particular those that are related to the informed consent/assent in the decision making process and the communication of results (general and individual) as well as the disclosure of incidental findings. Thus, grounded in our experience acquired in the FP7 EU project MeDALL[1] (Mechanisms of the Development of ALLergy) and practices from the project partners, we argue that the most appropriate way to ensure adequate protection for child participants in paediatric genetic research regarding the issues mentioned above remains the adapted communication of information about the study. We propose models for documents to be used and underline the importance of involvement of patient/family associations in the preparatory process.

Footnotes

[1] http://www.medall-fp7.eu/

1

V. Anastasova: None. **A. Mahalatchimy:** None. **E. Rial-Sebbag:** None. **A. Cambon-Thomsen:** None.

P18.41

EuroGentest E-Courses, a new 'time and money saving' platform for the training of genetic testing laboratories.

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The aim of EuroGentest Workshops is to aid laboratories in the process of implementing and developing a quality system, improving existing quality systems and working towards accreditation (ISO 15189). The workshops also contribute to harmonize the approaches to accreditation of genetic testing services in Europe.

The subjects vary from general topics for those who would like to start up or improve their quality system, to more specific and advanced topics such as validation of diagnostic tests, internal audits, management review and IT support for quality management.

Since 2007, 747 participants took part in the 31 workshops organized up to now. These workshops were organized in 13 different countries, to reach as many participants as possible.

From now on we will try to target a bigger audience of participants worldwide through the organization of e-courses. In this era of fast technological advance and minimization of distance through the use of internet, e-courses provide a 'time and money saving' platform for the training of genetic testing laboratories.

A first pilot e-course on quality management consisted of an online presentation, a quiz and an interactive forum. A total of 36 participants attended, representing 23 countries worldwide. We will evaluate if this pilot and future e-courses represent an added value for participants in sharing experiences with other people and experts in the field and allow them to gain knowledge for improving their current quality systems.

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P18.42

Attitudes toward genetic testing in Japan: National surveys in 2005, 2009 and 2013

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[Aim] The aim of this study is to explore attitudes toward genome research applied to medicine among the general population of Japan, focusing on willingness to undergo genetic tests for disease susceptibility and pharmacogenetic tests.

[Methods] Postal questionnaire surveys were conducted in 2005, 2009 and 2013 in random samples of the general public in Japan. The questionnaire included items on genetic knowledge, attitudes toward the application of genomic studies to medicine and crop science, basic genomics research, and technology. We analyzed the data with a focus on the attitudes toward the application of genomic studies to medicine.

[Results] There were 2171 participants in 2005 (response rate; 54%), 2005 in 2009 (response rate; 50.1%) and 1161 in 2013 (response rate; 60.3%). In these surveys, most respondents had positive attitudes toward these genetic tests (66.4% of respondents had positive attitudes in 2013). The logistic regression analysis showed that significant predictors of the willingness to undergo both genetic tests were to perceive more benefit and having positive impression and interest in the research. Considering that the use of genetic testing is likely to increase in the near future, we propose that more information about genome and communication between scientists and the public should be provided in Japan.

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P18.43

Psychosocial approach in ophtalmic genetics

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The Reference Center for Rare Diseases in Ophthalmology in Strasbourg is taking care of patients of all ages with progressive conditions. The latter are diseases such as retintis pigmentosa, Leber's neuropathy, Stargard's maculopathy, leading to visual impairment with sooner or later a major handicap. To date no straight forward therapy is accessible for neither of them eventhougth massive research, often sponsored by patient groups, are currently on going.

In this context, we have observed numerous effects on the psychological and social life of the patients. Indeed, the progression of the disease induces a reorganization of emotional, relational, professional or school life(s). The psychological effects are major as the disease induces a real grief work. The acceptation of a genetic ocular condition is usually very slow and generates a psychological distress that can be major. During the last years, we have offered individual follow up by a social worker and a psychologist. However, the importance of the every-day life problems have led us to consider another therapy option by way of a support group therapy. This novel approach has led us to question about the efficiency of the different methods. We have noticed rapidly that the support group was efficient in respect to the acceptation and care of the disease. The positive impact of the support group seem to us higher compared to the individual follow-up that however should be pursued. The dynamics of the support group appears to be very beneficial for the grief work of most of the patients.

C. Plumere: None. **S. Pfeiffer:** None. **Y. Perdomo:** None. **V. Pelletier:** None. **F. Studer:** None. **S. Rudzki:** None. **H. Dollfus:** None.

P18.44

Teaching Physicians about Genomic Medicine

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Background: Due to new discoveries in genomic medicine, clinical guidelines are increasingly recommending the incorporation of genomic tests or therapeutics into routine care, especially as the transition of genetic knowledge from research laboratories into clinical practice is becoming more and more a part of Western health care systems. Primary care practitioners suffer from inadequate knowledge and skills in medical genetics and many are unaware of the technical, ethical, legal and psychosocial implications of genetic testing.

Methods: We initiated a „genomic education" program for the purpose of teaching primary care practitioners new advanced knowledge on genomic medicine. We emphasized the main take-home messages for physicians, which were: risk calculation for various genetic diseases, recognition of the mode of inheritance from the pedigree, guidelines for decision-making on

which molecular tests to use, and the interpretation of test results and their clinical implications.
Results: To date, 16 physicians have participated in our „genomic education" program, which included lectures, workshops and guided tours in genetic laboratories. In the „pre-course" examination the average score was 54.5% (range 20%-80%), whereas in the „post-course" examination it was 84.5% (range 74%-100%). The average improvement in score as a result of the course was 36% (range 15%-80%). The physicians who participated in our program reported a very high level of satisfaction from the content as well as the concept of a „one-week update".
Conclusion: A one-week „genomic education" program is an effective strategy to update primary care physicians in order to improve their care of patients.

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P18.45
Update of the spectrum of GJB2 gene mutations in Tunisian families with autosomal recessive nonsyndromic hearing loss

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Hearing loss is the most frequent sensory disorder. It affects 3 in 1000 newborns. It is genetically heterogeneous with 60 causally-related genes identified to date. Mutations in GJB2 gene account for half of all cases of non-syndromic deafness. The aim of this study was to determine the relative frequency of GJB2 allele variants in Tunisia. In this study, we screened 138 patients with congenital hearing loss belonging to 131 families originating from different parts of Tunisia for mutations in GJB2 gene. GJB2 mutations were found in 39% of families (51/131). The most common mutation was c.35delG accounting for 35% of all cases (46/131). The second most frequent mutation was p.E47X present in 3.8% of families. Four identified mutations in our cohort have not been reported in Tunisia; p.V37I, c.235delC, p.G130A and the splice site mutation IVS1+1G>A (0.76%). These previously described mutations were detected only in families originating from Northern and not from other geographic regions in Tunisia. In conclusion we have confirmed the high frequency of c.35delG in Tunisia which represents 85,4% of all GJB2 mutant alleles. We have also extended the mutational spectrum of GJB2 gene in Tunisia and revealed a more pronounced allelic heterogeneity in the North compared to the rest of the country.

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P18.46
European guidelines on prenatal diagnostic testing

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Despite a long history of prenatal testing in Europe, there are no overall guidelines to ensure minimum standards of care for women. With increasing use of non-invasive testing, there is a concern that prenatal testing will become 'routinized'. As part of the EuroGentest2 project, we developed European guidelines on prenatal diagnostic testing, using an Expert Workshop methodology. Sixteen professionals from disciplines including medical genetics, fetal medicine and laboratory genetics from nine different European countries attended, as well as patient representatives.
The guidelines were focussed on prenatal diagnosis, for women with a fetus at increased risk of a specific condition. Screening was therefore not included. The material was organised under four topics: objectives, general principles, logistical issues, and topics for counselling.
The *objective* of prenatal diagnosis counselling is to enable families to make informed choices consistent with their needs and support them in dealing with the outcome. *General principles* include the need for appropriately trained multi-disciplinary teams; ensuring informed decision making and the availability of pre- and post-test counselling. This type of service requires effective communication between multi-disciplinary teams and family, sensitive counselling with interpreters if needed, and consideration of wider family implications. Counselling *topics* should include psychosocial issues as well as information about the condition, the test and available options. The Expert Group concluded that these guidelines for prenatal diagnosis were applicable to both invasive and non-invasive testing.
The guidelines have been subjected to wide consultation in and beyond Europe and are now available on the EuroGentest website.

L. Goldsmith: None. **L. Jackson:** None. **H. Skirton:** None.

P18.47
Family matters: How do relatives receive hereditary cancer information? A qualitative study

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Background
Genetic counseling for hereditary breast or colon cancer has implications for both counselees and relatives. Counselees are encouraged by genetic counselors to disclose hereditary cancer risk information to their relatives. Yet the question is how at-risk relatives appreciate being informed by counselees and to what extend they understand the information. The aim of this qualitative study was to gain insight in relatives' experiences with receiving hereditary cancer information from counselees.
Methods
A heterogeneous purposefully selected group of first and second degree relatives of counselees visiting the Genetics Department regarding the possibility of hereditary breast or colon cancer were invited to participate in face-to-face, in-depth semi-structured interviews. Counselees could have had a confirmed genetic mutation or an uninformative test result. Relatives' experiences were identified by two independent coders from transcribed interviews. In-depth inductive analysis of 8 interviews provided a coding scheme of major thematic categories used for the analysis of the remaining interviews.
Findings
Overall, interviews with 25 relatives from 16 families (8 colon and 8 breast cancer) were included. Preliminary results show that informing relatives about genetic cancer information is a complex process since the genetic information is difficult to understand for relatives. Especially for relatives of counselees' with an uninformative test result. Family characteristics (perceived closeness) and cancer history play an important role in the communication process between counselees and relatives.
Discussion:
Informing relatives about genetic cancer information is a complex and multi-facetted process. Understanding relatives' experiences may aid clinicians in their provision of family centred genetic counseling.

E. de Geus: None. **J. van der Vloodt:** None. **C.M. Aalfs:** None. **F.H. Menko:** None. **J.C.J.M. de Haes:** None. **E.M. Smets:** None.

P18.48
Rare autosomal recessive syndrome in Yakut children

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In the Medical and Genetic counseling from 2006 were counseling 10 children with Hurler-like phenotype (7 girls and 3 boys). Eight children died before the 1,5 year old. All the children were from Yakut unrelated families. These children had identical clinical features:
Dysplastic physique. All the children were born at term, full-term.
- A gradual change of the type of person gargoilizm: hydrocephalic head, enlarged parietal tuber, low growth of hair on the forehead, hypertelorism, thick and thick eyebrows, long eyelashes, periorbital edema of the eyelids, broad bridge of the nose, short nose with open nostrils forward, dense at touch the cheeks and nose, macroglossia.
- Skin: thick
- Skeletal deformities: shortened neck, chest wall deformity: barrel-shaped or bell-shaped, the shortening of the chest, stiff, wide aperture, mainly thoracolumbar kyphosis, lumbar spine, stiffness progression of small and large joints to contractures, change the brush-type ,claws „deepening palmar furrows.

- The presence of bronchial obstruction: noisy breathing, shortness of breath rise, auscultation hard breathing, various dry and moist rales, the need for oxygen therapy.
- Cardio-vascular system tachycardia, hypertension, systolic murmur auscultation.
- Gastro-intestinal tract: an enlarged abdomen due to hepatosplenomegaly,different types of hernia: inguinal, umbilical, non-operated.
- The nervous system of all children had delayed motor and static-psycho-verbal development.

Were excluded congenital hypothyroidism, cystic fibrosis, deficiency of alpha-1-antitrypsin, a chromosomal abnormality, MPS I, II, III, IVB, VI, VII types, GM 1, 2-gangliosidosis, Gaucher's disease,classical form of mucolipidosis 2 and 3, Niemann-Pick disease type C / B / A, Zhakena syndrome.

E.E. Gurinova: None. **N.R. Maximova:** None. **A.L. Suhomyasova:** None.

P18.49

The Progress of the Human Variome Project

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The Human Variome Project (www.humanvariomeproject.org) was formed to facilitate the collection, curation, interpretation and sharing of genomic knowledge worldwide and to assist its continued integration into research and clinical care. The Human Variome Project Consortium believes that the global knowledge capacity in medical genetics and genomics can be significantly improved if local knowledge is shared in a free and open manner to become global knowledge.
The role of the Human Variome Project is to expand the technical and knowledge capacity around medical genetics across the globe by establishing and maintaining standards, systems and infrastructure; promoting ethical behaviour in the field of medical genetics and genomics; sharing knowledge about our genome and its function in determining health; and assisting individuals and nations build their capacity to address genetics aspects of individual and global health.
This presentation will focus on:
- The Project's vision for a Global Collection Architecture;
- Current standards development work underway;
- Progress by pilot member initiatives: the InSiGHT Database and the Human Variome Project Australian Node;
- Activities of disease-focussed interest groups: cardiac genetics, mitochondrial genetics, neurogenetics and nutrigenomics;
- An ethical framework for data collection and sharing;
- The Project's approach to education and training in response to global pressures and expanding need for skills and knowledge;
- Capacity building work through the Human Variome Project/China Country Development Program.

R.G.H. Cotton: Other; Modest; Wiley - Liss (Role: Communicating Editor of Human Mutation journal).

P18.50

Does Presymptomatic testing influences Age at Onset in a Negative manner (PAON): follow up of at risk persons for Huntington disease

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In PAON we studied prospectively the impact of presymptomatic testing on the onset of symptoms in gene carriers for Huntington disease. 1554 at risk persons requested the test (mean age at request 34.9 ± 11.9 years) between 1992 and 2008 and entered multistep and multidisciplinary care and counseling. 367 (32%) did choose not to take the test, 476 were non-carriers and 307 carriers of an abnormal expanded CAG repeat in the HD gene. Follow-up in 2013 was available for 191 carriers (62%, mean age at follow up 45.3 ± 11.0 years) including 3 who died (1 cancer, 2 suicides). Estimated age at onset will be calculated (Langbehn formula). We collected 48 questionnaires and 46 underwent physical examination, using the UHDRS evaluation score (max worse value 124) at the same time. Mean UHDRS was 12.9 ± 12.7. There were 16 carriers with values 5 years ago, anxiety was still frequently reported (48%) and 8% were currently depressed. Interestingly, 41 % of carriers with signs at examination (UHDRS >4) declare not to show symptoms and 31% of those with normal examination felt that they had signs. All are relying on family/medical support (94%) but knowing their genetic status interfere with daily life activities in 69% of the unaffected carriers and in 33% of the carriers with signs (p<.05). Regardless of the fact that 76% showed increased auto-observation, neurological follow-up was not systematically requested (56%). In addition, 69% declared a need for psychological support.

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P18.51

Doing the right thing of one's children: deciding whether to take the genetic test for Huntington's disease as a moral dilemma.

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A range of reasons are cited for requesting a predictive test for Huntington's disease (HD). One reason which is mentioned frequently is the "need to know for my children". We have analysed transcripts of interviews from 9 participants considering a predictive test for HD and subjected them to interpretative phenomenological analysis. Four participants wanted to take the test and three of them claimed it was to assist their own children's reproductive decision making. Two participants currently opposed to predictive testing cited the same reason as a key factor in their decision making. Three participants were undecided and 2 of these explicitly cited their own children's reproductive decision making as a consideration.
We will present a detailed analysis of this single theme from 3 of the participants to show how they thought through this issue. Four imperatives emerged: wishing to stop HD, doing the right thing for my child, right to chose to have a family, and giving information to others. We will report on the evidence of the participants endorsing, rejecting or not mentioning these. Consideration of these issues resulted in them reaching different decisions regarding predictive testing. We will suggest some ways in which a counsellor may explore the theme of „need to know for my children" during pretest discussions. In addition, we will discuss this result in relation to similar work on communication of genetic information in families with Hereditary Breast and Ovarian Cancer.

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P18.52

Reflecting on earlier experiences with unsolicited findings: Points to consider for next generation sequencing and informed consent in diagnostics

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With the recent advent of high-throughput nucleotide sequencing in the clinic for diagnostic purposes, unsolicited findings and informed consent are receiving increased attention. To share experiences and explore dilemmas, an international and multidisciplinary meeting entitled "Exome sequencing in diagnostics: exploring needs for the informed consent procedure" was organized by Workpackage 8 of Eurogentest2 in Amsterdam, the Netherlands in March 2012. The aim was to provide input for the development of guidelines to optimize the informed consent procedure.
Based on the meeting and by exploring recent experiences with unsolicited findings in other medical settings -for example in the context of disclosure of carrier status in neonatal screening, feedback of actionable results in genetic research and the changing informed consent when using array CGH- an attempt is made to describe what can be learned for implementing next ge-

neration sequencing in standard genetic diagnostics. The results show that both ethical and practical dilemmas are encountered when contemplating informed consent for next generation sequencing in diagnostics. Issues that were discussed include: patient rights and professional duties, management of patient expectations, attunement between different actors, a changing role for the lab, feedback of results and involvement of an advisory board for unsolicited findings.
Based on the discussions, a framework has been developed in order to guide decision-making with regard to the return of unsolicited results and the implications for informed consent in clinical diagnostics.

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P18.53
Australian life insurance and genetic testing policies 2013 - implications for families and research

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The Life Insurance industry in Australia's genetic testing policy became a standard (#11) in 2005 making it mandatory practice: genetic testing would not be requested for applicants for new life insurance products or for increases to existing policies for income protection, trauma and permanent and total disability insurance. If however an applicant knew their, or their blood relative's test result, they must disclose it in the application as the contract is one of good faith: the insurer needs to know what the applicant knows. In this context, family health history also needed to be disclosed. In 2011 amendments to standard #16 (Family Medical History) were instituted whereby requirements for disclosure of family history was limited to **only** first degree relatives in recognition of the difficulties some applicants were experiencing. This has significant implications for those with a family history of a genetic condition: it may enable in some cases policies to be assessed at average risk on the basis of family history alone. A further change that however balances this benefit relates to research. While the insurers undertake that their participation in such research will not be into account when underwriting a policy, there is the potential that the requirement will impact on willingness to take part in research projects given issues of public trust and perception regarding life insurance companies. Information about the life insurance requirements for research participants will have to be included in the consent forms. Implications of these changes to policy will be discussed.

K.K. Barlow-Stewart: None.

P18.54
Genetic counselling remains challenging in Leber's optic neuropathy

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Leber's hereditary optic neuropathy (LHON) is usually characterised by initial unilateral subacute central visual loss with rapid bilateralisation leading to a severe visual handicap.
This optic neuropathy is transmitted exclusively by women because of its mitochondrial inheritance. More than 18 homoplasmics mutations are identified today.
The age of onset varies from infancy to late adulthood. The neuropathy is usually isolated but can be rarely associated to heart conduction disorders or neurologic abnormalities.
Risk of developing this condition differs between sexes from 30% to 83% in men to 5% to 32% in women.
The homoplasmy, the risk of an abrupt severe visual prognosis (four to five times higher in men than in women), the « guilty » feeling of the women who carry LHON mutations and the feeling of a „sword of Damocles" that threaten their children are major issues for genetic counselling that remains a challenge for women who wish to have children.
We present four clinical situations that illustrate the complexity of genetic counselling for LHON : in a context of a prenatal project or during a pregnancy, in the context where another genetic affection coexists. In all cases, questions about prenatal diagnosis (PND) and preimplantation genetic diagnonsis (PGD) were addressed.
What is the place for sexe determination by PGD? What about PND? We will detail the outcomes of each situation that learns us about the deep impact of LHON in the families.

V. Pelletier: None. **P. Gosset:** None. **M. Miguet:** None. **Y. Perdomo:** None. **F. Rezaiguia-studer:** None. **C. Plumere:** None. **S. Pfeiffer:** None. **H. Dollfus:** None.

P18.56
Midwifery issues on induced abortion in Japan

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Abstract
Background: Induced abortion is one of stressful procedure for both patients and medical specialists. There are a lot of researches on midwifery model care for the patient who is having induced abortion in the world. However, there are only few studies about psychological issues of midwife themselves. In Japan, we cannot give up the pregnancy because of fetal reason and we cannot perform any induced abortion after 22 weeks. Midwives sometimes hold difficult feelings during or after complicated deliveries. The purpose of this study was to explore the concerns of midwives through their duty especially in induced abortion. Methods: A focus group explored issues in induced abortion from the midwife's point of view for twice. Participant midwives were recruited from the maternity ward and their year of experience is three to twelve. Recorded their deliveries were assessed with Interpretative Phenomenological Analysis (IPA). Results: Analysis of the focus group data identified three superordinate themes: 'Ethical Issues as a human', and 'Issues as a midwife, caregiving role' and 'Difficulties in working environment'. Conclusion: These data provide evidence that the midwife have guilt feelings, sorrow and sense of awe in the living life when they care patients having induced abortion. Talking to patients more and talking with colleagues could help them to take care of themselves as sublimation.

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P18.57
Misoprostol and teratogenicity. review of 38 cases of vascular disruption defects in Colombia

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Introduction: Misoprostol is a prostaglandin E1 analogue, is used for the prevention of gastric ulcers and has been registered in about 80 countries. Because of Its abortive properties, is used as a method for abortion in countries where abortion is illegal. It has been documented that prenatal exposure to misoprostol has been associated with vascular disruption defects (VDD), mainly Moebius syndrome and transverse reduction defects.
Materials and methods: A case series study where 38 number of cases of VDD (gastroschisis, Moebius sequence, hydranencephaly,) with prenatal history of exposure to misoprostol are reviewed. The cases are mainly identified from the records of the birth defect surveillance system and clinical consultation. A teratogenicity analysis of this drug is made, taken into account dose, gestational age of exposure and type of VDD.
Results: Of the 38 cases of VDD, 27 were Moebius sequence, 4 hydranencephaly, 3 Klippel Feil, 1 Pentalogy of Cantrell, 1 VACTERL association, 1sirenomelia with cyclopia and 1 disruption by amniotic bands. In 7 cases other types of VDD were presented. The average dose of exposure was 652 grams, and gestational age of exposure was 7.2 weeks.
Discussion: The association of prenatal exposure to misoprostol and different types of VDD support the hypothesis of a similar physiopathological mechanism and that the disruptive event is secundary to the teratogenicity of this drug. The presented case series is one of largest in the world and it should raise concerns to the control and surveillance entities in the countries where abortion is illegal.

H. Pachajoa: None. **W. Saldarriaga:** None. **C. Isaza:** None.

P18.58
Two cases of Noonan syndrome with neonatal JMML due to PTPN11 mutation p.E139D

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Noonan syndrome (NS) is a frequent condition characterized by short stature, congenital heart defect, and developmental delay of variable degree. Furthermore, NS is accompanied with an elevated risk of hematologic malignancies, especially juvenile myelomonocytic leukemia (JMML).

Gain of function mutations within the RAS-MAPK pathway have been identified to contribute to NS, among which mutations of PTPN11 play a key role and account for approximately 50% of cases. Genotype phenotype correlations revealed hotspots within exons 3 and 13 of PTPN11 as well as within other genes where mutations specifically predispose to JMML.
We report on two boys with neonatal diagnosis of NS. Both patients showed characteristic features of the disorder including JMML. In both cases, molecular genetic analysis revealed the known de novo mutation PTPN11 p.E139D. According to the literature, only two NS patients carrying this mutation have developed JMML, two further patients developed other malignancies (acute lymphoblastic leukemia and oligodendroglioma). Thus, our two patients strengthens the previously assumed hypothesis of a potentially high risk for hematologic malignancies associated with PTPN11 p.E139D.

C. Courage: None. **M. Zenker:** None. **R. Ammann:** None. **S. Lüer:** None. **S. Gallati:** None. **J. Lemke:** None.

P18.59

Osteogenesis imperfecta (type IV) with dentinogenesis imperfecta: 10-year dental follow-up

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Osteogenesis imperfecta (OI) is a genetic disorder characterized mainly by varying degrees of bone fragility, osteoporosis, and predisposition to fractures. Common features of affected individuals include blue sclera, hearing loss, dentinogenesis imperfecta, osteoporosis, growth deficiency and joint laxity. It is classified into 13 subtypes and type IV, which is an autosomal dominant form is caused by the mutations in COL1A1 and COL1A2 genes. Here we present 10 year dental follow-up of a 17-year-old female with OI type IV. She had a history of multiple fractures, which have started at 1-year old. She was referred to pediatric dentistry department initially at age 7 because of yellow/brown discoloration of primary teeth with the attrition of the exposed dentin, early loss of primary teeth because of deep caries, anterior cross-bite and class III malocclusion. The clinical and radiographic examinations confirmed the diagnosis of dentinogenesis imperfecta. Throughout the follow-up period all restorative treatments and orthodontic treatment were performed. Physical examination at age 17 showed short stature and pale blue sclera besides dental findings. DEXA revealed osteoporosis. The molecular analysis of the COL1A1 and COL1A2 genes revealed the presence of a heterozygous transition G>C in exon 17 of the COL1A1 gene at position c.1084. In conclusion, it is suggested that dental status of OI cases should be examined as soon as teeth are erupted to prevent loss of tooth structure and to maintain their oral health.

D. Cogulu: None. **E. Yetkiner:** None. **I. Unal:** None. **H. Ulucan:** None. **O. Cogulu:** None.

P18.60

Unexpected Outcomes: Impacting Higher-Education Teaching Practice via Secondary School Outreach

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Funding agencies and the science-education research community have collaborated to implement programs designed to increase outreach from scientists to secondary-school students and teachers, with the goal of improving science learning by younger students. Simultaneously, but independently, they have collaborated on educational research projects to determine effective ways to improve instructional practices and learning in higher-education. However, improving university instruction through secondary-school outreach has never been the explicit goal of an educational research program in the United States. We will present results from a three-year outreach project in the U.S. that demonstrate how secondary school outreach, via partnerships between genetics researchers and high school teachers, can have the unintended and beneficial consequence of motivating instructional change by the research faculty in their own institutions. Although this was a gratifying and positive outcome that should be adopted more broadly, substantive and widespread change in university instruction is unlikely without systemic changes in higher education, such as the incentive system for tenure and promotion.

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P18.61

Pallister-Killian syndrome due to mosaicism of two supernumerary isochromosomes (hexasomy 12p) in a girl with relatively mild phenotype

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Pallister-Killian syndrome (PKS, OMIM #601803) is a rare and sporadic genetic disorder due to tissue-limited mosaicism for a small supernumerary marker chromosome (sSMC) (isochromsome 12p / i(12p)), which is usually present in fibroblasts but may be absent or at very low level in cultured lymphocytes. PKS was initially described in adults (Pallister et al., 1977) and children (Killian et al., 1981) comprising features of profound mental retardation, diaphragmatic defects and pigmentary skin anomalies.
Vogel et al., 2009 reported a girl with PKS and noted that this was the second case of mosaic hexasomy 12p due to two additional isochromosomes 12p. Van der Veyver et al., 1993 attributed a more severe phenotype in patients with hexasomy to a stronger gene-dosage effect compared to tetrasomy 12p. Since 2009, there have been no further reports on cases of hexasomy 12p.
We report on a 4.5-year-old girl with submucous cleft palate, cranio-facial dysmorphism (hypertelorism, broad nasal root, blepharophimosis, high forehead, sparse hair bilaterally, dental crowding), areas of hyper-/hypopigmentation on trunk and extremities and very mild developmental delay.
The detection of two isochromosomes 12p in 46% of skin fibroblasts but not in cultured lymphocytes confirmed diagnosis of PKS due to mosaic hexasomy. The relatively mild phenotype of our patient is in contradiction to the pure gene-dosage hypothesis previously proposed by others but strengthens the observation from Vogel that the severity of symptoms cannot be predicted by either gene-dosage or the percentage of mosaic cells alone.

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P18.62

Family history of cancer among Ashkenazi pancreatic cancer patients with a BRCA1/2 mutation compared to non-carriers

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Aim: This study investigated the family history of cancer in Ashkenazi pancreatic cancer patients, in carriers compared to non-carriers of a mutation in BRCA1/2 genes. Methods: Cancer family history was available for 90 pancreatic cancer patients, consecutively referred to our hereditary breast-ovarian clinic. Results: Fifteen of them (16.7%) carried a founder Ashkenazi mutation in either of BRCA1or BRCA2: nine (60%) carried the 6174delT mutation in BRCA2; five (33.3%) and one (6.7%) harbored the 185delAG and the 5382insC mutations in BRCA1 gene, respectively. The table illustrates the cancer family history of all 90 patients. Discussion: A family history of breast, ovarian and hepatic cancer is associated with a BRCA1/2 mutation in pancreatic cancer patients. Patients with a family history of breast-ovarian and pancreatic cancer showed a marginally stronger association to BRCA1/2 mutation compared to patients with a family history of cancer other than breast-ovarian-pancreatic cancer (p=0.065). Conclusion: These findings support the addition of pancreatic cancer within the entity of „hereditary breast-ovarian cancer syndrome“ associated with BRCA1/2 mutations.

Type of cancer	BRCA1/2 (n=15)	Non-Carriers (n=75)	Significance
Breast ≤3rd degree ≥4rd degree/none	8 (30.8) 7 (10.9)	18 (69.2) 57 (89.1)	0.022
Ovarian ≤3nd degree ≥4rd degree/none	4 (100.0) 11 (12.8)	0 (0.0) 75 (87.2)	0.001
Pancreas ≤3nd degree ≥4rd degree/none	3 (30.0) 12 (15.0)	7 (70.0) 68 (85.0)	0.216
Colon ≤3nd degree ≥4rd degree/none	3 (17.6) 12 (16.4)	14 (82.4) 61 (83.6)	0.575
Gastric ≤3nd degree ≥4rd degree/none	2 (25.0) 13 (15.9)	6 (75.0) 69 (84.1	0.398
Prostate ≤3nd degree ≥4rd degree/none	1 (16.7) 14 (16.7)	5 (83.3) 70 (83.3)	0.739
Brain ≤3nd degree ≥4rd degree/none	2 (50.0) 13 (15.1)	2 (50.0) 73 (84.9)	0.128
Liver ≤3nd degree ≥4rd degree/none	5 (50.0) 10 (12.5)	5 (50.0) 70 (87.5)	0.010

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P18.63

Demonstrating patient benefits from clinical genetics services: pilot studies in six UK centres

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Background and aims: Patient Reported Outcome Measures (PROMs) have recently taken centre-stage in UK healthcare evaluation, reflecting the NHS quality improvement agenda. PROMs are short self-completion questionnaires that measure healthcare quality from the patient's perspective. UK clinical genetic services have routinely been evaluated using process measures e.g. number of genetic tests done, waiting times, numbers of patients attending. To evaluate services more effectively a new clinical genetics-specific PROM, the Genetic Counselling Outcome Scale (GCOS-24) was developed and validated. This paper reports on pilot studies in six NHS clinical genetics centres that implemented GCOS-24 for service evaluation.

Methods: Patients were asked to complete GCOS-24 before and 4-6 weeks after clinic attendance. Satisfaction data was also collected. Data were analysed using analysis of variance and bivariate correlation. Centres provided feedback on feasibility of using GCOS-24 for service evaluation.

Results: Five centres demonstrated statistically significant improvement in GCOS-24 scores, following clinic attendance with usable samples sizes of 42, 45, 54, 55 and 74 patients respectively ($p<0.001$). Useful sample sizes were limited by less than optimal processes for matching patient's pre-clinic and post-clinic questionnaires, and one centre had insufficient matched pre-clinic and post-clinic questionnaires to enable a useful analysis. GCOS-24 improvement scores correlated significantly with patient satisfaction.

Conclusions: Findings demonstrated that NHS clinical genetics services are delivering significant measurable patient benefits and that GCOS-24 has potential to be a useful supplement to existing methods to evaluate quality of routine clinical genetics services. Consideration needs to be given to how to improve patient response rates.

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P18.64

Vertical transmission of a somatic PKLR mutation leading to non-spherocytic anaemia due to piruvate kinase deficiency: implications on genetic counselling

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INTRODUCTION

Chronic hemolytic anemia due to pyruvate kinase (PK) deficiency (MIM 609712) is a rare autosomal recessive disorder caused by mutations in the *PKLR* gene. An estimated 3% of PK-deficient patients present with a *de novo* mutation. We report for the first time a case of PK deficiency associated to vertical transmission of a *de novo* somatic mutation in *PKLR*.

METHODS

DNA analysis of *PKLR* was performed by conventional Sanger sequencing. Massive parallel sequencing of *PKLR* was performed on DNA from peripheral blood, mucosal swabs, 24 hours urine, and sperm samples from selected family members.

RESULTS AND DISCUSSION

The proband was an 18 month old boy suffering from severe transfusion-dependent chronic hemolytic anemia due to PK deficiency. PK activity in the mother resembled the PK-deficient carrier state whereas in the father was normal. DNA sequence analysis revealed the proband to be compound heterozygous for two missense mutations in *PKLR*: c.359C>T (p.Ser120Phe) and c.1168G>A (p.Asp390Asn). The c.359C>T change was found to be inherited from the mother. However, the c.1168G>A mutation could not be detected in blood father's DNA, despite confirmed father-mother-child allelic inheritance. Subsequent massive parallel sequencing of the region encompassing nt c.1168 on DNA from different tissues of the father indicated that this mutation has arisen postzygotically, thereby producing parental mosaicism. The methodology used allowed the detection of a somatic mutation in the father that was vertically transmitted to his son. This enabled us to unravel an exceptional case of a rare autosomal disease and, thereby, enabled proper genetic counseling.

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P18.65

A step-wise approach for the implementation of pharmacogenomics in developing countries in Europe

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Pharmacogenomics promises personalized treatment. However, many developing countries currently lack the knowledge or the resources to individualize drug therapy. Here, we propose a multi-step approach for the implementation of pharmacogenomics in developing
countries in Europe, including (a) Collection of DNA samples from healthy volunteers to determine the various pharmacogenomic markers allele frequencies in this population, using a 2-tiered approach (b) Conduct a comprehensive health economic analysis to illustrate the cost-effectiveness of pharmacogenomic testing, (c) Survey the level of awareness of several stakeholders, including genetic laboratories, pharmacists, the general public and healthcare professionals, (d) Organization of education activities to disseminate pharmacogenomics knowledge to society, and (e) Establishment of national guidelines for medication prioritization. Since 2009, we are implementing this approach using the Hellenic population as model. DNA from 45 healthy donors of Hellenic origin was isolated with consent, and subsequently genotyped to determine the allele frequencies in 1,936 pharmacogenomic markers in 220 pharmacogenes, using the DMET+ microarray (Affymetrix, Santa Clara, CA, USA).

We found 46 pharmacogenomic markers that display divert allele frequencies compared to those of the Caucasian population ($p<0.05$), that are currently being confirmed in 500 healthy donors of Hellenic origin. We are also concluding a cost-benefit analysis to demonstrate the usefulness of integrating pharmacogenomics in everyday clinical decision-making process, to adjust the acenocoumarol-dosing scheme, which would lead in the reduction of adverse reactions and the resulting healthcare costs. This approach could be replicated in other developing countries enabling integration of pharmacogenomics in healthcare decision-making at the country level.

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P18.66

Couples' attitudes toward PGD in Greece - Reproduction in crisis

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Objectives: To assess attitudes towards Preimplantation Genetic Diagnosis (PGD) among high-risk or infertile couples in Greece in relation to underlying demographic, medical and reproductive history predictors.
Method: 50 couples who were referred for PGD were interviewed in person after receiving genetic counseling. A semi structured questionnaire was answered based upon socio-demographic, educational, medical and reproductive history variables. The couples' awareness, opinion on advantages/disadvantages of the procedure and opinion of PGD comparing to Prenatal Diagnosis (PND) were also investigated.
Results: The majority of couples have a very burdened medical and reproductive history with β-thalassemia being the main reason for PGD referral (38%). 40% of couples were informed for medical matters directly by doctors, while medical geneticists or another medical specialty initially offered counseling for PGD in 90% of the cases. When comparing PGD to PND most couples consider PGD as a better alternative; for most of the couples having a healthy child and avoiding a selective termination of pregnancy are perceived as the main advantages of PGD. The main recorded disadvantages of PGD are low pregnancy rates and high cost given that financial difficulties were reported in 54,2% of the couples.
Conclusion: Couples' attitudes towards PGD in Greece are mainly positive as are very eager to have a healthy child avoiding PND despite PGD's high cost. Surprisingly they would prefer to avoid having another PGD cycle in the future given the anxiety of the whole procedure.

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P18.67

Translational impact of VEGFA variants on the prediction and follow-up of ocular complications in pseudoxanthoma elasticum.

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Background. Among the phenotypic characteristics of pseudoxanthoma elasticum (PXE), a rare ectopic mineralisation disease affecting skin, eyes and cardiovascular system, the ocular complications (neovascularisation, subretinal haemorrhage and vision loss) result in important morbidity. In the absence of genotype-phenotype correlations with the causal gene ABCC6, it has been suggested that variants in the VEGFA gene, encoding vascular endothelial growth factor, may influence the ocular phenotype. The aim of this study was to evaluate the clinical usefulness of such VEGFA variants in PXE counseling and ophthalmological follow-up.
Methods. The VEGFA coding region, introns and promotor were analysed in 66 molecularly confirmed PXE patients with mild, respectively severe retinopathy. Three categorizing methods were applied to define severe retinopathy: i) visual acuity (VA) <10/10, ii) VA < 5/10 (legal blindness) and iii) VA <5/10 and/or multiple anti-VEGF injections.
Discussion and conclusion. Significant association of 4 VEGFA SNPs with severe retinopathy was found only when using the third categorizing method which also takes into account anti-VEGF treatment, even with (near) normal VA. As such, the identification of these SNPs in a patient does not necessarily imply vision loss with significant impact on activities of daily living but rather an increased necessity for anti-VEGF treatment. This subgroup of patients might thus benefit from a more strict follow-up, emphasizing the importance of regular self-testing, and a more pro-active initiation and duration of anti-VEGF treatment. Whether this will eventually change the outcome will have to be evaluated prospectively.

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P18.68

Measuring maternal anxiety during child's development and its effects on psychosocial health and partner relationships

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Study aims: This study explores the consequences of being worried about the development of one's child and its association with psychosocial health.

Participants: The Norwegian Mother & Child Cohort Study (MoBa) is a vast resource of epidemiological and biological data collected throughout pregnancy and childhood. Pregnant women from 1999-2009 (N=approximately 100 000) were invited to participate in a national study regarding a diverse number of serious health issues in mother and child.

Methods: This is a cross-sectional study focusing on data (questionnaire) submitted by the mother when their child was 18 months of age. We applied the following tools: The Hopkins Symptom Check-List to measure psychosocial health and Relationship Satisfaction Scale to measure partner relationships quality.

Results: Preliminary results show that being worried about one's child's development has a negative influence on relationship satisfaction and mothers' psychosocial health. There is a statistically significant association between worry about developmental delay in the child and psychosocial problems (OR=5.39).

Conclusions: Findings apply to genetic counsellors, medical doctors and health care workers meeting these parents and assessing the child's development. Parents' psychosocial health needs should be addressed as anxiety may influence understanding and compliance, and to avoid long term implications for the mother and in turn the child. Communication techniques to lower levels of worry and facilitate coping are discussed.

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P18.69

Professional´s views on quality issues of genetic counselling offered for presymptomatic testing for late-onset neurodegenerative disorders: what is relevant?

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Quality assessment of genetic counselling practice for improving of healthcare is a challenge for genetic services worldwide. However, there is a scarcity of literature regarding quality issues in genetic counselling offered in the context of presymptomatic testing for late-onset neurological diseases. Neither common definitions of quality indicators, nor appropriate guidelines for effective practice exist internationally. The aims of this qualitative study were (1) to explore professionals' views of relevant quality indicators in their own genetic counselling practice; and (2) to describe how presymptomatic testing protocols are currently being implemented in Portugal. We undertook interviews with 18 professionals from the major genetic counselling services for these diseases in Portugal (85% of the professionals currently involved). Core components of genetic counselling encompass providing information and decision-making support, informing the consultand about the protocol and exploring their motivations, anticipated changes, results expectations and personal and familial experience with the disease. Identification of quality indicators for effective practice was less clear. Professionals also discussed some specific challenges in practice, such as the ambiguity of the health/illness status and confirming consultands' autonomy during the process. When questioned about the consultands' views of what would be effective in practice, some reported they had not reflected on the topic previously. Although national guidelines and legislation exist, professionals described concerns regarding heterogeneous standards of practice, mainly due to the lack of regulation regarding genetic counselling outside credited centres and in private services. We conclude that a credible set of quality indicators for presymptomatic testing is required.

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P18.70

Centers for rare diseases at the University Hospital Brno, Czech Republic (CZ)

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In accordance with the „Council Recommendation on an action in the field of rare diseases" the Government of the CZ approved the National Strategy for rare diseases.
„Interdepartmental and interdisciplinary working group on rare diseases" runs under the czech Ministry of Health, and coordinates the establishment of centers for rare diseases. The main co-ordination center is located in the University Hospital Motol, Prague cooperating with the center at the University Hospital Brno.
In University Hospital Brno the EB Center was established for all 183 patients with Epidermolysis bullosa congenita (EBC) from CZ and CF Center for patients with Cystic fibrosis (CF), one of five CF Centers in the country.
A team of 19 EB specialists provides health care, including genetic counseling and prenatal and postnatal DNA diagnostics. The EB Center cooperates with EB Haus Austria and with Debra CZ, which supports families of patients with EB. EB Center and Debra CZ are involved in various educative activities.
A team of experts from CF Center Brno currently cares for 63 children and 30 adults with CF, provides genetic counseling and DNA analysis of the CFTR gene. University Hospital Brno is also responsible for newborn screening for CF (NSCF) for the Moravia region (about 40,000 newborns/year).
Centers for Orphan cooperate with patient organizations associated in the Czech Association of Rare Diseases. A important part of the work of all experts on rare diseases is an effort to raise awareness about rare diseases among specialists and the public.

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P18.71

Medicinal products for rare diseases in Europe

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Having knowledge of the development of medicinal products from their preclinical phase to their marketing authorization is of great relevance for the rare disease community. It is for this reason that Orphanet developed a database of orphan designations, medicinal products and clinical trials related to them, allowing for the analysis of trends in clinical research and development. Today 144 drugs have been granted a European Marketing Authorization (MA) through the centralized procedure. Of these, 66 drugs, targeted nearly 70 diseases, are orphan medicinal products (OMP, so-called orphan drugs) that have been granted a European orphan designation (according to the Regulation (EC) No141/2000), and that have then been granted a European MA and - if applicable - a positive evaluation of significant benefit. Ten orphan medicinal products have been granted a European Marketing Authorization in 2012. There are also 78 drugs bearing one or more indications for a rare disease or a group of rare diseases. These OMPs have received a European marketing authorization for one or more indication(s) of use for a rare disease, but they have not been granted a European orphan designation or their designation was withdrawn. In total, more than 120 diseases have a product with an official indication to treat them. On the top of the list of diseases concerned by clinical development are rare cancers, which are widely represented (acute myeloid leukemia, multiple myeloma), as well as some low prevalence (<1/10,000) diseases such as cystic fibrosis, Duchenne muscular dystrophy and pulmonary arterial hypertension.

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P18.72

Support to the International Rare Diseases Research Consortium

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The International Rare Diseases Research Consortium (IRDiRC) joins members that share common goals and principles and have agreed to work in a coordinated and collaborative manner within a multinational consortium. The objective is to team up researchers and organisations investing in rare diseases research in order to achieve two main objectives by the year 2020, namely to deliver 200 new therapies for rare diseases and means to diagnose most rare diseases. The members are research funding organizations dedicating over 10 million US$ to research into rare diseases. A number of challenges will need to be addressed through collaborative actions: establishing and providing access to harmonised data and samples, performing the molecular and clinical characterisation of rare diseases, boosting translational, preclinical and clinical research, and streamlining ethical and regulatory procedures. The consortium has established three Scientific Committees. The Diagnostics committee advises on research related to the diagnoses of rare disease, including sequencing and characterization of these diseases. The Interdisciplinary committee provides expertise on cross-cutting aspects of rare diseases research including issues related to ontologies, natural history, biobanking, and registries. The therapies committee gives guidance for the pre-clinical and clinical research aiming to deliver new therapies for rare diseases. The guiding principles, the plan for action and the achievements so far will be presented, as well as the way for the genetic research community to get engaged in this global effort. The scientific secretariat of this International consortium is established at the rare Diseases Platform in Paris.

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P18.73

Orphanet.co.uk and Orphanet.ie are for you

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Orphanet is the reference portal for information on rare diseases and orphan drugs for all audiences. Orphanet provides free and direct online access listing around 6,000 rare diseases, a classification of rare diseases elaborated using existing published expert classifications, a directory of specialised services, an inventory of orphan drugs, an 'assistance to diagnosis' tool, emergency guidelines, the EUCERD's newsletter and the Orphanet Report Series.
The Orphanet entry points for the UK (www.orphanet.co.uk) and Ireland (www.orphanet.ie) were launched on February and March 2011 respectively. They provide specific national information including: the Orphanet team and its Scientific Advisory Boards, collaborators, patient organisations and private not-for-profit funding bodies.
The sources of information and inclusion criteria for the UK were recently added to clarify the registration process. Online registration forms for patient organisations, expert centres, research projects, clinical trials and registries have also been added to simplify and accelerate this process.
Aiming to increase national awareness of rare diseases and the availability (or not) of services, we are now planning to add information about national activities; any patient organisation, funding body, research/clinical group, laboratory or other relevant stakeholder is invited to submit an article for publication on the national home page.
We also aim to promote interaction between the Orphanet national team and the rare disease community (patients/families and/or professionals); work is underway to collect feedback from users, such as "like" buttons, "suggestions" fields, or gathering satisfaction levels on different national events or Orphanet-related matters.

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P18.74

Representing the evolution of genetic testing: new services at Orphanet to adequately serve the end users

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Techniques and processes used to diagnose diseases evolve quickly, creating a challenge for providing access to stakeholders. Over the years, Orphanet has maintained a database of genetic tests in 38 countries, in collaboration with EuroGentest. This must now evolve to meet the professional's needs. Besides the classical representation of the test -gene(s)- disease(s) triplet, which allows one to search for a test by the disease or gene name, it is now necessary to represent new generation of techniques capable of testing an entire panoply of genes.

The techniques/processes will be grouped into 8 categories: molecular genetics, molecular cytogenetics, biochemical tests, immunology, microbiology/virology, hematology, pathology, and Imaging. More specifically, "Molecular genetics" will be split into: mutation targeting or scanning, sequencing of total or partial gene sequences, CNV by array or MLPA, whole genome or exome sequencing, methylation analysis, uniparental disomy study, allowing for a search by techniques.
Furthermore, the purpose for testing will be annotated: antenatal diagnosis, pre-implantation diagnosis, post-natal diagnosis, pre-symptomatic diagnosis, pharmacogenetics, risk assessment and newborn screening.
The search tool currently allows Orphanet websites users to retrieve a specific test for a disease or a gene as well as the quality assessment and the testing site. It will now also offer the possibility to choose the technique / process, or the purpose of the test. Additionally, the ability to find a laboratory by its EUGT number, name of institution, location, technique/process or purpose, without any need for the entry for a specific disease, will be possible.

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P18.75

The marketing of gene therapy medicinal products: What are the regulatory challenges?

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In 2003 Gendicine (injection to treat head and neck cancers) has been approved by the Chinese State Food and Drug Administration. It became the first gene therapy medicinal products (GTMP) to be marketed in the world. However, the equivalent of Gendicine, Advexin did not reach the European authorisation. Almost ten years later, in October 2012, Glybera (to treat lipoprotein lipase deficiency) was finally granted marketing authorization by the European Commission. After a chaotic regulatory process it became the first GTMP to be approved in Western countries. Although approvals of these GTMP have mainly been seen as a huge step for patients and for the future of gene therapy, both regulatory processes have been challenged. On the one hand, the relevance of assessment's criteria by the relevant institutions is questioned. China and the European Union do not have the same eligibility criteria for marketing authorization. But in any case, the balance benefit/risk is particularly difficult to assess for GTMP. Beyond the safety criteria, efficacy raised high concerns. It led Gendicine to be seen as a quite controversial drug for Western countries while Glybera suffers from several negative opinions at the European Medicine Agency's relevant committees. On the other hand, going through the whole regulatory process requires a very substantial investment for companies, especially in time and costs. Based on the experience of Gendicine, Glybera and Advexin, this paper aims to highlight the regulatory challenges that GTMP faced to be marketed.

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P18.76

Experiences and attitudes of Flemish pharmacists towards (genetic and non-genetic) self-testing. An interview study

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An increasing number of medical 'self-tests' with the goal of detecting the risk for certain conditions are becoming available to consumers. Although various of these (genetic and non-genetic) self-tests are available through the Internet, pharmacists are increasingly confronted with the availability of these self-tests and the consumers who purchase them.The objective of this study was to explore the experiences of community pharmacists with regard to (genetic and non-genetic) self-tests and their attitudes towards those self-tests, as well as their role towards consumers and other health care professionals. 29 community pharmacists spread over Flanders were interviewed. A semi-structured interview was designed that incorporated open-ended questions regarding their knowledge about self-tests, their experiences with self-tests, their views with regard to self-tests, and their perspectives on their role related to the provision of self-tests as well as their relation to other health care professionals. Overall, community pharmacists had only limited experiences with consumers asking for self-tests. Although community pharmacists advanced potential benefits of self-tests, they emphasized as well potential concerns such as the limited predictive value, false positive and false negative test results, misuse of the device, the psychological impact of the result, or the absence of a medical framework. Most community pharmacists see an important role for their profession in relation to self-tests, especially in the provision of information when delivering a self-test and in the interpretation of the results. However, they underlined as well the non-diagnostic purpose of their role and the importance of consumers interacting with physicians.

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P18.77

When a patient sends you a 'friend request': Social networking in the context of Genetic Counselling.

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Internet-technology (IT) is a major part of everyday-life, both professionally and personally. As an "engine" of globalisation, its use revolutionised the way we communicate with others, distribute and access information. Although beneficial, just like any other innovation, IT
has certain disadvantages. The use of IT has been integrated into healthcare through novel approaches like *internet-based-telemedicine (IBT)*. IBT creates opportunities for underserved patients to access healthcare which can positively influence the patient/healthcare professional (HCP) interaction. Unfortunately, there are instances that can risk the dynamics of this interaction. HCPs have ethical responsibilities towards their patients including the preservation of a professional relationship with a patient within moral boundaries. Social-networking websites, such as facebook, can put HCPs in difficult situations e.g. being "facebook-friends" with patients can potentially blur the boundaries of their relationship. The small bi-communal composition of Cyprus, culturally, has a "close-nit" characteristic, where everyone knows everyone". The Clinical Genetics Clinic (CGC) has substantial experience in providing genetic services in a relatively small community, where balancing the equilibrium of patient/HCP relationship can be a delicate issue. In the recent years, with the popularity of facebook, a new dilemma has emerged in terms of whether it is appropriate for HCPs to be "facebook friends" with patients. Although this issue is addressed in medical literature mainly for physicians, it hasn't been addressed for genetic specialists such as genetic counsellors and/or clinical geneticists. This poster will reflect on the experiences of CGC-team in the added effect of facebook upon genetic counselling in a small-community.

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P18.78

15 years of education and volunteering service with Down Syndrome groups

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Introduction: Aim of this paper is to describe the changes in life quality of people with this syndrome, after 15 years of multidisciplinary volunteer work with groups from Western part of Romania. We work together with other NGO from Romania and founded an association - ANBRaRo - dedicated to all support groups of people that start occupied with genetic transmitted diseases.Material: In 1997 Save the Children - Timisoara formed support group for children with Down syndrome- a "club". We worked with high school volunteers from 1997 and after 2005 together with students from our medical university - they made teams around 5-10 persons. Around 35 people with Down syndrome were our beneficiaries in all these years. In our Down club we start one by one relation with volunteers. In specific moments we performed psychological evaluation and motor testing using kinethoterapic observation. Results: Assessment of their advancement might establish the exact role of communication among the Down Club. Parents have observed important cognitive changes in their children - at the beginning was the only method of evaluating their progress, but emotional and social improvement. Psychological evaluation was performed in specialized centers. Conclusions: The former children from "Down club" evolved in social life easier and have established professional contacts and school relationships much faster. Continue monitoring and participation to support programs led to easy integration in the community. In the absence of a coherent state policy, input from various NGOs is crucial for good independent life skills to this category of beneficiaries.

M. Gafencu: None. **V. Stan:** None. **S. Per:** None. **Z. Andrei:** None. **G. Doros:** None.

P18.79

Telemedicine use in Clinical Genetics; a European survey

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Introduction: The demand for genetic counseling is increasing, while simultaneously health care resources are scarce.
US and Australian reports show that Telecounseling (TC, real-time counseling via internet and webcam) can save time and costs, when it is used as an alternative for traditional face-to-face outpatient counseling. TC also allows for a more flexible planning of consultations for both patient and counselor. We successfully completed a TC pilot study in oncogenetics and cardiogenetics at our department. Our aim was to inventorise if comparable TC-programs in Europe are initiated and what their characteristics are, to allow for exchange of experiences and collaboration.
Methods: We set up a cross-sectional survey in collaboration with the American Telegenetics Workgroup. The survey was spread amongst 927 clinically and non-clinically working ESHG-members of whom email-addresses were available for use between November 2012 and January 2013.
Results: 120 ESHG members (92 institutions in 39 different countries) completed the survey. Members reported the use of telephone-only counseling (20%) and various web-based counseling methods (6-14%). 24% of respondents indicated videoconferencing facilities being available at their departments, though 78% of them do not use these. 56% of respondents indicated to be interested in joining a meeting or participating in a working group on Telegenetics.
Conclusion: While TC seems a promising modality to meet increasing health care demands, only about 20% of respondents use some form of TC. Setup of an ESHG working group is recommended to support the use of TC, collaboration and exchange of experiences.

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P18.80

Congenital lamellar ichthyosis in Tunisia is caused by a founder nonsense mutation in the TGM1 gene

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Lamellar ichthyosis (LI, MIM# 242300) is a severe autosomal recessive genodermatosis present at birth in the form of collodion membrane covering the neonate. Mutations in the TGM1 gene encoding transglutaminase-1 are a major cause of LI. In this study molecular analysis of two LI Tunisian patients revealed a common nonsense c.788G>A mutation in TGM1 gene. The identification of a cluster of LI pedigrees carrying the c.788G>A mutation in a specific area raises the question of the origin of this mutation from a common ancestor. We carried out a haplotype-based analysis by way of genotyping 4 microsatellite markers and 8 SNPs flanking and within the TGM1 gene spanning a region of 6 Mb. Haplotype reconstruction from genotypes of all members of the affected pedigrees indicated that all carriers for the mutation c.788G>A harbored the same haplotype, indicating common ancestor. The finding of a founder effect in a rare disease is essential for the genetic diagnosis and the genetic counselling of affected LI pedigrees in Tunisia.

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P18.81

Thalassemia genetic counselling in Greece: an initial evaluation.

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Genetic counselling consists a vital part of prenatal decision making and this should be performed according to international guidelines, adopted to ethnic necessities. Thalassaemia and haemoglobinopathies are the most frequent genetic diseases in Greece. The National Thalassaemia Prevention Program, established thirty years ago, proved to be quite effective as it led to a severe reduction of affected births. A slight increase in affected newborns, observed recently, prompt us to evaluate the efficacy of the genetic counselling offered. Towards evaluation of the counselling services provided, two questionnaires were completed by 100 individuals requesting prenatal testing for thalassaemia. Remarkably, a noteworthy proportion of the participants did not adequately comprehend the provided information. Our results showed that certain fields require more detailed, intensive and simplified communication. The percentages of areas less understandable and thus requiring improvement are: mode of inheritance (~60%), combination of disease causing mutations (~25%) as well as knowledge about severity of the disease in terms of life expectancy (~30%) and lack of radical treatment (~35%). Furthermore, the majority of people declared not informed about prenatal diagnosis options as well as the necessity of prenatal testing for other high prevalence genetic diseases.
These results are contradictory to the long life time of the national prevention program in the country. It is worth mentioning that genetic counselling in Greece is provided by healthcare professionals, not specialised accordingly. These data suggest the urge for the official establishment of genetic counselling specialty in Greece, as a distinct specialty in human / medical genetics.

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P18.82

Somatic mosaicism in trichorhinophalangeal syndrome: a lesson for genetic counselling

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Trichorhinophalangeal syndrome type I (TRPSI) is a genetic disorder characterized by sparse hair, a bulbous nasal tip, short stature with severe generalized shortening of all phalanges, metacarpal and metatarsal bones and cone-shaped epiphyses. This syndrome is caused by autosomal dominant mutations in the TRPS1 gene. However because recurrence has been observed in siblings from healthy parents, an autosomal recessive mode of inheritance has also been suggested.
We report on a male patient, born to healthy unrelated parents, with TRPSI. Using Sanger sequencing, we identified a mutation in the TRPS1 gene (c.2735G>A, p.Cys912Tyr). The same mutation was detected as a 10% mosaic mutation by Pyrosequencing in blood-derived DNA from his healthy mother.
To our knowledge, this is the first time that somatic mosaicism has been identified in TRPSI. This data combined with the observations of recurrences in siblings from healthy parents modifies the genetic counselling for TRPSI which should discuss a 5 to 10 percent recurrence risk for healthy parents with an affected child because of the possibility of germinal mosaicism.

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P18.83

TRPC6 mutation is a rare cause of steroid-resistant nephrotic syndrome in children

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Heterozygous mutations of the TRPC6 gene have been reported in ~5% of autosomal-dominant podocytopathies with focal segmental glomerulosclerosis (FSGS). Most patients present with nephrotic range proteinuria in their thirties-fifties, and progress to end-stage renal failure within 10 years. Recently, TRPC6 mutations have been identified in 4 children; one of them had severe collapsing FSGS at 2 years of age.
A boy, born to non-consanguineous parents presented at the age of 6 years with nephrotic syndrome, chronic kidney disease and hypertension. The renal biopsy showed collapsing FSGS with 50% of sclerotic glomeruli. The

renal function rapidly worsened despite steroid therapy, and peritoneal dialysis was started within 2 months. He was transplanted one year later, with no recurrence. The eGFR remained stable around until the last follow-up 20 years later.
Genetic testing revealed a de novo heterozygous mutation c.2678G>A (p.Ser893Asn) in the exon 13 of the TRPC6 gene. This missense mutation is predicted to be "probably damaging" by the Polyphen2 software with a score of 1. The TRPC6 gene encodes a non-selective cation-channel highly expressed in podocytes. It mediates intra-cellular calcium influx and plays a major role in signaling cascades of the slit diaphragm where TRPC6 interacts with nephrin and podocin. The mutation involves a highly-conserved residue located in the last cytoplasmic domain of the protein, that could participate in the assembly of subunits of TRPC6 and its interaction with membrane phospholipids.
In conclusion, TRPC6 mutations can be detected in early-onset and severe forms of familial and sporadic
steroid-resistant nephrotic syndromes.

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P18.84
A systematic approach to clinical classification of DNA sequence variants in mismatch repair genes: the InSiGHT initiative

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Sequence variants of uncertain significance are common in genetic test reports. Several parameters can be evaluated to assess their significance, but usually none of them can be used on its own to obtain clinically useful interpretation. The Variant Interpretation Committeee of the International Society for Gastrointestinal Hereditary Tumors (InSiGHT), comprised of a multidisciplinary panel of 41 experts, has undertaken a collaborative effort to establish interpretation guidelines, to encourage data submission and to provide objective assessment of available variant information for the mismatch repair (MMR) genes. Guidelines matching the five-tiered classification scheme proposed by the IARC Unclassified Genetic Variants Working Group were devised based on scientific evidence and expert opinion. The scheme was tested on different sets of MMR variants. Data for evaluation included co-segregation with phenotype, family history, co-occurrence with clearly pathogenic variants, frequency in controls, tumor molecular characteristics (microsatellite instability and immunohistochemistry of MMR proteins), effects on RNA (assessed on patient samples or by minigene splicing assays) and in vitro functional consequences. Data were retrieved from the LOVD database (http://www.insight-group.org/mutations/); submission of unpublished information from InSiGHT members was encouraged, leading to accrual of substantial amounts of novel data. The approach was applied to all 2,759 variants listed in the database, including 129 with discordant interpretation provided by difefrent submitters. This large-scale endeavour has important implications for the clinical management of suspected Lynch syndrome families and provides an important model of international multidisciplinary collaboration for DNA variant interpretation.
Presented on behalf of the InSiGHT Variant Interpretation Committee.

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P18.85
The Challenges of Genetic Counselling in Vascular Ehlers Danlos Syndrome

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Vascular Ehlers Danlos Syndrome (Vascular EDS) is a dominantly inherited connective tissue disorder. Mutations in the COL3A1 gene affect collagen type III which is found mainly in the walls of hollow organs and blood vessels. The main clinical features of Vascular EDS include:
- a tendency to very easy bruising
- thin skin
- fragile blood vessels which can lead to complications due to rupture of arteries
- hollow organ rupture
- premature death

The National Diagnostic Service for EDS has now been running since April 2009 and was set up to diagnose complex cases of EDS. Genetic testing for Vascular EDS has been available in the UK since 2007 with a 99% pick up rate. Over 30 patients have now been diagnosed with Vascular EDS by the National Diagnostic Service. Some of these individuals did not present with the classical phenotype or clinical history. We have also seen a number of individuals who were referred with the diagnosis for specialist advice on the condition. We review recent data gathered from the clinic and discuss cases which highlight some of the issues that have emerged and to share our experiences of genetic counselling with patients who are newly diagnosed with Vascular EDS.

J.H. Tocher: None. **J.M. Bowen:** None.

P18.86
Challenges of clinical whole exome sequencing and practical considerations for genetic counseling

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Objective: Our objective is to demonstrate how clinical whole exome sequencing (WES) testing has impacted our genetic counseling (GC) practice.
Methods: Between Johns Hopkins Hospital (JHH) and Kennedy Krieger Institute (KKI), 93 cases have been sent for clinical WES from Nov 2011-Feb 2013. For each case data was gathered for result types and time spent in case management. Data was analyzed using STATA Statistical Software.
Results: Of the 29 genetic counselors at both JHH and KKI, only 6(21%) have thus far sent clinical WES for various indications. The use of this testing has been utilized by the pediatric, general genetics and neurogenetics clinics. Of the 93 cases sent for WES, results of 33 have come back thus far: 12 Negative (36.36%); 13 Positive (39.39%); and 8 with Variants of Unknown Clinical significance (VUS) (24.24%). The time spent in each category required for case management is as follows: time spent pre-test counseling N=84 (Mean 56±16.0 min); time spent researching results in light of patient phenotype N=18 (88±58.4 min); time spent post test GC N=13 (65.8±28.4 min); time spent writing result notes N=16 (71.2± 43.3 min).
Conclusions: The above preliminary data demonstrate an increased time required in patient case management. An increased amount of time was spent in interpreting the results in light of the patient's phenotype and medical documentation of results, which are non-billable time. Though major conclusions cannot be generated from this preliminary data, it suggests that many results, whether classified as "Positive" or "VUS", only partially explained patients' phenotypes.

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P18.87
Genetic basis of Xeroderma pigmentosum in MENA region: consequences for patient management and care

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Xeroderma pigmentosum (XP) is a rare autosomal recessive disorder characterized by a high skin sun-sensitivity predisposing to skin cancers. In Tunisia, the frequency of the disease was estimated to 1/10000. Tunisian XP patients have been classified in three forms: severe, intermediate and moderate.
In the present study, 114 patients were investigated by direct sequencing or genotyped using STR to confirm linkage by homozygosity mapping strategy. Our preliminary molecular investigation showed homogeneity of the mutational spectrum for *XPA* and *XPC* genes. Indeed, among studied cases, 24 patients (21%) affected by intermediate cutaneous and neurological manifestations shared the same mutation in the *XPA* gene: p.R228X and 44 patients (38.6%) with the severe clinical form have the same mutation in the *XPC* gene: p.V548AfsX25. These are two founder mutations as they segregate each in a single haplotype. Twelve patients (10.5%) with the moderate clinical form are linked to the *XPV* gene with four different haplotypes. For

the remaining cases, the aetiology of XP is still unknown.
Having only two recurrent mutations that are responsible for the majority of explored patients made it easier to propose genetic counselling and prenatal diagnosis for the investigated families. Indeed, six consanguineous XP Tunisian families (4 XP-A and 2 XP-C) and one XP-C Libyan family have benefited from a prenatal diagnosis.
Based on a common history of settlement and the share of a similar genetic background, the mutations identified in the present study could be prioritized for mutation screening in other similar populations particularly from the MENA region.

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P18.88

Heterogeneity of XP-C group in Tunisian families: two novel mutations

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Xeroderma Pigmentosum (XP) is a rare recessive autosomal cancer prone disease, characterized by UV hypersensitivity and early appearance of cutaneous and ocular malignancies.
In the present study we investigated four patients suspected to be affected by XP-C group. These patients were from consanguineous unrelated families. Among them, three patients developed cancers: two patients (XP45 and XP50) developed melanoma. Internal tumors was also observed in patient XP28 and XP45: Thyroid cancer and benign articular tumor of the knee respectively.
Sequencing of the exon 9 showed absence of the frequent mutation XPC p.V548AlafsX25 in these patients. However genotyping and linkage analysis revealed possible involvement of XPC gene, this gene was screened for mutation by direct sequencing
Mutation analysis revealed the presence of two novel mutations. The first mutation is a splice site transition in intron 6 present in XP50 patient that abolishes the splice donor site and leads to a premature stop codon. The second one is a nonsense mutation located in exon 7 that has been identified in the three remaining patients. These originating from three cities of Southern Tunisia and bearing the same haplotype, in favor of a founder effect. Reverse Transciptase PCR revealed the absence of the mRNA of the XPC gene in all patients, thus confirming the hypothesis of mRNA degradation by the Non sense Mediated mRNA Decay system.
We concluded that in Tunisia, XP-C group seems to be homogeneous with some pockets of heterogeneity, that should be taking into account to improve molecular diagnosis of this disease.

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P19.01

Corpus Callosum Malformations: a neuropathological description and classification in a series of 105 fetuses

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Corpus callosum (CC) is the major brain commissure connecting the homologous areas of both hemispheres at the midline. CC malformations (CCM) are the most frequent brain malformations with an incidence of 1/4000 newborn often associated with chromosomal anomalies or mendelian syndromes with recessive and dominant inheritance. Recurrence is observed in 5 % of cases. Children with CCM have an uncertain neuro-developmental outcome. Therefore, counseling remains challenging. A monocentric retrospective study was carried out between 2000 and 2012, on fetuses whose pregnancy was terminated for CCM observed on US and/or MRI. In 105 fetuses, neuropathological examinations permit distinction of four groups: I: abnormal decussation of callosal fibres (48 cases); II: cortical malformations (34); III: abnormal growth of CC (7) and IV: dysplasia of CC (16). Most importantly, among 61 cases where CCM was thought to be isolated antenatally, another brain and/or extraneurological signs were found in 32 cases (50%). To date, chromosomal and molecular screenings permit identification of a cause in 18 cases of this cohort (17%). This includes trisomy 18, der(7)t(7;8), add(1)(q43), add(8)(q11.21), 1q44, 3q13.2, 11p13, 14q12 and 17p (*LIS1*) deletions respectively, dup(19)(p13), tetrasomy 9p, as well as mutations in *TUBA1*, *TUBB2B* and *OFD1* genes. In this heterogeneous cohort, morphological approach based on steps of callosal development provides valuable insight into the underlying mechanisms of CCM. This study will be instrumental for genotype-phenotype correlations and identification of the causal underlying molecular defect in unexplained cases that will be undertaken using a high throughput sequencing approach.

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P19.02

Prenatal diagnosis of 22q11 deletions: a collaborative, retrospective analysis by 27 laboratories

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Objective: The 22q11 deletion (del22q11.2) is one of the most common human microdeletions and is associated with a wide spectrum of abnormalities. The objective of the present study was to evaluate the number of pregnancies diagnosed with a del22q11.2 in French laboratories and to describe the corresponding prenatal, clinical features.
Methods: 27 laboratories collaborated to perform a retrospective analysis of data on fetuses with a del22q11.2.
Results: 204 fetuses with a del22q11.2 were identified. The mean time of diagnosis was 25.6wg. Diagnosis was mainly performed after an ultrasound examination. The most frequent malformations were congenital heart defects (CHDs, in 82.4% of cases). The other typical 22q11 deletion syndrome features were polyhydramnios (n=21), kidney abnormalities (n=16), thymus abnormalities (n=8) and cleft palate (n=6). The mean time of diagnosis was significantly shorter for the 69.8% of couples who decided to terminate their pregnancy than for those who decided to continue (24.8wg vs 28.2wg; p=0.0015). Interestingly, the del22q11.2 was inherited in 24.3% of cases. A fetal pathological examination was performed in 56 cases. Even though CHD was still the most frequently observed disease feature, thymus abnormalities were detected more frequently in the pathological examination (n=26 (46.4%), only two of which had been identified on ultrasound). The facial dysmorphism observed in 42 fetuses (75%) with del22q11.2 had not been detected on ultrasound.
Conclusion: Out of CHD that prompt a del22q11 analysis, we found that polyhydramnios and kidney abnormalities are the most frequent. Furthermore, the time of pregnancy termination depended on the time of diagnosis.

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P19.03

Application of molecular methodologies for the identification of genetic abnormalities in aborted fetuses/ intrauterine deaths that failed to grow in vitro

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Of all the recognized pregnancies, about 10-15% ends in clinical miscarriage/spontaneous abortion, usually towards the end of the first trimester. Of these 50% have a chromosomal abnormality, if they are all successfully cultured. Cytogenetic analysis of abortion samples can be limited by culture failure and this is attributed to the in vivo death of tissue. Genome-wide array-CGH in combination with QF-PCR can be implemented as an alternative to chromosomal analysis for samples that failed to grow. In addition, using array-CGH it is possible not only to detect aneuploidies but also aberrations that are beyond the resolution of chromosomal analysis.
Here we present our results from application of combined genome-wide array-CGH and QF-PCR analyses in 66 selected samples from first and second trimester Product of Conception/Intrauterine deaths/Stillbirths which failed to grow in vitro. Abnormalities were identified in 32 samples (48.4%).
Twenty-one autosomal full trisomies, seven sex-chromosome aneuploidies and four triploidies were detected. No submicroscopic copy number changes were detected.
The benefits these methods offer, in POC/intrauterine death/stillbirths samples are evident considering the fact that around 30% of the total of these samples received by the laboratory over a year would have failed. Moreover, the turnaround time is dramatically decreased compared to chromosomal analysis.
Finally, a very small amount of DNA is required for both of the analyses to be carried out. The limitations of the combined methodology lie with the fact that they cannot detect balanced rearrangements, therefore chromosomal analysis of parental samples is required when an aneuploidy is detected.

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P19.04

Diagnosis by a Camerounese-Swiss collaboration of a 610 kb MECP2 duplication leading to a prenatal diagnosis in a Camerounese woman.

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A 610Kb duplication in the Xq28 region encompassing *L1CAM, IRAK1* and *MECP2* genes resulting in a functional disomy, was diagnosed by Array-CGH in a 5 years old boy with global severe devopmental delay, hypotonia, recurrent infection and hypoplastic genitalia/cryorchidism. He was the second of a sibship of 2 and the first boy, affected by the same clinical symptoms, died within a seizures context. The mother was found to be carrier of the same Xq28 duplication with a biais of X-chromosome inactivation of 100%. Prenatal diagnosis was done for a pregnancy showing a girl carrier of the maternal duplication and X-chromosome 100% inactivated. At 6 month's old, the girl has a satisfactory development. More of 45 patients have been described in the literature showing Xq28 duplications ranging from 0.2 to 22 Mb. *MECP2*, is a dosage sensitive gene described in X-linked mental retardation syndrome, with typical features of infantile hypotonia, poor speech development, recurrent infections, epilepsy, and progressive spasticity. Duplications Xq28 lead to a slighter phenotype in women, as a consequence of a normal random inactivation.
The clinical service of Genetic Medecine exits in Cameroun since 2007. The consultations identified a large number or patients with mental retardation. The coverage of the affected patients quickly highlighted a lack of local resources also technical as human. This case put in evidence the efficiency of an international collaboration tightening the links between European laboratories and the African country. The training of local teams and the patient's access to diagnosis remain the priority.

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P19.05

Prenatal Diagnosis by aCGH on Uncultured Prenatal Samples, Validation Study

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Objective: We aimed to assess the suitability of using small aliquots of uncultured prenatal samples for routine aCGH analysis using a targeted BAC array.
Methods: The Cytochip Focus Constitutional array (Cambridge Bluegnome) was evaluated with an automated DNA extraction method (Qiagen) using 70 consecutive amniotic fluids (2ml) and CVS samples (0.5ml of cell suspension). DNAs were tested by QF-PCR to confirm extraction efficiency and to exclude maternal contamination. Hybridisations were performed with slight modifications of the manufacturer protocol and data analysed using Bluefuse V2.6 software. All samples were also cultured and karyotyped.
Results: Auto-passed QC check were obtained in all cases, suboptimal pass was observed in 5% (up to 2 parameters outside optimal range) while 3 samples failed. Five chromosome abnormalities were identified 3 of which were also detectable by cytogenetic analysis (one mosaic trisomy and 2 unbalanced rearrangements). Two abnormalities (1 microdeletion and 1 mosaic trisomy) were detected in the group of samples with suboptimal data. Results were confirmed by either FISH or cytogenetic analysis. Seven benign CNVs were observed, none of uncertain significance using default smoothing values of 3 clones.
Conclusions: The Cytochip Focus provided clear and easily interpretable results. It allowed detecting 2 chromosome abnormalities otherwise invisible by cytogenetic analysis without exposure of CNV with uncertain clinical outcome. The assay was robust enough to also detect abnormalities in presence of suboptimal data. In our experience only 2ml uncultured AF provided enough DNA to perform up to 4 hybridisations thus easily allowing confirmation of abnormal results.

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P19.06

Non-invasive prenatal detection of achondroplasia using cell-free fetal DNA: 2 case reports from the prenatal centre in Czech Republic.

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Background: Achondroplasia is one of the most common form of short limb dwarfism with incidence ranges from 1/10 000 to 1/30 000 life birth. The majority of cases is sporadic and results from a *de novo* mutation c.G1138A in *FGFR3* gene (98% cases). Prenatal ultrasound examination of achondroplasia often fails but sometimes it is suspected during the last trimester of pregnancy mainly by evidence of short limbs. Our aim is to improve prenatal diagnosis of this condition by implementation of cell-free fetal DNA analysis (cffDNA).
Methods: cffDNA was extracted from maternal plasma between 17 and 36 gestation weeks. PCR combined with restriction analysis and followed by fragmentation analysis was used for c.G1138A mutation detection. The presence of cffDNA was confirmed by real-time PCR detection of DYS14 marker (male fetus) or methylation status analysis of marker RASSF1A (female fetus).
Results: Nine cases at risk of achondroplasia were scanned in our centre. In two pregnancies (27 and 36 gestation week) mutation c.G1138A was detected in cffDNA. Both cases exhibited reduced ossification and shortening of long limbs, slight hypoplasia of the chest and frontal bossing. Mutation was confirmed by genomic fetal or newborn DNA analysis.
Conclusion: Non-invasive prenatal diagnosis of achondroplasia using cffDNA is a safe alternative approach to invasive testing. Accurate diagnosis is important for parental counselling as well as for clinical management in time of delivery and for the newborn with achondroplasia. It has also an important psychological aspect since the family can be prepared for the child with this condition.

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P19.07

Recurrence of an adjacent-2 segregation of a non-Robertsonian reciprocal translocation t(14;21)(q11.2;q11.2)

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Adjacent-2 segregation is a very rare type of segregation of balanced reciprocal translocations during meiosis. One of the derivative chromosome is transmitted with its counterpart. Thus, there is a trisomy and a monosomy for the centric segments of the derived chromosomes without genomic imbalance of the translocated segments. We report on the recurrence of an adjacent-2 segregation of a reciprocal translocation. Ultrasound examination in the first pregnancy of a 30-year old woman at 30 weeks' gestation showed intrauterine growth restriction associated with a right-sided pelvic kidney. The fetal karyotype performed on cultured amniocytes showed an abnormal chromosome 21. This chromosome was shown to be a derivative chromosome 14 of a non-Robertsonian translocation between a chromosome 14 and of a chromosome 21. Thus, the fetus had a partial proximal 14q trisomy and a partial proximal 21q monosomy. The pregnancy was terminated at 33 weeks' gestation. The father's karyotype showed a reciprocal balanced translocation t(14;21)(q11.2;q11.2). Thus, the fetus had inherited the derivative chromosome 14 from an adjacent-2 segregation. During the fourth pregnancy, after two miscarriages, the fetal karyotype performed on trophoblastic cells showed the same derivative chromosome 14. In carrier of balanced reciprocal translocation, the formation of unbalanced gametes due to adjacent-2 segregation is rarely observed. This type of segregation has been observed when the translocation involves at least one acrocentric chromosome or a chromosome with a short centric segment. This observation shows that this cytogenetic diagnosis should be considered when an abnormal acrocentric chromosome is detected, especially in prenatal diagnosis.

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P19.08

Prenatal diagnosis using array-CGH: a French experience.

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Array-CGH or Chromosomal Microarray Analysis (CMA) is increasingly used in prenatal diagnosis throughout the world. However, routine practices are very different among centers and countries, regarding CMA indications, design and resolution of microarrays, notification and interpretation of Copy Number Alterations (CNA). We present our data and experience from our Fetal Medecine Center on 224 prospective prenatal diagnoses, using FISH, karyotype and CMA. Rapid FISH analyses enabled to identify 64 common aneuploïdies (trisomies 13, 18, 21, Turner and triploïdies) in 213 fetus presenting with ultrasound abnormal features (30%). Conventional karyotyping confirmed these 64 aneuploïdies and detected 7 other chromosomal anomalies (+3%). In parallele, conventional karyotyping identified 11 other chromosomal anomalies among a group with other prenatal indications (advanced maternel age, abnormal first or second trimester screen). CMA was carried out in 160 samples: in the 18 fetus with chromosomal anomalies identified by karyotyping in order to characterize these rearrangements, and in the 142 fetuses with no karyotype anomaly. Among the 142 fetus with ultrasound abnormal findings, the anomaly rate fluctuated from 5.3% (Group of fetuses with isolated increased nuchal translucy) to 16.6% (Group of fetuses with 2nd and 3rd trimester minor ultrasound anomalies). Our approach is practical, and aims to propose a strategy to offer Chromosomal Microarray Analysis (CMA) to selected fetuses, and to help to interpret CNA. We hope that this publication could encourage development of CMA in centers that have not started yet this activity in prenatal routine, and could contribute to edict guidelines in this field.

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P19.09

A very rare case detected by array-CGH

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Extensive experience gained in the past decades has shown that prenatal karyotyping is a robust technique to detect the majority of chromosomal anomalies. But there is a growing request for arrays in the prenatal setting. Array-CGH showed an increased diagnostic yield compared to karyotyping, varying from 1-5%, depending on the reason for referral.
The higher abnormality detection yield and its amenability to automation render array-CGH suitable for prenatal diagnosis.
We report a case with pathological cytogenetic results and normal ultrasound scan at 16+2 weeks pregnancy woman. A cytogenetic study in amniotic fluid cell cultures revealed structural abnormality, consisting of an isochromosome of the short arm of an X chromosome: 46,X,i(X)(p10). This diagnostic was performed in another laboratory.
Array-CGH from fetus DNA, revealed a terminal deletion of about 33.66Mb in the long arm of chromosome X, and a partial trisomy of 535 Kb in the terminal segment of the long arm of chromosome 21.
Subtelomeric FISH probes showed that trisomy of 21qtel region is located in long arm of deleted X chromosome.
Karyotype: 46,X,der(X)t(X,21)(?;q22.3).ish der(X)t(X;21)(q28-;q22.3+).arr 21q22.3(46,358,908-46,894,371)x3,Xq25q28(121,214,280-154,878,101)x1.
Parents cytogenetic studies were normal, therefore fetus abnormalities were de novo.
Genetic counselling was performed by a Clinical Genetics Consultation and an obstetrician, and parents decided to continue the pregnancy, which is still on course.
Specific techniques revealed the existence of several chromosomal abnormalities than conventional karyotype study could not be found to observe a isochromosome Xp wrong.
Combination of cytogenetic techniques, FISH and CGH-array must be considered as the best choice in difficult cases.

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P19.10

Perinatal & childhood outcomes in ART children: Experience from a Medical Genetics Department

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In developed countries, 1-3% of births involve some form of assisted reproductive technology (ART). However, ART has been associated with a 30-40% increased risk of major congenital anomalies, compared with natural conceptions.
Over a period of 10 years, 147 children and fetuses who had been artificially conceived were examined in our Medical Genetics Department.
Regarding the demographic features, 53.7% of the patients were male and 46.2% female. The median age at diagnosis was 19 months. The mean maternal age was 34.7 ± 5.5 (mean ± SD) and the mean paternal age was 38.7 ± 5.8 (mean ± SD).
Most patients presented with congenital anomalies, the most common being craniofacial, musculoskeletal and genitourinary. Psychomotor delay was reported in 51.02% of cases. Several patients were diagnosed with syndromes such as Sotos, Noonan, Down, Williams, Prader - Willi, Angelman, Kabuki or others. A positive family history was reported in 21.9% of cases.
In most cases (64%), a total of two embryos were transferred. Almost in all cases (96.7%), a cesarean section was performed, with the main reasons being premature rupture of membranes, IUGR or oligohydramnios. Perinatal complications occurred in 51.7% of cases and included respiratory distress syndrome, jaundice and neonatal infections.
The increased risk of congenital anomalies in children who have been artificially conceived has been partly attributed to the ART itself, micromanipulations during ICSI, periconceptional drug administration or underlying genetic reasons of infertility. Therefore, more research needs to be conducted in order to help counseling couples who are considering treatment for infertility.

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P19.11

Atypical prenatal case of cat eye syndrome

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Cat-Eye syndrome (CES) is a rare malformation syndrome mainly due to a partial tetrasomy of chromosome 22. CES is characterized by a high phenotypic variability. The main characteristic signs are : abnormal ears, coloboma, and anal malformations.
CES is often discovered postnatally when characteristic signs are associated. In the litterature, only one case was antenatally diagnosed based on ultrasonographic anomalies.
We report the second fetal case of CES who presented several signs at the ultrasound examination : intra-uterine growth retardation (cephalic circummference and femoral length below the 10th percentile), miroretrognathia, cerebellum hypoplasia, and low-set ears at the three-dimensional ultrasonography.
An amniocentesis was performed. The fetal karyotype was established using RHG banding and result was : 47,XX,+idic(22)(q11.21). This result has been confirmed by array-CGH, which concluded to a typical type I CES chromosome.
Fetal autopsy confirmed the ultrasonographic features. No coloboma and no anal malformation were found.
Fetal diagnosis of CES is difficult because of variability of the phenotype. Compared to the other reported cases, our observation is atypical. The fetus did not present coloboma or anal malformation. He had a cerebellum hypoplasia which was reported in one case only. Prenatal diagnosis of CES is even not easy because of difficulty to detect the characteristics signs at ultrasonography. This explains why only one prenatally case has been reported up today. On these two cases, ears anomalies detected on three-dimensional ultrasonography were crucial for the diagnosis.
Diagnosis of CES and other polymalformation syndromes will be facilitated by progress in fetal ultrasound examination.

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P19.12

Heterochromatin variants of chromosome 9: clinical aspects and method of molecular cytogenetic examination

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Heterochromatin abnormalities of human chromosomes are mostly believed to be clinically insignificant variants of the human karyotype. However, several authors have studied the possible association of heterochromatin variants (including those of chromosome 9 which are the most common) with certain clinical diagnoses, primarily with reproduction failure (sterility and/or repeated abortions).
We provide an overview of diagnoses in the large group of 405 chromosome 9 variant carriers that were karyotyped between 1986 and 2012. Idiopathic sterility was the most common clinical indication with frequency 43.5%. In the control group of 700 individuals with normal karyotype (without any kind of variant), the referral incidence of idiopathic infertility was significantly lower - only 30.0% (p<0.0001).
We also present a special molecular cytogenetic procedure which we use for more precise analysis of heterochromatin area of chromosome 9. Three different FISH probes - centromeric alpha-satellite, centromeric III-DNA satellite and a specific BAC probe (hybridizing on 9p12 and 9q13 homologous sequences) - are involved. By this method we investigated in detail the chromosome 9 variant in 12 carriers. We have proven, that this molecular cytogenetic examination is able to distinguish among different (sub)variants of chromosome 9 much better than standard G-banding. Although believed to be harmless, the variants of chromosome 9 have been repeatedly mentioned as potentially associated with reproduction failure. Since the majority of these variants are undoubtedly truly benign, we present a possible tool for their further investigation.
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P19.13

Detection of high recurrence risk ciliopathies in a cohort of intrauterine fetal death conceptuses of unknown cause: a functional genomic approach

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The wide application of prenatal ultrasound often leads to the detection of multiple congenital anomalies and consequently to termination of pregnancy (TOP) without a definitive diagnosis.
Severe congenital anomalies at ultrasounds, such as (cranial) neural tube defects, ventriculomegaly, cerebellar and kidney dysplasia, skeletal abnormalities and heart-loop lateralization defects are also frequent in defects of the primary cilium (ciliopathies), mostly recessively inherited, such as Meckel syndrome or hydrolethalus syndrome, but establishing a diagnosis in a fetus can be challenging. Ciliopathies have an incidence of >1 in 1,000 conceptuses and around 2500 genes are expected to be related to function and/or structure of the cilia. We therefore sought to develop a diagnostic screening assay and subsequent workflow for ciliopathies in fetuses of pregnancies, terminated on the basis of the ultrasound findings. Initially, immunostaining of the primary cilium and basal body in cultured skin fibroblasts proved to accurately diagnose patients with known ciliopathies (such as Joubert syndrome, Meckel syndrome, Rotatin mutation). Additionally, Shh-mediated Gli response was used as functional test for cilia-related pathways and confidently detected patients with known mutations, even with minor/no cilia structural anomalies. In a cohort of TOP fetal fibroblasts derived from fetuses with above mentioned malformations, we detected a high percentage of structural and functional cilia abnormalities. In fetuses with undefined ciliopathy, this approach complements the use of targeted NGS panels for ciliopathy genes, useful for both patient selection and confirmation of genomic variants. This approach also detects high recurrence risk pregnancies, offering the possibility of early prenatal diagnosis.

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P19.14

Managing a complex chromosomal rearrangement in prenatal diagnosis

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Complex chromosomal rearrangements (CCR) are structural rearrangements, involving at least three breakpoints on two or more chromosomes. A de novo cytogenetically balanced CCR have been associated with increased risk for developmental delay/intellectual disability (DD/ID) and multiple congenital anomalies, however normal children have also been born. The risk increases with the number of breakpoints and is estimated to be approximately 3% per breakage.
Herein, we report on an amniocentesis performed because of increased nuchal translucency that revealed a cytogenetically de novo balanced complex translocation involving chromosome 7, 8 and 12 (46,XY,t(7;8;12)(q34;q21.1;q12)dn). Array CGH analysis revealed a 3.7Mb deletion of 12p12, unrelated to the breakpoint (arr 12p12.1(21,356,582-25,062,714)×1), which involved 8 genes (SLC1A2, RECQL, GYS2, LDHB, ABCC9, ST8SIA1, ETNK1 and SOX5). Deletions containing whole SOX5 and some adjacent genes have been associated with DD/ID, speech delay, dysmorphic features, skeletal abnormalities and congenital heart defects. The ultrasound scans (USS) of fetal morphology and heart were normal. Nevertheless, the risk for abnormal phenotype was high and the parents decided to terminate pregnancy. Post mortem examination was normal.
To our knowledge this is the first case of de novo 12p12.1 deletion in the prenatal setting. This report highlights the usefulness of array CGH for the specification of de novo CCR in prenatal diagnosis, facilitating the detection of other genomic imbalances and the establishment of risk for abnormal phenotype.

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P19.15

5 probes PGD-FISH strategy for a surprising complex chromosomal rearrangement.

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Pre-implantation Genetic Diagnosis (PGD) can benefit to couples carrying chromosomal anomalies, to avoid the birth of children with imbalanced rearrangements.
Fluorescent in situ hybridisation (FISH) is the technique of choice to detect chromosome imbalances in one or two biopsied blastomeres. Usually, 2 probes are used for PGD of Robertsonian translocations and 3 for reciprocal ones. PGD is more difficult for complex chromosomal rearrangement (CCR) because more probes are required, simultaneously or sequentially, to detect all imbalances.
A couple was referred to our PGD centre for CCR. The woman carries a balanced three-break rearrangement, ISCN: 46,XX,t(4;11;6)(q28;q14;q16).
Five probes were combined to adjust our FISH technique: three directly fluorescent labelled probes on 11 centromere (blue), 11q (green), 6q (red) and two hapten-labelled probes on 6 centromere and 4q which are detected by fluorescents antibody (green) and streptavidin (red) in a second step. This set can identify any possible imbalance in the offspring.
When testing these probes on patient's lymphocytes chromosomes, we identified an unexpected additional anomaly: a small piece of the translocated 11q region was found inserted at the breakpoint of derivative chromosome 4. In total, this leads to a complex anomaly involving 5 breakpoints on 3 chromosomes. Here we report the workup for the final PGD test, combining only 5 probes but detecting almost all possible imbalances in embryos.

C. Retter: None. **J. Lauer-Zillhardt:** None. **S. Casalanguida:** None. **C. Moutou:** None. **P. Gosset:** None.

P19.16

Confined placental mosaicism and pregnancy outcome: a distinction needs to be made between types 2 and 3

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Objective: To study the influence of types 2 and 3 confined placental mosaicism (CPM) on pregnancy outcome. *Method*: From 13 809 chorionic villus samplings (CVSs), karyotype after long-term cultured villi (LTC-villi) was systematically performed. Next, in case of suspicion of CPM, karyotype after short-term cultured villi (STC-villi) was established to define type 2 CPM (chromosomal abnormality limited to the mesenchymal core) or type 3 CPM (chromosomal abnormality found both in the cytotrophoblast and the mesenchymal core). Confirmatory amniocentesis was performed to exclude fetal mosaicism. Uniparental disomy (UPD) testing was carried out when the abnormal cell line involved chromosomes with imprinting genes. Fetal growth was assessed using term and birth weight, and adverse pregnancy outcomes were defined by the occurrence of fetal loss or perinatal death. Results were compared with those obtained in a control population of 198 patients. *Results:* Fifty-seven CPM cases were observed (57/13 809 = 0.41%) and of these, 37 were type 2 and 20 were type 3 CPM. Incidence of preterm infants, neonatal hypotrophy and adverse pregnancy outcome were comparable between patients in whom type 2 CPM was demonstrated and the control population. In contrast, for the type 3 CPM the incidence of these factors was higher than for the control population. *Conclusion:* When a CPM is suspected, it appears essential to determine type, since type 2 has no effect on fetal development and type 3 is associated with preterm infants, low birth weight and adverse pregnancy outcome, even in the absence of UPD.

J. Horovitz: None. **J. Toutain:** None. **F. Pelluard:** None. **D. Lacombe:** None. **R. Saura:** None.

P19.17

Characterization of novel GNRHR gene mutations underlying congenital hypogonadotropic hypogonadism

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Mutations in GNRHR, the gene encoding for GnRH receptor, cause autosomal recessive form of normosmic congenital hypogonadotropic hypogonadism (nHH). Prevalence estimates of biallelic GNRHR mutations in nHH patients vary from 3.5 to 40%, and to date, 24 different mutations have been reported. Patients with biallelic GNRHR mutations display variable reproductive phenotypes ranging from severe hypogonadism to reversal of nHH later in life.
We are characterizing functional consequences of two recently identified GNRHR mutations. A heterozygous c.924_926delCTT (p.del309F) mutation, located in the 7th transmembrane-domain of the receptor, was first identified in a Finnish family, in which it segregated with delayed puberty. Subsequently, the same mutation was found in a compound heterozygous state (del309F/R262Q) in a Finnish male with nHH who underwent reversal of HH. The c.714T>G (p.L238R) mutation, located in the 3rd intracellular loop of GNRHR which is responsible for G-protein coupling, was found in a compound heterozygous (L238R/R262Q) state in a Finnish female nHH patient. Assessment of cell-surface expression, ligand-binding properties and intracellular signaling properties of the mutant receptors are on-going.
We expect that characterization of the functional properties of these mutations will help in understanding how these mutations contribute to the nHH phenotype of the patients and whether the del309F mutation alone is severe enough to cause delayed puberty. In addition, these results provide us with a better knowledge on the roles of different domains in receptor function.

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P19.18

Population-based carrier screening for Cystic Fibrosis in Sicily: Three years experience

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Background: Cystic Fibrosis (CF) is an inherited disease caused by mutations in the *CFTR* gene (OMIM #602421). In this study we report our cases performed during a 3-year period on couples as preconception carrier screening, or on infertile couples from Southern Italy (Sicily). The aim of this paper is to report our experience of carrier screening for CF.
Materials and methods: Data were collected from January 2010 through January 2013 on 295 individuals. Screening of the most important mutations and polymorphisms into the CFTR gene was performed by molecular analysis on strips (reverse dot blot).
Results: A total of 295 individuals were screened. Out of them, 275 were wild type (93,2%).
We identified 20 heterozygous carriers (6,8%) for the following gene mutations: F508del (8; 2,7%), N1303K (4), I148T (3), W1282X (1), G542X (1), 621+3 A>G (1), 711+1G>T (1), 3849+10kbC>T (1).
Poly-T variant in intron 8 (IVS-8 poly-T) was also analyzed and we found: 7T/7T (186), 7T/9T (73), 5T/7T (22), 5T/9T (6), 5T/5T (4), 9T/9T (4). 5T variant was carried by 28 individuals (9,5%).
Conclusion: To date, more than 1800 mutations and polymorphisms have been identified in the CFTR gene. The combination of various mutations can give different phenotypes. Polymorphisms associated with poly-T variant of the CFTR gene may have an important prognostic value.
The goal of CF carrier screening is to identify couples at risk. Genetic counseling is important to discern whether the combination of mutations and variants would cause classic or atypical CF.

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P19.19

Best practice guidelines for Cystic Fibrosis Preimplantation Genetic Diagnosis

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Cystic Fibrosis (CF) is the most common lethal autosomal recessive disease affecting the Caucasian population. For couples at risk of having children affected with CF, preimplantation genetic diagnosis (PGD) is an alternati-

ve to prenatal diagnosis, giving couples the opportunity to have unaffected children without having to consider termination of pregnancy. The rapid increase of PGD over the last 20 years and the wide practical experience of different PGD laboratories over the world on CF, one of the most common indication of PGD, prompted the EuroGentest Network (www.eurogentest.org) to grant the organization a meeting dedicated to establish European Best Practice Guidelines for CF-PGD. Even though general best practice guidelines for amplification-based PGD have been already established by ESHRE (European Society of Human Reproduction and Embryology), the goal of this meeting was to edit CF-specific guidelines in order to harmonize inclusion criteria, genetic counselling, PGD strategy and technical protocols, and to provide the high standards of result reporting, across Europe (EGT2, Unit 3, WP 10). Sixteen experts in PGD and molecular diagnosis of CF from 7 countries attended the meeting, which was held in Montpellier, France, on December 14th 2011. Several topics were discussed and focused on mutation nomenclature, inclusion criteria (according to severity of mutations), genetic counselling (according to transferable embryos), PGD strategy (direct and/or indirect diagnosis) and results reporting (there is currently no formal consensus regarding the PGD reports, as it depends on legislation of each country). Hereby we present a summary of the consensus achieved.

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P19.20

Noonan syndrome spectrum and pathological aCGH abnormalities in fetal nuchal cystic hygroma with normal karyotype

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OOBJECTIVE: To determine the incidence of abnormal array CGH and Noonan syndrome spectrum mutations in fetal nuchal cystic hygroma with a normal karyotype.
METHODS: We analyzed the results of array CGH and DNA mutation analysis for Noonan syndrome spectrum on cases with fetal nuchal cystic hygroma and normal karyotypes, detected prenatally on 11-14 weeks ultrasound, between January 2011 to December 2012.
RESULTS: 49 cases of fetal cystic hygroma with normal karyotypes were included in the data collection. 46 cases had DNA analysis for both Noonan syndrome spectrum and microarray analysis. In 3 aCGH was not done since a mutation was detected on DNA analysis on Noonan syndrome spectrum.
6/49 (12.2%) were found to have mutations associated Noonan syndrome spectrum: 2 PTPN11, 2 RAF 1, 1 SOS1 and 1 MAP2K1 mutations. In addition a novel missense change, M117R was identified in the BRAF gene, predicted to be a benign variant. 2/46 (4.3%) cases were identified as having clinically significant array CGH abnormalities.
CONCLUSION: In our population with karyotypically normal fetal nuchal cystic hygroma detected prenatally 12.2 % were found to have a mutation in the Noonan syndrome spectrum and 4.3% were found to have aCGH abnormalities.

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P19.21

Optimized droplet digital PCR measurement of male fetal DNA in maternal plasma.

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Circulating fetal DNA (cfDNA) constitutes approximately 6-10% of the cell-free DNA in maternal plasma and is a proper source of fetal material for non-invasive prenatal diagnosis (NIDP). Since 2008, a routine facility for prenatal fetal sex determination based upon the detection of Y chromosome-specific gene SRY by quantitative PCR (qPCR) has been proposed in Cochin hospital (AP-HP, Paris, France). However, cfDNA circulating concentrations are close to the detection limits of PCR methods, especially during first weeks of pregnancy (<7 weeks). The objective of this study was to determine the suitability and reliability of using digital PCR (dPCR) for NIPD of fetal gender against already established qPCR Taqman assay. The fractional cfDNA concentration was assessed for each case, and the male DNA was detected and/or quantified with both standard qPCR and dPCR, which were compared for sensitivity and specificity. Fetal gender was confirmed by the result of ultrasound observation. Until now, fetal gender was determined correctly in 100% of the cases with cfDNA concentrations >7% using either method, but dPCR allowed a more accurate quantification. We confirm that, after an essential step of optimization of the fractional fetal DNA concentration, digital PCR can be used successfully for non invasive fetal sex determination, and may be adapted for single gene disorder detection.

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P19.22

Reduction of bias in non-invasive prenatal karyotyping

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Karyotyping for trisomy aberrations in non-invasive prenatal diagnostics using maternal cell free DNA (cfDNA) has proven to be difficult. There are probability tests on the market, but still no diagnostic test. The main problem is to distinguish fetal cfDNA from the large background of maternal cfDNA. The bias inherent in current library preparation methods, and especially library amplification, prohibits accurate testing.
We have developed a method reduce amplification bias by making all molecules unique before library amplification. This can be done either by attaching a unique molecular identifier (UMI), or by diluting the sample so that each molecule gets a unique starting position with high probability. The library is then amplified and sequenced to a depth where each molecule is seen at least two times, to make sure that all molecules present in the pre-amplified library are seen.
Using UMI we have shown that we can remove a large part of the GC bias from amplification and we get an even distribution of reads on all chromosomes including high GC content chromosome 19 and low GC content chromosome 13, not just from chromosomes with a normal GC distribution (e.g. chr 21). We have also shown that using UMI we can distingiush fetal trisomy samples down to 3% fetal cfDNA content.

K. Karlsson: None. **S. Linnarsson:** None.

P19.23

Fetoplacental discrepancy involving complex structural rearrangement of chromosome 13 : first case of constitutionnal chromothripsis ascertained by prenatal diagnosis

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Karyotype disparities between cytotrophoblast and fetal cells are detected in 1 to 2% of pregnancies undergoing first-trimester chorionic villi sampling (CVS). Cases involving structural rearrangement are very rare.
In the present report, CVS was performed on a 31-year-old woman because of a 3,37 mm cystic hygroma at 15 weeks of gestation (WG). Direct preparations showed a female karyotype harboring a robertsonian translocation between chromosomes 13 and 15 with duplication of the der(13;15), leading to trisomy 13 and trisomy 15 : 46,XX,rob(q10;q10),+der(13;15)(q10;q10). Cytogenetics analysis after long-term CVS culture displayed balanced karyotype with rob(13;15), except for one metaphase with supernumerary chromosome 13 : 45,XX,rob(13;15)(q10;q10)[20]/46,idem,+13[1]. Mosaic trisomy 13 was confirmed on FISH analysis (15% of nuclei). Amniocentesis was therefore performed and karyotype from amniocytes showed a robertsonian translocation between a structurally abnormal chromosome 13 and a chromosome 15. Array-CGH analysis (Agilent®, 4x44K) performed

on DNA extracted from amniotic fluid cell cultures confirmed the highly complex rearrangement of chromosome 13 with seven duplicated regions and four deleted regions.
After genetic counselling, parents opted for termination of the pregnancy at 19 WG. Fetal autopsy showed a eutrophic female fetus presenting with dysmorphic features, cerebral abnormalities, congenital heart defect with transposition of the great vessels and skeletal abnormalities.
Recently, it has been suggested that chromothripsis, a chromosome catastrophe phenomenon described in 2%-3% of cancers, in which numerous genomic rearrangements are acquired in one single catastrophic event, could explain constitutional complex genomic rearrangements. Thereby, this report could be the first case of constitutional chromothripsis ascertained in prenatal.

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P19.24
N680S polymorphism on the FSHR gene and its effect on ovarian donor follicle stimulating hormone stimulation.

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INTRODUCTION: Genetic factors could explain differences in response to drugs. Clinical studies have demonstrated that N680S polymorphism on FSHR gene determines ovarian response to FSH stimulation in patients undergoing IVF. Patients with the S680 allele need more FSH during the stimulation phase. The aim of this work was Investigate whether N680S FSHR polymorphism has a predictive value for ovarian response to stimulation with gonadotropins and cycle outcome in our egg donor program.
MATERIAL AND METHODS: FSHR polymorphism N680S has been studied in 145 oocyte donors at Instituto Bernabeu and underwent ovarian stimulation (n=355). The main outcome measures were oocyte yield, stimulation days, gonadotropin dosages, biochemical pregnancy, ongoing pregnancy and miscarriage rates.
RESULTS: Significant differences were reported in antral follicles count (16.5 + 5.0 for NN, 14.5+4.7 for NS and 14.1+3.8 for SS), number of eggs retrieved (21.5 + 9.2 for NN, 18.5 + 8.2 for NS and 19.8 + 8.9 for SS) and gonadotropin doses (2098.5 + 639.4 IU for NN, 2023 +490.1 IU for NS and 2149.5 + 552.3 IU for SS) between genotypes. Differences in cycle outcome were not affected by the N680S polymorphism on FSHR gene.
DISCUSSION: In spite of the final clinical outcome is not different, this investigation reveals that in a population of fertile egg donors, FSHR gene polymorphism at position 680 is associated with different ovarian response to COH. Genotyping FSHR N680S together with some additional markers may therefore provide a means of identifying a group of poor responders before infertility treatment is initiated.

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P19.25
Study of triple CGG repeats (*FMR1* gene) in 157 patients with ovarian failure of unknown origin

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Fragile X-associated primary ovarian insufficiency (FXPOI) is characterized by a large spectrum of ovarian dysfunction phenotypes and possible early menopause as the end stage. FXPOI is due to a premutation (PM) - 55 to 200 CGGs- in the *FMR1* gene and recent studies have even suggested that women with ovarian failure might be at risk of carrying alleles in the intermediate range.
This study was designed in order to find out the frequency of PM and intermediate alleles in our population of women with different types of ovarian failure.
We studied 157 women with ovarian failure, 67 of them already had early menopause and the others still don't. All participants were informed regarding the purpose of the study and signed the written informed consent.
DNA from patients was analysed using a commercial triplet CGG assay (AmplideX FMR1 PCR kit from Asuragen).
Nine (5,73%) of the 157 women studied had FMR1 alleles >44 repeats; five of them (3,18%) with PM and four (2,55%) with intermediate alleles. Three of the women with PM (95, 88 and 75 CGG repeats) presented with a very early menopause (21, 28 and 28 years old respectively). The other two women with PM (64 and 56 CGG repeats) had an ovarian poor response.
This study demonstrates that women in the midsize range of the PM have the highest risk of having FXPOI and the need to study the triplet CGG repeat in patients with ovarian failure and/or early menopause.
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P19.26
Paternal *NF1* germ cell mosaicism: should parental sperm analysis be offered for recurrence risk assessment in de novo cases of NF1?

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The neurofibromatosis type 1 (*NF1*) gene exhibits one of the highest mutation rates of human disease genes, and ~ 50% of NF1 index patients occur sporadically. At least 10% of them are suspected mosaics. Somatic and gonado-somatic NF1 mosaicism may clinically present as (mild) generalized, segmental or oligo-symptomatic NF1. However, germline mosaicism typically only comes to attention in families with more than one affected child born to unaffected parents. Very few of such families have been described and only in two of them germline mosaicism was molecularly confirmed.
We identified a third case in the course of prenatal testing. The first child of unaffected parents was diagnosed with NF1 caused by a recurrent *NF1* mutation (p.Arg304*). Having been made aware of the rare possibility of germline mosaicism, the couple opted for prenatal testing in the second pregnancy despite the absence of the mutation in their blood lymphocytes. Unexpectedly, the *NF1* mutation p.Arg304* was also present in chorionic villi sampling of the second pregnancy. Sanger sequencing confirmed presence of the mutation in the father's sperm cells. Quantitative evaluation showed that ~9% of sperm cells carried the mutation. Even a more sensitive allele-specific PCR-assay was unable to detect the mutation in somatic cells of the father's blood, buccal mucosa and urothel.
The majority of intragenic *NF1* mutations are expected to arise in paternal germ cells. Hence, offering analysis of the father's spermatozoa should improve recurrence risk assessment in future cases and will uncover the frequency of NF1 germline mosaicism.

A. Wernstedt: None. **F. Laccone:** None. **S. Tinschert:** None. **J. Zschocke:** None. **K. Wimmer:** None.

P19.27
HMGA2 deletion and complex translocation t(1;12;14)(q42;q14;q32) in a fetus with intra-uterine growth delay and bilateral cataract.

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HMGA2 is known to have multiple functions in development and cellular growth. Here we report the prenatal detection of its deletion, associated with a complex chromosomal rearrangement, in a fetus with growth delay and phenotypic abnormalities.
A nonconsanguineous couple was referred to our center at 30 weeks of gestation after an ultrasound revealing growth retardation and bilateral cataract. Cytogenetic analyses revealed a complex *de novo* rearrangement. After genetic counseling and according to the French law the pregnancy was terminated at 32.5 weeks of gestation. Fetopathologic examination confirmed ultrasound abnormalities.
Standard karyotype and FISH analyses on amniotic fluid revealed a complex *de novo* translocation, resulting in a formula 46,XY,t(1;12;14)(q42;q14;q32).

CGH-array study showed one significant deletion of 387Kb on chromosome 12 in q14.3 only 200 to 700Kb distant from the breakpoint in 12q14, that encompassed the *HMGA2* gene and occurred *de novo*. To date, this is the smallest described, that confirms the already suspected role of *HMGA2* loss of function in growth retardation.
The link between cataract and genotype is less easy to draw. Among the genes disrupted by the breakpoint in 12q14, *GRIP1* has been associated with mice abnormal eye development, including lens degeneration (Schwiergiel *et al* 2000). Interestingly enough, *HMGA2* could also be involved: Peng *et al* (2012) showed a decreased *HMGA2* expression in elderly's lens, correlated with the severity of lens opacity, and Lord-Grignon *et al* (2006) identified *HMGA2* expression in the mice developing lens. Further studies are in process to better determine correlation between genotype and lens opacification.

L. Raymond: None. **B. Francou:** None. **F. Petit:** None. **L. Tosca:** None. **A. Briand-Suleau:** None. **C. Métay:** None. **J. Martinovic:** None. **A.G. Cordier:** None. **J. Bouligand:** None. **A. Mantel:** None. **M. Goossens:** None. **G. Tachdjian:** None. **S. Brisset:** None.

P19.28
Severe prenatal renal anomalies associated with mutations *HNF1B* and *PAX2* genes

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Introduction: Mutations in *HNF1B* and *PAX2* can be associated with congenital anomalies of the kidney and urinary tract of variable severity and with extra-renal anomalies.
The aim of our study was to estimate the prevalence of these mutations in a series of 103 foetuses with severe isolated prenatal renal anomalies, which led to termination of pregnancy, and to describe associated extra-renal anomalies and genotype-phenotype correlations.
Patients: Renal phenotypes of the 103 foetuses were the following: bilateral multicystic dysplasia in 22, bilateral dysplasia with cysts in 24, bilateral hypodysplasia in 5, a combination of the previous phenotypes in 5, unilateral agenesis in 18 and bilateral agenesis in 27.
HNF1B was analyzed in 90 unrelated foetuses. A mutation was identified in 12 cases from 11 unrelated families: 3 complete and 1 partial heterozygous deletions, and 8 point mutations. Parents were tested 9 times: mutations were inherited in 6 cases.
PAX2 was tested in 75 unrelated foetuses. A point mutation was identified in 4 cases. All were inherited, one parent carrying a somatic mosaicism for the mutation.
Conclusions: Our study shows that mutations in genes involved in syndromic CAKUT with a Mendelian inheritance are not rare in fetal cases with severe CAKUT appearing as isolated at prenatal US. The severity of the renal phenotype was not correlated with the type or the position of the mutation. This phenotypic variability makes genetic counselling particularly difficult in the inherited forms.

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P19.29
A 3q27.1q28 microdeletion detected by micro-Array in a fetus with isolated growth retardation. Delineation of a new syndrome and discussion about the indication of micro-array in prenatal diagnosis.

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Understanding the genetic mechanisms of intra-uterine growth retardation (IUGR) constitutes a major issue in prenatal diagnosis and fetal medicine. We report the observation of the second pregnancy of a non-consanguineous healthy couple. The beginning of the pregnancy was uneventful. No drug or toxic was reported. Fetal hypotrophy (biometry below the 5th percentile) was detected at 23 weeks of amenorrhea. Sonographic examination was normal furthermore and doppler investigations excluded a vascular etiology. Karyotype from amniocytes and *in situ* hybridization of 4p16.3 locus (Wolf-Hirschhorn syndrome) were performed at 28 WA and showed normal results.
A fetal CGH-array was decided to be performed by the Multidisciplinary Centre for Prenatal Diagnosis. Micro-Array using Affymetrix Cytoscan HD Array detected a *de novo* 5.2 Mb microdeletion arr[hg19]3q27.1q28(182.877.291-188.090.034)x1 (3q26.33) to 185.786.898
(3q27.2). This deletion was confirmed by FISH and occurred *de novo*. After information, the parents opted for termination of pregnancy.
To our knowledge, we describe here the first prenatal case of 3q27.1q28 microdeletion. Very recently, a first report of three children has been reported about three children with growth retardation and moderate to severe mental retardation and an overlapping 3q26.33-3q27.2 microdeletion. This disorder is considered to constitute a possible contiguous gene deletion. At least 40 genes are located within this region, involved in brain development and function, but also body growth.
Finally, this report raises the question of the diffusion of micro-arrays technology in prenatal diagnosis, not only for polymalformative fetuses, but also for diagnostic strategy of isolated IUGR.

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P19.30
First case of prenatal inv dup del 11q in a fetus presented with isolated growth retardation.

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Interstitial inverted duplication associated with terminal deletion (inv dup del), have been identified on an increasing number of chromosomes arms. We report on the first prenatal case of inv dup del involving the long arm of chromosome 11 in a fetus presenting isolated intra uterine growth retardation (IUGR).
An amniotic fluid exam was performed at 33 SA because of an IUGR. The fetal karyotype combined with FISH studies showed a subtelomeric 11q deletion associated with additional material on the long arm of chromosome 11 derived from the chromosome 11. After reserved genetic counselling, a termination of pregnancy was proposed. Nevertheless, the mother gave birth prematurely at 36 weeks to a boy presenting growth retardation, microcephaly and joint contractures. At the age of six months, we also noted developmental delay and minor dysmorphic features. To map the chromosomal rearrangement, a SNP-array (HumanCytoSNP-12, Illumina) analysis on post natal sample was performed. It revealed a 1.45 Mb telomeric deletion associated with an adjacent 11q24.1q25 duplication of 10.26 Mb.
Distal 11q deletions are involved in Jacobsen Syndrome, a well known contiguous gene syndrome.
Regarding the duplication, only ten cases of isolated 11q duplication have been reported, six overlapped with the duplication of our patient. Common findings among these patients include developmental delay, growth retardation, microcephaly, abnormal ears, microretrognathia, and congenital heart defect.
We compare the clinical features of our patient with abnormalities previously described in overlapping 11q rearrangements to better determine the correlation genotype-phenotype.

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P19.31
X chromosome related heterotaxy in a family with two consecutive Ivemark syndrome pregnancy

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Objective: Familiar heterotaxy is a genetically heterogeneous condition and could be inherited on autosomal dominant, autosomal recessive, or an X-linked trait. Heterotaxy is defined as any deviation from the normal situs solitus. A family with two consecutive pregnancies with male fetus having signs of heterotaxy on ultrasound examination visited our department.
Materials and methods: During the second trimester ultrasound screening (20week of gestation) fetal heterotaxy was revealed in two consecutive pregnancies of the same couple. Fetal echocardiography was also performed, where severe cardiac malformation was found. Both pregnancy was terminated and fetopathological examination was performed. DNA was iso-

lated from the fetus. Protein coding exons and immediate flanking intronic regions og the ZIC3 gene where sequenced.
Results: In both pregnancies the ultrasound anatomy of the fetus showed signs of heterotaxy: the fetal heart on right side of the chest, fetal stomac on the right side of abdomen etc. Fetal echocardiography showed malposition of great arteries, atrioventricular septal deffect. During fetopathological investigation all this conditions where prooven. By sequencing of ZIC3gene c.869delC was detected in the exon 1 of the gene, the c.869delC.
Conclusion: The hemizygous mutation in the ZIC3 gene could be behind the heterotaxy observed in the family. Prenatal diagnosis from the CVS sample at 12th gestational week could be used in the next pregnancy to detect the observed genetic alteration.

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P19.32
Identification of a human fertility gene required for normal sperm number in semen

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It is estimated that 10% of couples are unable to conceive a child without medical intervention, and that in almost half of these cases a sign of male factor infertility is present. Despite its high prevalence, few of the genes involved in male infertility have been identified, and consequently, in most cases, the risks associated with medically assisted reproduction cannot be fully assessed. We have studied a consanguineous family from the Lebanon in which five of the six sons have severe oligozoospermia, 50 000 sperm per ml (normal = > 20 million sperm per ml). Genotyping at the French National Genotyping Centre (CNG) identified a critical region of homozygosity on chromosome 15, containing 134 genes (LOD score is Z=3.1 at θ=0.01). Sequencing the entire region revealed a single coding variant that was homozygous in the five oligozoospermic sons, heterozygous in the father and absent from public databases or 190 Lebanese controls. This variant introduces a stop codon that is predicted to ablate expression of the protein. We have named this gene OZF1 (Oligozoospermia Factor 1. Preliminary expression analysis reveals that OZF1 is expressed predominately in the testis, and in the mouse its transcription increases as meiotic stages populate the post-natal testis. We conclude that loss of OZF1 expression during human spermatogenesis results in oligozoospermia, possibly through an effect on meiotic germ cell progression.

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P19.33
Genetic screening in Tunisian globozoospermic men: chromosomal abnormalities, Y chromosomal deletions and screening for mutations of DPY19L2. globozoospermic Tunisian men: chromosomal abnormalities, Y chromosomal deletions and screening for mutations of DPY19L2.

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Infertility is an important health problem affecting 10-15% of couples. The contribution of male factors to infertility is about 30-50%. Recent studies have indicated that both environmental and genetic factors are involved in the decline of reproductivity in males. The main genetic factors playing a role in male infertility are constitutional chromosomal abnormalities and Y chromosomal microdeletions. Among the male factors, globozoospermia is a rare type of teratozoospermia accounting for <0.1% of male infertility. Mutations or deletions in three genes, SPATA16, PICK1 and DPY19L2, have been shown to be responsible for globozoospermia.
In this cohort study, ten globozoospermic patients from 20 independent families were recruited during routine infertility treatment in a Tunisiain fertility center. The semen samples from these were analyzed according to the WHO criteria. Their constitutional blood karyotype was performed on cultured lymphocytes, according to standard techniques. Microdeletion analysis of the Y chromosomes used a sequence tagged site-polymerase chain reaction technique. DPY19L2 is the third gene identified as being associated with globozoospermia and the most frequently mutated in this phenotype. In order to better estimate its contribution, we performed PCR and found that four of them were homozygous for the DPY19L2 deletion.
A molecular diagnosis can now be proposed to affected men; the presence of the deletion confirms the diagnosis of globozoospermia and assigns a poor prognosis for the success of in vitro fertilization. The realization of a molecular diagnosis for globozoospermic men would permit providers to adopt the best course of treatment for these patients.

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P19.34
A retrospective study of outcomes of 223 cases of prenatally detected micrognathia at two tertiary obstetric centres in London.

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Micrognathia is a feature common to a number of congenital syndromes but it can also occur in isolation. Although associated defects are correlated with a poorer outcome, accurately predicting outcomes of fetuses with isolated micrognathia on prenatal scans is challenging, owing to the paucity of literature on this subject. We undertook a retrospective analysis of 223 cases of micrognathia detected on antenatal scans at two tertiary obstetric units. This represents the largest reported case series of prenatally detected micrognathia.
21 cases (9%) had isolated micrognathia, of which there were 12 live births, 3 terminations and 6 cases with unknown outcome. Karyotyping was undertaken in 9 cases; 8 were normal. Of the 12 live births 7 were found to have Pierre-Robin sequence postnatally, but no additional anomalies.
The remaining 201 (90%) cases of micrognathia occurred in conjunction with other anomalies. 130 cases were terminated, and another 22 ended in intrauterine death. There were 29 live births, of which 13 died at less than one week of age. Summary results of karyotyping are tabulated below.

Karyotype result of fetuses with multiple anomalies	Number
Trisomy 18	36
Trisomy 13	6
Trisomy 9	2
Trisomy 22	2
Trisomy 15	1
Trisomy 21	1
Triploidy	5
Tetrasomy	1
Other chromosomal deletions/ duplications	14
Normal	79
Not done	54

These findings indicate that prenatally detected micrognathia is an ominous finding, which warrants careful examination of the fetus and growth parameters. However, in cases of isolated micrognathia, with a normal karyotype, a positive prognosis can be offered.

H.E. Grote: None. **M. Koopmans:** None. **L.C. Poon:** None. **B. Thilaganathan:** None. **S. Mansour:** None. **T. Homfray:** None.

P19.35
High-Throughput miRNA sequencing reveals two endometriotic foci-specific miRNAs

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Introduction: Studies of eutopic and ectopic endometria have suggested microRNAs (miRNAs) are involved in endometriosis development, but the full miRNome of eutopic and ectopic endometria is a field that needs to be explored. In this study we used high-throughput sequencing to characterize miRNA expression in endometriosis foci by comparing adjacent healthy tissue to ectopic endometrium of the same patient. Also, our study aimed to reveal in what extent the miRNA expression profile in endometriosis foci differs from eutopic endometrium.

Material and methods: Deep sequencing technology was used to obtain miRNome data of five peritoneal endometriotic foci, five adjacent healthy tissues and two eutopic endometrial samples of two women with moderate-severe endometriosis. To test for differential miRNA expression between endometriosis foci, healthy tissue and eutopic endometrium, the edgeR package for R was used. miRNAs with adjusted p-value ($p < 0.05$) and false discovery rate (FDR) < 0.1 were considered statistically significant. Differentially expressed miRNAs were further validated using qRT- PCR.
Results: Endometriotic foci and adjacent healthy tissue had similar miRNA expression patterns that differed largely from eutopic endometrium. Two miRNAs (both p-values< 0.001, FDR 0.004) were significantly up-regulated in endometriotic foci compared to adjacent healthy tissues. Validation analysis confirmed differential expression of these miRNAs in endometriotic foci.
Conclusions: The comparision between endometriotic foci and healthy tissues revealed two miRNAs that could play an important role in the pathogenesis of endometriosis. miRNA expression profile of endometriosis foci and adjacent healthy tissue is more similar compared to endometrial miRNA profile.

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P19.36
The expression of miR-200 family in the endometrium of women with and without endometriosis

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A number of studies have shown that miRNAs, including the miR-200 family, are aberrantly expressed in endometriosis and are therefore believed to play a role in the pathogenesis of the disease. Our study aimed to determine the expression levels of miRNAs from miR-200 family in the endometrium of women with endometriosis compared to healthy controls. The expression levels of miR-200a, miR-200b and miR-141 in the endometrial biopsies from patients with endometriosis and healthy women were determined by real time PCR using TaqMan microRNA assays. Endometrial biopsies were matched by menstrual cycle day. Our results indicated that the endometrial expression of studied miRNAs varied greatly between the individuals. No statistically significant differences in miRNA expression levels between the endometria from women with and without endometriosis were observed. Based on our results, we suggest that endometrial expression of miR-200 family members does not play a crucial role in the pathogenesis of endometriosis.

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P19.37
Screening for and functional assessment of miscarriage CNVs

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Detection of unique DNA copy number variations (CNVs) in miscarriages suggests that their integral genes have a role in maintaining early pregnancy. Previously, we identified 11 unique miscarriage CNVs in ~30% of studied miscarriages, which were predominantly familial in origin. Using qPCR, we have now screened for copy number changes of 13 genes from these miscarriage CNVs in 250 couples with recurrent miscarriage (RM). RM couples showed more frequent copy number changes in *PNPLA4* (Xp22.31) (0.6% vs 0.3%) and *STX6* (1q25.3) (0.2% vs 0%) compared to controls. For three genes (*TIMP2*, *OFD1* and *TRAPP2C*) the RNA and protein expression was altered in miscarriages that had CNVs, in keeping with their genomic copy number changes. In depth analysis of a maternal duplication disrupting the *TIMP2* gene was performed in four miscarriages from one female with RM, due to the involvement of *TIMP2* in placental remodeling and embryo development, and the previous suggestion that it is maternally expressed in placenta. Methylation of upstream promoter sites in the miscarriages and control early pregnancy tissues, as well allelic expression levels, are consistent with preferential maternal expression of the *TIMP2* gene in early pregnancy; however, the regulation of its expression and imprinting appears complex. Further functional studies of miscarriage CNVs may elucidate their role in causing miscarriage.

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P19.38
A monozygotic twin with TRAP sequence and an incomplete mitotic trisomy rescue.

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Here we report a case where two rare events occurred almost simultaneously during the first 2 weeks of pregnancy.
A 38 old woman with a monozygotic twin pregnancy was referred because structural ultrasound analysis at 12 weeks of gestation revealed a twin reversed arterial perfusion (TRAP). In addition, large transsonic spaces without any flow were present in the placenta and the fetus showed intra-uterine growth retardation without any additional structural abnormalities. The QF-PCR revealed an additional, relatively lower peaks for the chromosome 18 markers, suggestive for low mosaicism for trisomy 18. Fluorescence in situ hybridization with uncultured amniocytes revealed trisomy 18 in 14% of the cells. In addition, QF-PCR analysis of both parents showed maternal uniparental disomy for chromosome 18 in the cell line with disomy 18. With karyotyping we could not confirm the trisomy 18 in 28 colonies. The pregnancy was terminated and follow-up studies were performed. QF-PCR analysis revealed a trisomy 18 in the placenta, a high mosaicism for trisomy 18 in the acardiacus and confirmed low mosaicism for trisomy 18 in several tissues of the pump twin.
We describe a complex case where in the first 2 weeks after conception two separate events occurred. The combined results from different diagnostic tests strongly suggest that first a trisomy rescue occurred in an early post-zygotic mitotic division followed by a division of the zygote which lead to the formation of a monochorionic, diamniotic twin with trisomy 18 mosaicism.

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P19.39
Reproductive options for women with affected offspring due to an mtDNA mutation

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Mitochondrial diseases are among the most common inborn errors of metabolism, in at least 15% caused by maternally transmitted mitochondrial DNA (mtDNA) mutations. Severe mtDNA mutations are often heteroplasmic. Because of the clinical severity and the lack of treatment, couples with a previous child with an mtDNA disorder may request prevention of transmission to a subsequent child. PND is technically possible, but due to limitations in predicting the phenotype, only suitable if a high likelihood exists of offspring with either no mutation or a mutation load below the threshold of expression. This is the case for *de novo* mtDNA disease, based on the absence of the mutation in multiple tissues of the mother. Correct counselling is critical, as often wrong estimates for recurrence are given, based on the high mutation load in the affected child and not on the absence of the mutation in the mother. PND could be offered for reassurance. We present some illustrating cases. PND is also an option for carriers with a low mutation load, in case these mutations demonstrate skewing. Preimplantation Genetic Diagnosis (PGD) can be offered to carriers of heteroplasmic mutations with a high recurrence risk, followed by transfer of an embryo below a mutation-specific or general threshold of expression. A 95% or higher chance of being unaffected was found at mutant level of 18% or less, irrespective of the mutation. PGD was applied for the m.3243A>G, m.8993T>G and m.8344A>G mutations. All carriers tested produced oocytes below the threshold.

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P19.40

Copy number variations and their role in the etiology of Müllerian aplasia

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Müllerian aplasia (MA) is a reproductive disorder characterized by congenital loss of uterus and vagina. Without treatment, intercourse is difficult and reproduction is only possible through surrogacy. Only a few MA patients have been reported with mutations in the *WNT4* and *LHX1*, but for the majority, the cause is still unknown.
We investigated copy number variations (CNVs) that could contribute to the etiology of MA using aCGH (180 kb) in 50 Finnish MA patients, whose diagnoses have been confirmed by laparoscopy. We identified nine CNVs, not commonly found in the control population, in eight (16%) patients. Seven of the CNVs were deletions and two were duplications, both detected in the same patient. Five of the observed deletions (5p14.3, 9q21.13, 11q13.4, 15q26.1, 16p13.3) and the duplications (19q13.11, 19q13.12) were novel, whereas two deletions (16p11.2 and 17q12, including the *LHX1* gene) have been previously described in MA patients. We confirmed the sizes of the CNVs by SNP genotyping (HumanOmni2.5-8). Subsequently, we could confirm the same 16p11.2 deletion in four other MA patients. The deletion expands ~0.53 Mb and includes at least 26 genes.
To date, altogether 12/112 Finnish patients (10.7%) were found to carry CNVs. Our results lend support to complex genetic etiology of MA. Further studies are needed to identify the cause in the majority of patients.

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P19.41

Best practice guidelines for Preimplantation Genetic Diagnosis of triplet repeat disorders

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Myotonic dystrophy (MD), Huntington disease (HD) Fragile X syndrome (FXS), spinocerebellar ataxia (SCA1, SCA2, SCA3, SCA6, SCA7, SCA17), Friedreich ataxia, Oculopharyngeal Muscular Dystrophy and Kennedy disease are caused by the expansion of unstable polymorphic trinucleotide repeats. For couples at risk of transmitting one of these diseases, preimplantation genetic diagnosis (PGD) has become a reproductive option. According to the European Society of Human Reproduction and Embryology (ESHRE) PGD consortium, MD, HD and FXS are three of the most common indications for PGD for monogenic disorders. In order to define the basis for an international harmonization of technological protocols for PGD of triplet repeat disorders, EuroGentest2 granted a workshop that was held in February 2013 in Montpellier, France : experts from 9 PGD centers in Europe (Athens, Brussels, London, Maastricht, Madrid, Montpellier, Paris, Rome and Strasbourg) as well as the scheme director of EQA (UK NEQAS) for monogenic disease PGD attended the meeting, producing a first draft of guidelines.
Here we report the data discussed for the three most studied diseases for PGD : MD, HD and FXS. Several general points were addressed including :
-Strategies to use (direct or indirect analysis);
-Definition of « informative » microsatellite markers;
-Localization and genetic map of the markers used;
-Inclusion criteria depending on the number of informative markers;
-Strategies to use in the case of a lack of informativity.
Finally, depending on the triplet repeat disorder, specific recommendations were also reported.

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P19.42

15 years of epidemiology and genetics of Neural Tube Defects. Registry of Congenital Malformations of Alsace (1995-2009)

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Objectives: To review clinical and epidemiologic data of neural tube defects (NTDs) and to evaluate the efficiency of prenatal diagnosis in Alsace (northeast of France).
Material and Methods: A population-based retrospective study from data of the Registry of Congenital Malformations of Alsace between 1995 and 2009.
Results: 272 NTDs were recorded (prevalence: 1.4 per 1000), divided in 95 cases of anencephaly-exencephaly (35 % of all cases), 18 cases of craniorachioschisis (7 %), 35 cases of meningo(encephalo)cele (13 %) and 124 cases of (myelo)meningocele/spina bifida (45 %). The prevalence has been quite stable throughout the period. The following data are presented concerning each NTD malformations: isolated or associated case, associated syndromes or sequence, prenatal diagnosis, outcome of pregnancy. The rate of prenatal diagnosis was 99 % in
anencephaly, 100 % in craniorachischisis, 77 % in meningoencephalecele and 79 % in myelomeningocele and the rate of termination of pregnancy after prenatal diagnosis was respectively 98 %, 100 %, 96 % and 97 %.
Concerning myelomeningocele, our study emphasized the circumstances of prenatal diagnosis, and especially the impact of evolution of Down's syndrome prenatal screening. If the increased alphafetoprotein (AFP) used in the second trimester screening constituted a NTD indicator, is there a risk of misdiagnosis with the diffusion of first-trimester (without AFP) screening ?

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P19.43

Introduction of cfDNA based screening of common trisomies in Spain

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Objective: To evaluate the suitability of introducing cfDNA based screening for common autosomal trisomies in Spain.
Methods: A pilot study was designed to evaluate the implementation of the Harmony prenatal test (Ariosa Diagnostics) within the largest network of private hospitals in Spain. Women at both high and low risk of aneuploidies were included and 232 consecutive clinical samples were retrieved from singleton pregnancies of at least 10 weeks of gestation, egg donor patients were excluded. A total of 213 more samples were then collected also including egg donation pregnancies. Patients received genetic counselling before and after the test, results were evaluated with all available clinical information to decide if further invasive genetic tests were necessary.
Results: Results were obtained in 429 cases (96.4%), in 2.7% of samples final report was delayed to 3 weeks. A total of 425 cases were reported as low risk (<0,01%) for fetal trisomies 13, 18 and 21. In 4 cases risk score >99% for Trisomy 21 were reported, these results were confirmed by QF-PCR analysis of fetal cells obtained by invasive procedures.
Conclusions: cfDNA based screening for common trisomies using the Harmony test proved efficient and reliable, in this preliminary study we did not observe false positive results. Although follow up on all cases is still in progress, the test revealed to be a powerful alternative to conventional trisomy risk assessment, providing prompt relief of maternal anxiety and potentially reducing unnecessary invasive procedures

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P19.44

Non Invasive Prenatal Diagnosis

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For more than 40 years, prenatal diagnosis of Down syndrome is performed using invasive procedures which are associated with a significant risk of fetal loss (~1%). Many attempts have been made in the last two decades towards the development of a non-invasive prenatal diagnostic (NIPD) test. One of the most promising is the application of the Methylated DNA Immunoprecipitation (MeDIP) real time qPCR based approach. The MeDIP methodology combined with high-resolution tiling arrays revealed thousands of Differentially Methylated Regions (DMRs) between maternal and fetal DNA across chromosomes 13, 18, 21, X and Y. The best 12 DMRs were used for the development of a novel NIPD test for trisomy 21 ($NIPD^{21}$). The method was validated using 80 maternal blood samples including 34 Down syndrome pregnancies and 46 normal pregnancies demonstrating 100% specificity and 100% sensitivity. The $NIPD^{21}$ method was further improved and validated using 175 new cases, demonstrating 99.2% sensitivity and 100% specificity. The improved $NIPD^{21}$ was created using old and new DMRs and taking into account their genomic position. An additional advantage is that it consists of only seven DMRs which simplifies the diagnostic assay and reduces the cost even further. In conclusion, the MeDIP real time qPCR based approach is an accurate, reliable NIPD test for Down syndrome. It is simple, fast and easy to perform in every genetic diagnostic lab worldwide as it does not require expensive equipment, software or special infrastructure. Additional research highlighted the potential use of the novel approach for other genetic disorders.

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P19.45

Targeted sequencing of SNPs results in highly accurate non-invasive detection of fetal aneuploidy of chromosomes 13, 18, 21, X, and Y: a validation study

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No available non-invasive fetal aneuploidy test leverages the increased information offered by single-nucleotide polymorphisms (SNPs) and parental genotypes, which identify fetal ploidy state and parental origin. Here, we detect fetal aneuploidy at chromosomes 13, 18, 21, X, and Y via analysis of fetal cell-free DNA (cfDNA) from maternal blood by targeting 19,488 SNPs covering chromosomes 13, 18, 21, X, and Y in a single multiplex PCR reaction. Maternal plasma samples (673 euploid; 52 trisomy 21; 17 trisomy 18; 8 trisomy 13; 13 45,X) were collected at ≥9 weeks gestation under an institutional review board-approved protocol. 351 samples were externally blinded. Isolated cfDNA was amplified using 19,488-plex PCR. Sequencing data was analyzed using the NATUS algorithm, which employs Bayesian statistics to analyze multiple copy number hypotheses, determine the Maximum Likelihood hypothesis, and calculate sample-specific accuracies without a reference chromosome. In samples that passed stringent quality control metrics (94.1%), NATUS detected trisomy 13 (n=7; sensitivity: 100% [CI: 59.0-100%]), trisomy 18 (n=15; sensitivity: 100% [CI: 78.2-100%]), trisomy 21 (n=47; sensitivity: 100% [CI: 92.5-100%]), and 45,X (n=11; sensitivity: 91.7% [CI: 61.5-99.8%]), with average calculated accuracies of >99%. Fetal ploidy state was determined as low as 3.9% fetal fraction and as early as 9 weeks gestation. This is the first large-scale study, including blinded sample analysis, of the NATUS algorithm, which non-invasively detected fetal ploidy state at chromosomes 13, 18, 21, X, and Y with high accuracy. SNP- and NATUS-based cfDNA analysis thus offers a clinically viable, highly accurate, non-invasive approach to identifying fetal aneuploidies.

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P19.46

Validation study of non-invasive procedures for fetal sex and RHD status determination

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Cell-free fetal DNA (cffDNA) in maternal plasma provides the basis for non-invasive prenatal diagnosis. The diagnosis of fetal sex is indicated in X-linked genetic conditions, while fetal RhD determination is useful in RhD- women to identify pregnancies at risk of haemolytic disease of the fetus and newborn. The methods consist in multiplex qRT-PCR targeting Y chromosome-specific sequences (SRY-DYS14) and RHD gene for fetal sex and RHD determination, respectively. Since both methods are based on presence/absence of amplification signals, the simultaneous analysis of fetal DNA-specific (epigenetic) markers is compulsory to attest the presence of cffDNA.
We aim to validate these procedures on a large cohort of pregnant women (n= 2000) at different gestational period (8-11 weeks). To date 500 maternal plasma samples have been collected and a first set of 50 samples (with known fetal sex) have been already analysed for fetal gender (table 1).
Results will be compared with QF-PCR/karyotype results and/or with follow up data to establish the sensibility/specificity of the methods, and therefore whether this test could be suitable for routine clinical settings. The introduction of these procedures will allow to: i) avoid unnecessary invasive testing for carriers of X-linked diseases in case of female fetus that would be, at worst, carrier of the disease; ii) reduce the administration of anti-D prophylaxis by avoiding the treatment in women with RhD- fetus.

Plasma Samples (n=500)					**Sex Determination Results (n=50)**			
Maternal age (mean ± SD)	Weeks" gestation (mean ± SD)	Invasive PD outcome (n=50)			Female (n)	Male (n)	Sensibility	Specificity
		46,XX (n)	46,XY (n)	Other (n)				
34±5	9±1	23	26	1 (47,XX,+21)	24	26	100%	100%

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P19.47

No association of free fetal DNA levels in maternal plasma with the correct classification of trisomy 21 using the MeDIP-qPCR methodology.

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Non-invasive prenatal diagnosis (NIPD) of chromosomal aneuploidies has been challenging. Nevertheless, experts in the field have overcome the limitations of previous methodologies through the implementation of novel alternative approaches. We have successfully implemented the MeDIP-qPCR methodology and have demonstrated 99.2% specificity and 100% sensitivity. We hereby present a study in which we aim to evaluate whether the amount of ffDNA, total DNA and "fetal fraction" found in maternal plasma influences the enrichment ratios of Differentially Methylated Regions (DMRs) and the correct classification of trisomy 21 using the MeDIP-qPCR methodology.
Absolute quantification of ffDNA using DYS14 and total DNA using β-globin was applied in 83 maternal plasma samples. The quantification values for all 83 samples were correlated with the enrichment ratios of all seven DMRs and D-values that were obtained from the diagnostic formula of MeDIP-qPCR method.
Our analysis concluded that trisomy 21 samples had significantly higher ffDNA and total DNA levels compared to normal samples. Enrichment ratios of the majority of DMRs studied exhibited no association with ffDNA, total DNA and "fetal fraction" and only a small portion of DMRs exhibited moderate association. Correlation studies of ffDNA, total DNA and "fetal fraction" with the diagnostic D-value showed weak to no association but without affecting the classification of trisomy 21. Therefore, he hereby demonstrated that the MeDIP-qPCR is the only methodology describes so far in which the variability in the amount of ffDNA and total DNA among maternal samples does not affect the correct non-invasive prenatal diagnosis of trisomy 21.

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P19.48

Non-invasive prenatal diagnosis (NIPD) of RHD and fetal sex using cell free fetal DNA (cffDNA) from maternal plasma

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Cell free fetal DNA (cffDNA) in maternal blood is a valuable source of fetal material for non-invasive prenatal diagnosis (NIPD). The major clinical use of cffDNA genotyping has been for the NIPD of fetal RHD status and fetal sex in the pregnancies at risk of haemolytic disease of the fetus and newborn (HDFN) or X-linked disorders, respectively. The aim of our study was to evaluate the specificity and sensitivity of the real-time quantitative PCR method for NIPD of RHD status and fetal sex determination in a clinical diagnostic setting. A total of 71 pregnancies at risk of HDFN were enrolled in the study (66%-T3, 26%-T2 and 8%-T1). RHD genotyping was done using real-time PCR amplification of exon 5 and 7 of RHD gene while fetal sex was determined by amplification of multi-copy DYS14 marker on Y-chromosome. In addition a multiplex PCR amplifying the 10 exons of RHD gene was performed on maternal and fetal DNA to exclude partial/weak RHD phenotypes. CffDNA results were compared to the results from DNA from amniocentesis/chorionic villi sampling. NIPD showed that 34% (24/71) of the fetuses were RHD negative and 35/71 (49.3%) were males. NIPD results were fully concordant with those of invasive tests, giving a 100% sensitivity and specificity of the test. We didn't detect any RHD variants in our study group. In conclusion, this method can be used in clinical practice for targeted anti-RhD prophylaxis and improvement of management of RHD fetomaternal incompatibility and in pregnancies at risk for X-linked disorders.

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P19.49

Noninvasive detection of fetal trisomy 21: systematic review and report of quality and outcomes of diagnostic accuracy studies performed between 1997 and 2012

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Background: Research on noninvasive prenatal testing (NIPT) of fetal trisomy 21 detection is developing fast. Commercial tests have become available. To provide an up-to-date overview, an evaluation of the methodological quality and outcomes of diagnostic accuracy studies published between 1997 and 2012, was made.
Methods: Of 79 abstracts, 16 studies were included as they evaluated the diagnostic accuracy of a molecular technique for NIPT of trisomy 21, and test sensitivity and specificity were reported. Diagnostic parameters were analyzed and possible bias and applicability were evaluated utilising the QUADAS-2 tool.
Results: Recently published large cohort studies (7/16) examined massively parallel sequencing (MPS) with or without pre-selection of chromosomes, and reported sensitivities between 98.58% (95% CI 95.9-99.5%) and 100% (95% CI 96-100%), and specificities between 97.95% (95% CI 94.1-99.3%) and 100% (95% CI 99.1-100%). None of the studies had overall low risk of bias and low concerns regarding applicability. MPS with and without preselection of chromosomes exhibits an excellent negative predictive values (100%) in conditions with disease odds from 1:1500 to 1:200. However, positive predictive values were lower, even in high-risk pregnancies (19.7%-100%).
Conclusion: NIPT of trisomy 21 by MPS is promising and likely to replace the prenatal serum screening test that is currently combined with nuchal translucency measurement. Before NIPT can be introduced in a social insurance health care system, more evidence is needed from large prospective diagnostic accuracy studies and further assessment, of whether NIPT can be provided in a cost-effective, timely, and equitable manner, is required.

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P19.50

R72P p53 single nucleotide polymorphism in patients with repeated implantation failure and pregnancy loss: influence in the outcome of IVF cycle.

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INTRODUCTION: Significant differences in the codon 72 polymorphic form p53 might affect its activity, R72 is markedly better than P72. Previous studies shown R72P polymorphism is a risk factor for implantation failure. The aim of this work was investigate whether R72P p53 polymorphism has a higher prevalence among women with recurrent implantation failure (RIF) and pregnancy loss (RPL) and its influence in their IVF cycle outcome.
MATERIAL AND METHODS: p53 polymorphism P72 has been studied in 181 women. The control group included 83 oocyte donors. In the study group 98 women were included: 44 with RIF and 54 with RPL. From the study group, 70 patients underwent IVF-cycles (43 RPL and 27 RIF) at Instituto Bernabeu.
RESULTS: The frequency of P72/P72 genotypes on p53 gene among women experiencing RIF was 11.4% vs 18.5% for those with RPL and 6% in controls ($p<0.01$). Significant differences were reported in pregnancy rate (73.3% for RR, 67.6% for RP and 33.3% for PP; $p<0.05$), embryo implantation rate (36.6% for RR, 43.3% for RP and 19.6% for PP; $p<0.05$) and ongoing pregnancy rate (46.7% for RR, 52.9% for RP and 14.3% for PP; $p<0.05$) among women suffering RIF and RPL.
DISCUSSION: Genotyping p53 gene is an important factor for determining the prognosis of IVF cycles on RIF and RPL women because P72 on p53 gene is more prevalent in RIF and RPL patients than fertile population. Moreover, patients carrying a PP genotype on p53 codon 72 will have less chance to achieve an ongoing pregnancy.

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P19.51

Evaluation of prenatal samples analyzed with the AmplideX FMR1 PCR assay

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Abnormal expansion of the CGG repeat in the 5' UTR of the FMR1 gene causes Fragile X syndrome. The CGG repeat lengths can be divided in four diagnostic categories: normal, grey zone, premutation and full (methylated) mutation alleles. In our lab, Fragile X testing is performed by sizing the CGG repeat with a commercially available assay, the AmplideX FMR1 PCR Kit (Asuragen), comprising primers for a sizing PCR and a CGG triplet-repeat primed PCR. Validation experiments confirmed Asuragen's results with detection of all FMR1 expansion types, with an analytical sensitivity of 100%, a detection limit of 10% for a full mutation and an accurate repeat sizing. The validation experiments were performed on different matrices, including previously analyzed prenatal samples. In routine, additional prenatal samples were analyzed. The results of these prenatal samples will be presented in more detail. Four CV samples showed an unexpectedly number of gene-specific sizing peaks: including one triploidy, one sample mosaic for a premutation/full mutation as a result of both contraction and expansion of the maternal repeat, and two samples with a low percentage of maternal contamination. In conclusion, the Asuragen assay was found very reliable and sensitive for detection of Fragile X (expanded) alleles in different matrices, including prenatal samples, with the additional advantages of a fast turnaround time and need for only a small amount of DNA. Moreover, implementation of the TP-PCR assay significantly reduces the need for Southern analysis or other methylation-sensitive assays.

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P19.52

A pilot study for prenatal and preconceptional Fragile-X Syndrome screening in the Balearic Islands

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The Fragile X-syndrome (FXS) is caused by the expansion of a CGG triplet located in the 5'UTR region of the *FMR1* gene and is one of the most frequent causes of hereditary intellectual disability. The estimated incidence of FXS in males in the Spanish population is 1 in 2500 for full mutation and about 1 in 250 for premutation (Fernandez-Carvajal et al. 2009). Although the prevalence of premutation in Spanish women has not been established, estimates in other western countries range from approximately 1 in 150 to 1 in 250. Given the severity of the disease, its high incidence in the general population, the exclusively maternal expansion, the familial and social impact of the FXS, and the high level of detection of current techniques (99%), we think that screening for FXS in women of reproductive age is a reliable and desirable option. Therefore, we have initiated a pilot study in the Balearic Islands to determine the feasibility and acceptability of prenatal and/or preconceptional screening in women of childbearing age. The results obtained so far, in a total of 422 women (117 preconceptional and 305 prenatal) indicate a high acceptability of FX screening in women that are referred for prenatal or preconceptional consultation. Surprisingly, the incidence of carriers of a premutation (55-200 repeats) is (to date) very high: 1 in 60, which may indicate a higher prevalence than previously thought. We will present updated results based on a total of approximately 2000 women.

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P19.53

Improving Preimplantation Genetic Diagnosis for Fragile X Syndrome: a new powerful single-round multiplex test.

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Fragile X (FraX) syndrome is the most common cause of inherited mental retardation, affecting around one out of 2500 individuals. FraX is caused by expansion of an unstable CGG repeat located in the FMR1 gene on Xq27.3. Preimplantation Genetic Diagnosis (PGD) can be proposed to couples at risk of transmitting the disease, i.e. when the female carries a premutation or a full mutation.
Technical conditions for FraX PGD are not trivial for many reasons: i) single-cell CGG-amplification is obtained for normal alleles only, limiting the use of a direct test to couples informative for this locus; ii) GC-rich DNA content of the repeats perturbs the amplification of other loci (i.e. microsatellites sequences) by single cell multiplex-PCR; iii) currently used polymorphic markers show limited to no informativity for a non-negligible part of the couples asking for PGD. Moreover, premature ovarian failure occurring in some premutated women often leads to poor oocyte retrieval and only few embryos analysed during PGD. Thus, a powerful PGD test is of fundamental importance to minimize the rate of non-diagnosed embryos.
We describe a new single-cell, indirect multiplex PCR using unpublished, highly heterozygous polymorphic markers. Globally increased informativity improves robustness of this test. Futhermore, it allows PGD feasibility for some couples for which previous strategies were not applicable. It was applied alone (on 2 cells) or concurrently with CGG repeats amplification (on one cell per test) for 4 PGD cycles: 10 embryos were analysed, all were successfully diagnosed, 6 were transferred, and 2 pregnancies are ongoing.

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P19.54

Attitudes toward preimplantation genetic diagnosis (PGD) procedure among couples performing PGD for autosomal dominant disorders

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PGD technology provides an alternative to prenatal diagnosis (PND), and since PGD obviates the need for pregnancy termination (TOP), some argue that the moral justification for using it should be less strict than for PND. Others claim that the ethical and social implications of using PGD resemble ‚eugenic' ideas and thus advocate minimizing or banning its practice.
PGD has been widely used in Israel, by many sectors of the population, for a broad range of conditions. While any couple at risk for genetic disease in their offspring face dilemmas regarding their reproductive future, one of the unique groups of PGD consumers are individuals living with a medical condition which they choose to prevent in their future offspring. As a part of a larger project on the attitudes of PGD users in Israel, semi-structured in-depth interviews were carried out with 13 PGD users with autosomal dominant disorders (Achondroplasia, Myotonic Dystrophy, Neurofibromatosis etc). The interviews explored decisions, experiences, concerns, and attitudes toward PGD. Issues concerning medical, ethical and sociological aspects of PGD were also analyzed.
Preliminary findings show that the main reason for using PGD was to prevent difficult lifetime experiences related to the genetic condition. However, more than half of the interviewees thought their condition does not justify TOP. For them, PGD is the only acceptable preventive procedure.
Understanding the experiences and attitudes of PGD consumers will contribute to the development of counseling programs for future PGD users and will assist in fostering a public debate concerning various aspects of PGD.

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P19.55

Reproductive life after preimplantation genetic diagnosis; the decisions couples make and why.

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Success rates for PGD are well documented, but little evidence exists about the impact on reproductive decisions. An understanding of the decisions couples make and why after PGD provides valuable information in counselling potential PGD couples.
Questionnaires were sent to 251 eligible couples who had PGD between 1999-2007 at Guys Hospital; 113/251 (45%) were returned. Of these 68/113 (60.2%) couples were successful and 45/113 (39.8%) were unsuccessful.
The main reasons for PGD were; avoidance of termination of pregnancy, recurrent miscarriages and fertility problems with genetic risk. Successful couples had 105 babies and 9 of the unsuccessful couples had miscarriages following positive pregnancy tests. There were 53 unaffected non PGD children across both groups, at least 12 of whom were born after PGD. The unsuccessful couples had 5 affected children after PGD.
The reasons behind post PGD reproductive decisions were complex. Irrespective of prior success: emotional and physical stress, cost, impact of treatment and success rates were given as reasons not to proceed with further PGD. Successful PGD gave couples the confidence to try naturally. Lack of alternative choices balanced against a strong desire for children lead to options chosen by unsuccessful couples. After treatment couples in both groups reviewed their perception of the underlying genetic risk.
The findings provide insight into the value of PGD as a reproductive option and the impact it has on couples. Evidence of the reproductive decisions made after PGD should contribute to the discussions clinicians have with couples opting for PGD treatment.

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P19.56

Molecular techniques as an alternative to FISH (fluorescence in situ hybridization) in preimplantation genetic diagnosis (PGD) of reciprocal translocation

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Objective: The standard method for PGD of reciprocal translocations is FISH using commercial probes. This associates with 5-7% misdiagnoses due to non-specific hybridization, overlapping or split signals. We sought to evalu-

ate whether accuracy may be improved by polymorphic marker analysis and array comparative genomic hybridization (CGH).
Methods: Balanced carrier of t(14;21)(q11.2;q22.1) has opted for PGD. Since commercial fluorescence probes were not available we designed a multiplex PCR protocol using polymorphic markers in 3 different regions along the relevant chromosomes and applied to PGD. For reanalysis, affected embryos were subjected to array CGH.
Results: Haplotype analysis of parental genomic DNA revealed that of 30 polymorphic markers tested, only 12 were informative (4 in each region). Ten embryos were biopsied for PGD and only one was diagnosed with balanced chromosomal constitution. Five embryos were unbalanced and since parental alleles were excessively overlapping in four embryos, diagnosis was inconclusive. These embryos were reevaluated by array CGH with 40 probes on average in each locus that confirmed the suspected chromosomal aberration.
Conclusion: CGH array may be the most accurate and reliable method for detection of chromosomal imbalances in PGD, but is relatively expensive. Polymorphic marker analysis is an accurate technique when there are at least 4 fully informative markers around the translocation sites. However, dozens of primers that must be tested make it time consuming and not cost effective approach. FISH is rapid, economical, relatively reliable, but, when no commercial probes are available, array CGH is favored over markers analysis for PGD of translocation.

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P19.57

Developmental neuropsychological assessment of 4-5 years old children born following Preimplantation Genetic Diagnosis

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Introduction: Preimplantation genetic diagnosis (PGD) is a technique that enables the prevention of affected embryo transfer for couples at risk of transmitting a genetic disorder to offspring. Due to its relative invasiveness it is essential to identify any adverse effects on the long term development of children born as a result of PGD.
Aim: The aim of this study was to measure and evaluate developmental neuropsychological profiles of 4-5-year-old children born after PGD relative to norms representing the general population.
Materials and methods: Twenty seven, 4-5 year old, PGD children were recruited. Participants were subjected to a battery of neuropsychological assessments including: WPPSI-III(cognitive development), PLS-4(language development), WRAVMA(visual motor abilities), CARS2(screening test for autistic spectrum disorders) and the Miles ABC Test(ocular dominance). Parental questionnaires regarding executive function and demographics were performed as well. Each subject's tests results were compared to each test's norms as provided by the test authors. When available, Israeli norms were used for comparison.
Results: Children born after PGD showed scores within the normal or above-normal ranges for all developmental outcomes. The mean WPPSI Full Scale Intelligence Quotient and Verbal Intelligence Quotient were significantly higher than the general population(P=0.013), although not clinically significant(100±15). All other tests performed within normal test ranges.
Conclusions: This pilot study shows that children conceived after PGD display normal developmental neuropsychological outcomes at age 4-5 years when compared to age-matched normal children in the general population. A larger controlled study is needed to confirm the validity of this conclusion.

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P19.58

Overcoming limitations of single cell analysis in duplicated genomic regions using multiple displacement amplification for Preimplantation Genetic Diagnosis (PGD)

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PGD on single cells requires concurrent analysis of a mutation together with multiple linked polymorphic markers from closely related family members in order to prevent misdiagnosis. In PGD cases involving childless *de novo* mutation carriers, linkage cannot be performed based on family members but rather must first be identified in single gametes. This is an especially difficult task if the mutation to be assayed lies in a duplicated genomic region because gene-specific long-range PCR must be coupled with short-range PCR analysis of genetic markers on single cells. Here we describe a novel method by which accurate PGD of pseudogene-homologous mutations can be achieved. Essentially, we performed multiple displacement amplification on single sperm or blastomeres followed by haplotype construction and long range PCR-based mutation analysis. This original and universal strategy was used to establish allelic association for two different mutations in genes with one or more pseudogene copies (*IKBKG* and *PKD1*). For *IKBKG*, the method was implemented during a PGD cycle which resulted in the birth of a healthy child. Regarding *PKD1*, our method detected unexpected germline mosaicism in single sperm from the mutation carrier. These results indicate that the methodology overcomes pseudogene masking while still facilitating the detection of single copy targets with high accuracy.

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P19.59

Introducing Preimplantation Genetic Screening (PGS) by array comparative genomic hybridization in Hungary: improving outcome of *in vitro* fertilization

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The aim of our study was to assess the aneuploidy patterns of embryos from Hungarian patients undergoing *in vitro* fertilization (IVF). Day three embryos had one cell removed as a biopsy specimen before DNA amplification and microarray comparative genomic hybridization (aCGH) analysis were performed.
Our retrospective study was performed on 421 human embryos from 107 Hungarian patients (ave. age: 36.69). Out of them 28.98% were normal, 34.44% had complex aneuploidy (embryos with >2 chromosome abnormalities), 11.88% were double aneuploidies, 13.78% monosomies and 10.93% trisomies. The number of complex abnormal embryos significantly increased with advancing maternal age while the occurance of normal embryos decreased, from 37.23% and 39.89% in patients ≤35 to 69,51% and 10.97% in patients 41 and older, respectively. Out of 107 patients, 73 had normal embryos. 16 of these patients have ongoing pregnancies and four of them delivered children already.
The results show similar tendencies with previous observations at numerous points; most importantly, the higher the maternal age, lower the euploid/aneuploid ratio is. The occurrences of the monosomy and trisomy events in the examined embryos decreased, while complex aneuploidies increased with elevated maternal age. Despite the finding of previous studies, which described chromosome X as a chromosome with the highest frequency of aneuploidy, in our study the aneuploidy of sex chromosomes were extremely rare. The most common aneuploidies were for chromosomes 15, 5, 16, 10, 20 and 1. The rarest aneuploidies were for chromosome Y, 4, 12, 3, 11 and 2.

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P19.60

Preimplantation genetic screening with used 24sure microarrays, our results and the success of in vitro fertilization cycles

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Chromosomal abnormalities are a major cause of the failure of embryos implantation and of miscarriages, also their number increases in oocytes with

age. Preimplantation genetic screening (PGS) is a principally new approach for the prevention of genetic disorders, which allows the selection of unaffected embryos in in vitro fertilization (IVF) cycles. PGS can be applied for chromozomal abnormalities such as aneuploidies using diagnostic protocols based on the Fluorescence in situ hybridization (FISH) or Array comparative genomic hybridization (aCGH).
In our work we studied cells of trophectoderm in 185 embryos. We analysed DNA of cells of trophectoderm with the use of 24sure microarrays BlueGnome. We obtained DNA from the few cells by the whole genome amplification. We found 30 % of abnormal embryos in group of women younger than 35 years and 49 % of abnormal embryos in group of women older than 35 years. Then the total 36 % in all analysed embryos was abnormal. Major part of abnormalities represented partial or total gains and losses of chromosomes 1, 7, 15, 16, 18 and 21.
The results of genetic analyses of embryos should serve as a supporting tool for the determination of chromosomal aberrations in embryos. Probability of pregnancy and birth of a healthy child improves the selection of embryos without aberrations. The succes rate of an IVF cycle is in our centre 68% with the use of PGS and strictly one embryo transfer. Cooperation is very important between embryology and andrology. Obtaining good-quality embryos is crucial for PGS.

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P19.61
Preclinical validation of oligonucleotide array-CGH method for preimplantation genetic screening

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The field of diagnosis of embryos for chromosome abnormalities, i.e. preimplantation genetic screening (PGS), has been recently re-energized by the introduction of microarray-based technologies allowing analysis of all 24 chromosomes in one test and thus providing much more information than approaches used in the past. There are two microarray platforms (BAC and oligonucleotide based) developed for use in PGS.
We describe preclinical validation of the oligonucleotide based arrays used for PGS performed on blastocyst biopsy. The validation was done in several steps: 1) validation of whole genome amplification (WGA) protocol; 2) validation of array platform on appropriate cell samples; and 3) determination of PGS array resolution.
The WGA and array platform validation was done by testing of 5-10 cells from lymphocytes of normal control samples and sample with a Down syndrome to ensure the consistent results on the array. In the second step, samples from blastocysts with known aneuploidies were tested. WGA method based on GenomePlex adjusted for single cell amplification (PicoPlex, Rubicon) was selected as the satisfactory amplification method and the Single Cell Aneuploidy Array 15K from Oxford Gene Technologie as the optimal array platform.
Array CGH resolution was tested using diluted DNA from samples with known chromosomal aberrations. Using our optimized protocol, we were able to detect multiple chromosome abnormalities including aneuploidies but also smaller structural abnormalities as, e.g. 2 Mb deletion.
Our results indicate that this oligonucleotide array platform can be reliably used for PGS in patients undergoing IVF.
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P19.62
Placental growth factor in prenatal diagnosis

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Placental Growth Factor (PlGF) improves 1st trimester trisomy 21 screening, detects risk of early preeclampsia (PE) and allows low-dose aspirin prevention before 16th week. The aim was to ascertain control levels within 9th-18th weeks in Czech women.
PlGF was examined in 315 sera within 8th-13th and 155 within 14th-18th weeks by Delfia Xpress. The aneuploidy and PE risk were ascertained by LifeCycle/Elipse and Preeclampsia Predictor (usable only for 1st trimester). Levels for 3rd, 5th, 25th, 50th, 75th and 95th precentiles within 9th-18th weeks correspond to the Perkin Elmer's percentiles published for 9th-13th week only. PlGF linearity increase within 9th-18th week was identical with published data. The cut-off levels for 9th-18th weeks were calculated for the PE/aneuploidy risks. PE 1st trimester risk was found in 2 women (<5th percentile). Decreased levels were found in 2 pregnancies with gestational hypertension. The levels in 16 pregnancies with severe PE referred for Caesarean section within 30th-40th weeks corresponded to medians for 9th-20th weeks. Beneficial consequence of PlGF in trisomy 21 1st trimester was confirmed by isolated PlGF decrease (<3rd percentile, 1 case) and isolated increase (>75th percentile, 2 cases). PlGF decrease (<5th percentile) was observed in 2/3 triploidies. In one trisomy 21 PAPP-A, βhCG and PlGF were normal in 11+6 week, increased βhCG and PlGF <3rd percentile in 15+1.
The results support the inclusion of the PlGF to the 1st trimester screening of aneuploidies and early PE treatment.
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P19.63
CASK gene Deletion in a Female Presenting with Cystic Hygroma, Echogenic Bowel and IUGR - Expanding the Fetal *CASK* phenotype

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CASK is a calcium/calmodulin dependent serine protein kinase that belongs to the membrane associated guanylate kinase (MAGUK) protein family. Located on the X chromosome, heterozygous mutations in the *CASK* gene have previously been described in females with severe intellectual disability, microcephaly and pontocerebellar hypoplasia (MICPCH)(MIM 300749) and FG syndrome 4 (MIM 300422).
To date, there has been no prenatal description of mutations or whole gene deletions encompassing this critical region. We report a female fetus with cystic hygroma, IUGR, cardiac malformation and echogenic bowel. Investigation done to delineate the etiology of the abnormalities included Noonan syndrome gene panel, chromosome analysis and parental DNA analysis for the 39 most common mutations in CFTR gene and TORCH analysis failed to identify an abnormality. Array CGH showed a *de novo* 5 Mb deletion at Xp11.4p11.3, encompassing: *CASK, NYX, MAOA, NDP* and *KDM6A*. Deletions of the genes in this region have been described with a phenotype that is clinically indistinguishable from loss of function mutations in *CASK*, suggesting that it is the main contributor to the clinical picture. Limited autopsy results from our patient showed facial features previously described in *CASK* gene mutations including faint philtrum, thin upper lip and retro-micrognathia.
Loss of function mutations of *CASK* and large chromosome deletions of this region described previously have been associated with MICPCH. In the prenatal setting many of these key features of *CASK* mutation cannot be readily assessed. The findings in our case expand the phenotype associated with *CASK* gene mutation/deletion to the prenatal setting.

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P19.64
The importance of offering targeted preconception carrier testing: First results in a small fishermens' village in the Netherlands

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Autosomal recessive disorders in general are rare, but may be high prevalent in subpopulations. Within a small community in the Netherlands relatively many children are born with a severe autosomal recessive disease (approximately 2-4 children of 220 live births a year). Founder mutations were identified for the four following diseases: pontocerebellar hypoplasia type 2 (PCH2), foetal akinenesia syndrome (FAS), rhizomelic chondrodys-

plasia punctata type 1 (RCDP1), and osteogenesis imperfecta type II/III (OI). Children with these disorders suffer significant morbidity and have a reduced lifespan. This warranted the start of a preconception outpatients' clinic in collaboration with the local midwifery practice, aimed at couples planning a pregnancy. Standard preconceptional advice is given, combined with genetic counseling.
From September 2012 till January 2013, 63 individuals (related to 32 couples) were counseled in this clinic and carrier testing for the above disorders was performed.
In total 19/63 individuals (30%) were identified as carrier: nine were carriers of FAS, eight of PCH2, three of RCDP1 and one of OI. One carrier couple at high risk (25%) was identified. Two persons were carrier of two diseases, thus 19 individuals were carrier. In 73% (n= 46) individuals no positive family history of carriers/affected persons within the family was known. Nine of these 46 (20%) individuals were carrier of at least one disease.
These first results stress the importance of offering preconception carrier screening within populations with high prevalence of specific genetic disorders, and can be used as a test case for other community-based carrier screening programmes.

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P19.65

Comparative gene expression profiling of placentas from patients with moderate and severe preeclampsia

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Preeclampsia is a pregnancy related disease associated with hypertension, proteinuria and increased maternal and perinatal morbidity and mortality, without known underlying mechanism and preventive treatment. To investigate how the expression of maternal genes contributes to the mechanisms underlying the progression of the disease, we investigate global placental gene expression in preeclampsia using microarray technology. Genome-wide transcriptional profiling was performed on placental tissue from moderate preeclamptic (n=5), severe preeclamptic (n=5) and normal (n=11) pregnancies. Among the 47000 transcripts that were screened, 63 were found to be differentially expressed between normal and pre-eclamptic tissues (Fold Change>1.5, FDR<0.1). Among these candidates, 53 were up-regulated and 13 were down-regulated. These differentially expressed genes were associated with such biological processes as response to stress, immune system process, regulation of cell communication, intracellular signaling cascade etc. Comparison between moderate and severe preeclampsia showed 8 differentially expressed genes. Two genes - BAG3, encoding Bcl-2-binding protein, whose main function is the inhibition of chaperone HSP70/HSC70, and HSPA1A, whose product is heat shock protein 70 (HSP70) are the most interesting for further study. These genes may therefore prove to be novel biological markers by which the severity of this condition could be predicted. This finding may provide insight into the pathophysiology of the disorder and lead to new therapeutic possibilities for this disease. This work was supported by the Russian Foundation for Basic Research.

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P19.66

Gene expression analysis in pregnancy-induced hypertension: the inverted response between mother and fetus

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Background: Pregnancy-induced hypertension (PIH) affects almost 10% of pregnant women and represents a major cause of morbidity worldwide for both mother and child. The major mechanisms currently associated with PIH are metabolic and immune processes, cell communications, and angiogenesis. We have further explored the molecular basis of PIH considering both the maternal and the fetal components.
Methods: Gene expression analysis (HumanHT-12 v4-Expression BeadChip; Illumina) was performed in placental tissue, comparing expression profiles between cases (pregnant women with PIH; n=23) and controls (pregnant women without PIH; n=49). Both maternal and fetal sides of the placenta were investigated. "Genome studio" software (Illumina) was used for the data analysis. A fold change ≥1.5 and a false discovery rate (FDR)-based multiple testing correction were applied in evaluating differential gene expression.
Results: Expression data demonstrated a significant dysregulation of extracellular matrix genes involved in cell migration and focal/cell adhesion (up-regulated in fetal, down-regulated in the maternal placenta side; $p=10^{-11}$) and secreted molecules of immune adaptation (up-regulated in maternal, down-regulated in fetal placenta side; $p=10^{-3}$). For each subset we focused on specific genes: inflammation (*AIF1,APLN,VCAM1*) and hormone (*CGB5*) genes in the maternal side; apoptotic genes (*IL33,CASP1,CADM1*) and degradation molecules (*HTRA4,RNASE4*) in the fetal side of placenta.
Conclusions: The concomitant identification of differential expression of genes across the placental wall strongly highlights the molecular crosstalk between mother and fetus. These data are consistent with a model where trophoblast invasion is defective as a consequence of an immune-associated mechanism affecting cell migration, invasion and survival.

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P19.67

Screening for NR5A1 gene mutations in cases of premature ovarian failure

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Sex determination, in 46,XY individuals, primarily occurs through the action of *SRY* gene. Whereas sex development in 46,XX individuals was thought to be merely a default pathway, several cases of XX female-to-male sex reversal have been described without presence of *SRY* gene. Further, loss of function mutations in some genes, such as *WNT4*, *RSPO1* e *FOXL2* have been considered to be ovarian promoting or anti-testis genes. Recently, *NR5A1* gene was associated with patients with abnormalities in ovarian development and function, like in premature ovarian failure (POF). Transcription of a large set of genes associated to reproduction and sex determination and differentiation is regulated by SF-1, a nuclear receptor encoded by *NR5A1* gene. The direct sequencing of the seven exons of *NR5A1*, including the promoter region, intron/exon boundaries and 3'UTR was performed in 50 patients (n=100 alleles) with POF. The study revealed 9 polymorphisms (SNPs): rs113048364 within 5'UTR (n=1), rs115601896 within intron 2 (n=3), rs138805488 within exon 3 (n=1), rs1110062 and rs1110061 within intron 4 (n=4), rs2297605 within intron 5 (n=14), rs7037254 within intron 7 (n=32) and rs5900617 and rs10120967 within 3'UTR (n=35 and n=26). The mutation p.Gly146Ala, considered a SNP (rs1110061), has now been associated with POF patients and in the present screening it was identified in 4 alleles. Considering the several interactions that *NR5A1* play and the fact that p.Gly146Ala leads to a decrease in the transactivation capability upon some gene promoters, this research can be important to elucidate the role of *NR5A1* in ovarian development and maintenance.

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P19.68

Repeat Primed PCR (RP-PCR) method for detection of FMR1 gene premutation in Primary ovarian insufficiency

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The primary ovarian insufficiency/premature ovarian failure (POI/POF) in women in fertile age can significantly influence the reproductive chances.
Objective: Introduction of a molecular genetic test method diagnostics of the POI/POF patients by which accurate and reliable results can be predicted concerned about the disease. The main research targets in patients of premature ovarian failure are the detection of the increase in the number of (CGG)n repeats in the FMR1 gene promoter region. **Patients and methods:** There was molecular genetic testing in 78 patients examined with suspected POI. Early ovarian depletion criteria were consistent with international protocols: secondary amenorrhea, ovarian failure up to the age of 40 years, levels of FSH≥ 40 IU/L, and low estrogen levels. As a first step the patients were tested by Southern blot analysis and we subsequently investigated the CGG trinucleotide repeats in the FMR1 promoter region by hybridization with radiolabeled DNA probes. In all cases, which confirmed the pre-mutation status by the Southern blot, we completed the so-called Repeat Primed PCR (RP-PCR) method used to determine the exact number of CGG repeats.
Results: In 8 cases out of 78 patients we could verify the increase of the CGG repeat number, it is 10.1% of the cases. In one cases we found mosaic form.
Conclusions: The genetic examination of the premature ovarian failure is very important for the patient and her family also, because the genetic results have serious influence on the reproductive possibilities and family planning of the premutation carriers.

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P19.69

Retrospective evaluation of prenatal diagnosis records in a reference genetics center: 18 years experience, 7935 samples

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The aim of this study is to review and evaluate the prenatal diagnosis records in terms of demographic data, distributions throughout the years, indications, cytogenetic results and discuss the results in different aspects. A total of 7148 amniocentesis (ASI), 505 chorionic villus sampling (CVS) and 282 cordocentesis (CRD) results were retrospectively evaluated between the period 1995 to 2012. The mean age of the study group was 33.2±5.88. The mean gestational week for ASI, CVS and CRD was 17.74±1.65, 12.16±1.13 and 22.69±2.59 respectively. The number of referrals significantly increased throughout the years. The most common indications were advanced maternal age (49.5%), abnormal maternal screening tests (26.0%) and abnormal fetal ultrasound findings (%10.2) which were increased throughout the years. The demand for the prenatal testing of single gene disorders such as beta thalassemia (4.1%) and spinal muscular atrophy (0.7%) has increased. Normal karyotype was observed in 93% of all samples. Autosomal trisomies were detected in 2.2%. Numerical sex chromosomal anomalies including 45,X; 47,XXY and others constituted 1.1%. Balanced and unbalanced structural anomalies comprised 0.8% and 0.1% of all samples respectively. Risk of having trisomy 21 significantly increased with advanced maternal age, particularly after the age of 40. Abnormal maternal serum screening tests over 1/200 were positively correlated with trisomy 21. Cystic hygroma and intrauterine growth retardation were the most significant ultrasound findings resulted with abnormal karyotype. In conclusion, our results have contributed to our knowledge of prenatal testing and genetic counseling which may provide to develop long term and effective strategies.

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P19.70

Offering pregnant couples at increased risk for Down syndrome a choice between 5-10 Mb and 0,5 Mb array analysis: a first impression of what pregnant couples really want

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Whole genome SNP array testing with a resolution of 0.5Mb is now available in our university hospital for patients with increased risk for aneuploidy and normal ultrasound examination results, referred mainly due to advanced maternal age or abnormal first trimester screening results. Thanks to higher testing resolution array technique reveals not only pathogenic abnormalities, but also so called risk factors or susceptibility loci for intellectual disability/developmental delay/autism spectrum disorders or epilepsy. Thus, more information about the (future) health of the unborn child can be obtained with this technique. We offered pregnant couples the choice between array results obtained by analysis at a 0,5 Mb or a 5 Mb resolution, the latter similar to that of karyotyping. The preliminary results of our pilot (n=30) are that 18% chose the 5 Mb resolution, 33% opted for 0.5 Mb resolution excluding risk factors and 47% opted for 0.5 Mb resolution including risk factors. Refusal to learn about risk factors was out of fear of becoming needlessly worried. Over 74% of couples indicated the wish to decide how much information they desired about the (future) health of their unborn child.
So far, our results indicate that pregnant couples differed regarding their preferences. In addition all couples appreciated being offered the choice. It seems that with the broadening of techniques for prenatal diagnosis, reproductive autonomy may only be accomplished by offering pregnant couples a choice regarding what they wish to learn about the (future) health of their unborn child.

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P19.71

Detection of chromosomal abnormalities in products of conception by Karyolite BAC-on-Beads assay

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Karyotyping is the gold standard method for detecting chromosome imbalances in products of conception (POCs) but this method based upon cell culture gives a high failure rate. The KaryoLite™ BACs-on-Beads™ (KL-BoBs™) assay is a new molecular cytogenetic test using a multiplex microsphere-based suspension array technology. KL-BoBs™ quantifies proximal and terminal regions of each chromosome arms.
We prospectively tested with KL-BoBs™ each POC sample (n=175) that we received at our laboratory for chromosome analysis. It was mainly polymalformed fetus but also in utero fetal deaths and terminations of pregnancy not previously characterized by karyotype. For each sample, a cell culture was done in parallel, in order to verify by FISH and/or karyotyping, any abnormality suspected on BoBs.
We found 11.5% (20/175) chromosomal imbalances in our cohort. The failure rate was 4.6%. BoBs profile showed visible maternal cell contamination in 2.3% of the male samples. Aneuploidies account for 60% of abnormalities detected, structural chromosome imbalances for 20%, triploidy (69,XXY) for 10%, and sex chromosome abnormalities for 10%.
Our results are concordant with those found in the literature. The technical performance and feasibility of this assay for rapid detection of chromosomal abnormalities in POCs are rather good. Our data showed best results concerning detection of structural abnormalities than those reported before. However, KL-BoBs™ had also limitations as the inability to well detect polyploidy or low mosaicism. But a good management of this assay, helped by FISH or microarray, makes this test a useful tool for chromosome analysis of POCs.

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P19.72

Prenatal screening for Noonan syndrome mutations: high frequencies of PTPN11 mutations in fetuses with elevated nuchal translucency

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Noonan syndrome is a common autosomal dominant disorder characterized by cardiovascular anomalies, short stature, broad neck, characteristic facial features, lymphatic dysplasia and variable degrees of intellectual disability in about 1/3 of patients. Ultrasonographic abnormalities for the prenatal diagnosis of Noonan syndrome are rather nonspecific: e.g. elevated nuchal translucency (NT), cystic hygroma, hydrops fetalis.
Pergament et al. (2011) reported the detection of Noonan syndrome mutations in the PTPN11, SOS1 and RAF1 genes in a total of 6.6% (n=120) of fetuses with elevated NT by array mutational sequencing after exclusion of a chromosomal imbalance.
Croonen et al (2012) report a higher detection of Noonan syndrome mutations (17.3%) by sequencing of all coding regions of the PTPN11, SOS1, KRAS and RAF1 genes. In these two publications PTPN11 mutations were detected in 4.1% and 12% of cases, respectively.
We performed PTPN11 sequencing of the whole coding region and array mutational sequencing of further known mutations after normal karyotyping and microarray testing in fetuses with NT >3mm. Although the number of cases we have screened so far is much lower (20 cases) the percentage of PTPN11 mutations of 25% is much higher. NT varied from 3 to 8 mm (mean: 6.04 mm). The PTPN11 mutations in our collective were all de novo and had already been described as being associated with Noonan syndrome. The aims of our study are to further collect phenotypic and genotypic data in prenatal cases and to investigate possible correlations between the NT measurements and the gene mutations.

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P19.73

Complex Fetoplacental chromosomal discrepancy detected by QF-PCR

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Quantitative fluorescent PCR (QF-PCR) have been introduced to perform rapid prenatal diagnosis of common chromosome aneuploidies that allows 24 hr diagnosis of the most prevalent trisomies (13, 18, 21) thus providing rapid reassurance for women and early reflexion on pregnancy management for abnormal foetuses.
Foetoplacental discrepancy occurs in 1-2% of cases with majority of chromosomal abnormality confined to the placenta while in rare instances, the placental karyotype may be normal and fetal cells show an abnormal karyotype.
We report the situation of a 35-year-old gravida referred at 15 weeks of gestation with an increased 1st trimester serum screening risk 1/80.
The QF-PCR testing showed triallelic profile for 4/5 informative microsatellites D21S1435, D21S226, D21S1411 and D21S1270 with unusual peak area ratios of 2:1:1. The most proximal D21S11 indicated a normal diploid allele with peak area ratio of 1:1. Based on the semi-quantitative approach of QF-PCR, we determined that all the triallelic microsatellite one peak area ratio value was twice of the others two and analysis of parental samples showed in the fetus sample, one paternal allele (peak area ratio 1) and two maternal alleles (peak area ratios 0,5:0,5). Cytogenetic analysis identified a normal karyotype 46,XY [15]. No ultrasound abnormality was seen and the pregnancy was carried on. After delivery, placental analysis revealed a trisomy 21 mosaicism in 56% of the cells with isochromosome 21: 46,XY,der(21;21)(q10;q10)[30]/46,XY[23]. We describe the potential mechanisms by which this uncommon situation of trisomy rescue occurred and underline the complexity to interpretate of QF-PCR electrophoresis profiles

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P19.74

Expanded prenatal genetic screening: Simultaneous detection of chromosome aneuploidies and mutations for monogenetic diseases

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Chromosomal aberrations are the main cause of severe developmental abnormalities during pregnancy. Quantitative fluorescent polymerase chain reaction (QFPCR) has established itself in recent years as an alternative detection method for prenatal chromosomal abnormalities which complements cytogenetic analysis . QFPCR offers a cheap and quick detection of the most common prenatal chromosomal aneuploidies which affect chromosomes 13, 18, 21, X and Y.
In this paper we present an improved QFPCR method, which allows the simultaneous detection of chromosome aneuploidies and common mutations for monogenetic diseases: cystic fibrosis, spinal muscular atrophy and congenital adrenal hyperplasia.
Allele specific PCR primers detecting following mutations for most common monogenetic diseases were incorporated into QFPCR oligonucleotide mixtures; the delta F508 mutation in CFTR gene (cystic fibrosis), the exon 7 mutation in SMN1 gene (spinal muscular atrophy), the I172N and IVS2-12A/C>G mutations and the deletion or gene conversion of the CYP21A2 gene (congenital adrenal hyperplasia). In addition, the presence of the RhD gene and Kell blood group T193M was detected as well.
A 100% concordance was obtained when 48 samples of genomic DNA with known genotypes were reanalyzed in a blind experiment. The quantitative nature of QFPCR for aneuploidy detection was not compromised. All mutations were correctly detected and non-specific signals indicating non-specific allele amplification were also absent,
We conclude that QFPCR can be amended for prenatal screening of frequent mutations which are characteristic for certain monogenetic diseases. Further mutations/genetic conditions may be added to the presented setting.

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P19.75

RASSF1A in maternal plasma as a molecular marker of preeclampsia

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Objectives: To quantitate cell free (cf) and cell free fetal (cff) DNA in maternal plasma by determining *RASSF1A* levels before and after enzyme digestion in women who subsequently developed preeclampsia (PE) and compare them to uncomplicated pregnancies.
Methods: 24 samples from pregnant women who developed PE and 48 samples from women with uncomplicated pregnancies were analysed. Blood samples were obtained at 11-13 weeks (mean 12.1±0.6). cfDNA was determined by quantifying *RASSF1A* using qRT-PCR. A second qRT-PCR was performed following methylation-sensitive enzyme digestion by *BstUI,* in order to quantitate hypermethylated *RASSF1A* sequences of fetal origin. *ACTB* gene was used as control to confirm complete enzyme digestion.
Results: cfDNA and cffDNA levels were significantly increased in women who developed PE as compared to uncomplicated pregnancies (median cfDNA: 9402 vs 2698, median cffDNA: 934.5 vs 62 respectively). Following ROC curve analysis a cut-off value of 7486Eq/mL for cfDNA and 512Eq/mL for cffDNA were chosen which provided a sensitivity of 75% and 100% and specificity of 98% and 100% respectively, to identify women at risk for PE.
Conclusions: The study demonstrates potential use of cffDNA and cffDNA in maternal plasma as markers for the early prediction of women at risk for PE. Early prediction during the first trimester of pregnancy could enable planning of an appropriate management and close surveillance of pregnant women at risk.
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P19.76

Genotype-Phenotype Analysis of Recombinant Chromosome 4 Syndrome: An Array-CGH Study and Literature Review

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Recombinant chromosome 4 is a rare constitutional rearrangement arising from pericentric inversion and consisting of a duplicated segment of 4p13/p15→4pter and a deleted segment of 4q35→4qter. The clinical spectrum commonly includes growth retardation, microcephaly, broad nose with anteverted nares, thin upper lips, abnormal ears, pointed chin, short neck, broad chest, and cardiac and genitourinary defects. We describe the second

case in which array-CGH was used in a 1-year-old boy patient with consistent clinical features. Conventional cytogenetics and FISH documented a recombinant chromosome 4, derived from a paternal pericentric inversion, leading to partial trisomy 4p and partial monosomy 4q. Array-CGH, performed to further characterize the rearranged chromosome 4 and delineate the breakpoints, documented a small (4.36 Mb) 4q35.1 terminal deletion and a large (23.81) Mb 4p15.1 terminal duplication. Genotype-phenotype analysis of the 10 reported cases and the present case indicated relatively consistent clinical features and breakpoints. This consistency was more evident in the two cases characterized by array-CGH, where both showed the common breakpoints of p15.1 and q35.1. A genotype-phenotype correlation study between rec (4), dup (4p), and del (4q) syndromes revealed that urogenital and cardiac defects are likely due to the deletion of 4q; other clinical features are likely due to 4p duplication. Our findings suggest that recombinant 4 syndrome is a discrete entity with relatively consistent phenotype that can be suspected on the basis of clinical features. Cytogenomic and molecular studies of patients with rec (4) may yield additional information on specific gene contributions to the phenotype.

M. Hemmat: None.

P19.77

Recurrent pregnancy loss - karyotyping products of conception should be of the first line of investigation

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Recurrent pregnancy loss (RPL) affects ~1% of couples. Determining the cause and recurrence risk of miscarriages impacts family planning and may alleviate feelings of guilt or inadequacy. Aneuploidy accounts for up to 60% of early spontaneous abortions in general. The purpose of this study was to evaluate the benefit of chromosomal analysis of products of conception (POC) in RPL.
A total of 131 POCs from women with RPL were referred for karyotyping to the Genetic Institute, Tel Aviv Sourasky Medical Center, Israel.
Cytogenetic results were compiled along with demographic and clinical data. Cultures were successfully obtained in 92.4% of samples (121/131). Maternal cell growth could not be ruled out in 10 non-chorion samples with a female karyotype. Of the 111 conclusive cases, abnormal karyotype was detected in 65 (58.6%). Numerical changes were found in 43 (38.7%) and structural abnormalities in 5 (4.5%): 3 inherited and unbalanced translocations, and 2 were de novo, one of each was accompanied by trisomy. Seven cases (6.3%) revealed triploidy. Sex chromosome aberrations were detected in 10 cases (9%). The remaining 46 samples displayed a normal karyotype. Interestingly, the incidence of abnormal karyotype (~60%) was similar regardless of the number of previous pregnancies or miscarriages.
Our study demonstrates that the majority of RPLs are due to sporadic chromosomal imbalances which will readily be detected by conventional cytogenetic analysis, making other causes for RPL (such as uterine anomalies or thrombophilia) far less frequent. We suggest that karyotyping of POC be the first line in evaluating RPL.

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P19.78

Improvement of the non-invasive fetal *RHD* detection method and application in the first trimester of pregnancy

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The detection of *RHD* genotype of the fetus in plasma of RhD-negative women is based on *RHD* gene deletion. However, in some cases the *RHD* deletion is not complete. Here, we use an upstream step including the maternal DNA testing for exons 7 and 10 of the *RHD* gene. Then after, the plasma genotyping was performed with primers for exons 7 and 10. We compared the Ct results of method I and II using extracted DNA concentrated from 1/7 plasma volume in 25 µl PCR volume and ¼ of the plasma volume in 30 µl PCR, respectively. The real-time PCR was performed in triplicates and used *HBB* for total and *SRY* for fetal DNA as controls. The *RHD* genotype was compared to the immunologically assessed phenotype of the newborn. Student *t*- test was used for statistical evaluation. The methods I and II were applied on plasma samples obtained from 37 and 20 pregnant RhD- women in II. trimester, respectively. All 33 were *RHD* positive newborns were confirmed immunologically. From 24 immunologically RhD negative newborns were 23 negative and one false positive. The mean Ct values for exon 7 were 39.98 and 36.35 ($P < 0.0001$). The mean Ct values for exon 10 were 38.99 and 38.47 ($P=0.32$). Testing in 19 samples from I. trimester showed 100% specificity and sensitivity. Here, we describe an upstream step of maternal DNA testing and improve the Ct values to enable the I. trimester
testing. Using more samples, we will perform ROC evaluation.

Z. Lasabová: None. **I. Švecová:** None. **A. Mendelová:** None. **M. Haško:** None. **I. Hudecova:** None. **V. Janušicová:** None. **M. Telková:** None. **K. Gemzová:** None. **J. Danko:** None.

P19.79

Quality of life of 19 patients with Mayer-Rokitansky-Kuster-Hauser Syndrome.

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Mayer-Rokitansky-Kuster-Hauser Syndrome (MRKH) is a congenital anomaly of the female genital tract, estimated to occur in approximately 1 in every 4500 females. Phenotype features include upper vaginal atresia and uterine abnormalities that range from an absent to small uterus. Affected females have functioning ovaries, normal external genitalia and normal female karyotype. It is often found associated to renal agenesis or adysplasia as well as skeletal malformations. MRKH Syndrome is the second cause of amenorrhea and is often discovered in adolescence due to primary amenorrhea. In this study, 19 MRKH patients from 18 non consanguineous families were identified through phenotype, hormonal, chromosome, echography, laparoscopic / laparotomy studies carried out between 1989 and 2009. The participants visit the Hospital annually. A semi-structured interview was held in order to obtain information about life progress and the following specific content areas were addressed: educational progress, relationships, employment, leisure activity and sexual identity. The condition of MRKH affects a woman's quality of living by narrowing some of life's possibilities such as intercourse and childbearing. However, almost all patients reported on marriage or cohabitation. Women who had had surgery did not experience better sexual wellness or sexual function than those who were untreated. The majority were well-educated, held full-time employment, involved in optimal and diverse recreation, were financially independent and all reported to have heterosexual identity.

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P19.80

SOX9 duplication in 46,XX infertile males SRY negative: a case report

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Male development is normally triggered by the transient expression of the SRY gene, which initiates a cascade of gene interactions orchestrated by SOX9, leading to the formation of testes from bipotential gonads. SOX9 is considered to be the direct target gene of the protein encoded by SRY and its overexpression in an XX murine gonad can lead to male development in the absence of Sry. Recently, two families were reported with respectively a 178 kb duplication and 96 kb triplication 500 kb upstream of SOX9 in the gene desert region ending in which 46,XY duplicated persons were completely normal and fertile whereas the 46,XX ones were infertile males. Here, we report a new case of 46,XX infertile male, SRY negative, and with SOX9 duplication. The patient, aged of 30 years, was referred to our department for genetic investigation of his azoospermia. Examination showed normal male secondary sexual characteristics and bilateral gynecomastia. The patient had low serum testosterone concentrations and increased follicle stimulating hormone and luteinising hormone. The scrotal sonography was normal. The karyotype revealed 46,XX chromosome constitution and SRY was negative. Array-CGH analysis showed a duplication of about 220-250 kb of the long arm of chromosome 17 [dup(17)(q24.3)]. The duplication maps about 400 kb upstream of SOX9 gene. Our results confirm the previously reports supporting that even in absence of SRY, complete male differentiation may occur, possibly driven by overexpression of SOX9 in the gonadal ridge, as a consequence of the amplification of a gene desert region.

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P19.81

Utility of sperm FISH analysis in patients with structural chromosomal abnormalities before pre-implantation genetic diagnosis.

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Objective: To optimize orientation of couples towards PGD due to paternal structural chromosomal abnormalities or towards the alternative ART with donor sperm.
Methods and Materials: 10 patients were included in this prospective study. 9 harbored a reciprocal translocation and 1 insertion. Sperm FISH was carried out on semen samples collected on the day of oocyte retrieval. Three subtelomeric probes were used, allowing distinction of meiotic segregation. For each semen sample, 250 spermatozoa nuclei were analyzed by an automated capture system. A retrospective study of 133 cycles and 108 ovarian punctures due to paternal reciprocal translocations was conducted in parallel.
Results: No correlation was found between chromosome segregation profiles in spermatozoa and embryos, except for the adjacent 2 segregation type. The rate of embryos with balanced or normal chromosomal profiles was less than that in spermatozoa in 8 out of 10 cases. The retrospective evaluation showed that it is not the age, but the number of oocytes injected that was positively correlated with the number of chromosomally balanced embryos obtained.
Conclusions: Combined sperm FISH and ovarian response to controlled hyperstimulation could be used as predictive criteria for PDG outcome which would allow orientation of couples towards the most adapted ART offering them the best chance of becoming parents.

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P19.82

Cryptic familial submicroscopic translocation t(9;17)(q34.3;p13.3) associated with multiple malformation pregnancies and an affected child.

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We present here the study of a familial submicroscopic reciprocal two-way translocation, discovered fortuitously through prenatal diagnosis.

A 12-week pregnancy was referred for chromosomal analysis and array-CGH due to increased nuchal translucency. Chromosomal analysis was normal but array -CGH (105K) revealed a double segmental imbalance; a terminal deletion on chromosome 9q (1.35Mb) and a terminal duplication of chromosome 17p (1.95Mb). Both imbalances fall within known syndromic regions; Miller-Dieker (includes YWHAE gene but not PAFAH1B1 gene) and 9q subtelomeric deletion syndrome. Parental subtelomeric FISH testing revealed the presence of a submicroscopic apparently balanced translocation between chromosomes 9 and 17 in the mother. Subsequent normal FISH analysis of the maternal grandparents revealed that the translocation occurred *de novo* in the mother. Following genetic counselling the couple elected to terminate the pregnancy. Family history revealed an affected son with mild intellectual disability, short stature, dysmorphic features and hypoplastic corpus callosum (no lissencephaly) and a previous pregnancy terminated based on multiple severe ultrasound findings including tetralogy of Fallot. The parents consented to perform array-CGH on stored genetic material from their previous pregnancy and their affected child which revealed the same unbalanced products of the translocation on both (a duplication on chromosome 9q and a deletion of chromosome 17p). Possibly both aberrations have an effect on the affected child's phenotype and the ultrasound findings from the terminated pregnancy. This case demonstrates that prenatal diagnosis by array-CGH is essential in cases with ultrasound findings and that obtaining detailed family history is of paramount importance.

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P19.83

Mosaic tetrasomy 21 in a female fetus with isolated complex heart malformation at second-trimester ultrasound screening.

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Tetrasomy 21 is a rare cytogenetic aneuploidy at birth, but is often reported in acute leukemias. Fewer than 10 cases have been published in the literature and tetrasomy 21 was frequently initially confused with tetrasomy 12p (Pallister-Killian syndrome). The phenotype largely resembles that of trisomy 21. We report a mosaic tetrasomy 21 diagnosed in a female fetus born to healthy unrelated parents (maternal age 39 years). First-trimester ultrasound showed no structural anomalies (nuchal translucency 2 mm and crown-rump length 77 mm), and maternal serum markers were at low risk for trisomy 21 (1/2500). Second-trimester ultrasound at 22.5 weeks' gestation (WG) revealed isolated complete atrioventricular septal defect (AVSD). Interphase FISH for trisomy 21 and fetal karyotype on amniotic fluid were normal (46,XX). Because of the severity of the heart malformation, termination of pregnancy was discussed with the parents and performed at 30 WG. Pathology showed facial dysmorphism (moon face, flat profile, upslanting palpebral fissures, hypertelorism, marked suborbital folds, lingual interposition, dysmorphic ears), short arms, broad hands, confirmation of the complexe heart malformation (AVSD), normal bone radiographies, features strongly suggestive of trisomy 21. Microarray analysis on lung DNA showed trisomy of all chromosome 21 markers. FISH on touch-preparation slides on lung, liver, and thymus showed mosaic tetrasomy 21/trisomy 21 (74.5%/4,5%), 4% tetrasomy 21, and no aneuploidy, respectively. The phenotype of low-grade tetrasomy 21 is very suggestive of trisomy 21. Consequently, when prenatal diagnosis for trisomy 21 is negative in the light of a characteristic phenotype, mosaicism for tetrasomy 21 should be considered.

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P19.84

Methylation specific Multiplex Ligation-dependent Probe Amplification: Utility for prenatal diagnosis of parental origin in human triploidy

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When a triploid pregnancy is diagnosed prenatally, the diagnosis of hydatidiform mole relies on the histopathological examination of the tissue from the terminated pregnancy. However, reproducibility is poor and variability is high when histopatologically diagnosing hydatidiform moles.
Triploid pregnancies can have either the chromosomal constitution of two maternal and one paternal sets or two paternal and one maternal sets, but only the conceptuses with two paternal sets has the potential to develop into hydatidiform moles. Therefore, it would be beneficial to introduce a method that reveals the parental origin of the genome of the triploid conceptus as early as possible, at best even before termination.
DNA marker analysis can been used to distinguish the two types of triploid pregnancy but has the disadvantage of requiring a sample from both parents in order to be fully informative, delaying the full diagnosis of the abnormal pregnancy.
Here we present a method, analysing imprinting-patterns, capable of determining the parental origin of the genome of triploid conceptuses within 24 hours; it is inexpensive, simple, and easy to use and parental samples are not needed.

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P19.85

Prenatal detection of fetal aneuploidy using bench top sequencing

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The clinical implementation of noninvasive prenatal testing (NIPT) for fetal aneuploidy detection via massively parallel sequencing has significantly increased the sensitivity and specificity with which these anomalies are detected prenatally. Despite the many benefits of this approach, one aspect which could be improved upon is the total time required for sample processing. Sample collection to clinical result takes approximately seven days, which can be problematic in special, time critical, situations or when repeat testing is required.

Technical advances have recently produced several low-throughput but fast bench top sequencers which may prove valuable for time sensitive applications. Here, we examine the performance of an NIPT fetal aneuploidy test using the MiSeq® sequencer (Illumina®, San Diego California) and Ion Torrent™ Personal Genome Machine (PGM™) (Life Technologies™, San Diego California). Ten patient samples, eight from women carrying a known euploid fetus and two from women carrying a known trisomy 21 fetus, as determined by fetal karyotyping, were analyzed using these DNA sequencers.

The total number of aligned reads and uniformity of genome coverage was sufficient in both cases to generate the necessary discriminatory power to indicate aneuploidy status. All ten patient samples processed on both sequencing platforms were correctly identified according to their karyotype results. In terms of the required labor, total time, and hands-on time, the MiSeq platform was significantly more efficient than the PGM, though the sequencing itself was faster on the PGM. These instruments may be attractive candidates in a deployable version of the aneuploidy detection assay.

T. Zwiefelhofer: A. Employment (full or part-time); Significant; Sequenom Center for Molecular Medicine. **P. Whitley:** A. Employment (full or part-time); Significant; Sequenom Center for Molecular Medicine. **K. Roy:** A. Employment (full or part-time); Significant; Sequenom Center for Molecular Medicine. **R. Jean-Jacques:** A. Employment (full or part-time); Significant; Sequenom Center for Molecular Medicine. **M. Ehrich:** A. Employment (full or part-time); Significant; Sequenom Inc.

P19.86

Gestational Age and Maternal Weight Effects on Fetal cfDNA in Maternal Plasma.

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Objective: To determine the effects of gestational age and maternal weight on fraction of fetal cell-free DNA (cfDNA) in maternal plasma and the change in fetal cfDNA amounts within the same patient over time.

Methods: cfDNA was extracted from maternal plasma from 22,384 singleton pregnancies of at least 10 weeks gestation undergoing the Harmony™ Prenatal Test. The Harmony Prenatal Test determined fetal fraction via directed analysis of cfDNA.

Results: At 10 weeks 0 days to 10 weeks 6 days gestation, the median fetal fraction was 10.2%. Between 10 and 21 weeks gestation, fetal fraction increased 0.1% per week ($p < 0.0001$) and 2% of pregnancies were below 4% fetal fraction. Beyond 21 weeks gestation, fetal fraction increased 1% per week (p<0.0001). Fetal fraction was proportional to gestational age and inversely proportional to maternal weight (p=0.0016). Of 135 samples that were redrawn due to insufficient fetal fraction of the initial sample, 76 (56%) had greater than 4% fetal fraction in the sample from the second draw.

Conclusion: Fetal cfDNA increases with gestation, decreases with increasing maternal weight and generally improves upon a blood redraw when the first attempt has insufficient fetal cfDNA.

E. Wang: A. Employment (full or part-time); Significant; Ariosa Diagnostics, Inc. **A. Batey:** A. Employment (full or part-time); Significant; Ariosa Diagnostics, Inc. **C. Struble:** A. Employment (full or part-time); Significant; Ariosa Diagnostics, Inc. **T. Musci:** A. Employment (full or part-time); Significant; Ariosa Diagnostics, Inc. **K. Song:** A. Employment (full or part-time); Significant; Ariosa Diagnostics, Inc. **A. Oliphant:** A. Employment (full or part-time); Significant; Ariosa Diagnostics, Inc..

P19.87

Short repeat tandem assay refines the diagnosis of molar trophoblastic diseases.

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Introduction: Complete hydatidiform moles (CHM) and partial hydatidiform moles (PHM) are gestational trophoblastic diseases. CHM are diploid and androgenetic, PHM are triploid with an extra set of paternal chromosomes. Molecular genotyping, which determines the parental origin of alleles, refines the diagnosis.

Materials and Methods: DNA genotyping analysis was performed using the kit AmpFLSTR ® SGM Plus ® PCR Amplification (Life Technologies, Foster City, CA). Genomic DNA of maternal decidua and conceptus was extracted from paraffin embedded tissue sections after macrodissection. 10 short tandem repeat sequences loci and the amelogenin locus (Sex chromosome marker) were amplified in multiplex for each extract. The amplification products were separated by capillary electrophoresis and DNA amplification profiles obtained for conceptus and maternal tissue were compared.

Results and Discussion: 43 cases were analyzed : 11 PHM, 14 CHM, 15 non molar specimens and 3 unclassified diploid molar specimens. In 38 cases molecular genotyping confirmed morphological and immunohistochemical diagnosis : 14 diploid CHM showed exclusively paternal alleles, 10 PMH displayed diandric triploidy and 14 nonmolar specimens were biparental diploid. One unclassified case was a mosaic or chimera. Genotyping was non contributive in 2 cases (due to DNA alteration).

Conclusion: Molecular genotyping allows a more accurate diagnosis of different subtypes of molar gestational trophoblastic diseases.

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P19.88

Reduced fertility of *TSPY* transgenic mice on a FVB/N genetic background

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The *TSPY* gene is conserved in placental mammals and encodes the **t**estis-**s**pecific **p**rotein, **Y** encoded. It is assumed that TSPY plays a role in proliferation and/or meiotic differentiation of testicular germ cells. Recently, *TSPY* copy number variations and mutations within the first exon have been identified as modifiers of spermatogenesis and risk factors for male infertility. Because the laboratory mouse is carrying only a single-copy Y-chromosomal *Tspy* pseudogene (*Tspy-ps*), we generated a *TSPY* transgenic mouse line on a NMRI-outbred strain genetic background (NMRI-Tg(TSPY)9Jshm) that carries a human *TSPY* gene in approximately 50 copies on the mouse Y chromosome. *TSPY* transgenic B6;NMRI-Kit^{Wv}/Kit^{Wv} mice on a mixed NMRI/C57BL/6J genetic background are able to partially rescue spermatogenesis and fertility of homozygous Kit^{Wv}-mutant males. In order to bring the human *TSPY* transgene on FVB/N and C57BL/6J inbred genetic backgrounds we backcrossed for over 10 generations *TSPY* transgenic males with FVB/N-females and C57BL/6J-females, respectively. While fertility and spermatogenesis of *TSPY* transgenic males on a C57BL/6J genetic background were normal, impaired spermatogenesis and fertility were observed in FVB/N-Tg(TSPY)9Jshm mice. *TSPY* transgenic FVB/N males older than 5 months are showing an increased vacuolation of the seminiferous germinal epithelium in comparison to age-matched FVB/N controls. Interestingly, sperm motility parameters such as velocities (VCL, VSL, VAP) and lateral head amplitude (ALH), and mean number of epididymal sperm of FVB/N-Tg(TSPY)9Jshm males were significantly decreased in comparison to FVB/N males, thereby leading to decreased fertility of *TSPY* transgenic FVB/N males.

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P19.89

Pregnancy outcome of prenatally diagnosed Turner syndrome: a 32 years collaborative french study including 1079 cases

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Background: Turner syndrome (TS) is a common sex chromosome aneuploidy (45,X) diagnosed prenatally with an incidence of 0.18%. It is mainly detected due to presence of abnormalities on ultrasound (US) (84%) but it

may be a fortuitous diagnosis. The associated genetic counseling is difficult and the parents' decision to continue or terminate the pregnancy depends on information provided which is different as to presence of US abnormalities, mosaicism with a normal cell line and diagnosis term. We sought to assess the pregnancy outcomes and the influence of multidisciplinary centers for prenatal diagnosis (MCPD) in France on parental decisions in cases of TS.

Methods : a cohort of 1079 french prenatal TS diagnoses between 1980 and 2012 was reported. 20 french laboratories participated to this study. In each case, the karyotype indication, maternal age, year of prenatal testing, sampling procedure, karyotype, associated US findings and outcome were recorded. We statistically (Student test) compared data before and after 1997, the year of application of MCPD.

Results and conclusions : The pregnancy termination rate fell significantly ($p<0.002$) from 90% before 1998 to 80% thereafter. This decline is significant in case of mosaicism (from 69% to 24%, $p<0.05$)), fortuitous diagnosis (from 78% to 26%, $p<0.05$) and especially along pregnancy period (93% on first trimester, 58% and 22% at second and third trimester respectively $p<0.05$), which underlines evolution in way of thinking thanks to MPCD. Nevertheless, due to an early diagnosis, mainly on first trimester, TOP rate remains high in case of lack of mosaicism.

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P19.90

Unusual pattern of segregation of a reciprocal translocation resulting in duplication of one of the derivative chromosomes

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Chromosomes involved in a reciprocal translocation form a quadrivalent at meiosis. These chromosomes segregate by alternate, adjacent-1, adjacent-2, or 3 : 1 modes to give gametes with various balanced or unbalanced chromosome complements. We report the prenatal diagnosis of an unusual segregation of a maternal reciprocal translocation. Ultrasound examination performed on a 24-year old woman at 22 weeks' gestation showed the presence of bilateral ventriculomegaly, short long bones and unusual aspect of the corpus callosum. Subsequently, a microarray performed on uncultured amniocytes showed a gain of 1q42.13q44 in combination with a gain of full 9p and a gain of 9q13q22.32. Karyotype and FISH studies revealed the presence of 47 chromosomes with an abnormal chromosome 1 and two abnormal chromosomes 9 derived from a reciprocal translocation between the long arm of chromosome 1 and the long arm of chromosome 9. After genetic counselling, the pregnancy was terminated at 24 weeks' gestation. Parental karyotypes allowed the detection of a balanced reciprocal translocation in the mother 46,XX,t(1;9)(q42.13;q22.32). Combination of two events is necessary to explain the observed chromosomal imbalances : an alternate segregation during maternal meiosis I in which the two translocated chromosomes are transmitted and the segregation of both sister chromatids of the derived chromosome 9 in a same cell. This segregation of both chromatids may result either from a premature separation of chromatids at meiosis I, or from a nondisjunction at meiosis II. To our knowledge, this unusual pattern of segregation of a common balanced reciprocal translocation has never been reported.

M. Beaujard: None. **B. Bessières:** None. **J. Bernard:** None. **M. Maurin:** None. **M. de Blois:** None. **G. Quenum:** None. **I. Dargery:** None. **C. Ozilou:** None. **F. Poulain:** None. **S. Romana:** None. **F. Encha-Razavi:** None. **T. Attié-Bitach:** None. **Y. Ville:** None. **C. Turleau:** None. **M. Vekemans:** None. **V. Malan:** None.

P20.01

Two cases of large DHCR7 gene deletion in Smith-Lemli-Opitz syndrome

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Smith-Lemli-Opitz Syndrome (SLOS, MIM 270400) is an autosomal recessive metabolic malformation syndrome with variable clinical severity. SLOS is caused by mutations in the delta7sterol-reductase gene (DHCR7), resulting in impairment of endogenous cholesterol biosynthesis. More than 100 mutations have been identified and about 380 SLOS patients are documented in the DHCR7 database (http://lovd.i-med.ac.at/home.php), but a second mutation remains unidentified in 4% of patients.

Patient 1 showed growth retardation at birth, short proximal limbs, 2, 3 toe syndactyly, typical heart, and renal defect and facial features. Plasma sterol analysis showed elevated 7- and 8- dehydrocholesterol.

Patient 2 showed also highly elevated 7DHC and typical clinical features of SLOS.

The patients´ and parental DNAs have been analyzed by genomic sequencing of DHCR7 and a self-designed multiplex ligation-dependent probe amplification (MLPA) of exons 2 to 8 of the DHCR7 gene.

In patient 1 a heterozygous deletion of exons 3 to 6, in addition to the heterozygous common mutation p.Arg352Trp (c.1054C>T) has been detected.

Patient 2 carries a heterozygous deletion of exons 1 and 2 inherited from his mother, confirmed by SNP analysis. The paternal mutation was p.Thr93Met (c.278C>T).

The pathogenicity of the two deletions (one involving the 5´UTR/promoter sequences) will be demonstrated. MLPA is recommended in SLOS cases with only one mutation and elevated 7DHC. These cases highlight the importance of including all SLOS individuals (with two, one or no detected mutation) in the locus database.

M. Witsch-Baumgartner: None. **K. Hinderhofer:** None. **B. Maas:** None. **D. Haas:** None. **K. Carter:** None. **M. Suri:** None. **J. Zschocke:** None. **H. Sawyer:** None.

P20.02

Identification of allelic imbalance in retinal expressed (disease) genes

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Reduced penetrance and variability in disease expression with respect to onset, course, and severity is a well-documented phenomenon in retinal diseases making reliable genotype/phenotype correlations as well as individual disease prognosis difficult.

Understanding the mechanisms behind such phenotypic variation is a key aim in human genetics.

We hypothesize that cis-acting variants regulating gene expression levels play an essential role in phenotypic variation and disease penetrance in hereditary retinal disorders.

To date the extent and mechanisms by which cis-acting polymorphisms influence the human phenotype are barely understood although they are common.

The aim of this project is the identification of such cis-acting gene variants and the determination of their impact on retinal disease expression. Individuals heterozygous for cis-acting polymorphisms that affect gene expression will show an allelic imbalance (AI).

Up to now more than 50 retinal genes were screened for heterozygous cSNPs applying PCR and sequencing in five different inbred mouse strains as a proof-of-principle experiment. Pyrosequencing assays were applied on RT-PCR amplified cDNAs generated from retinal RNA to assess AI based on the identified cSNPs.

Our results demonstrate an AI in ten retinal disease genes. However, in two genes the AI can already be seen on DNA level indicating a copy number variation. Screening of the Pde6c gene revealed a 116-bp insertion on cDNA level resulting in a premature termination codon leading, due to the nonsense mediated mRNA decay, to a downregulation of the mutant transcript. For the remaining genes the cause of the AI has to be clarified.

S. Balendran: None. **B. Wissinger:** None. **S. Schimpf-Linzenbold:** None.

P20.03

Nine new cases of de novo constitutional complex chromosome rearrangements (CCR) involving a single chromosome detected by aCGH: is there a unifying mechanistic model?

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Recently, next generation sequencing data shed light on complex chromosome rearrangements involving one or few chromosomal loci that may be due to shattering of chromosomes in a single catastrophic event, called chromothripsis and identified in 2-3% of cancers.
In the present work, we reviewed the array-CGH results from 7 centers among the French array-CGH network between 2009 and 2012. Among 7774 aCGH performed in constitutional setting, we identified 9 cases of constitutional CCR involving more than 4 CNV in a single chromosome (0,01 %). These 9 cases included were referred for intellectual disability and/or malformations in pre-natal (2/9) or post-natal (7/9) context. Eight different chromosomes were involved namely chromosome 1p, 5q, 6p, 11p, 11q, 13q, 19p/q, 21q, 22q. Number of CNV per chromosome varied from 5 to 12 and chromosomal imbalances from 6.91 to 37.95 Mb. All these CNV were confirmed by FISH or R/G-banded karyotype and were de novo. Multiple rearrangement patterns were observed including various combinations of deletion, duplication, triplication and normal segments. Interestingly, in six cases, chromosome abnormality was identified on conventional R/G banding (homogeneous ring, mosaic ring, apparently balanced translocation, dicentric chromosome, insertion, karyotype disparities between cytotrophoblast and fetus).
Our study point out that constitutional CCR on a single chromosome is a rare and heterogeneous event. Recently, the mechanism of chromothripsis has been suggested to explain also constitutional CCR. Nevertheless, alternative hypotheses, such as chromoanasynthesis involving replication-based mechanisms, have been proposed. Systematic identification and breakpoint characterization of these CCR will be necessary to understand the mechanism.

D. Sanlaville: None. **B. Keren:** None. **S. Chantot:** None. **S. Jaillard:** None. **N. Marle:** None. **C. Metay:** None. **R. Molignier:** None. **J. Puechberty:** None. **G. Lefort:** None. **N. Chatron:** None. **V. Jauffret:** None. **C. Muti:** None. **A. Briand:** None. **L. Tosca:** None. **M. Cordier:** None. **A. Labalme:** None. **A. Mosca:** None. **P. Callier:** None. **A. Rafat:** None. **S. Taviaux:** None. **A. Schneider:** None. **S. Whalen:** None. **A. Afenjar:** None. **S. Brisset:** None. **C. Thauvin-Robinet:** None. **M. Portnoi:** None. **S. Odent:** None. **M. Till:** None. **L. Faivre:** None. **C. Schluth-Bolard:** None.

P20.04
Four-break complex genomic rearrangement uncovered by FISH follow-up of an apparently de novo aCGH imbalance

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Array CGH testing is now in place in most cytogenetic laboratories, in many centres replacing traditional G-banded chromosome analysis. Follow-up of abnormal array findings is often carried out by parental array testing; when neither parent carries the proband's imbalance, this may represent a truly de novo event, or may have arisen due to a balanced parental chromosome rearrangement. FISH analysis in these cases can uncover the chromosomal status of the parents and establish reproductive risks.
To date we have carried out parental FISH studies for eleven cases where the proband had imbalance detected by array CGH that was beyond the resolution of karyotype analysis. FISH was first carried out on the proband alone, to confirm that the FISH probes were informative for the imbalance. In two of these eleven cases (18%) one parent carried a chromosome rearrangement which resulted in the imbalance seen in the proband. One case was a reciprocal translocation, with submicroscopic translocated segments. In the second case, array CGH analysis detected a 1.474Mb duplication of material from the long arm of chromosome 4 in a three year old boy. Array CGH analysis carried out on DNA from both parents showed normal copy number of this region; however, the father was found to carry a deletion of material adjacent to the region duplicated in the proband. FISH studies showed a four-break chromosome rearrangement in the father.
We will describe this latter case and speculate on possible mechanisms for these findings, and their significance for this family.

S.M. Bint: None. **J. Ahn:** None. **A.F. Davies:** None. **C. Mackie Ogilvie:** None.

P20.05
New technologies permitted rectification of a diagnosis 14 years later

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Putatively benign copy number variants (bCNVs) can be broadly defined as DNA copy number gains or losses that do not lead to a recognizable clinical phenotype. The interpretation of these CNVs has presented challenges, especially in determining whether or not they are pathogenic when analyzing patients with abnormal phenotypes. Our patient was first evaluated at 2 months old for dysmorphic features and axial hypotonia with moderate distal hypertonia. A standard karyotype showed a direct 14q31 duplication inherited from his phenotypically normal mother. This duplication was also detected in five relatives with a normal phenotype and maternal inheritance through three generations. At this time, three hypotheses were suggested to explain abnormal phenotype of the proband: a variable expression of this duplication, the role of imprinting genes, or a coincidental event. Follow-up at the age of 14 years revealed slight dysmorphism, slight overgrowth (+2 SD) and developmental delay with a greater impact in the verbal sphere. The use of high resolution technologies allowed us to pursue our investigations. Array-CGH confirmed the 8.5 Mb duplication in the 14q31.1q31.3 region but also revealed a 22q11.21 duplication (DiGeorge syndrome region) of at least 3.1 Mb. This duplication was confirmed by q-PCR studies and was shown to be *de novo*. This second anomaly provided the explanation of the phenotype while we could now conclude that the 14q31 duplication was a bCNVs. The contribution of new technologies such as array-CGH and NGS will allow us to revise diagnosis initially based on karyotype anomalies.

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P20.06
Inherited Cat-Eye Syndrome due to an unusual dicentric monosatellited derivative chromosome 22

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Cat eye syndrome (CES) is characterized by ocular coloboma, preauricular pits and/or tags, anal atresia, heart defect and renal malformations. It is due to tetrasomy for 22q11 region, secondary to the presence of a dicentric and bisatellited supernumerary marker chromosome in most of cases.
We report here a 2 months old boy with facial dysmorphism, total anomalous pulmonary venous return, anal atresia and renal malformation. He is the first child of a 26 years old woman with bilateral coloboma, atrial septal defect and renal malformation. Standard blood karyotype of both proband and his mother showed short arm asymmetry of chromosome 22. Array CGH displayed four copies of the proximal 22q11 region (chr22: 16,133,474-18,651,673, hg19) in the child. The derivative 22 was finally characterized by FISH using 14/22 centromeric and NORs probes and BAC clone CTD-3144O3 (22q11.1) showing a dicentric monosatelited chromosome 22 with one signal CTD-3144O3 on the long arm in 22q11.1 and two signals CTD-3144O3 on the short arm between both centromeres.
Although atypical cytogenetic presentation of CES have already been described including interstitial duplication and triplication, this is the first case of maternally inherited dicentric derivative 22 presenting two additional copies of 22q11 region on its short arm. This rearrangement resulted in tetrasomy for the CES I region, consistent with patients phenotype. Mechanism of formation of such derivative chromosome will be discussed.

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P20.07
CFTR interacts with metabotropic glutamate receptor and modulates IL-8 production in cystic fibrosis T-Lymphocytes

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The action of glutamate on the secretion of IL-8 by lymphocytes derived from healthy subjects and cystic fibrosis patients, as well as the expressi-

on of glutamate receptor and glutamate receptors binding partners in the membrane fractions of lymphocytes were investigated. The content of cystic fibrosis transmembrane conductance regulator-associated ligand, Na+/H+ exchanger 3 regulatory factor 1, as well as PDZ-binding proteins: metabotropic glutamate receptor subtype 1, ionotropic glutamate AMPA and NMDA receptor subtypes were analysed after immunoprecipitation of CFTR. Western blotting of co-immunoprecipitated proteins revealed that normal, non-mutated CFTR, as well as mutated forms of CFTR (F508CFTR mutation) were associated with NHERF-1 and CAL in the plasma membrane fractions. In the macromolecular complex of mutated, as well as of non-mutated forms of CFTR, NMDA NR2A type of glutamate receptors were found, whereas metabotropic mGluR1 in the supramolecular complex containing CFTR was detected only in the normal T-cells. Besides, in lymphocytes with DF508-CFTR mutation the amount of cell-surface expressed CFTR-CAL complex greatly decreased. Furthermore, our results have shown that CF-derived T-cells in the presence of IL-2 produce more IL-8, than T-cell from healthy control. However, only in normal lymphocytes was detected a significant increase (144%) in the IL-8 secretion during exposure to high concentration of glutamate (10-4 M). We have concluded that CFTR and mGluR1a compete for binding to CAL, which in turn can regulate levels of these two proteins. Reduced expression of surface exposed mGluR1a in lymphocytes can disarrange appropriate T-cell phenotype polarization and lead to aberrant immune responses in CF.

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P20.08
Study of long-range regulatory mechanisms of the CFTR gene

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The cystic fibrosis transmembrane conductance regulator (CFTR) gene was identified in 1989. Twenty years later, the regulatory mechanisms controlling its complex expression are still not fully understood. Although, more than 1930 mutations have been identified, many cases of cystic fibrosis or CFTR Related Disorders remain still of unknown origin.
The promoter which binds transcription factors and drives some aspects of CFTR gene expression, cannot alone account for tissue specific control. This implicates other distal cis- or trans-acting elements in cell-type-specific regulation of CFTR expression.
The aim of our project is to study long-range regulatory mechanisms of the CFTR gene.
Our first approach consisted to map potentials regulatory elements located within conserved non-coding sequences, which could interact specifically with the CFTR gene by tri-dimentional folding mechanism. These interactions have been detected by Chromosome Conformation Captures (3C).
Subsequently, we enhanced our analyses with a high-throughput adaptation of 3C: the 3C-Carbon Copy (5C) technology. This approach allows the analysis of millions chromatin interactions.
Thanks to 3C and 3C-derived studies, we could identify new possible mutations far from the gene, which may lead to its dysfunction by modifying the chromatin conformation.
These analyses will be pursued on patients affected by cystic fibrosis in whom either a single mutation or none was found in the CFTR locus.

S. Moisan: None.

P20.09
DCTN4 as a modifier of *Pseudomonas aeruginosa* bronchial chronic infection in cystic fibrosis

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In Cystic Fibrosis (CF), *Pseudomonas aeruginosa* (PA) chronic airway infection is associated with faster rate of lung function decline, increased number of exacerbations and shorter survival. Recently, using exome sequencing and extreme phenotype design, it has been shown that isoforms of dynactin 4 may influence PA infection by reducing PA autophagic clearance in the airway of individuals with CF or by altering macroautophagic clearance of class II mutant *CFTR*, leading to more severe airway disease. The purpose of this study was to investigate the role of missense variants in DCTN4 on PA infection incidence, on age at first PA colonization, and chronic PA infection prevalence. PCR and direct sequencing were used to screen DNA samples for *DCTN4* variants. 109 adult CF patients from the CF Centre at Cochin Hospital have been included (patients with lung transplantation were excluded): 60 patients had chronic PA infection (CPA) and 49 had no CPA. *DCTN4* variants were identified in 22 CF patients with CPA (22/60, 37%), and in only 6 CF patients with no CPA (12%). *DCTN4* variants were predominantly observed in individuals with early age at first PA colonization (before 15 years-old). In this study, we confirm the association between chronic PA infection and *DCTN4* missense variants. The *DCTN4* genotype distribution differed significantly between the two groups (p=0.004). In conclusion, our observations suggest that p.Phe349Leu of DCTN4 may be involved in the pathogenesis of PA infection in CF.

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P20.10
Telomerase mutations in families with idiopathic pulmonary fibrosis : 23 new mutations of TERT and TERC

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Germline telomerase mutations have been reported in approximately 10% of familial idiopathic pulmonary fibrosis (IPF). The T allele of the rs35705950 in MUC5B promoter has been associated with idiopathic pulmonary fibrosis (IPF) in American and European cohorts.
We report here our French experience until 2008 in molecular exploration of TERT and TERC in familial cases of IPF and sporadic case of IPF with associated bone marrow disease or unexplained liver disease suggestive of a telomerase disease. We identified 22 heterozygous TERT mutations in 24 patients including 20 mutations never reported. We also found 4 heterozygous variations of TERC in 6 patients. Assessing pathogenicity of TERT and TERC mutations is critical to evaluate risk of telomerase disease in carriers. By considering -the co segregation study when possible, -the presence of the variant in databases, -the predicted impact on the protein structure or splicing step and -the telomere length of mutation carriers, we conclude that most of the variants can be classed as loss of function mutations, whereas the results for new TERC variants remain of unknown significance. Discriminating between mutant and neutral variants not only provides information that can be used for genetic counselling but also adds to our understanding of the functionally critical residues or nucleotides in TERT. Moreover, we found that MUC5B promoter snp genotype of IPF patients carrier of TERT or TERC mutations (excess of G allele of the rs) is significantly different than MUC5B genotype of IPF patients from the COFI French cohort of IPF patient.

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P20.11
Telomeres are normally elongated and maintained in ICF syndrome derived iPSCs

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Human telomeric regions are packed as constitutive heterochromatin, characterized by extensive subtelomeric DNA methylation and specific histone modifications. In somatic cells, telomerase is inactive and telomeres shorten during proliferation. When human induced pluripotent stem cells (hiPSCs) are generated, telomerase is activated and telomeres elongate. In addition, subtelomeres are hypermethylated in hiPSCs and levels of TERRA, an RNA transcript emanating from telomeric regions, are elevated.
ICF (Immunodeficiency, Centromeric instability, Facial anomalies) type I patients, carry mutations in DNMT3B encoding an enzyme that de novo methylates repetitive sequences. ICF cells display hypomethylated subtelomeric regions associated with short telomeres, advanced telomere replication time and abnormally high levels of TERRA.
We study the role of de novo subtelomeric methylation during development. We used hiPSCs as a model for the developmental stage at which de novo methylation occurs and ICF syndrome cells as a natural model of almost ab-

sent subtelomeric DNA methylation.
We have generated iPSCs from ICF-fibroblasts and observed the potential capacity for elongation and maintenance of telomeres in the pluripotent state. Following differentiation, very rapid telomere shortening occurs, leading to early senescence in fibroblast-like cells derived from these ICF syndrome-derived iPSCs, similar to the pre-iPSC fibroblasts. In addition, the subtelomeres of the ICF-iPSCs remain significantly hypomethylated. Ongoing experiments, including DNA and RNA analyses are aimed to elucidate the mechanism whereby hypomethylation is linked to telomeric abnormalities in ICF syndrome.

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P20.12
Positions of HSA6, HSA12, HSA18 and HSAX in interphase nuclei of human MSC depend on cultivation time and differentiation

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Chromosome territories' (CT) positioning is not random and seems to be an important mechanism of epigenetic regulation. Study of CT is of particular interest in stem cells such as mesenchymal stem cells (MSC) because they are widely used in regenerative medicine and are of great value as a biological model capable of differentiation in different cell-lineages. The aim of the research was to identify the differences in the positions of chromosomes 6, 12, 18 and X in MSC. Cells in early (up to 4) and late (6-10) passages were analyzed as well as cells after differentiation in osteogenic and adipogenic directions. Over 4000 nuclei from 19 cell cultures were analyzed using FISH with centromere probes. Radial distances (RD) were measured for each chromosome centromer. At late passages the median RD values of the distal HSA6 homologue changed its position from 0,78 to 0,81 while centromere of HSAX in male cultures moved centrally (median values are 0,7 and 0,61 for early and later passages respectively). After adipogenic differentiation proximal homologues of both HSA18 and HSA12 moved to the periphery (median values are 0,34 and 0,39 for HSA18, 0,45 and 0,5 for HSA12 before and after differentiation respectively). In addition centromeres of HSAX showed statistically different position in male and female cultures which might be explained by different levels of activity of HSAX in male and female cells. The detected changes in the structure of the nucleus are new karyological characteristics of human MSC.

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P20.13
Family-based genome wide association study in Patagonia, a region with high prevalence of oral clefts: preliminary results

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The etiology of cleft lip and/or palate (CL±P) is complex and several SNPs and chromosomal regions had been associated with this condition. We hypothesized that in a CL±P high prevalence region, as the Patagonia, one of these associated factors may be prevalent over the others. We aim the identification of polymorphic markers that may contribute to CL±P in our population with a family-based design for genome-wide association (GWA) analysis. The CL±P high prevalence population in Patagonia was identified by the ECLAMC and 150 families were until now ascertained and studied. SNPs from Genome-Wide 6.0 array from Affymetrix were tested (TDT) for association with isolated CL±P in 11 trios, 2 duos, and 10 sibs using PLINK. We found statistical evidence of association between polymorphic markers on chromosome 1, 4, 5, 7, 10, 12, 13, 17 and 18 with CL±P ($p < 0.0009$). None of these SNPs were previously described as associated with CL±P. The STRING 9.0, a tool used to know and predicted protein-protein interactions, shows that the *MACF1* gene protein (1p34.3) appears directly related to the *IRF6* product. These preliminary results suggest some *loci* on chromosomes 1, 4, 5, 7, 10, 12, 13, 17 and 18 as candidate regions for CL±P. Although a relation between one of those SNPs (on MACF1 gene) and IRF6 could be of interest to CL±P etiology in this population, more families must be analyzed in order to confirm our preliminary results.

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P20.14
Somatic mosaicism for DNA Copy Number Variations: a rare phenomenon in human genome

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Somatic mosaicism is defined by the presence of genetically distinct populations of somatic cells in a single organism. Mosaicism can be caused by DNA mutations, epigenetic alterations, chromosomal abnormalities and spontaneous reversion of inherited mutations. DNA copy number variations (CNVs) ranging from kilobases to megabases are a well-known type of genetic variation in human. However, the somatic mosaicism for CNVs in humans is not well studied. It is unknown whether CNVs arise in somatic cells, but it is, however, generally assumed that normal cells are genetically identical. It is suggested, that mosaicism may result from a mutation during postzygotic development which is propagated to only a subset of the adult cells.
The aim of the present study was to test the hypothesis that cells from different fully differentiated tissues could carry different CNV patterns. We tested 48 tissue samples from four subjects, 12 different tissues from each subject, applying Illumina HumanOmniExpress-12 BeadChip (Illumina, Inc; San Diego, USA) for copy number variation detection and observed at least three CNVs, affecting more than one tissue in one of the four individuals studied.
Our results suggest that somatic mosaicism for CNVs is not very common in normal human cells. The data also indicates that further examination of samples derived from a larger cohort may be needed to reveal the frequency of CNV mosaicism. CNVs occurring in a substantial fraction of normal cells might predispose certain types of cells or a tissue to a specific disease-related phenotype.

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P20.15
A complex chromosome rearrangement involving four chromosomes, nine breakpoints and a cryptic 0.6 Mb deletion in a boy with hypoplasia cerebellar and defects in skull ossification

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Complex chromosomal rearrangements (CCRs) are considered rare cytogenetic events. Most apparently balanced CCRs are de novo and are usually found in patients with abnormal phenotypes. In these cases, high-resolution techniques are unveiling genomic imbalances in a great percentage of cases. In this paper, we report a patient with growth and developmental delays, dysmorphic features, nervous system anomalies (pachygyria, hypoplasia of the corpus callosum and cerebellum), a marked reduction in the ossification of the cranial vault, skull base sclerosis and cardiopathy who presents a CCR with nine breakpoints involving four chromosomes (3, 6, 8 and 14) and a 0.6 Mb deletion of 14q24.1. Although the only genomic imbalance revealed by the array technique was a deletion, the clinical phenotype of the patient most likely cannot be attributed exclusively to haploinsufficiency. Other events must also be considered, including the disruption of critical genes and position effects. A combination of several different investigative approaches (G-banding, FISH using different probes and SNP-array techniques) was required to describe this CCR in full, suggesting that CCRs may be more frequent than initially thought. Additionally, we propose that a chain chromosome breakage mechanism may have occurred as a single rearrangement event resulting in this CCR. This study demonstrates the importance of applying different cytogenetic and molecular techniques to detect subtle rearrangements and to delineate the rearrangements at a more accurate le-

vel, providing a better understanding of the mechanism involved in CCR formation and a better correlation with phenotype. (Financial support: FAPESP and CAPES, Brazil; Else Kröner-Fresenius-Stiftung).

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P20.16

Molecular study of Congenital Erythrocytosis in 70 unrelated patients revealed new mutations in EPOR, VHL and PHD2 genes.

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Background: Congenital erythrocytosis results from 1) inherited intrinsic defects in red blood cell precursors that cause increased responsiveness to EPO due to a mutation in EPO receptor gene (*EPOR*); 2) inherited defects in hypoxia sensing pathway due to mutation in the Von Hippel-Lindau gene (*VHL*), in the prolyl hydroxylase domain 2 gene (*PHD2*) or in the hypoxia-inducible factors gene (*HIF2α*); 3) high affinity hemoglobin variants (HBB and HBA); 4) 2,3-bifosfogliceratomutase (*BPGM*) enzyme deficiency. The inheritance is autosomic dominant for Congenital erythrocytosis associated to *HBB*, *HBA*, *EPOR*, *PHD2* and *HIF2α* mutations and recessive for *BPGM* and *VHL* gene mutations. Conversely, in a great number of congenital erythrocytosis the underlying defect remains unknown.
Material and Methods: With the main objective of describing the aetiology and molecular basis of congenital erythrocytosis, we studied 70 unrelated patients.
According to a proper algorithm, we have sequenced the genes described as associated with congenital erythrocytosis.
Results and discussion: Erythrocytosis molecular aetiology was identify in 27 (39%) of the 70 subjects. High-affinity Hb variants were the most common causes, present in 20% of the cases. New mutations were identified in the *EPOR* (c.1310G>A, p.Arg437His; c.1311_1312delTC, p.Pro438Metfs*6; c.1235C>A, p.Ser412*), *VHL* (c.586A>G, p.Lys196Glu) and *PHD2* (c.1000 T>C, p.Trp334Arg) genes. None of the subjects showed mutations in *BPGM* or *HIF2α* genes.
Conclusion: We were able to identify the congenital erythrocytosis molecular aetiology in 27/70 unrelated subjects. In spite of the genes already known to be involved in congenital erythrocytosis, the majority of the cases still has unknown etiology.

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P20.17

Co-occurrence of 4p16.3 microdeletion and 11p15 microduplication in a boy carrying a der(4)t(4;11)(p16.3;p15.4)mat

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We report on a boy carrying an unbalanced translocation der(4)t(4;11)(p16.3;p15.4)mat. He was referred for genetic evaluation at 1 year of age because of severe prenatal and post-natal growth retardation (<3rd centile), developmental delay, seizures, and facial dysmorphisms. MS-MLPA was initially carried out due to the diagnostic possibility of Silver-Russell syndrome (SRS), and revealed an 11p15 microduplication on the maternal chromosome, including both imprinting centers. When refining the microduplication limits by a-CGH (180K whole-genome platform; Agilent), in addition to a 3.3 - 3.5 Mb 11p15.4-pter microduplication, we detected a terminal microdeletion of the short arm of chromosome 4, spanning about ~3.85 Mb. Subsequent FISH analysis confirmed the array findings, and revealed a balanced translocation t(4;11)(p16.3;p15.4) in the patient's mother. Clinical re-evaluation of the patient indicated features of both Wolf-Hirschhorn syndrome and SRS, but most of them could be attributed to the 4p16.3 microdeletion. Two previously described patients carrying similar chromosomal imbalances due to maternally inherited der(4)t(4;11) presented a similar phenotype (South et al. Am J Med Genet A. 2008;146A:2691; Ou et al. Genome Res. 2011;21:33). Both breakpoints in the translocation here described fall within segments rich of segmental duplications sharing high sequence identity between chromosomes 4 and 11, pointing to a rearrangement originated through non-allelic homologous recombination, as previously demonstrated for other t(4;11) (Ou et al. 2011). Financial support: FAPESP (CEPID 98/14254-2; Student fellowship 2011/12486-0)

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P20.18

Application of array comparative genomic hybridization (array-CGH) for detection of chromosomal imbalances in children with Developmental Delay/congenital malformations in Saudi Arabia

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Microarray - based Comparative Genomic Hybridization (a-CGH) has enabled wide investigation of the genome at high resolution and has been implemented in different centers as a clinical diagnostic tool. Chromosomal imbalances are implicated in the etiology of Developmental Delay (DD)/Intellectual disability (ID)/ congenital malformations. However, most of these cases could not be diagnosed by conventional cytogenetic techniques. We aimed to establish (a-CGH) technique and assess its potential as a diagnostic tool of chromosomal imbalances and to detect known and novel chromosomal aberrations in patients with DD/ ID. Subjects & Methods: A total of 72 patients presented with DD/ ID with or without congenital malformations were referred to the CEGMR for cytogenetic analyses. We used both conventional cytogenetic G-banding and Fluorescent in-situ hybridization techniques, besides we applied (array-CGH) high resolution Agilent scanner with 1X244 K array format. Chromosomal aberrations could be detected in 10/72 (13.8%) patients by G-banding technique and 4/50 (8%) by FISH technique, however, 17/72 (23.6%) were diagnosed by a-CGH technique. All microdeletion syndromes and partial duplications were detected by the chromosomal microarray technique. However, one patient with unbalanced translocation and another with mosaicism could not be detected by this technique. We noticed the increased number of CNVs detected by a-CGH which need further investigation for contribution to phenotypes. Our results indicated the strength of high resolution genomic arrays in diagnosing cases of unknown etiology and in detection of contiguous genomic alterations in the wide spectrum of cases with DD/ID/congenital malformations.

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P20.19

Mitotic correction of trisomy 21 occurred in human induced pluripotent stem cells from Down Syndrome

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Down Syndrome (DS) is the most common aneuploïd genetic disorder caused most frequently by trisomy of whole chromosome 21. Thus, correction of trisomy 21 and autologous cell-replacement therapies would be a promising therapeutic strategy. We explored this hypothesis by using induced pluripotent cells (iPS) technology to model DS and follow the ploïdy of the iPS during extended culture. Amniotic fluid cells (AFC) harboring a complete trisomy 21 from three donors were reprogrammed into iPS and DS-iPS lines (polyclonal and clonal iPS). The pluripotency was tested in vitro and in vivo and the trisomy 21 confirmed by karyotype. We observed that upon iterative manual passage, one polyclonal iPS has completely lost the supernumerary chromosome 21 during extended culture. Correction was reproducible (n=3) and due to chromosome mis-segregation and pronuclei formation mechanisms. We eliminate the hypothesis of a normal unrela-

ted iPS contamination using microsatellite assay. In addition, we showed by ch21p polymorphism both parental contributions of the two remaining chromosome 21. CGH-array (135K Roche-Nimblegen) did not reveal major genomic instability. We reported minor alterations with a dup12q24.33 of 277.55Kb (including the genes POLE, PXMP2, PGAM5, ANKLE2, GOLGA3, CHFR, ZNF605) and a qupYp11.32 of 167.11Kb (SHOX gene).This is the first demonstration that iPS can reproduce in vitro a spontaneous mis-segregation procedure resulting in a trisomy 21 correction. The generation of related isogenic disomic iPS should rescue the phenotype of disease and will allow evaluating consequences of phenotypic reversion in DS by a potential autologous normal substitution cell therapy.

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P20.20

Altered expression of immune-related genes in children with Down syndrome

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Down syndrome (DS) individuals have high incidence of immunological alterations with increased susceptibility to bacterial and viral infections besides high frequency of hematologic malignancies and autoimmune disorders. In this study, we investigated the expression pattern of 92 immune-related genes in DS children aiming to identify candidate genes that might be involved on the immunological alterations frequently associated to DS. Peripheral blood mononuclear cells (PBMC) samples were obtained from six healthy individuals with full trisomy 21 and six healthy control individuals (ages 2-6 years). Gene expression was profiled in duplicate according to the manufacturer's instructions provided by commercially available TaqMan® Human Immune Array representing 92 immune function genes and 4 housekeeping genes on a 96-plex gene card (Applied Biosystems, Carlsbad, CA). Of the 92 genes, 15 did not meet criteria for statistical analysis. Thus, 77 genes were subjected to statistical analysis and the comparison between children with DS and controls resulted in 16 differentially expressed genes after correction for multiple tests. Four of them (*CCR2*, *BCL2L1*, *IL10* and *CCR5*) were up-regulated (adjusted *P*-values: < 0.001, 0.006, 0.008 and 0.047; respectively), while twelve genes (*BCL2*, *CCL3*, *IL6*, *EDN1*, *CD40LG*, *CD80*, *CCR7*, *IKBKB*, *CD28*, *CD19, SKI* and *CD40*) were down-regulated (adjusted *P*-values: 0.001, 0.001, 0.001, 0.003, 0.007, 0.008, 0.010, 0.019, 0.026, 0.046, 0.05 and 0.05; respectively). This study identified a modest dysregulation in the expression of some genes that may be related to the immunological alterations seen in DS and indicates candidate genes for further investigation into the molecular mechanism underlying DS pathology.

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P20.21

Alterations in gene expression microarray in oesophageal atresia tissues - the role of SHH and Wnt pathway signalling

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Introduction: Esophageal atresia (EA) may occur as an isolated (IEA) or syndromic anomaly (SEA). It is supposed that combination of multigenic factors and epigenetic modification of genes can play a role in its aetiology. The aim of work was to assess the gene expression in microarray study in esophageal tissue samples.
Material and methods: RNA was extracted from 26 esophageal tissue collected during thoracoscopic EA repair in neonates with IEA and SEA. Lower pouch of esophageal tissues were analysed. Control esophageal tissues were taken during autopsy from aborted fetuses and stillborn neonates without macroscopic defects. We used Agilent Human Gene Expression Microarrays to determine gene expression profiling. Quality analysis and scale normalization for results were performed. Analysis of differential expression (DE) was done (R limma package) and functional pathway analysis were performed (globaltest method, KEGG and DAVID database).
Results: We identified 787 down- and 841 up-regulated transcripts between SEA and controls, and about 817 down- and 765 up-regulated probes between IEA and controls. 50% of these genes showed DE specific for either IEA or SEA. Functional pathways analysis revealed substantial enrichment for Wnt and SHH as well as cytokine and chemokine signaling pathways. We performed reverse transcription PCR study in a group of SHH and Wnt genes with DE in microarray profiling to confirm the microarray results. We verified the altered expression in SFRP2 gene (Wnt) as well as SHH, GLI1, GLI2 and GLI3 (SHH).
Conclusion: The results suggest a important role of SHH and Wnt pathways for EA etiology.

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P20.22

Luciferase assays of 5' untranslated region polymorphisms of the alpha-galactosidase gene

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INTRODUCTION:
Lysosomal alfa-galactosidase (α-Gal) is the enzyme deficient in Fabry disease. The 5' untranslated region (5'UTR) of the GLA gene shows a remarkable degree of polymorphic variation, with single nucleotide polymorphisms (SNPs) described at nucleotide positions 44 (g.1136C>T), 30 (g.1150G>A), 12 (g.1168G>A) and 10 (g.1170C>T) relative to the AUG start codon. In vivo studies in hemizygotes have shown that SNP 30AUG is associated with high plasma α-Gal activity and that SNP 10AUG is associated with moderately decreased α-Gal enzyme activity in leucocytes, whereas SNP 12AUG does not seem to affect enzyme activity. Limited data suggest that the rarer SNP 44AUG might be associated with increased α-Gal plasma activity. We used a luciferase reporter system to assess the relative transcription efficiencies of the different GLA 5'UTR variant isoforms in vitro, as compared to the wild-type sequence.
METHODS:
The wild type 134bp GLA 5'UTR and the four variant isoforms were amplified from genomic DNA and inserted into pGL3 Luciferase Reporter Vector. All five plasmids were cloned into recombinant Escherichia coli and transfected into HeLa and ND7 cells with luciferase and β galactosidase reporter vectors, and their relative expressions were measured.
CONCLUSIONS:
SNPs 12AUG and 44AUG significantly raised luciferase activity in both cell types, while the activities observed with the 10AUG and the 30AUG SNPs were within the wild type range of variation. These results are in contrast with the in vivo findings in hemizygotes and do not support the explanation that these are due to 5'UTR related differences in GLA gene transcription.

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P20.23

Study of ACVR1 gene expression regulation: a way to discover a „druggable" target toward Fibrodysplasia Ossificans Progressiva (FOP) treatment.

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The ACVR1/ALK2 gene encodes a type I receptor for Bone Morphogenetics

Proteins (BMPs). ACVR1 gain of function mutations are associated with FOP (OMIM 135100), a rare disease characterized by congenital malformation of great toes and progressive heterotopic ossification in soft tissues leading to severe disability. No treatment is available for FOP and much interest is devoted to find targets for novel therapies.
Given our interest in understanding the poorly investigated molecular mechanisms regulating ACVR1 gene expression, we have characterized its promoter region. We identified a Transcription Start Site for ACVR1, and focused on the 2.9 kb genomic region upstream of it. We analyzed the sequence by bioinformatic means and performed a transfection-based functional study.
We found that the region is able to drive a strong transcriptional activity in different cell lines. We characterized the contribution of different parts of this region to the transcriptional control of the gene and defined a GC-rich core promoter. Finally, we used this functional region as target for High-throughput screening (HTS) of chemical compounds with potential pharmacological effect on the ACVR1 mediated pathway, by targeting its expression at the transcriptional level. Thus, we generated a cellular system in ATDC5 cells stably expressing Luciferase reporter gene under the control of the identified ACVR1 promoter and we adapted cell culture conditions to the 96-well format useful for HTS.
We report here the results of the functional characterization of the ACVR1 promoter and the results obtained by testing our cellular system with a small library of chromatin-modifier compounds.

S. Cappato: None. **F. Giacopelli:** None. **L. Tonachini:** None. **R. Ravazzolo:** None. **R. Bocciardi:** None.

P20.24
Two novel variants affecting splicing of *FLNA* exon 18 cause a severe PVNH phenotype

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Loss of function mutations of *FLNA* are responsible for X-linked periventricular nodular heterotopia (PVNH) which generally leads to prenatal lethality in affected males.
We report 2 novel *FLNA* mutations identified in exon 18 by direct DNA sequencing, a synonymous variant in one female (P1) and a missense substitution of the last nucleotide in one male (P2), displaying a severe PVNH phenotype. *In silico* analysis predicted a splicing effect: activation of a cryptic donor site in P1 and abolition of the canonic donor site in P2.
FLNA transcripts were studied using RT-PCR of total RNA extracted from lymphocytes and agarose gel electrophoresis. Amplicons were then cloned and sequenced.
In both cases, at least two transcripts were visualised. The sequencing of the clones confirmed the presence of the same aberrant transcript lacking 34 bp, in both patients, due to the preferential utilization of the predicted cryptic donor splice site in exon 18. A longer transcript was also detected in P2 resulting from the use of a cryptic splice site in intron 18.
The detection of these aberrant transcripts, containing a premature stop codon is consistent with a partial NMD. Unlike in P1, the canonic donor splice site was functional in P2 maintaining a full length mutated transcript. Male patient survival could be explained by the production of a mutated full length FLNA protein.
These results point to a sensitive regulation of exon 18 splicing and stress the relevance of mRNA studies in filaminopathy A molecular diagnosis especially in case of synonymous variants.

N. Houcinat: None. **I. Coupry:** None. **S. Moutton:** None. **J. Deforges:** None. **J. Amiel:** None. **A. Dieux:** None. **D. Lacombe:** None. **B. Arveiler:** None. **C. Goizet:** None. **P. Fergelot:** None.

P20.25
FOXL2 and its coregulators in ovarian function

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FOXL2 is a forkhead transcription factor and a major determinant of ovarian development and function. FOXL2 germline mutations are responsible of the BPES syndrome, a genetic condition involving facial malformations and premature ovarian failure. Recently, a unique FOXL2 mutation (p.C134W) was discovered in more than 95% of adult-type granulosa cell tumors, suggesting a role for FOXL2 in cancer.
FOXL2 regulates a wide variety of cellular processes in the granulosa cells of the ovary, including steroidogenesis, cell cycle, apoptosis and oxidative stress response. To better understand how FOXL2 integrates extra and intracellular signals, we used a yeast two-hybrid screening approach that led us to the discovery ten new protein partners of FOXL2. We next characterized their effect on FOXL2 activity using an array of target genes. Importantly, we found that FOXL2 pro-oncogenic mutant may be impaired in its ability to integrate pro-apoptotic signals from its partners. In parallel, we have used mass spectrometry to identify eight sites of post-translational modification on FOXL2 protein, suggesting that FOXL2 modification affects its stability and activity.
Many transcription factors of the nuclear receptor family are FOXL2 partners and are of major relevance in ovarian biology. We therefore started to characterize how FOXL2 regulates the cellular response to the sex steroids through nuclear receptors. Our findings suggest that FOXL2 is a major regulator of estrogen and androgen responses in granulosa cells of the ovary. This multifaceted approach will bring new insights into the role of FOXL2 both normal and pathological conditions.

A. Georges: None. **D. L'Hôte:** None. **A. Todeschini:** None. **J. Kim:** None. **R.A. Veitia:** None. **J. Bae:** None.

P20.26
DNMT3B, encoding a *de novo* methyltransferase, as a new candidate gene for Hirschsprung disease.

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Hirschsprung´s disease (HSCR, OMIM 142623) is a developmental disorder characterized by the absence of the enteric ganglia in variable lengths of the hindgut, resulting in severe intestinal dysfunction. HSCR is attributed to cell proliferation, migration and/or differentiation failure of the enteric neural precursors along the gut during normal embryonic development. Although some genes involved in this pathology are known, many aspects remain poorly understood. In this study we have characterized the precursors of human Enteric Nervous System (ENS) and have identified a group of genes with differential expression patterns between ENS precursors from HSCR patients and controls, being worth of mention the DNMT3B gene, which encodes a a DNA methyltransferase that performs *de novo* DNA methylation during the embryonic development, and that is involved in the expression of crucial genes for the proper human tissue growth. Through the experiments designed to study the role of *DNMT3B* in HSCR disease, we have observed a decrease of DNA methylation in enteric precursors from HSCR patients resulting in lower pluripotency. Taken together, our results demonstrate that in the context of HSCR, ENS precursors seem to have a limited capacity to response to stimuli implicated in neuronal differentiation. We show, for the first time, that DNMT3B could be involved in the ENS development, and therefore would play a role in the onset of HSCR. Moreover, DNMT3B may be regulating the expression of known genes implicated in this pathology through DNA methylation.

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Abstract P20.27 withdrawn

P20.28

Chromosomal analysis of induced pluripotent stem cells (iPSCs) derived from senescent cells of elderly persons

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Reprogramming of adult somatic cells into induced pluripotent stem cells (iPSCs) provide a unique opportunity to generate patient-specific stem cells with potential application in regenerative therapies and without the ethical concerns of human embryonic stem cells (hESCs). However, important question persist about the safety of iPSCs and their chromosomal stability.

In order to assess the chromosomal consequences of reprogramming process, we performed classical and molecular cytogenetic analysis on 4 iPSC lines generated from 74- and 96-years-old men's fibroblasts. Cytogenetic analysis was carried out using standard methods. FISH assays with both centromeric and painting probes were used for chromosomal detection on interphase nuclei. A minimum of 20 metaphase spreads and 100 interphase nuclei per cell line were analyzed. The 4 cell lines exhibited normal karyotypes. In three iPSC lines, these investigations were completed by an analysis of the telomere sizes since senescence is characterized by telomere shortening. We measured in situ the length of telomere repeat domains using PNA pantelomeric probes and Metasystems image analyzer software Isis for quantifying the fluorescence intensity of telomeres from metaphases and nuclei from each cell line. In the 3 iPSC lines, we observed a significant increase in telomere length, when compared with their parental fibroblasts and control hESCs. These data were confirmed by telomere restriction fragments analysis.

The perspective of using iPSCs in regenerative medicine is of particular interest in the context of age-associated disorders, but cytogenetic and genetic screening of iPSCs need to become standard practice before these cells might be used clinically.

F. Pellestor: None. **L. Lapasset:** None. **F. Becker:** None. **J. Puechberty:** None. **P. Sarda:** None. **J. Lemaitre:** None. **G. Lefort:** None.

P20.29

Characterization of unbalanced chromosomal abnormalities in three Jacobsen syndrome using array-CGH and FISH-BACs

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Context: Jacobsen syndrome occurs in approximately 1 out of every 100,000 births and the majority of cases are *de novo.* Classic phenotypes of this syndrome includes mental retardation, characteristic facial trait and thrombocytopenia. Its karyotype shows a terminal 11q deletion, which can vary from 7 to 20 Mb, with phenotypic expression related to the lost size.

Objective: Characterization of the 11q deletion using array CGH (aCGH) and FISH with BACs in three patients with Jacobsen syndrome have been carried out in order to establish a more accurate genotype-phenotype association. These 11q deletions had been previously identified by G-banding and high resolution CGH (HR-CGH).

Results: The aCGH analysis confirms the terminal 11q deletion in three patients and redefined the size deleted in each patient (15Mb, 14.6Mb and 11.53Mb). Additionally, the aCGH revealed the presence of one microduplication (dup 16p13.11 of 784 Kb) in a patient and one microdeletion (4p15.33 of ~ 244 Kb) in another patient. Moreover, the observation of BACs in interphasic nuclei demonstrates that the duplication 16p13.11 presents a direct orientation. So far it is the smallest duplication in which their orientation has been identified.

Conclusion: The 11q deletions identified in all three patients are within the range of the size associated with the syndrome. These are the first two Jacobsen syndrome cases showing other structural chromosome alterations in addition to the 11q deletion. These findings suggest that a more complex genetic disorder could be involved in heterogeneity of clinical features associate to Jacobsen syndrome.

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P20.30

Reactivation of Foetal Haemoglobin by Chemical Inducers

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Pharmacogenomic studies aimed at understanding how particular compounds are able to induce foetal haemoglobin (HbF) may help to elucidate the molecular control of globin gene switching. In this study, a collection of Maltese healthy adults (n=5), β-thalassaemia homozygotes and compound heterozygotes (n=5), and KLF1-haploinsufficient adults (n=3) from two families were explored. The effects of hydroxyurea (HU), thalidomide (Thal) and 5-Aza-2'-deoxycytidine (Aza), all well-known HbF inducing agents, were explored to quantify the HbF effect. HbF augmentation was minimal across the groups (< 1.5 FC) with the exception of one healthy adult that was hyper responsive to all three drugs after 24hrs (for Thal 1μM FC 2.26, Thal 10μM FC 2.34, Thal 100μM FC 2.50, with Aza 1μM FC 2.16, Aza 10μM FC 3.47, Aza 100μM FC 2.28) and maintained with two drugs even after 72hrs. The HbF of β-thalassaemia homozygotes and compound heterozygotes was inducted late after 72 hours in culture with thalidomide. Western blot assays for KLF1 showed an attenuation of the protein by the drugs (In cultured cells without drugs phosphoimager intensity 1,176 while in some cultured cells with drugs decreased to 880) hence suggesting a role of inhibition by drugs in culture (in vitro) in human haematopoiesis. The KLF1 gene may be an important pharmacogenomic marker to predict efficacy of HU, thalidomide or 5-Aza-2'-deoxycytidine treatment. HU, thalidomide and 5-Aza-2'-deoxycytidine mechanisms might be acting on HbF control by down regulation of KLF1 but the effect on Hb F may be inhibited by "Foes of KLF1".

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P20.31

Prelamin accumulation in primary endothelial cells induces premature senescence and activation

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Defects in lamin A maturation result in premature aging syndromes and severe atherosclerosis as observed in Hutchinson-Gilford Progeria Syndrome. In age-related atherosclerosis, several features of cell senescence have been characterized in endothelial cells including telomere shortening and increased oxidative stress, but lamin A alterations have been little investigated. To study lamin A-related senescence in primary endothelial cells and consequences in the activation status of cells, normal primary endothelial cells from human umbilical vein (HUVEC) or cord blood (ECFC) were used. Lamin A defects were induced by treatment with the protease inhibitor Atazanavir during 48h.We showed that PI treatment led to the accumulation of farnesylated prelamin A and induced nuclear shape abnormalities and premature senescence in both HUVEC and ECFC. ICAM-1-dependent activation was present and monocyte adhesion was increased in HUVEC whereas ability to generate a microvascular network in matrigel was decreased for ECFC. The effects of PI treatment were reversed when cells were PI-treated in combination with Pravastatin and Zoledronate in both mature and progenitor endothelial cells. Reversion was also demonstrated with 2 antisense oligonucleotides targeted toward lamin A specific splice sites.This study showed that PI treatment reproduces premature senescence due to lamin A defects in primary endothelial cells after a 2 day exposure. The cells used were extracted

from neonates, allowing considering that other senescence pathways were not activated and that the observed alterations were specific of prelamin A accumulation. This constitutes a valuable model to test different approaches aimed at reversing specifically lamin A-related cellular senescence.

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P20.32

Identification of an additional Prelamin A deleted transcript in a cohort of patients affected with progeria-like syndromes due to LMNA mutations

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Premature ageing syndromes, and especially Hutchinson-Gilford progeria syndrome, which is associated with mutations in LMNA, are rare genetic disorders mimicking some clinical and molecular features of ageing. They are associated with numerous alterations of cell homeostasis. LMNA pre-mRNAs physiologically undergo an alternative splicing event leading to the production of Prelamin A (the Lamin A precursor) and Lamin C. Several deleted Prelamin A transcripts (prelamin AΔ50, Δ35 and Δ90) have been implicated in the physiopathology of lamin-related diseases (Navarro et al 2004, Hishima et al 2011, Fukushi et al 2004). Most patients affected with typical HGPS carry an heterozygous de novo mutation located in exon 11 (c.1824 C>T; p.Gly608Gly) which leads to an in-frame deletion of the last 150 base pairs of the exon, and the production of prelaminAΔ50, a truncated and permanently farnesylated precursor. The purpose of this work was to study the presence and the effect of Prelamin A truncated transcripts, including prelaminAΔ90, which lack the entire exon 11, on clinical phenotypes in a cohort of patients affected with progeria-like syndromes. PrelaminAΔ90 was exclusively identified in patients carrying mutations close from the donor splice site of exon 11. In these patients we attempted to correlate the clinical phenotypes with either: the presence of the rs4641 polymorphism (p.His566His), the impact of the ratio between all the deleted prelamin A isoforms and wild type lamins A and C or the level and the severity of cellular abnormalities observed. Finally, we try to demonstrate the existence of prelaminAΔ90 at the protein level.

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P20.33

Functional characterization of genetic variations in *MATE* promoter in Koreans

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BACKGROUND: Multidrug and toxin extrusion transporters (*MATE1* and *MATE2-K*) play important roles for the elimination of many cationic drugs and their metabolites from the body. To date, a few genetic variants have been described in *MATE1* and *MATE2-K*. The goal of this study was to identify and functionally characterize novel genetic variations of *MATE1* and *MATE2-K* promoters in Koreans.
METHODS: DNA samples from 48 healthy Korean subjects were screened for variants in the promoter regions of *MATE1* and *MATE2-K*. To measure promoter activities of *MATE1* and *MATE2-K* haplotypes, reporter assays were performed. Potential transcription factors that regulate the transcription of *MATE1* and *MATE2-K* were identified using TFSearch and confirmed by electrophoretic mobility shift assays.
RESULTS: There were five variants in the *MATE1* and nine variants in the *MATE2-K* promoter regions. Two of the *MATE1* and seven of the *MATE2-K* variants were polymorphic. In the case of *MATE1,* haplotype 3 containing a variant, V1 showed a 72% increase in reporter gene expression, compared to that of the reference. Results from electrophoretic mobility shift assays showed that three transcriptional factors are involved in the regulation of *MATE1* transcriptional activity. Two haplotypes of *MATE2-K*, H1 and H2 also showed a significant increase in reporter activity.
CONCLUSION: Our studies revealed that novel promoter genetic variations of *MATE1* and *MATE2-K* result in changes in transcriptional activity of these genes. These variants can potentially affect the pharmacokinetics or drug response of many drugs that are substrates of MATE1 or MATE2-K.

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P20.34

Gene expression modifications in Wharton's Jelly mesenchymal stem cells promoted by prolonged in vitro culturing

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The umbilical cord matrix, represented by the Wharton's Jelly (WJ), contains a great number of mesenchymal stem cells (MSCs), which have been characterized as expressing the best suited MSCs markers, shared by both human and animal models. The abundant amount of WJ makes it an attractive source of MSCs for cell-based therapies. However, as in other stem cells models, a deeper investigation about the biological properties of WJ-MSCs related to their long expansion and fast growth abilities is required before their use in a clinical setting. In this context, in order to analyze the gene expression modifications occurring in WJ-MSC promoted by the prolongation of culture, we investigated the transcriptomic profile of WJ-MSCs cultures at 4th and 12th passage in vitro expansion by microarray analysis Hierarchical clustering analysis of the data set originated from a total of 6 experiments revealed that 12 passages in vitro expansion of WJ-MSCs promote the selective over-expression of 157 genes, while 440 genes were down-regulated as compared to 4 passages . Ingenuity Pathway Analysis was carried out to investigate the main functions played by the selected genes disclosing that several transcripts are related to inflammatory and cell stress response, cell proliferation and maturation, and apoptosis. Taken together, these modifications may lead to an impairment of the cell expansion ability and resistance to apoptosis, two hallmarks of aging cells. In conclusion, the results provided by this study suggest the necessity of novel culture protocols able to preserve the stem cells plasticity.

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P20.35

Expression of a linear reporter construct in isolated human mitochondria

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Transfection of reporter genes is a standard method to study nuclear gene expression. For mammalian mitochondrial DNA, such a genetic manipulation has so far been elusive. Thus, disease causing mutations in the mitochondrial DNA cannot be functionally characterised and basic genetic mechanisms of the mitochondria still remain unknown. Isolated mammalian mitochondria possess the ability to import DNA from their environment. We used this ability to study reporter constructs. After isolation of active mitochondria from cultured rat Zajdela hepatoma cells and human HepG2 cells, we monitored DNA import using quantitative PCR and confocal microscopy. Import of linear double-stranded DNA from 100 bp up to 3.3 kbp revealed time-dependent uptake. After DNA import, mitochondria were then sustained in respiration buffer for expression of imported constructs. Expression of human reporter genes in isolated rat hepatoma mitochondria was successfully achieved. Using a circular RNA (CR)-RT-PCR assay, accurate maturation, i.e. adding of the CCA trinucleotide post-transcriptionally to the heterologues expressed human tRNAArg was demonstrated. By generation of constructs containing all regulatory elements adapted to the human expression system, we could show that a rat-specific tRNALeu(UUR) is fully processed at its 3`end with CCA trinucleotide addition, but aberrantly processed at its 5`end. Furthermore, mRNA expression of an imported GFP reporter could be demonstrated. Establishing such a system for human cells not only allows the characterisation of mitochondrial gene expression (e.g. maturation, cis-acting elements, polyadenylation, turnover of mRNAs, regulation), but also the elucidation of the systemic impact of mitochondrial DNA mutations at a functional level.

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P20.36

Mechanisms of rearrangements at 1p36

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Deletions of 1p36 occur in approximately 1 in 5,000 newborns. To date, we have ascertained more than 150 cases with monosomy 1p36, representing four possible classes of rearrangements: pure terminal deletions, interstitial deletions, unbalanced translocations, and complex rearrangements (involving duplications in monosomy 1p36). For each individual, deletion and duplication sizes, and parental origin of the rearrangements were determined using array CGH and genotyping. To further understand the mechanisms of 1p36 aberrations the rearrangements breakpoints were investigated using molecular cytogenetics and molecular biology methods. Deletion sizes as well the breakpoints locations were different in each patient. Our results show higher than accepted complexity of the studied rearrangements and indicate involvement of multiple mechanisms in the DNA breakage and repair process during rearrangement formation. Although the causes of the chromosomal breaks that initiate the rearrangement formation are unknown, the joining mechanisms were consistent with features of the non-homologous end-joining pathway. Examples of the rearrangements and the mechanisms will be discussed.
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M. Gajecka: None.

P20.37

Detection of splicing-affecting mutations in the primary-immunodeficiencies related genes.

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Mutations that influence splicing of precursor mRNA are conservatively estimated to cause 15 % of Mendelian disorders. They generally have severe impact on gene expression. However, their detection is not always elementary.
In order to test the incidence of splicing-affecting mutations in our patients, we took 92 point mutations we found in the genes BTK, SerpinG1, STAT3, IL2RG, WAS and CD40LG. At first, we analysed these mutations using in silico prediction tool NNSplice. Accordingly, we selected 16 mutations most probable to disrupt splicing (including 13 mutations affecting authentic splice sites) and we tested them in vitro by splicing minigene analysis. Minimal score change between these wild type and mutant sequences was 7 %.
Effect of all 13 mutations predicted to disrupt splice sites was confirmed by in vitro analyses. Two of these mutations simultaneously created de novo splice sites that were utilized in vitro. Of three other mutations predicted to create de novo splice sites, only one disrupted splicing in vitro. Surprisingly, this novel site was predicted with lower score than the authentic splice site, as opposed to the other two de novo splice sites.
Overall, we found out that 14 of the 92 originally tested mutations (15 %) disrupted splicing of pre-mRNA. RT-PCR from RNA of seven mutations carriers (all of the available) confirmed results of minigene analysis, showing no false positive or negative results. When assessing the predictions, generally recommended 10 % cut-off limit of score difference would lead to omission of one of the splicing affecting mutation.

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P20.38

NPHS2 mutations in Brazilian children with nephrotic syndrome

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Nephrotic syndrome (NS) is defined by heavy proteinuria, hypoalbuminemia, edema and hyperlipidemia. It is divided into two categories based on the response to steroid therapy: steroid-sensitive (SSNS) and steroid-resistant (SRNS). *NPHS2* gene mutations cause autosomal-recessive NS. In this study we report the molecular evaluation of the *NPHS2* gene of 111 Brazilian patients (n=222 alleles) with infantile NS. The eight coding exons, promoter region and intron/exon boundaries of *NPHS2* were direct sequenced. A total of 11 nucleotide variations (11/222, 4.95%) had been identified. Five out of 11 were located in the promoter region in the heterozygous or homozygous state. Three of them (c.-51G>T, c.-116C>T and c.-535TTTTTT$_2$/TTTTTT$_3$) were described as determining downregulation of the reporter gene expression when transfected in podocytes. Two others (c.-196C>G and c.-267C>G) had been identified here for the first time. Six already described heterozygous missenses were identified: p.P20L, p.R229Q, p.A242V, p.E264Q, p.A284V and p.E310K. We analyzed the possible impact of the amino acids substitutions based on *in silico* prediction sites and all of them returned in a damaging outcome. This is the first study in a Brazilian cohort and emphasizes the relevance of the molecular analysis of NS in childhood since it is a clinically heterogeneous disease in which informations on the genotype may guide further treatment. Additionally, living related donor transplantation might be considered promptly since SRNS patients with homozygous or compound heterozygous mutations in *NPHS2* have reduced risks of recurrence of focal segmental glomerular sclerosis after renal transplant compared with children without mutations.

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P20.39

NF1 molecular characterization and neurofibromatosis type I genotype-phenotype correlation: the French experience

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Neurofibromatosis type 1 (NF1) affects about one in 3,500 people in all ethnic groups. Most NF1 patients have private loss-of-function mutations scattered along the *NF1* gene. Here, we present an original *NF1* investigation strategy and report a comprehensive mutation analysis of 565 unrelated patients from the NF-France Network. A *NF1* mutation was identified in 546 of the 565 patients, giving a mutation detection rate of 97% never reached before. The combined cDNA/DNA approach showed that a significant proportion of *NF1* missense mutations (30%) were deleterious by affecting pre-mRNA splicing. Multiplex ligation-dependent probe amplification and/or custom array-*comparative* genomic hybridization allowed the identification of restricted rearrangements that would have been missed if only sequencing or microsatellite analysis had been performed. In four unrelated families, we identified two distinct *NF1* mutations within the same family. This fortuitous association points out the need to perform an exhaustive *NF1* screening in the case of molecular discordant related patients. A genotype-phenotype study was performed in patients harboring a truncating (N=265), splicing (N=145), or missense (N=41) mutation. The association analysis of these mutation types with 12 common NF1 clinical features confirmed a weak contribution of the allelic heterogeneity of the *NF1* mutation to the NF1 variable expressivity.

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P20.40

Spectrum of single and multiple *NF1* exons copy number variations in neurofibromatosis type

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Neurofibromatosis type 1 (NF1) is one of the most frequent autosomal dominant genetic disease. NF1 is caused by loss of function mutations of the tumour suppressor gene *NF1*. A large spectrum of *NF1* molecular abnormalities is found: large locus deletions, missense or nonsense mutations, splice mutations, and intragenic deletions/insertions. In our laboratory, *NF1* molecular investigation is performed by intragenic microsatellite pre-screening study, quantitative PCR, and a combined approach of cDNA/DNA sequencing. Here, we have evaluated the usefulness of the multiplex ligation-dependent probe amplification (MLPA) approach, which allows a simultaneous gene dosage of nearly all *NF1* exons. Among 564 NF1 index

cases of the French NF1 cohort, thirty patients were identified as „negative" patients with no molecular abnormality found using the molecular strategy of our laboratory. These „negative" patients were subsequently tested by MLPA. Quantitative abnormality of one or more exons was identified in 12 of them (40% of "negative" patients). This MLPA approach also allowed the confirmation of nine deletions and one tandem duplication that were previously identified by the cDNA/DNA analysis. In total, MLPA identified a *NF1* molecular defect in nearly 4% of the 564 patients clinically affected by NF1. The abnormalities found were single exon deletions (eight cases including four affecting the *NF1* promoter and exon 1), multiple exons deletions (11 cases including one mosaic deletion), one double deletion, and two duplications. All results were confirmed using a custom array-CGH which allowed characterisation of the duplication/deletion boundaries and identification of recurrent breakpoints.

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P20.41

Partial monosomy 22qter resulting from a paternal chromosome 22 inversion: a cytogenetic pitfall

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Pericentric inversions are rare chromosomal rearrangements leading to a variable risk of aneusomic recombination depending on the chromosome involved and the inversion size.

We report the case of a child affected with 22q13 deletion syndrome resulting from a paternal pericentric inversion of chromosome 22 which was first missed by using solely molecular and FISH analyses.

In the proband, R banding prenatal chromosome analysis was normal but telomere screening by MLPA revealed a 22q13 deletion which was confirmed by FISH analysis. FISH analysis of both parents was interpreted as normal leading to the diagnosis of de novo 22q13 deletion. The proband's phenotype was in keeping with the Phelan-McDermid syndrome. A favourable genetic counselling for a next pregnancy was given.

Subsequently, investigation of both parents for infertility by high resolution R and G banding chromosome analysis on peripheral blood lymphocytes revealed in the father a pericentric inversion of chromosome 22 [46, XY, inv(22)(p11.2q13.3)]. This was confirmed by Ag-NOR staining in the proband and his father. As expected, Agilent® human genome 60K CGH microarray analysis showed in the proband a terminal 7.8 Mb deletion from 22q13.2 to 22qter with no other genomic imbalance, particularly no duplication.

This observation confirms that, in the absence of a previously known familial chromosome anomaly, conventional cytogenetic analysis remains the only method able to diagnose balanced chromosomal rearrangements which cannot be detected by the powerful and modern molecular techniques currently considered as the first-rate strategy in MCA/ID syndrome investigation.

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P20.42

Inactivation of Pif1 helicase causes late-onset mitochondrial myopathy with multiple mtDNA deletions in mice

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Mitochondrial helicases such as Twinkle and DNA2, play a crucial role in replication and repair of the mitochondrial genome (mtDNA) and are involved in late-onset mitochondrial myopathies with mtDNA instability. Here, we show that inactivation of mPif1, a third member of this family, causes a similar phenotype in mouse. Pif1-/- animals develop a mitochondrial myopathy after 1 year of age with accumulation of mtDNA deletions. Muscle weakness is objectified by a decreased exercise capacity in Pif1-/- mice with a daily running distance significantly lower compared to wild-type animals. The mouse PIF1 mitochondrial isoform, that occurs via downstream alternative translation initiation (dATI), is present inside mitochondria and binds to mtDNA. Furthermore, we show that mPif1 inactivation is responsible for a deficiency to repair oxidative stress-induced mitochondrial DNA damage, thus confirming the role of this helicase in mtDNA maintenance. These results open new perspectives for the exploration of patients with mtDNA instability disorders and for the study of mitochondrial helicases that play a critical role in the maintenance of mtDNA stability, possibly preventing age-associated accumulation of mtDNA mutations.

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P20.43

Prader Willi : a typical case with a 118 kb paternally inherited microdeletion of the snoRNA SNORD116 cluster

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Prader Willi syndrome (PWS) is a neurobehavioral disorder caused by the loss of expression of the paternal allele of several imprinted genes including snoRNA within 15q11-q13. Large interstitial deletions encompassing the SNRPN gene of paternal origin, chromosome 15 maternal uniparental disomy and rare imprinting defects are the classical mechanisms reported in almost all cases of PWS. Because these etiologies result in the lack of the unmethylated allele at the SNRPN locus, methylation analysis is the first genetic test used for the diagnosis of PWS. However, three individuals with features of PWS were recently reported as having a normal SNRPN methylation status. Remarkably, these patients displayed a micro-deletion overlapping the paternally expressed SNORD116 gene cluster. Here, we describe the smallest paternally inherited microdeletion of the SNORD116 in a patient with typical features of PWS, including, notably, severe neonatal hypotonia. Our study strengthens the notion that the lack of SNORD116 expression genes contributes to the pathogenesis of PWS.

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P20.44

Breakpoints of non allelic homologous recombination (NAHR) events at the *DPY19L2* locus are centered around a *PRDM9* recognition sequence

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We demonstrated previously that 75% of infertile men with round, acrosomeless spermatozoa (globozoospermia) had a homozygous 200 Kb deletion removing the totality of *DPY19L2*. We showed that this deletion occurred by Non Allelic Homologous Recombination (NAHR) between two homologous 28 Kb Low Copy Repeats (LCRs) located on each side of the gene.

NAHRs are believed to occur during meiotic prophase, induced by the crossing-over machinery on misaligned homologous sequences. Recent work showed that PRDM9 apposed post-translational histone modifications preferentially in the vicinity of a 13 nucleotide consensus sequence, which has been found to be associated with recombination hotspots. These epigenetic marks likely contribute to the recruitment of the recombination initiation complex, thus favouring the occurrence of recombinations at these loci.

Here, we developed two PCR assays that allowed us to detect approximately 90% of the recombined alleles (deletions and duplications) occurring at the *DPY19L2* locus. We amplified and sequenced a total of 46 recombined alleles from globozoospermia patients and heterozygous individuals and 139 *de novo* recombined alleles from control sperm DNA. We identified 5 distinct breakpoints and showed that they all cluster within a 1153 bp region roughly located in the middle of the 28 Kb LCR. We found a *PRDM9* consensus site at the epicentre of all identified breakpoints. Our work therefore reinforces the theory indicating that the *PRDM9* recognition sequences represent a strong signal for the occurrence of double strand break and recombination.

C. Coutton: None.

P20.45

Comparison of conventional cytogenetic techniques with molecular cytogenetic techniques in analysis of chromosomal imbalances in spontaneous abortion and fetal demise

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Traditionally, chromosomal study of product of conception (POC) has been conducted on specimens received following spontaneous abortion. It is also possible to perform chromosomal study on tissue obtained following IUFD and perinatal fetal death. There are major limitations in successful chromosomal analysis as it requires viable cells for culture.

In 15 years, we have performed chromosomal analysis on 2053 samples obtained from chorionic villi, placental tissue and/ or muscle biopsy following abortion or fetal demise. Tissue culture failed in 384 of these samples and in the remainder, approximately 23% show chromosomal abnormalities.

For the past 3 years, as an alternative to chromosomal study we have started to use molecular cytogenetic techniques for analysis of these samples. Following DNA extraction, we have performed QF-PCR for common 5 chromosome aneuploidy (13. 18. 21. X and Y). Thereafter in those cases where a chromosome abnormality has not been detected we have performed array comparative genomic hybridization, initially on BAC array and more recently on oligoarray platforms. Of the more than 400 samples thus studied, only 15 have failed to label and of the successfully studied cases, approximately 30% have shown chromosomal abnormalities.

The techniques are compared and the age related ratios of chromosomal aberrations are further discussed in detail. Overall, it appears that the application of the molecular cytogenetic techniques is much more efficient in detecting chromosomal aberrations, specially in later gestational age fetuses.

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P20.46

Lamin A expression is downregulated by miR-9 in the brain of a progeria mouse model, a mechanism that protects HGPS patients from cognitive impairment

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Hutchinson-Gilford Progeria syndrome (HGPS) is an extremely rare disease characterized by segmental premature aging leading to premature death at the mean age of 13.5 years. Clinical features are severe dermal and bone abnormalities, a general lipoatrophy, sarcopenia, as well as major cardiovascular defects. HGPS is typically caused by a *de novo* transition (c.1824C>T) in *LMNA*, encoding major components of the nuclear lamina and matrix, lamins A and C. This mutation activates a cryptic splicing site which delete 150 base pair, leading to accumulation a truncated and aberrantly farnesylated form of lamin A, called progerin. Nonetheless, despite a wide spectrum of affected tissues, no cognitive defect was never reported in HGPS patients. Recent studies proposed a brain protective role of mir-9, a brain specific microRNA targeting Lamin A. To better characterize *in vivo* relation between mir-9 and low levels of lamin A and progerin in brain tissues, we studied KI $Lmna^{G609G}$ Progeria mouse model which closely reproduces the human pathology both at the molecular and phenotypic level. First, we monitored cognitive processes. Then we performed MRI and volumetric analyses on a panel of cerebral structures. Altogether these explorations evidenced absence of cognitive impairment in mice $Lmna^{G609G}$. In parallel we analyzed Lamin A/C and progerin expression by western blot and immunofluorescence in several cerebral regions and showed reduced lamin A and progerin levels correlated with an overexpression of miR-9 in the same regions. This developmental and biological study confirmed the protective role of mir9 identified in neuronal cells derived from HGPS iPS.

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P20.47

Submicroscopic deletions at GNAS locus and their putative underlying causative mechanisms

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Introduction: Different genetic/epigenetic defects within *GNAS* locus result in a human disorder called Pseudohypoparathyrodisim type 1 (PHP-I), characterized by hypocalcemia and hyperphospatemia due to resistance to parathyroid hormone (PTH). Heterozygous mutations within the *GNAS* locus lead to pseudohypoparathyroidism type Ia (PHP-Ia), which is caused by maternally inherited inactivating mutations affecting the exons encoding Gsα. In very few cases, submicroscopic deletions including part or the whole gene have been reported. The MS-MLPA technique is a powerful tool for evaluating genetic deletions that cannot be detected using PCR based sequencing techniques, in addition to detect aberrant methylation patterns.

Objective: In this study, we aimed to establish the subjacent genetic mechanisms of large deletion(s) identified by MS-MLPA in patients with PHP-Ia.

Design: First, dosage and methylation analyses of *GNAS* locus were carried out by MS-MLPA using SALSA ME031 kit. An Illumina Human660W-Quad BeadChip was run for patients with whole gene deletions. Finally, breakpoints delimitations at nucleotide level of the different deletions were performed by Semiquantitative multiplex PCR of short fluorescent fragments and confirmation was done by large PCR and posterior direct sequencing.

Results and Conclusion: Two patients presented large deletions at *GNAS* locus. The breakpoints at nucleotide level of these deletions show that they are located inside non-long terminal repeat (Non-LTR) retroposons. Several consequences of activation of these non-LTRs have been described, including DNA's breaks. So, the breakpoints location around GNAS locus in our patients could be associated with the presence of this kind of elements.

I. Garin: None. **F.M. Elli:** None. **J. Clarke:** None. **G. Mantovani:** None. **G. Perez de Nanclares:** None.

P20.48

ROR-Alpha Target Genes in Monocyte and Endothelium

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Aim: ROR-alpha is a member of nuclear receptor family of transcription factors, and therefore has ligand-dependent activity. Natural ligands of ROR-alpha include cholesterol and melatonin. Due to importance of ROR-alpha ligands in atherosclerosis, we aimed to identify ROR-alpha target genes in monocyte and endothelium.

Methods: After administration of THP-1 and HUVEC cells with ROR-alpha specific ligand, chromatin immunoprecipitation (ChIP) was performed. Following fixation and fragmentation, chromatins were precipitated with anti-ROR-alpha antibody to perform enrichment of DNA fragments bound by ROR-alpha. Enriched DNA fragments were then amplified using whole genome amplification kit. Identification of DNA fragments was performed with whole-genome tiling array (NimbleGen).

Results: Whole-genome tiling array analysis of enriched ChIP DNA gave 11,600 and 13,000 peaks for THP1 and HUVEC cells, respectively. Considering the FDR score, p value and genomic location of these peaks ($p<0,05$, FDR<0,01), we found 3,790 genes for THP1 and 4,290 genes for HUVEC which had ROR-alpha peaks between -5000 bp and +2000 bp accordance with TSS. Of these genes, 1,330 were found to be common to both cell types. Annotation clusters with highest enrichment score were glycoprotein and cell/plasma membrane (4.26 for HUVEC and 4.02 for THP1). Other common annotation clusters were cell motion, motality, migration, adhesion and actin-binding, actin cytoskeleton.

Conclusion: Our results showed that ROR-alpha peak freaquency tends to increase at -1500 and +1500, while decreasing around TSS. Functional annotation of target genes suggests that ROR-alpha may involve in cell-cell and cell-matrix interactions through regulating membrane glycoproteins, cell cytoskeleton, cell movement and cell migration.

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P20.49

Inhibitory Effect of SR1001 on ROR-Alpha Activity Requires Intracellular Cholesterol

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Aim: ROR-alpha is a member of nuclear receptor family of transcription factors. In addition to natural ligands, like cholesterol and melatonin, some synthetic compounds have also been shown to bind ROR-alpha as a ligand. One of these synthetic ligands is SR1001 which was demonstrated to inhibit ROR-alpha activity. We aimed to investigate the involvement of cholesterol in the antagonistic effect of SR1001 on ROR-alpha activity.
Methods: Target gene identification was performed by ChIP-PCR. ROR-alpha activity was inhibited by administration of SR1001 in the culture medium. Cholesterol synthesis was inhibited at various steps by the administration of zaragozic acid (ZA), neridronate or simvastatin. RNA levels of *SPP1* were measured using UPL probe in LC480 device.
Results: Both ChIP-PCR and expression analyses showed that *SPP1* is a target gene of ROR-alpha in THP1 monocyte. In cultured THP1, SR1001 suppressed *SPP1* expression in concentration-dependent manner. In the presence of distal cholesterol synthesis inhibitors, antagonistic effect of SR1001 on *SPP1* expression decreased or disappeared. This effect was more obvious in ZA than neridronate. In simvastatin, however, antagonistic effect of SR1001 was independent of inhibition of cholesterol synthesis. Absence of LDL-derived cholesterol enhanced the disappearance of antagonistic effect of SR1001 in the presence of neridronate. In the presence of ZA, however, absence of LDL-derived cholesterol converted the antagonist effect of SR1001 to agonistic.
Conclusion: Our results suggest that the cholesterol is not only a ligand for the ROR-alpha, but also it may be a component which arranges the relationship of ROR-alpha with other synthetic/natural ligands.

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P20.50

New point mutations and exon deletions of the EP300 gene in patients with Rubinstein-Taybi syndrome

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Rubinstein-Taybi syndrome (RSTS, #180849, #613684) is a congenital neurodevelopmental disorder characterized by postnatal growth deficiency, characteristic skeletal abnormalities, dysmorphic features and cognitive deficit. Mutations in two genes, CREBBP (16p13.3) and EP300 (22q13.2), have been identified in ~50% and ~3% affected individuals, respectively.
CREBBP and EP300 are ubiquitously expressed homologous proteins acting as transcriptional co-activators with intrinsic histone and non histone acetyltransferase activity.
So far, only seven EP300-mutated RSTS patients have been described and other 13 mutations including four exon deletions are reported in the LOVD database.
EP300 analysis by DHPLC/direct sequencing and MLPA of 25 CREBBP-negative cases showed four new germline EP300 alterations including two early inactivating point mutations, an indel in exon 1 (c.41_51delinsT) and a duplication in exon 2 (c.668dupT) both leading to a frameshift and premature stop codon (p.K14Ifs*31 and p.Q223Sfs*19) and two novel exonic deletions involving exon 12 and both exons 17-18. The transcripts analysis of patient carrying the exon-2point mutation revealed the presence of the aberrant one, but at reduced level, suggesting its possible instability.
According to the limited literature about all the four EP300-mutated patients show a convincing RSTS phenotype, but at the mild level with minor skeletal anomalies, a slight cognitive impairment and absence of major malformations. Beyond expanding the RSTS EP300 mutations repertoire, our study points to a contribution of the "minor" gene EP300 less negligible than predicted. Results, implemented by extending the study to our accumulated 52 CREBBP-negative patients, will also help the clinical practice in genotype-phenotype correlation doing.

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P20.51

Xq chromosome duplication, including SOX3 gene, in a male: clinical and molecular characterization

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Duplications of the distal long arm of the chromosome X are rare events. Clinical manifestations widely vary depending on the gender of the patient and on the gene content of the duplicated segment. In XY males, structural X disomy always results in functional disomy.
We report on a 39-year old male with hypogonadotropic hypogonadism associated with short stature and obesity. In childhood, he was diagnosed with growth hormone deficiency. On re-evaluation, he was found to have panhipopituitarism, low levels of TSH and gonadotropins as well as an abnormal adrenal response after ACTH stimulation test. He was of normal intelligence and his brain MRI was unremarkable.
Chromosome analysis on peripheral blood lymphocytes showed a male karyotype with pericentric inversion of chromosome 10: inv(10)(p11.2q21.1) and additional material on the long arm of the X chromosome. Fluorescence in situ hybridization using whole chromosome painting probes showed that additional material is derived from chromosome X.
To define the exact duplicated region on chromosome X an array-CGH was performed, indicated two copies of Xq27.1q28, 12,4 Mb in range. After aCGH, the karyotype was re-written: 46,Y,dup(X)(q27.1q28)inv(10)(p11.2q21.1).arr Xq27.1q28(139412100-151852048)x2.
The duplicated region contains 53 genes, among them SOX3. In males both under and overdosage of SOX3 are associated with similar phenotypes, ranging from isolated growth hormone deficiency to panhipopituitarism, and sometimes also with mental retardation. We have compared the clinical, cytogenetic and molecular findings of our patients with those previously reported. According to the literature reported pericentric inversion of chromosome 10 is a normal chromosome variant.

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P20.52

First report of a small supernumerary marker chromosome derivative from chromosomes 8 and 14

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/>Small supernumerary marker chromosomes (sSMC) are usually detected unexpectedly in routine cytogenetics with no correlation with a specific clinical outcome. We present the first report of a patient with a *de novo* complex sSMC derived from chromosomes 8 and 14. G-banding, SNP-based array and FISH revealed: 47,XY,+der(14)t(8;14)(p23.2;q22.1)dn.arr 8p23.3p23.2(46,385-4,301,995)×3,14q11.2q22.1(19,002,111-51,106,110)×3 resulting in a 4.3 Mb 8p duplication and a 51 Mb 14q duplication. Our propositus, born at preterm with 2550 g, 47 cm and Apgar 4/7/9, mother 44 years-old. At age 21 months, presented common characteristics of partial duplication and triplication 14q: intellectual disability, seizures, neuropsychomotor development delay, brachycephaly, low set ears, high palate, macrostomia, tongue protusion and cryptorchidism. According to OMIM there are about 14 and 250 genes duplicated at 8p and 14q, respectively. The 8p22→p23.3 duplication have been reported to be clinically normal or have mild mental retardation with no dysmorphic features, suggesting a minor role in the development. We propose that the clinical features in our patient mainly result from the trisomy 14q. Most of the patients described in the literature with microduplication 14q12 including the *FOXG1* gene suffered of seizures or infantile spasms similar to our patient. The clinical phenotype of our patient may be the result of the additional genes present in the duplicated region and not only the *FOXG1* gene, which represents the most interesting candidate to explain the abnormal neurodevelopmental phenotype. The sSMC originated from maternal chromosomes 8 and 14 probably from errors in meiosis that could be related to mother's advanced age.<br FAPESP, Brazil.

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P20.53

The genetic basis of TAR syndrome: Compound inheritance of a low-frequency regulatory SNP and a rare null mutation /deletion. A model for microdeletion syndromes.

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Thrombocytopenia -Absent radius syndrome (TAR) is characterised by bilateral radial ray defects, thrombocytopenia and other congenital abnormalities. Previously shown to be associated with a chromosome 1q21.1 deletion, this was also found in 50% of unaffected first degree relatives, suggesting that this was not sufficient alone to cause TAR. Exome sequencing identified a SNP in either the 5'UTR or intron 1 of the RBM8A gene from the deleted TAR 1q21.1 region in the majority of TAR cases. The SNP were inherited from different parents. Sequencing of RBM8A in two TAR cases without the TAR 1q21.1 deletion but with the 5'UTR SNP, identified null mutations. We also showed that TAR 1q21.1 deleted cases ascertained by array CGH with non-TAR phenotypes lack the RBM8A SNP. Genotype-phenotype correlation has been assessed in 60 cases including one sib pair each witha different SNP/deletion combination and marked phenotypic differences
The RBM8A gene encodes Y14 one of the four exon-junction complex (EJC) units which performs essential RNA processing tasks. The SNPs result in reduction of transcription and Y14 expression is reduced in platelets from TAR cases.
We conclude that compound inheritance of a rare null allele and one of two low-frequency non-coding SNPs in RBM8A causes TAR syndrome. This novel bi-allelic inheritance may be a mechanism which explains the variable penetrance and expression in other microdeletion syndromes which currently hinders accurate genetic counselling..
Recurrence risks for TAR affecting siblings and offspring can now be predicted based on family testing for the 1q21.1 deletion and RBM8A SNP.

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P20.54

Towards the identification of novel palindrome-mediated translocations

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Constitutional t(11;22)(q23;q11.2) is the most frequent recurrent non-Robertsonian translocation in humans. The breakpoints on both 11q23 and 22q11.2 are located at the center of palindromic AT-rich repeats (PATRR). To date all the palindrome-mediated translocations involve the PATRR22. To identify novel PATRR on translocation partner chromosome, we analyzed breakpoints of four translocations from patients with multiple congenital anomaly with mental retardation; 45,XX, der(14)t(14;22)(q32.3;q11.2),-22, 45,XY,der(2),t(2;22)(q37.3;q11.2),-22, 45,X,der(X)t(X;22)(p22.1;q11.2),-22, and 47,XX,+der(22)t(15;22)(q26.1;q11.2). All were unbalanced translocations via 3:1 malsegregation in meiosis I. Cytogenetic array revealed that each translocation had a breakpoint at the different location on 22q11. Only the t(14;22) was found to have a 22q11 breakpoint at the same interval with that of the recurrent t(11;22). The breakpoint on chromosome 14 was narrowed down within 7kb region. However, no PATRR-like sequence was identified within the 7kb region according to the reference human genome sequence. However, it is well documented that PATRR is often deleted from the genome database due to unclonability or inability to sequence. Further, PATRR often manifests deletion polymorphism due to the genomic instability. We are now looking for missing gap within the 7kb region to find a novel PATRR that induced the t(14;22) translocation.

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P20.55

Involvement of the same TNFR1 residue in Mendelian and multifactorial inflammatory disorders

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Objectives. TNFRSF1A is involved in a Mendelian autosomal dominant auto-inflammatory disorder called TNFR-associated periodic syndrome (TRAPS). Most TNFRSF1A mutations are missense changes and, apart from those affecting conserved cysteines, their deleterious effect remains often questionable. This is especially true for the frequent R92Q mutation, which might not be responsible for TRAPS per se but represents a susceptibility factor to multifactorial inflammatory disorders. This study investigates TRAPS pathophysiology in a family exceptional by its size (13 members).
Methods. TNFRSF1A screening was performed by PCR-sequencing. Comparison of the 3-dimensional structure and electrostatic properties of wild-type and mutated TNFR1 proteins was performed by in silico homology modeling. TNFR1 expression was assessed by western blotting and ELISA in lysates and supernatants of HEK293T cells transfected with plasmids encoding wild-type and mutated TNFR1.
Results. A TNFRSF1A heterozygous missense mutation, R92W (c.361C>T) perfectly segregated with typical TRAPS manifestations within the family ($p<5.10^{-4}$), and was associated with very high disease penetrance (0.9). Prediction of its impact on protein structure revealed local conformational changes and alterations of electrostatic properties. In addition, R92W leads to abrogation of the receptor shedding, whereas TNFR1-R92Q behaves like the wild-type receptor.
Conclusions. These data demonstrate the pathogenicity of a mutation affecting arginine 92, a residue whose involvement in inflammatory disorders is deeply debated. Combined with previous data on arginine 92 mutations, this study discloses an unusual situation in which different amino acid substitutions at the same position in the protein are associated with a clinical spectrum bridging Mendelian to multifactorial conditions.

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P20.56

Hamartin and Tuberin expression studies: Further insights into *TSC1* and *TSC2* genes in a cohort of patients with well define Tuberous Sclerosis phenotype

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Tuberous sclerosis complex (TSC) represents an autosomal dominant genodermatosis with multisystemic involvement. In 20% of patients with a definite diagnosis, no point mutation, deletion or duplication in TSC1 or TSC2 gene could be identified. No other mechanism or anomaly in another gene has so far been identified as contributing to the cause of TSC. To further evaluate the implications of the TSC1and TSC2 genes, we conducted expression studies among a cohort of 132 index patients referred with definite, possible or probable TSC diagnosis. 25 did not carry a deletion/duplication or a mutation. Among these 25 patients, two were familial. RT-PCR was used to quantify TSC1/TSC2 mRNA (DNA and RNA from blood samples and from 5 normal and/or abnormal fibroblast cultures). Gene expression was assessed after normalization with the β-actin housekeeping gene and with the DU145 cell line as a qPCR calibrator using the 2-ΔΔCt method. Sequencing of TSC1/TSC2 promoters and flanking 5'/3'UTR regions was performed in 8 patients with a definite diagnosis. In one familial presentation, segregation was associated with the TSC2 locus. After validation with healthy controls expression studies identified either elevated or decreased hamartin or tuberin level (in one only or both TSC alleles): this was recorded in all patients with a 'definite diagnosis' and in 50 % with 'possible' or 'probable' diagnosis. Any sequence distortion was found both in promoters and in the 5'/3'UTR regions

of TSC1/TSC2 genes. The present results allow to postulate on additional heterogeneity or epigenetic mechanism as causative for TSC.

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P20.57

Variant ataxia telangiectasia: clinical and molecular findings and evaluation of radiosensitive phenotypes in a patient and relatives

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Variant ataxia telangiectasia (A-T) may be an underdiagnosed entity, as it is associated with milder neurological impairment and fewer systemic symptoms compared to the classic form. We have investigated a patient with generalized mild dystonia, moderately dysarthric speech, increased serum α-fetoprotein but no ataxia nor telangiectasias, no nystagmus or oculomotor dyspraxia. She has a severe IgA deficiency, which is unusual in atypical A-T patients, but does not suffer from recurrent infections or other immune-related diseases.
Molecular analysis showed that the patient is compound heterozygote for *ATM* c.8122G>A (p.Asp2708Asn) and c.8851-1G>T, leading to *in frame* loss of 63 nucleotides at the cDNA level. In the patient ATM protein expression is strongly reduced, but a trace amount of protein is translated from both alleles. Residual kinase activity is derived only from the p.Asp2708Asn allele.
The conventional G0 micronucleus test, based on irradiation of resting lymphocytes, revealed a radiosensitive phenotype for the patient, but not for the heterozygous relatives. As ATM is involved in homologous recombination and in the G2/M cell cycle checkpoint, we developed a novel test, evaluating micronuclei in lymphocytes irradiated in the S and G2 phases. This test showed increased radiosensitivity for both the patient and heterozygous carriers. Intriguingly, heterozygous carriers of c.8851-1G>T (mutation associated with absence of kinase activity) showed a stronger radiosensitive phenotype with this assay than heterozygous carriers of p.Asp2708Asn (mutation associated with residual kinase activity). Our novel S-G2 micronucleus assay provided phenotypic insight to complement the diagnosis of this atypical A-T patient

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P20.58

VariantMaster: a tool to analyze High Throughput Sequences to identify causative variants in family trees, unrelated individuals and matched tumor-to-normal design.

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The availability of high throughput DNA sequencing technologies has significantly advanced the discovery of the sea of genomic variants per individual. Complex pipelines can map billion of sequences up to the identification of single nucleotide variants (SNVs) and insertion and deletions (indels). In parallel, international research projects have resulted in a rich annotation of common and rare genomic variants with population frequencies, damaging potential, and association with genomic features (i.e. transcription factor binding sites, epigenetic and chromatin marks). Cancer genomics too has greatly advanced since somatic variants driving tumor initiation and progression could be identified.
VariantMaster is a novel software that apply several modes of inheritance (dominant, recessive, X-linked) and identify denovo mutations to detect putative causative variants in affected individuals. It can also extract the additional somatic or lost germline variants in tumor cells in order to estimate the recurrence of mutations in unrelated patients. VariantMaster uses as input annotated or unannotated variants, aligned reads (bam files) and/or phased haplotypes to reliably assess the presence of a mutation. Prioritization of variants with respect to allelic frequencies (1000 Genome project, dbSNP and Exome Variant Server) as well as pathogenicity scores and other features is easily customizable by the user. Additionally VariantMaster can evaluate unrelated families with similar phenotype, searching for variants in one or more mutated genes. This unique software has been successfully employed in clinical genetics to identify putative causative variants in trios, recessive diseases in consanguineous couples, X-linked disorders and to identify somatic driver mutations in tumor cells.

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P20.59

Expanding the Wolf-Hirschhorn syndrome critical region to a 1.5 Mb region on 4p16.3: a model for contigous gene deletion syndromes.

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Wolf-Hirschhorn syndrome (WHS) is a contiguous gene syndrome caused by partial 4p deletion, with critically deleted region, WHSCR-2, restricted to an about 400 kb interval on 4p16.3. Great variability of the WHS phenotype exists, however there is a consensus in considering the core phenotype as defined by severe growth delay, intellectual disability, peculiar facial dysmorphisms, and seizures. Pathogenesis of the WHS phenotype was already assumed to be multigenic, with *WHSC1* being the candidate gene for the facial characteristics and growth delay, and *LETM1* as the major gene for seizures.
We report on four ID subjects with atypical 4p16.3 deletions without true clinical evidence of WHS. Deletions were interstitial and encompassed the WHSCR-2 in two. They spanned 1.2 Mb with preservation of the terminal 1.75 Mb region, and 0.81 Mb with preservation of the terminal 1.06 Mb region, respectively. Affected individuals never had seizures, nor had the peculiar WHS facial appearance. The last two subjects, brother and sister, were referred because of epilepsy and learning difficulties. They had a terminal 0.55 Mb deletion preserving the WHSCR-2, inherited from their healthy father, who suffered from febrile seizures and learning difficulties as a child.
Our observations provide evidence that *LETM1* is not the only gene for seizures and *WHSC1* not the only gene for growth delay and facial dysmorphisms. The WHSCR should be rather expanded throughout a 1.5 Mb segment. It is proposed as a model for other contigous gene syndromes, for the benefit of proper genetic diagnosis and search for candidate genes.

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P20.60

Incidence of large VWF gene deletions and duplications in the French cohort of 1182 patients with von Willebrand disease (VWD)

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The French reference center for VWD (CRMW) organizes a national biologic platform for the phenotype/genotype characterization of VWD. After an exhaustive sequencing of VWF gene, at least one deleterious sequence variation was identified in 1153 patients (635 families). However, the direct sequencing failed to detect any causative mutation in 17 index cases (IC). Then we search in these patients a large VWF gene deletion/duplication using multiple ligation-dependent probe amplification (MLPA) (MRC Holland). Nine distinct large gene alterations (6 novel*) were identified at a heterozygous state in 10 IC: deletion spanning exons 1-3, exons 6-18*, exons 19-20*, exons 32-34*, exons 33-34, whole gene deletion; duplication exon 6*, exons 35-37*, exons 38-42*. VWF gene alterations occur in about 2% of our large cohort of patients, and around 5% of patients with type 3 VWD. Hypothesis concerning the deleterious process of these defects may be (1) a frame shift which abolish VWF gene expression (feature of type 3 mutations), (2) the maintenance of the frame: then the truncated or the longest translated VWF affects the structure of the multimeric VWF and provides a dominant negative effect interacting with the normal VWF allele, (3) prevent the correct dimerization or multimerization process and subsequent extracellular secretion of VWF inducing a type 1 VWD. Finally, only 7 patients out of our 1182 patients (~ 0.5%) remain without identified VWF gene abnormality. Our results demonstrate that the screening of large deletions and duplications is an essential way to explain some VWD phenotype.

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P20.61

Gonadal dysgenesis in a women with an unusual t(X;9) and partial trisomy of chromosome 9 centromeric region

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Women with balanced X-autosome translocations constitute a heterogeneous group of patients whose most frequent phenotypic alteration is premature ovarian failure. Since the long arm of X-chromosome is a critical region for ovarian function, it has been postulated that this phenotype might result from epigenetic modifications in the region involved in the translocation, as a consequence of a chromosomal position effect. A woman with gonadal dysgenesis and an apparently balanced translocation of chromosomes X and 9 was studied. The SNP-array analysis did not detect any pathogenic genomic imbalances. Reverse-FISH (Fluorescence in situ hybridization) after microdissection of the derivative chromosomes suggested pericentromeric breakpoints. FISH with whole-chromosome paint and single-sequence probes showed the breakpoints in Xq12 and 9q10. Surprisingly, FISH with centromeric probes resulted in three signals of 9-chromosome centromere: one in the normal 9-chromosome and one in each derivative chromosomes of the translocation, one of them being dicentric. Then, the patient´s katyotype was determined as 46,X,dic(X;9)(XpterXq12::9p11.19qter),der(9)(9pter9q11.1::Xq12Xqter). The SNP-array was unable to detect this trisomy. Immunostaining of centromeric proteins (CENP) B and C in the dicentric chromosome detected signals only in the X-chromosome centromere, suggesting that CENP DNA-biding domain of 9-chromosome could be subjected to an epigenetic modification. These results indicate that the presence of the chromosome 9 centromeric region in both derivative chromosomes could interfere in the expression profile by the spreading of heterochromatic characteristics to Xq, causing the gonadal dysgenesis in this patient. This hypothesis needs to be demonstrated by further investigation.

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P20.62

Fabry disease : skewed X inactivation leads to full-blown expression of the disease and feminine phenotype in a patient with a 16 Mb deletion in a der(X)t(X;Y)(SRY+)

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BACKGROUND: Fabry disease (FD; OMIM #301500) is an X-linked (Xq22.1) inborn error of glycosphingolipid catabolism resulting from deficient lysosomal α-galactosidase A activity due to mutations in the GLA (OMIM #300644) gene, leading to the systemic accumulation of globotriaoslyceramide (Gb3) in lysosomes. Females with FD often develop clinical manifestations. However, the disease generally follows a milder course in heterozygotes. PATIENT AND METHODS: The 27-year-old daughter of a FD male patient presented with full-blown expression of the disease. Alpha-Gal A enzymatic assay, GLA gene sequencing, karyotype, fluorescent in situ hybridization (FISH), comparative genomic hybridization (CGH) and X-chromosome inactivation (XCI) analysis using the androgen receptor (AR) methylation assay were done. RESULTS: Alpha-Gal A activity was totally absent and Gb3 urinary excretion was highly elevated. A missense mutation (c.154T>C, p.C52R) was detected in the GLA gene. Karyotype found 46,X,der(X)t(X;Y)(p22.3?;p11) formula while FISH showed der(X)t(X;Y)(p22.3?;p11).ishYp11.3(SRY+). The t(X;Y) translocation was confirmed by CGH which also identified a 16.6 Mb deletion spanning from Xp22.13 to Xp22.32 in the derivative. Using polymorphic markers at the AR locus, complete skewed X-inactivation of the der(X)t(X;Y)(p22.13;p11.2)(SRY+) was demonstrated. Maternal inheritance of the derivative was confirmed through its identification in the patient's mother. Sequencing of the translocated SRY gene revealed a normal sequence. DISCUSSION: Complete skewed inactivation of a der(X)t(X;Y)(p22.13;p11.2)(SRY+) due to the presence of a large 16.6 Mb deletion led to full-blown phenotype of the paternally inherited FD mutation in trans and to feminine external and internal genitalia despite the presence of a wild-type SRY gene in the patient.

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X-Inactivation studies in females heterozygotes for Fabry disease

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Fabry disease (FD; OMIM 301500) is a lysosomal X-linked disorder caused by the deficiency of the lysosomal α-galactosidase A (α-GalA) enzyme. The deficient enzymatic activity leads to the progressive accumulation of globotriaosylceramide (Gb3) in the plasma and lysosomes of many cell types. Due to X-linked inheritance, heterozygous females had been traditionally described as asymptomatic carriers of the disease. Nevertheless, the phenotype in females ranges from nearly asymptomatic to the classical phenotype observed in males. The phenotypic spectrum expressed in heterozygotes has been attributed to the random inactivation of the X chromosome. However, the contribution of X-inactivation skewing to disease manifestations remains controversial. The aim of this study was to investigate whether correlations between the clinical phenotype and the X-inactivation pattern exist in heterozygous patients in an age-dependent way. In a cohort of 40 consecutive heterozygous females, the diagnosis of Fabry disease was confirmed by GLA sequencing. In all patients, clinical symptoms and phenotype were assessed using exhaustive check-up (renal, cardiac and cerebrovascular) along with two validated global severity scores (Mainz Severity Score Index (MSSI) and DS3). In all patients, methylation assays of the AR gene were carried out in 4 different tissues from different embryonic origins (skin, blood, urinary cells and buccal cells) in order to assess X-inactivation status. Results have shed insight in better understanding the expressivity of the disease in relation with X-inactivation skewing and will help to monitor enzyme replacement therapy in heterozygous female patients.

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Published Abstracts

J01.01

Mechanisms underlying non-recurrent microdeletions causing Incontinentia pigmenti

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Incontinentia Pigmenti (IP, OMIM#308300) is a genomic disorder, X-linked dominant affecting neuroectodermal tissues in females and lethal in males. IP is caused by NEMO/IKBKG gene mutations that result in alterations of NF-kB (Nuclear Factor kappaB) activation pathway. Indeed, NEMO/IKKgamma protein represents the central hub in the regulation of this signaling.

The NEMO gene partially overlaps the G6PD gene on the 5'-side and Low-Copy-Repeat (LCR1) on the 3'-side. This LCR contains an high frequency of repeated sequences, micro/macro-homologies and tandem repeats that increases the genomic instability of the locus by unusual molecular mechanisms (NAHR, NHEJ) generating de novo rearrangements. In the duplicated Low-Copy-Repeat (LCR2), closely linked to gene, lies the non-functional NEMO-pseudogene, involved in rare inversion/gene-conversion events with the LCR1 that cause the repositioning/copying of non-pathologic mutations from pseudogene to gene.

We have recently reported that such genomic instability produces about 80% of IP-causing deletions while de novo point mutations account for only the 12% of IP-causing alterations.

Here, we report a novel rearrangement (del36bp), that alters the open reading frame of NEMO protein synthesis producing a premature stop codon (p.Q145X). The breakpoint sequence analyses suggested that a DNA-replication-based mechanism, Fork-Stalling-and-Template-Switching (FoSTeS), has occurred during parental germline.

Moreover, we show 7 new mutations (2 missense, 3 splicing, 2 small deletion variants) found in IP patients.

Taken together these findings highlight how the vulnerability of the IP-locus produces novel alleles generating new sporadic IP cases. This study is supported by the Association Incontinentia Pigmenti France(http://www.incontinentiapigmenti.fr).

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J01.02

The italian craniosynostosis biobank: a national "hub" for the International Craniosynostosis Consortium (ICC)

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Craniosynostosis (CS), the premature fusion of one or more cranial sutures, is the most common craniofacial malformation (1/2000 live births). Nonsyndromic forms (NCS, i.e., without unrelated, major birth defects or developmental delay) represent 85% cases and are heterogeneous conditions, classified according to the involved suture. Gene mutations have been reported in very few cases, thus the genetic/environmental etiopathogenesis is still largely unclear. The International Craniosynostosis Consortium (ICC) was established in 2000 to organize the efforts of physicians and researchers studying CRS worldwide, in the attempt to better characterize the phenotypes, the genetic background, the environmental factors and the biological pathogenesis of NCS.Our research group based in Rome recently joined the ICC. In 2010 we started collecting calvarial suture specimens and blood. This allowed creating a biological repository that currently includes samples from over 230 CS patients, from the whole italian territory including 195 (84%) NCS cases. For each patient, we extract

genomic DNA from blood, for mutational screening in CS-associated genes, along with total tissue RNA and calvarial cells, for expression profiling, flow citometry and in vitro assays. DNA from parents are being collected to create a significant italian sample of case/parent trios for whole exome sequencing by the

ICC. The goal of this presentation is to show in details our sample database and protocols, and to inform geneticists who are willing to send us specimens to implement the biobank and cooperate in the mission of the ICC.

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J01.03

Genetic variation in toll-like receptor genes and atopic dermatitis in Volga-Ural region of Russia

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Atopic dermatitis is the common chronic inflammatory cutaneous disorder that often precedes asthma and allergic diseases. Number of studies has shown an association between pattern recognition receptor, particularly toll-like receptor genes variations and allergic diseases development. We have screened 62 SNPs in TLR1, TLR2, TLR4, TLR5, TLR6, TLR9, TLR10, NOD1, NOD2, CD14 and LRRC32 genes and C11orf30 gene that was associated with AD in GWAS. The AD group consisted of 335 AD patients from Volga-Ural region of Russia (Russians, Tatars and individuals of mixed origin). The control group included 330 non-atopic individuals of the same ethnic origin. Genomic DNA was isolated by phenol-chloroform extraction. The genotyping of SNPs was performed by OpenArray SNP Genotyping System (Applied Biosystems).

In our study we have found an association of AD development with SNPs in the TLR1 (rs5743604*A, p=0.022 and rs5743571*C, p=0.0004), TLR6 (rs5743794*C, p=0.0115) and TLR10 (rs10004195*T, p=0.0325 and rs4543123*A, p=0.0426) genes map closely together on chromosome 4p14, and with SNPs of the TLR2 gene (rs1816702*C, p=0.0016 and rs4696483*C, p=0.0367). The haplotype analysis revealed statistically significant difference in frequencies of TTATACG haplotype (consists of rs11466617, rs10004195 and rs4543123 of TLR10 gene and rs4833095, rs5743604, rs5743571 and rs2101521 of TLR1 gene) between AD patients and controls (p=0.0124). The C11orf30 gene SNPs (rs2508755 and rs2508747) was also associated with AD development in this investigation (p=0.0022 and p=0.0312).

The results of our research show that TLR1, TLR2, TLR6, TLR10 and C11orf30 genes variants are important risk factors of atopic dermatitis in the Volga-Ural region of Russia.

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J01.04

Genomewide linkage and exome sequencing analyses of a Dowling-Degos disease pedigree

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Reticular pigmented anomaly of the flexures (also known as Dowling-Degos disease) is an autosomal dominant pigmentary disorder. Dowling first delineated this genodermatosis as a distinct entity in 1938. In 1954, Degos and Ossipowski described a patient with a similar case. DDD is slowly progressive, appearing in adolescence or adulthood, affecting the axillae, neck, inframammary and sternal areas, etc. Betz et al, adopting linkage scan followed by fine mapping, discovered that loss-of-function mutations in KRT5 (on chromosome 12q) caused this condition. The advance of next generation sequencing technology makes the pinpointing of causal genes for Mendelian diseases much more effective. Here we investigated the molecular genetic architecture of DDD by genomewide linkage scanning of a Chinese DDD pedigree and target sequencing 50 Mb of exomes for each of the four members from the same pedigree using Illumina GAII platform. The mean coverage depth is 102.2, and on average, 83.4% of targeted bases were covered by >=15x. 47860 high quality SNPs and 5451 short indels were discovered among the 4 persons. Assuming autosomal dominant inheritance mode, we identified four candidate genes that were supported by both the linkage signals and sequence evidence. Further validations are being carried out. These candidate genes, besides KRT5, will shed more light on the pathological mechanisms of DDD.

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J01.05

Molecular Genetic Investigation of a Large Iranian Pedigree Affected with Sever Multiple Synostosis Syndrome2 (Farhud Type)

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Background: Bone Morphogenetic Proteins and the related Growth&Differentiation Factors (GDFs) are much conserved signaling proteins. GDF5 is pivotal for skeletal development. Several skeletal dysplasias and malformation syndromes are known as a result of mutations in GDF5. Multiple Synostosis Syndrome2 (SYNS2) for the first time was identified in a large Iranian family. SYNS2 is characterized by tarsal-carpal coalition, humeroradial synostosis, brachydactyly, and proximal symphalangism.
Method: We studied a large Iranian pedigree affected with SYNS2 (Farhud Type) in five successive generations (58patients,61relatives). Genetic linkage analysis of this pedigree excluded the locus on chromosome 17q21-q22 in our previous study. Thus, we focused on GDF5. Molecular genetics investigation was performed on 25individuals from the last generation that 16 members of them affected with SYNS2. Also, 40healthy Iranian individuals of the age, sex, and origin matched were included.
Results: Direct DNA sequencing results showed no mutation in NOG gene, whereas a heterozygote missense mutationc.1424G>A, S475N was identified in GDF5 gene. This mutation was found in all affected members of the family but not in the unaffected individuals and controls. This substitution has located in highly conserved and active mature domain of the protein and may has unknown interaction with normal active 3rd and 4rd structure of the product. Also the S475 was conserved in all 23 different species (according to multiple alignments).
Conclusion: We concluded that our findings may elucidate some aspects of discausative nature of such a mutation. We propose further etiological considerations for GDF5 as a secondary responsible gene for Multiple Synostosis syndrome2.

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J01.06

Multiple osteochondromas in Russian Federation

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Multiple hereditary exostoses (multiple osteochondromas (MO)) is an autosomal dominant skeletal disease characterized by the presence of multiple benign cartilage-capped tumors that develop mainly from the juxtaepiphyseal regions of long tubular bones. MO is genetically heterogeneous, and is associated with mutations in Exostosin-1 (EXT1) or Exostosin-2 (EXT2) genes. To date, an EXT1 or EXT2 mutation is detected in 70-95% of affected individuals. EXT1 mutations are detected in 65% and EXT2 in 35% mutations in MO patient cohorts. We investigated 17 patients with MO and found mutations in 10 (60%) patients. To detect mutations in EXT1 and EXT2 genes have been used sequence analysis and multiple ligation-dependent probe amplification (MLPA) analysis. EXT1 mutations were detected in 9 (90%) and EXT2 -in 1 (10%) patient. In EXT1 we found three novel mutations: c.771_772insT, c.1296_1301delCAGAAT and deletion of exons 10 and 11. In EXT2 we also found novel mutation c.678C>A (Tyr226Stop). Among our patients we investigated two families in which MO combined with hereditary motor and sensory neuropathy type 1B or primary pulmonary hypertension, confirmed by mutations in MPZ gene and BMPR2 gene.

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J01.07

G1138A mutation in FGFR3 gene in patients with achondroplasia

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Introduction. Achondroplasia is a common form of chondrodyplasia with a frequency of 1/15.000 - 40.000 new borns. It is transmitted by autosomal dominant trait with full penetrance but approximately 75-80% of the cases are caused by de novo mutations. The disease is determined by mutations in receptor-3 gene of the fibroblast growth factor (FGFR3), mapped to band 4p16.3, resulting in decreased endochondral ossficication, inhibited proliferation of chondrocytes in growth plate cartilage and decreased cartilage matrix. The most frequent mutations (up to 99%) are G1138A and G1183C. The aim of this study is to establish the prevalence of G1138A mutation in FGFR3 gene in patients with achondroplasia in our care.
Patients and method. The study group consisted of 24 patients (16 girls and 8 boys), aged between 1 year 8 months - 22 years, who were registered in the Centre of Genetic Diseases of First Pediatric Clinic Cluj in the period 2007-2012. The method consisted in: clinical assessment and radiological examinations (radiograms of the skull, upper and lower limbs, spinal column and pelvis). The DNA analasys was performed by PCR-RFLP technique.
Results. Between 2007 - 2012, 85 patients were diagnosed with bone dysplasia; 24 of them (28,23%) were diagnosed (on clinical and radiological basis) with achondroplasia. Out of this group, 16 patients (66,66%) were identified as heterozygotes for G1138A mutation in FGFR3 gene; this prevalence is very low comparatively with other studies.
Conclusions. It is the first study that reports the prevelance of this mutation in Romania.

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J01.08

Novel *CGI-58* mutation in an Iranian boy with ichthyosiform erythroderma, mental retardation, hearing loss and hepatomegaly

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Neutral lipid storage disease with ichthyosis (Chanarin-Dorfman syndrome) (OMIM 275630) is a rare disorder of lipid metabolism characterized by nonbullous congenital ichthyosiform erythroderma, hepatomegaly and other systematic manifestations that are associated with accumulation of triacylglycerol lipid droplets in many tissues. It has an autosomal recessive pattern of inheritance and is caused by mutation in the *CGI-58* gene (also known as *ABHD-5*) located on chromosome 3p21. We report a five-year-old male Iranian child with intellectual disability, ichthyosiform erythroderma, bilateral hearing loss and hepatomegaly, who was born to first cousin parents. Peripheral blood smear showed lipid vacuoles in his leukocytes. Sequencing of *CGI-58* from the proband revealed a novel homozygous mutation (c.838C>T, p.R280X) in exon 6; his parents were heterozygous for the mutation. This mutation results in truncation and loss of 69 amino acids from the carboxy-terminal region of the protein. Chanarin-Dorfman syndrome should be considered in all patients with mental retardation and ichthyosis. Checking for lipid vacuoles in peripheral leukocytes is an inexpensive and relatively easy method for a presumptive diagnosis of this disease.

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J01.09

A novel mutation in the coding region of OSTM1 gene in lethal infantile osteopetrosis

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Introduction: Osteoporosis is a heritable disorder of the skeleton characterized by increased bone density on radiographs. The autosomal recessive osteopetrosis (ARO) has an incidence of 1 in 250000 births. Osteopetrosis is caused by failure of osteoclast differentiation or function and mutations in at least 10 genes have been identified as causative in humans. In severe neonatal and infantile forms, TCIRG1, CLCN7, OSTM1, RANKL and RANK genes could be considered.
Case report: There were two cases of osteopetrosis in investigated family. This couple had first cousin marriage; and there were 4 similar cases in the their familial pedigree. The first pregnancy lead to a female neonate with birth weight 2800 gr. Clinical manifestations were: poor feeding, debilitation from first month, disturbed swallow from sixth month, urinary infections , hepatomegaly, hydrocephaly and finally death in 1 years old. *Osteosclerosis was seen in radiographic aspects. The second neonate had* almost similar signs and symptoms too.
We analyzed the second infant for promoter, splicing site and coding regions of TCIRG1gene by sequencing method. No mutation was found. The next candidate gene was OSTM1. There was a small two nucleotide deletion (CT)

at c. 231 (ACAAAACT^ 231 GAGTAGT) of exon 6. This mutation was novel.

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J01.10
Molecular-genetic diagnostic thrombocytopenia-absent radius (TAR) syndrome.

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Thrombocytopenia-absent radius (TAR) syndrome is a congenital malformation syndrome characterized by bilateral radial hypoplasia or aplasia with preservation of thumbs and congenital thrombocytopenia. TAR syndrome affected fewer than 1 in 100,000 newborns. TAR - syndrome caused by submicroscopic deletion of 1q21.1 combined with one of two low-frequency SNPs in the regulatory regions of RBM8A gene, encoding the Y14 subunit of EJC.

We have studied three unrelated patients with TAR syndrome and both parents of one dead patient with TAR syndrome. All patients presented with bilateral radial aplasia/hypoplasia with preserved thumbs and thrombocytopenia.

The rs 201779890 was found in homozygous/hemizygous state for two patients with TAR syndrome and father of dead patient with TAR syndrome in heterozygous state, the rs 139428292 was found in homozygous/hemizygous state for one patient with TAR syndrome using Sanger sequencing of the RBM8A gene.

For registry deletion at the RBM8A locus we created the multiplex system for MLPA PCR-analysis. Multiplex probemix contains MLPA probes for exons 1, 4 and 6 of the RBM8A gene and fragments of the TBP, USP3 and SIRT genes, as controls.

Now we carry out deletion in the RBM8A locus for all patients with TAR syndrome and parents of one dead patient with TAR syndrome.

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J01.11
Common polymorphisms in glutathione-S-transferases are not major determinants of psoriasis in the Central-European population

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Introduction: It is generally known that there is large interindividual variability in response to psoralen-ultraviolet A (PUVA) photochemotherapy that is routinely used for treatment of psoriasis. The glutathione S-transferases (GSTs) are detoxifying enzymes capable of inducing cutaneous defence against UV radiation-induced oxidative stress and as such represent elegant candidate biomarkers of UV sensitivity. Human GSTs, e.g. GSTM1, GSTT1 and GSTP1, are known to be polymorphic, and specific variants have been associated with increased UVB erythemal sensitivity and skin cancer risk. The aim of the study was to investigate possible association of common polymorphisms in GSTs (GSTM1, GSTT1 and GSTP1) with psoriasis and its severity.

Materials and methods: We investigated common polymorphisms in GSTs (deletion variants in GSTM1, GSTT1 and an Ile105Val substitution in GSTP1) in a cohort of 692 Central-European patients (402F/297M, 52.47+-15.53y) with psoriasis vulgaris chronica diagnosed according to criteria defined by AAD and compared them with those of a population sample of 749 control non-psoriatic individuals (193M/556F, 47.89+-13.90y). Staging of psoriasis was based on PASI score.

Results: No significant differences of the investigated polymorphisms were observed between the cases and the control cohort, moreover, we observed no associations of the investigated polymorphisms with psoriasis severity, neither in the univariate or multivariate modelling.

Discussion: Based on our observations, the investigated polymorphisms in the GSTs do not serve as major determinants of the psoriasis in the Central-European population.

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J01.12
Association of Catalase gene polymorphism to Vitiligo susceptibility in Gujarat, India Population.

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Vitiligo is a skin condition characterized by development of white patches on the skin resulting from the loss of functional melanocytes and melanin from the skin and mucous. Its incidence ranges from 1 in 100 to 1 in 400 with the onset of disease is usually ~20 years old, and it affect men and women equally. In India; more Gujaraties and Rajastanies are affected (8.8%) than other parts of the country (0.5-2.5%). The etiology of vitiligo remains unknown; both genetic and environmental factors have been identified as potential causative causes.Oxidative stress plays a vital role in etiology of depigmentation in skin by cellular loss, while catalase (CAT) is proven enzymatic defense system, catalyzing break down of hydrogen peroxide. Altered activity of the enzyme and increased stress markers have been reported in vitiligo patients of different geographic locations. We have analyzed the single nucleotide polymorphisms (SNPs) of CAT gene including one in the promoter region rs7943316 (T/C) and the other in the exon 9 rs769217 (A/T) with recognition site of BstXI and Hinf I respectively, in 54 vitiligo patients from Gujarat, India and 45 normal controls from the same geographic region. The genotype distribution and allele frequency of promoter region were not significantly different between vitiligo patients and normal controls. However, exon 9 showed significant correlation between affecteds and normal controls. While the haplotypesof these two polymorphisms also showed association with vitiligo. The present study suggests possible association between the CAT gene and the vitiligo susceptibility.

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J01.13
Congenitally missing upper lateral incisors and their orthodontic approach. Cases Reports

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Hypodontia is defined as the developmental absence of one or more teeth. It is one of the most common dental anomalies in permanent dentition. Heredity is recognized to be one of the possible factors associated with congenitally missing upper incisors. Several studies have shown that MSX1 and PAX9 genes play a role in early teeth development. PAX 9 is a paired domain transcription factor that plays a critical role in odontogenesis. The mutation of the gene MSX1 on chromosome 4p was identified, on a large family with several teeth agenesis.

Hypodontia creates significant challenges to the clinicians in both diagnosis and management. Comprehensive management often requires a multidisciplinary approach.

Three cases-a mother's (42 years) and two sons' (10 years and 8 years), with upper lateral incisor agenesis, are presented. Diagnosis was based upon clinical examination, study model examination, panoramic and retroalveolar radiographs and cephalometric analysis.

Different treatment modalities have been used to treat the malocclusions. Fixed orthodontic therapy was used for space opening and implant supported restoration inserted in the case of the mother. For both sons mesial eruption of the maxillary canines was facilited in order to obtain space closure.

These cases reports illustrate the need for a multidisciplinary team approach, not only at the treatment planning stage, but also throughout the entire course of treatment.

S.I. Pop: None. **B.D. Cristina:** None. **P.V. Radu:** None.

J01.14
Clinical and neuroimaging management of children with achondroplasia

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Aims: Children with achondroplasia may develop a broad panel of symptoms from subtle central apnea, respiratory distress, motor impairment, to quadriplegia and sudden infant death secondary to cervicomedullary

compression.
Methods: We evaluated, 6 children with clinical genetic and radiologic confirmed achondroplasia, for 18 months, quarterly. The average age was 11 months (range 1-36). The follow-up protocol included neuromotor developmental exam - evaluation with Peabody Developmental motor scale (0-6years) 2nd ed. and sleep studies. Imagistic evaluation was made by cranial ultrasound for infants under 18 months. Additionally, all patients (except the newborn) had cranio-cervical magnetic resonance imaging (MRI).
Results: Benign hydrocephalus and moderate ventriculomegaly has been present in all children since the moment of enrolling. The sleep study showed obstructive apnea in 66.7% of the children, showing a negative correlation with the developmental and neurologic status. Cerebral and cervical MRI revealed, in all children, symptoms of spinal cord compression, a hypoplastic cranial base with a narrowing of the foramen magnum, compression on the cervical cord and a combination of abnormal cord signal intensity myelomalacia and trunkal edema. Conclusion: Our study showed that complex evaluation including is needed to define disease status and indicate if more complex neuroimaging is necessary. Cranio-cervical MRI offered mandatory information directing the therapeutic attitude to neurosurgical decompression of the cord.

S. Dumitriu: None. **A. Chirita-Emandi:** None. **C. Haivas:** None. **S. Arghirescu:** None. **C. Gurban:** None. **M. Puiu:** None.

J01.15

A rare skeletal dysplasia Acromesomelic Dysplasia, type Maroteaux

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Acromesomelic Dysplasia, type Maroteaux (AMDM) is characterized by disproportionate shortening of skeletal elements, predominantly affecting the middle segments (forearms and forelegs) and distal segments (hands and feet) of appendicular skeleton. In addition, axial skeletal involvement occurs characterized by wedging of vertebral bodies, with dorsal margins being shorter than the ventral margins. Mode of inheritance of AMDM (MIM 602875) is an autosomal recessive with a prevalence of 1/1,000,000. Acromesomelic dysplasia was mapped on chromosome 9p13-q12 identified mutations in gene NPR2, encoding natriuretic peptide receptor B.
We here report a 4-year-old girl and 16-year-old boy, both products of consanguineous marriages, from different families with classical features such as short stature, disproportionate, acromesomelia, wedging of vertebral bodies, short broad fingers, normal intelligence. Clinical diagnosis of both patients will be confirmed by molecular analysis of the *NPR2.*

E. Koparir: None.

J01.16

Influence of VKORC1 c.-1639G>A, CYP2C9*2 and CYP2C9*3 variant alleles on the acenocoumarol dosage requirements in the group of patients under anticoagulation therapy in Serbian population

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During the last decade genetic factors affecting coumarin therapy have been extensively investigated. Among genetic determinants that have been studied, so far, variant alleles of the *VKORC1* and the *CYP2C9* genes have made the greatest impact on coumarin dose variability. Numerous studies have proved the impact of the *VKORC1* c.-1639G>A, *CYP2C9*2* and *CYP2C9*3* variant alleles on coumarin therapy, and benefits of genotyping before the start of the therapy. The frequency of *VKORC1* c.-1639G>A, *CYP2C9*2* and *CYP2C9*3* alleles differs considerably between ethnic groups and populations - consequently, the benefit of genotyping these alleles is expected to depend on the investigated population.
We set the goal to investigate influence of *VKORC1* c.-1639G>A, *CYP2C9*2* and *CYP2C9*3* variant alleles on the acenocoumarol dosage requirements in a group of 197 patients under stable anticoagulation therapy.
Our results showed that patients with AA genotype required 2.6 times lower dose, than carriers of GG genotype. Relative to the homozygous wild-type genotypes, individuals with *CYP2C9*2* or *CYP2C9*3* variant alleles required significantly lower (25- 59%) doses of acenocoumarol. Simultaneous presence of variant alleles of the both genes, represent a strong predictive factor for extreme response during the initiation of anticoagulation therapy associated with bleeding complications, especially in elderly patients.
In the studied group, 83% patients were carriers of the least one of variant alleles associated with low dose requirements of coumarin derivates, suggesting high percentage of subjects predisposed to low doses requirements of coumarins in our populations.

D. Radojkovic: None. **L. Rakicevic:** None. **J. Kusic-Tisma:** None. **M. Kovac:** None.

J01.17

Atherosclerosis is associated with reduced peroxisome proliferator-activated receptor gamma and liver X receptor beta gene expression

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Peroxisome proliferator-activated receptor (PPAR) gamma and liver X receptors (LXR) alpha/beta regulate genes involved in the control of cholesterol homeostasis and reverse cholesterol transport and are expressed in all major cell types of atherosclerotic lesions. Lipid-loaded macrophages are important during the early development of atherosclerotic plaques. The characteristic component of the atherosclerotic plaque is the differentiation of monocytes to macrophages that accumulate lipoprotein-derived cholesterol to form foam cells within the arterial wall.
Our aim was to study correlation between *PPAR gamma* and *LXR alpha* and *beta* gene expression and its possible contribution to atherosclerotic lesion development.
M-CSF–differentiated human macrophages was used for measure expression *PPAR gamma* and *LXR alpha* and *beta* in a group of patients with angiographically determined atherosclerosis (N=11) and in a control group (N=11).
Macrophage *PPAR gamma* expression was reduced in patients with atherosclerosis comparing with controls 0.27±0.11 and 1.42±0.48 ($p<0.001$), respectively and it was paralleled by a significant decrease in *LXR beta* expression in the same groups 0.38±0.11 and 0.65±0.16 ($p<0.001$), respectively. LXR alpha mRNA levels in macrophages did not differ in studied groups (0.53±0.27 0.79±0.44 ($p=0.17$)).
Our results suggest that the PPAR gamma and LXR beta mRNA levels may serve as a novel molecular target in the treatment of atherosclerosis since it can promote cholesterol efflux from macrophages and prevent foam cell formation.

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J01.18

Factor VIII gene inversions in Serbian patients with Hemophilia A

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Hemophilia A (HA) is an X-linked recessive bleeding disorder caused by heterogeneous mutations in FVIII gene. The recurrent inversions which cause 50% of severe HA cases are inversion of intron 1 (inv1) and intron 22 (inv22) with reported frequency of 2-5% for inv1 and 45% of severe HA patients for inv 22. The goal of the present study was to assess the presence of inv22 and inv1 inversions in the F8 gene in Serbian severe HA patients and to compare these frequencies with published data from other populations. This study describes the first HA mutation series from Serbia. Since May 2009, we started at our Laboratory for Medical Genetics, Mother and Child Health Care Institute of Serbia "Dr Vukan Cupic" (IMD) with analyses for the presence of inv1 and inv22 using Inverse Shifting PCR (IS-PCR). The cohort of 50 patients has been analyzed and inv1 was detected in 6% of patients while inv22 in 42% of them (inv22 type I in 34% of and inv22type II in 8%). These frequencies were not statistically different from other populations. Analyses for carrier status of mentioned inversions were also performed for 65 of family members of HA patients and according to that the *de novo* inversion of intron 22 were found in one family. Genetic counseling unit of IMD provide the adequate genetic advice to all HA affected patients and their family members.

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J01.19

Connective tissue disorders: mutation screening of genes associated with Marfan and marfan-like syndromes in patients from different regions of Russian Federation

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Marfan syndrome (MFS) is an inherited autosomal dominant connective tissue disorder. Abnormalities appear in skeletal, ocular and cardiovascular systems. Marfan-like syndromes (MFLS) combines a group of diseases with symptoms that resembles Marfan syndrome but doesn't quite fulfill all the criteria. The main cause of MFS and MFLS are mutations in the several genes - fibrillin1 gene (FBN1) and the transforming growth factor beta receptor 1 and 2 gene (TGFBR1, TGFBR2) Currently we analyzed some regions of FBN1 and TGFBR2 genes in 106 patients with MS and marfan-like syndromes from different areas of Russia by several methods: SSCP, TTGE, Heteroduplex Analysis on the BioRad DCODE Universal Mutation Detection System and HRM-method on Bio-Rad CFX96 system; and sequencing on MegaBace 1000. Several mutations have been detected in FBN1 gene in patients with Marfan syndrome (G1176Y, C2489Y), which affects cbEGF-like motifs of fibrillin-1 protein in two patients with classical MFS symptoms and mutation c.1455 G>A in patient with MFLS. Also 14 polymorphisms have been found throughout FBN1 gene, which 4 are not described. One novel mutation (670C>T; T223M) has been found in TGFBR2 gene in two unrelated patients with marfan-like syndrome who did not fulfill Ghent nosology and who did not have mutations in FBN1 gene. Mutation T223M affects highly conserved serine/threonine protein kinases catalytic domain that leads to change phospho transferring status of TGFBR2 protein. In addition two novel polymorphisms have been found in intronic regions of TGFBR2 gene.

R. Valiev: None. **R. Khusainova:** None. **R. Valiev:** None. **E. Khusnutdinova:** None.

J01.20

Noonan-like syndrome with loose anagen hair in 2 cases: clinical history and investigation results

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Noonan-like syndrome with loose anagen hair (NS/LAH; OMIM 607721) is the autosomal dominant disorder related to the SHOC2 missense mutation predicting p.Ser2Gly. Phenotype of this syndrome is reminiscent of Noonan syndrome but specific ectodermal features are characteristic. We present two patients with molecularly confirmed NS/LAH diagnosis, and comparison of clinical history since 3to 29 years of their life. Both patients manifested severe growth retardation and short stature, delayed psychomotor development, mitral valve stenosis ASD, feeding difficulties in the infancy, relative macrocephaly, short and webbed neck, pectus excavatum, hoarse voice and Noonan like dysmorphic features (high forehead, hypertelorism, posteriorly rotated ears, palpebral ptosis). Characteristic skin involvement with sparse, thin, very slow growing silver-blond hair, sparse eyebrows, dry skin and dystrophic /thin nails were also observed. Tendency to darkly skin pigmentation was present only in older girl in whom neonatal lymphatic edema, coagulation abnormalities with thrombocytopenia, and respiratory distress following premature birth in 28 week of gestation were documented. Her intellectual development was assessed as a mild degree of impairment but specific attention deficit and hyperactivity disorder was diagnosed. In both patients pathogenic, heterozygous c.4A>G (p.Ser2Gly) mutation in exon 1 of the *SHOC2* (10q25.2) gene was identified by NGS and confirmed by sequencing analysis. Detailed clinical, anthropometric and molecular studies will be discussed with natural history of the disorder in each of the cases.

E. Obersztyn: None.

J01.21

Novel splice site mutation in LEPRE1 gene cause severe recessive OI in patient from Russia

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Osteogenesis imperfecta (OI) is a clinically and genetically heterogeneous disorder characterized by susceptibility to fracture. Most OI cases are inherited in an autosomal-dominant manner and are caused by mutations in COL1A1 and COL1A2 genes, these 2 genes encode for the 2 α chains of collagen type I the predominant protein component of the bone matrix. For OI patients, an autosomal-recessive inheritance is described as including underlying mutations in CRTAP, LERPE1, PPIB, SERPINH1, SERPINF1, FKBP10, SP7 and BMP1 genes. Mutations in COL1A1 or COL1A2 genes result in primary structural or quantitative defects of collagen I molecules, whereas genetic mutations cause for recessive forms, which mainly lead to defects in collagen I modifications. Earlier we analyzed COL1A1 and COL1A2 genes in OI patients from Russia and only in 20% of cases mutations in COL1A1 gene were found.
The aim of our study was to identify mutations in LEPRE1 gene in OI patients from Russian populations.
We investigated 41 patients with OI from 33 families and 50 healthy control individuals. The LEPRE1 gene was sequenced including all coding and flanking intron regions.
Previously unreported splice site mutation c.1724+4G>A in LEPRE1 gene was identified in heterozygous state in one patient with recessive OI type 8. All family members were screened for this mutation by Sau96I restriction digestion. Mutation was confirmed in heterozygous configuration in proband's mother.
The present study revealed one novel mutation in LEPRE1 gene in OI patient from Russia, which hasn't been reported before.

D. Nadyrshina: None. **R. Khusaiova:** None. **E. Khusnutdinova:** None.

J01.22

Interleukin 1 Gene Polymorphisms Positively Influence NFκB Expression In Psoriasis

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Background: Psoriasis is a chronic inflammatory disease which is thought to be driven by an exaggerated production of pro-inflammatory cytokines, such as tumour necrosis factor (TNF-α, Interferon-γ, interleukin IL-1β, IL8, and IL-12. This disorder has a heterogeneous genetic background genetic polymorphisms are believed to be associated with the disease. NFκB regulates the expression of an exceptionally large number of genes and is believed to be involved in the pathogenesis of psoriasis. Methods: 112 cases of psoriatic skin biopsies were studied. Histology was done on the sections and genotyping was done for the IL-1β and IL-1RA genetic polymorphisms. In addition, NFκB immunostain was performed on 89 sections and the intensity of staining was evaluated in the epidermis, basal cells and the lymphocytes. Results: There was a strong association of IL-1β-511C/T polymorphism with psoriasis with both genotypes and alleles. There was a strong correlation between the IL-1β genotype and the grade of NFκB immunostaining (p - 0.012). The grade of lymphocyte staining showed a strong correlation with the IL-1RA genotype (p - 0.025) but not with the IL-1β genotype (p - 0.226). The genetic polymorphisms did not show any correlation with the histological features. Conclusions: Our study suggests that the Interleukin-1 genetic polymorphisms are not critical in the pathogenesis of psoriasis. However, NFκB appears to be activated more in cases that show pro inflammatory genetic polymorphisms. NFκB then influences the severity of psoriasis. The study underlies the central role played by NFκB and interleukin 1 genetic polymorphisms in the pathogenesis of psoriasis.

N. Moorchung: None. **B. Vasudevan:** None. **B.R. Achyut:** None. **B. Mittal:** None.

J01.23

Gene Variants of the Catalase, Gutathione Peroxidase 1, Endothelial Nitric Oxide Synthase in Vitiligo

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Vitiligo is a relatively common skin disease characterized by development of patchy depigmented macules and the cause is still unknown. Previous studies of vitiliginous melanocytes showed that an imbalance between oxidative and antioxidative patterns. For this reason, we aim to investigate the variants of detoxification genes, including catalase (CAT), glutathione peroxidase 1 (GPX 1), endothelial nitric oxide synthase (eNOS) in patients with vitiligo and healty controls groups by PCR and/or PCR-RFLP method. One hundred twenty-five patients with vitiligo and 100 healthy volunteers with no personal and family history of vitiligo were enrolled in the study. There were no significant differences in the two variants of eNOS, between vitiligo patients and healthy controls. TT genotype in CAT A/T promotor region are significantly increased in patients with vitiligo in comparison with controls.

However, there were no significant differences in the variants of CAT T/C codon 389 in exon 9 gen, between vitiligo and controls. TT genotypes in GPX 1 codon 200 are significantly increased in patients with vitiligo in comparison with controls (p=0.027). When all gene variants were analyzed also according to the clinical types and family history of vitiligo there were no significant differences between these parameters. In conclusion, TT genotype of catalase A/T promotor region and GPX 1 codon 200 may be associated with vitiligo susceptibility.

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J01.24
Clinical aspects and treatment options in a case of hemifacial microsomia

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Hemifacial microsomia (HFM) is the most frequently encountered form of isolated facial asymmetry and the second most common facial anomaly. It is a syndrome of the first branchial arch, involving underdevelopment of the temporomandibular joint, mandibular ramus, mastication muscles and the ear. The exact etiology of HFM is not fully understood, but teratogenic and genetic components have been examined by many investigators. However, a few cases have had a positive family history suggesting autosomal dominant, autosomal recessive, or multifactorial etiolgy.
A case of 13 year old patient with right hemifacial microsomia is reported by us. Also, the management steps to improve facial and oclussal aspects are described. The orthodontic diagnostic was class II division 1 malloclusion, with frontal open bite, narrowed maxilla on the involved side, decreased palatal width and unilateral crossbite. Three-dimensional CT reconstruction showed hypoplastic, malformed right mandibular body, underdevelopment of the condyle, incomplete zygomatic arch, small maxilla and squamous temporal bone.
Treatment of the anomaly included orthodontic therapy and facial reconstruction (face contouring plasty with silicon grafts). Esthetic appearance and social integration of the patient were improved.
In our case the financial situation of the patient did not allow other complex reconstructions (ear reconstruction,distraction osteotomy of the mandible,etc).
The treatment of patients with HFM always requires an interdisciplinary approach including at least maxillofacial surgery, plastic surgery and orthodontics. Co-operation not only within the team, but also with the patients and their family is essential in order to achieve the best results.

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J01.25
Polymorphisms P53 gene in children with juvenile idiopathic arthritis.

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Background: reduced capacity (sensitivity) of cells to apoptosis is one the possible mechanisms maintaining synovial inflammation in juvenile idiopathic arthritis (JIA). Polymorphisms *Arg72Pro 4ex*, *ins/del16bp 3in*, *G/C 6in* can determine activity of the *P53* protein - the key protein of intrinsic apoptosis pathway. (P.Dumont et al 2003; Sallivan A. et al, Ghosh A et al 2004)
Objective: we evaluated role of *P53* gene polymorphism in JIA pathogenesis.
Methods: clinical, serological and x-ray manifestations were analyzed in 125 JIA children. 60 healthy children without family history of any autoimmune disease were controls. The *P53* gene polymorphisms was detected by PCR-RFLP.
Results: we haven't revealed significant differences in genotypes distributions of *Arg72Pro ex4*, *ins/del16bp in3* and *G/C in6* between JIA children and controls. We identified significant difference of exon genotype Arg72Pro in girls who still persistently in «active» arthritis more 2 years compared with girls who achived clinical remission (AA - 36,7% и 83,3%, AP – 51,1% и 16,7%, PP – 12,2% и 0%). Boys havn´t any difference of exon polymorphism. We received no information about influence of intronic polymorphisms on the JIA course. Gaplotypes AA*dGG и AAddG* were significantly higher in girls with «inactive» oligoarthicular (OA) and polyarthicular (PA) disease course. Girls who still «active» OA and PA course had gaplotypes AP*dG*, PPi*GC и PPiiCC.
CONCLUSION: presence of AA genotype of Arg72Pro was associated with achieving clinical remission in JIA girls with OA and PA course.

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J01.26
Vitamin D receptor gene polymorphism influences on bone mineralization and metabolism in juvenile idiopathic arthritis.

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Objectives: We evaluated bone mineralization and metabolism changes related to vitamin D receptor (VDR) polymorphic genotypes in children with juvenile idiopathic arthritis (JIA).
Methods: 198 children (82 boys and 116 girls) were included in our study. Bone mineral density (BMD) was measured by lumbar spine DXA. Osteocalcin, CTX, parathyroid hormone, total and ionized calcium, inorganic phosphate, total alkaline phosphatase activity was utilized for assessment of bone metabolism. Molecular testing: TaqI (rs731236) and Cdx2 (rs11568820) polymorphisms of VDR were detected by RFLP.
Results: no differences in TaqI and Cdx2 haplotypes, genotypes and alleles distribution related with normal and low BMD (<-2SD) were found. Girls with TT TaqI VDR, who never been treated by glucocrticoides had lower BMD-Zscore than C allele carriers (TT =-0.94SD [IQR:-2.1;-0.5], TC+CC =-0.62SD [IQR:-1.26;0.39], p=0.03). Girls with Tanner I with TT had higher total and ionized Ca level than carriers of C allele (Ca: TT = 2.43±0.15 mmol/l, TC+CC = 2.28±0.2 mmol/l, p=0.024; Ca2+: TT = 1.15±0.08 mmol/l, TC+CC = 1.06±0.13 mmol/l, p=0.026). Presence of TT genotype negatively correlated with BMD-Zscore (r=-0.28, p=0.04), and positively with frequency of LBMD (r= 0.3, p=0.037).
Boy with GG Cdx2 genotype had lower total Ca (GG = 2.3±0.17 mmol/l, GA+AA = 2.43±0.17 mmol/l, p=0.004) compare with carriers of A allele. Pubertal boys (Tanner IV-V) with GG had higher CTX (GG = 1.75±0.11 ng/ml, GA+AA = 1.06±0.07 ng/ml, p=0.04.
Conclusion: TT genotype of TaqI and GG genotype of Cdx2 could be possible negative factor impact bone mineralization and metabolism in JIA children.

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J01.27
Management and genetic profile in an Apert syndrome case

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Apert syndrome is a type of acrocephalosyndactylia that belongs to the group of craniofacial synostoses.
It is characterized by craniofacial dysmorphism and syndactyly of hands and feet.
We present a six years old boy referred for genetic analysis for Apert syndrome. Family history revealed that he is the first child of a young couple. At birth, he presented the specific signs that emphasized the clinical diagnosis, craniosynostosis and symmetric syndactyly of all digits of the hands and feet. For craniosynostosis, a surgical intervention was performed at the age of one year old, and repeated surgical interventions for syndactyly were performed since. On the examination date, syndactyly was present in the left hand between the fingers II-IV and between the fingers II and III in the right hand. In lower limbs the syndactyly is present in all the fingers where correction was not performed.
A prominent metopic suture, middle facial floor hypoplasia, extended palpebral slants down oriented, less significant exophthalmia, palpebral ptosis, half-open mouth and low inserted ears were observed from a craniofacial point of view. The oral examination emphasized the absence of teeth erupti-

on in the central part of the upper maxillary, with open occlusion.
The genetic evaluation revealed a p.P253R mutation in fibroblast growth factor receptor type 2 consisting in a c.758C>G. This mutation is responsible for 26 % of cases.
According to literature data, the patients with Apert syndrome and p.Pro253Arg mutation have a more improved craniofacial appearance after a craniofacial surgery, fact confirmed also in our case.

A. Belengeanu: None. **M. Stoian:** None. **S. Farcas:** None. **D. Belengeanu:** None. **D. Misceo:** None.

J01.28

Detection and characterisation of Molecular biomarkers associated with low bone mass in Thalassemia major patients.

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Background: Osteoporosis represents an important cause of morbidity in β-thalassemia major (TM) and its pathogenesis has not been completely understood. Genetic factors play an important role in the pathogenesis of osteoporosis and several candidate gene polymorphisms have been implicated in the regulation of this process. A G→T polymorphism in the regulatory region of the collagen type I alpha 1 (COLIAI) gene and Parathyroid hormone (PTH) is well known to play an important role in calcium and bone metabolism.
Aim: To find out the distribution of COLIAI polymorphism and BST B1, RFLP of the PTH gene and its relationship with bone mineral density (BMD) in TM patients.
Material and Methods: G→T polymorphism and BST B1, RFLP of the PTH gene was detected in 150 TM patients and their BMD was measured in by Dual Energy X ray Densitometry (DEXA). Biochemical levels were estimated by ELISA.
Result : A total of 19.8% of the β -thalassemia patients were homozygous for G/G (SS) 35.8% were heterozygous for G/T (Ss) polymorphism and homozygous mutant (ss) 43.4% .There was significant difference between Z-score of patients hip (p=0.047) and at lumbar spine (p=0.001) region. We have observed frequency of each genotype_BB, Bb, and bb_was 43.1 %, 39.7%, and 17.2%, respectively and significant association was found with BMI (P=0.026) , PTH level (P=0.032) ,Vitamin D level (P= 0.02) and Z score at spine (P=0.001).
Conclusion: Our results raise the possibility that genotyping could be of clinical value in identifying chances of low bone density.

K. singh: None. **S. agarwal:** None.

J01.29

Ankyloblepharon-Ectodermal Defects-Cleft Lip/Palate Syndrome due to a new heterozygous missense mutation in the SAM domain of P63

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Ankyloblepharon-ectodermal defects-cleft lip/palate (AEC) syndrome is a rare autosomal dominant disorder with multiple congenital anomalies. Clinical manifestations include ankyloblepharon filiforme adnatum, ectodermal defects (sparse frizzy hair; skin defects; nail alterations; dental changes; decrease in transpiration capacity) and cleft lip/palate. The diagnosis is based on clinical criteria and molecular genetic testing of *P63* gene, the only gene known to be associated with AEC syndrome. All mutations result in an amino acid substitution in the Sterile Alpha Motif (SAM) domain, and are predicted to alter protein-protein interactions. Here, we report a case of Moroccan girl aged 2 years with clinical diagnosis of AEC syndrome. The molecular studies and bioinformatics tools showed a novel heterozygous mutation c.1798G>C (p.Gly600Arg) in *P63* gene. The molecular analysis and the early diagnosis of this syndrome are important to implement appropriate genetic counseling for parents, as well as clinical and dermatological treatment of the patient.

M. Tajir: None. **J. Lyahyai:** None. **S. Guaoua:** None. **M. El alloussi:** None. **A. Sefiani:** None.

J01.30

Mutational analysis of NF1 gene on Greek patients with Neurofibromatosis Type I.

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Neurofibromatosis Type I is a genetic disorder that affects 1 in 3,000 individuals worldwide and is characterized by cafe-au-lait spots, Lisch nodules in the eye, multiple cutaneous neurofibromas and increased susceptibility to the development of benign and malignant tumors. It is inherited in a autosomal dominant fashion and is caused by mutations at NF1 gene located at chromosome 17q11.2. While penetrance is 100%, expressivity of the disease is extremely variable. The gene is large and codes for at least three alternatively spliced transcripts. NF1 pseudogenes also occur in various genomic locations complicating the design of molecular assays for NF1 mutations.
In our study we focused on the elucidation of the genetic causes underlying the disease. A multi step protocol based on genomic DNA has been established for molecular diagnosis in Greek patients fulfilling the diagnostic criteria for Neurofibromatosis Type I. This protocol included multi-step PCR, sequencing of all NF1 gene exons and multiplex ligation-dependent probe amplification (MLPA) for deletions/ duplications.
We found a great number of genetic variants in coding and non-coding regions, the majority already reported in databases. However we have also found novel variants in coding and non-coding regions of NF1, stop codon mutations and variants which may affect the splicing process. All were analyzed with the use of bioinformatic tools. Different mutations were found in patients within the same family, indicating the genetic complexity of the disease. We have also attempted possible phenotype/genotype correlations.

M. Tsipi: None. **M. Tzetis:** None. **S.T. Kitsiou:** None. **K. Kosma:** None. **E. Kanavakis:** None.

J01.31

Novel mutations in FBN1 causing Marfan syndrome in Portuguese patients

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Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder, generally caused by a pathogenic mutation on the *FBN1* gene, encoding fibrillin-1, an extracellular glycoprotein essential for connective tissue structure. Its major manifestations include tall stature, long thin limbs and arachnodactyly, *pectus excavatum/carinatum*, myopia, mitral valve prolapse and regurgitation, and dilation of the aortic root (occasionally leading to dissecting aneurysm and death). Symptoms can appear at any age and vary greatly between patients, even within the same family.
We have screened 6 patients, with a clinical phenotype suggestive of MFS, for mutations in the *FBN1* gene. All exons and flanking intronic regions were amplified by PCR followed by bidirectional sequencing. MLPA was also performed to detect large gene rearrangements.
Mutation screening revealed the presence of three novel mutations in three of our patients: one indel mutation, c.2422delinsCTGTTT, leading to a frameshift and a premature stop codon (p.Ile808LeufsX41); one missense mutation, p.Tyr2149Asn (c.6445T>A); and another missense mutation, p.Met1? (c.3G>A), probably resulting in the loss of the protein start codon. Until now, we did not find any large gene rearrangements.
Our results suggest that *FBN1* mutations may also be the main cause of MFS in Portuguese patients. *FBN1* testing is useful to confirm the clinical diagnosis and to allow proper genetic counselling to patients and families. Regarding the three patients in whom no mutations were found, clinical information must be reviewed, in order to evaluate the need to pursue with mutation screening in other genes.

A.R. Marques: None. **A.F. Brandão:** None. **I. Ayhan:** None. **P. Brandão:** None. **A. Barros:** None. **J. Pinto-Basto:** None. **J. Sequeiros:** None. **I. Alonso:** None.

J01.32

Relationship between vitamin D receptor and collagen type I alpha-1 chain gene polymorphism with bone metabolic markers in children with congenital scoliosis

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Background: genetic polymorphisms of gene which operated in bone remodeling can influence on levels of bone metabolic markers.
Objectives: we evaluated changes of bone metabolic markers in children with congenital scoliosis due to TaqI and -3731A/G vitamin D receptor (VDR); SpI and -1997G/T collagen type I alpha-1 chain (COL1A1) gene polymorphic genotypes.
Materials and methods: 154 children (63 boys and 91 girls) with congenital scoliosis aged 6 moths to 17 years were included. Molecular testing: detecting TaqI and -3731A/G VDR; SpI and -1997G/T COL1A1 polymorphism were performed by polymerase chain reaction with restriction fragment length polymorphism. We evaluated serum biomarkers of bone formation: osteocalcin, amino pro-peptide of type I collagen (P1NP), serum biomarkers of bone resorption - C-terminal telopeptides (CTT), and hydroxyvitamin D 25(OH)D3.
Results: in children with TaqI VDR polymorphic genotypes we have revealed differences in osteocalcin levels (p=0.006) and levels of 25(OH)D3 (p=0.003). We detected increased osteocalcin levels (p=0.012) in carriers of GG genotype -1997G/T COL1A1 compare with T allele carriers (TT+GT). No differences in markers of bone turnover related to -3731A/G VDR and SpI COL1A1 polymorphic genotypes were found.
Conclusions: we found differences in levels of bone metabolic markers, associated with polymorphic VDR and COL1A1genotypes.

I. Kazarian: None. **M. Kostik:** None. **S. Vissarionov:** None. **A. Voitovich:** None. **M. Mnuskina:** None. **I. Nichunaeva:** None. **V. Larionova:** None.

J01.33
Marfan syndrome combined with balanced translocation

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Our case is a family, where Marfan syndrome was diagnosed during genetic counseling in pregnancy. Marfan syndrome is a systemic disease of connective tissue characterized by a variable combination of cardiovascular, musculo-skeletal, ophthalmic and pulmonary manifestations. The incidence of Marfan syndrome is approximately 1 in 5,000 worldwide and there is no difference between men and women. It is associated with autosomal dominant inheritance - child has 50% risk of inheriting the disorder.
We saw our family because of genetic and drug risk in pregnancy for the first time. The father had clinical signs of Marfan syndrome.The father´s height was 208cm, he had arachnodactyly, he was observed because of mitral valve prolapse, an ectopia lentis, an abnormal joint flexibility, a high palate and he had osteoporosis. The risk for the children was 50%.
In the father we found normal karyotype 46,XY. In the mother we found balanced translocation 46,XX,t(3;10). Molecular genetic testing of Marfan syndrome started in the father. First tests did not find any causal mutation in the TGFBR1,2, FBN1genes by the single strand conformation polymorphism and multiplex ligation-dependent probe amplification. Examination was supplemented by next generation sequencing and mutation in the FBN1, c.5578T>C(p.Cys1860Arg) was found. Daughter of the pair was four years old and had some features of Marfan syndrome - arachnodactyly, high palate, mitral valve prolapse, ascending aorta dilatation. Molecular genetic testing was performed and the same mutation as in the father was found and balanced translocation 46,XX,t(3;10) inherited from the mother was found simultaneously.

A. Klímová: None. **M. Soukupova:** None. **J. Lastuvkova:** None. **P. Cibulkova:** None. **M. Wilimska:** None.

J01.34
Comparative study of allele frequencies of MTHFR, F2 and F5 gene polymorphisms in children with primary systemic vasculitis, vasculitis associated with autoimmune diseases and in healthy children.

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Objectives: Comparative analysis of alleles and genotypes distribution of methylenetetrahydrofolate reductase (MTHFR) 677C/T polymorphism, prothrombin (F2) A20210 G/A polymorphism and blood coagulation factor V (F5) 1691G/A polymorphism in children with primary systemic vasculitis (1), vasculitis associated with autoimmune diseases (2) and healthy children (controls).
Patients and methods. 1st- group consisted of 27 patients (8 boys and 19 girls), 2nd- group - 34 patients (13 boys and 21 girls). Control group - 31 apparently healthy children (20 boys and 11 girls). MTHFR 677C/T, F2 and F5 1691G/A polymorphisms were analysed by real-time PCR using microchips containing lyophilized PCR mixture method. Statistical significance of differences between groups was assessed using $\chi 2$ tests.
Results. There was no difference($p>0.05$) in allele and genotype distribution of analyzed polymorphisms between groups. The genotype distribution was in accordance with Hardy-Weinberg equilibrium for all variants. Genotype F5 1691GA was found in 3,6% (n=1) in 1st group and in 2,9% (n=1) in 2nd group.
Conclusion. Despite the lack of statistically significant differences in the groups studied polymorphisms, we should continue to study more patients.

N. Shirinbekova: None. **M. Nikitin:** None. **G. Novik:** None. **M. Slyadnev:** None. **V. Larionava:** None.

J01.35
Prothrombotic Gene Polymorphisms in Young Patients with Cerebrovascular Accident

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Cerebrovascular diseases which are affected by genetic and environmental factors are complex multifactorial disorders. Their incidence increases with age and these are the most frequently encountered ones among neurological diseases. The objective of this study was to investigate thrombotic gene polymorphisms that may take place in formation of cerebrovascular diseases in young adults. In our study in 43 patients with CVA (cerebrovascular accident) below age of 51, thrombotic gene polymorphisms were evaluated. Anamnesis of the patients (including CVA story and risk factors in the family) were taken. DNA was obtained from peripheral blood samples and the related gene regions were amplified by multiplex PCR method. Of the patient samples, twenty were studied by CVD strip assay, five were studied by FV-PTH-MTHFR strip assay, eighteen were studied by real time PCR method and the common polymorphisms were evaluated. Twenty six of 43 cases with CVA were women (mean age:35.12±9.2) and 17 were men (mean age:34.56±10.07). Most of the etiologic reasons were idiopathic and the most important ones were smoking and hypertension. MTHFR C677T polymorphism heterozygous frequency was 46.1%, homozygous frequency was 9.3 %; MTHFR A1298C polymorphism heterozygous frequency was 39.47%, homozygous frequency was 26.31%; prothrombin polymorphism heterozygous and homozygous frequency was 2.3%; Factor V Leiden polymorphism heterozygous frequency was 9.3% in our patient group. It is thought that MTHFR gene C677T and A1298C polymorphisms might be risk facors in CVAs. We observed that together with these polymorphisms smoking hypertension and existence of family story increased the available risk.

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J01.36
Polymorphism gene angiotensinogen M235T with clinical, structural and functional indexes of the heart in patients with chronic heart failure (CHF)

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Purpose: To detect the association of polymorphism gene AGT with clinical features, structural and functional indexes of the heart in patients with CHF of ischemic etiology. **Methods:** 105 men with left ventricle ejection fraction ≤45 % (Simpson). Mean age 59,4±8 y.o **Results:** 25 patients were homozygous for the M-allele, 60 had the MT polymorphism and 20 patients were homozygous for the T-allele. All patients did not differ by CHF f.c. The incidence of MI in group of patients with T-allele had significantly earlier than in group of patients with M-allele (58.0±9.0 vs 51.9±9.1 y.o correspondently;

p<0,05). 18% of patients with T-allele had history of stroke and group of patients with MM-genotype didn't have stroke at all. Patients with M-allele had more significantly increased left ventricular end-diastolic and end-systolic diameters (LVDD/LVSD), significantly elevated pulmonary artery pressure (PAP) than patients with TT-genotype (LVDD/LVSD 59.1 ±10.0/60.4±10,0 mm vs 54.7±10.1/55.7±10.0 mm correspondently; p=0.03; PAP 41.6 ±18.1 vs 28.3 ±11.0 mmHg correspondently; p=0.02). Group with M-allele gene AGT had 12% events of paroxysmal atrial fibrillation and group with TT-genotype had no paroxysms. There was no difference in prevalence and onset of hypertension, presence of hypercholesterolemia and obesity. **Conclusion:** There was association of the TT-genotype with earlier cases of myocardial infarction and significantly more events of stroke than in patients with M-allele. Patients with ischemic CHF and M-allele had significantly higher sizes of left ventricle, degree of pulmonary hypertension in spite of equal duration of CHF and f.c. of CHF.

O. Krasnova: None. **V. Larionova:** None. **M. Sitniciva:** None. **B. Smirnov:** None.

J01.37
Clinical study of C677T polymorphisms of methylentetrahydrofolatreductase gene in children with Juvenile Idiopatic Arthritis

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The children with juvenile idiopatic arthritis (JIA) have an increase risk of early atherosclerosis development under the influence of systemic inflammation, endothelial dysfunction and homocisteine. Low activity of methylentetrahydrofolatreductase (MTHFR) can cause the elevation of homocysteine level. MTHFR activity changes due to mononucleotide polymorphisms (SNP) of its gene.
The aim of study was the determination of SNP of MTHFR.
Materials: 13 children with polyarthritis, 11 oligoarthritis, 3 systemic JRA were examined. High activity was observed in 4, moderate in 9, low in 14 patients. χ2-criterion was used for comparison and odds ratio was calculated.
Results: polymorphic variant C677C MTHFR was observed in 32%, C677T in 68% patients, T677T was absent.
Allele C677T was determined in11 children with polyarthritis (85.7%) (p = 0.0322; OR = 7.2, 95% CI:1.07- 48.64). In children with oligoarthritis with almost equal frequency C667T and C677C were observed. Only C677T genotype was observed in all cases of systemic form of JIA. It was determined in all causes of III degree and in 75% cases of II degree activity of JIA.
In our study observed protective influence of C677C polymorphism on development of polyarthritis (OR= 0.14, 95% CI: 0.02-0.94) and 7 time higher risk of polyarthritis in cases of C677T genotype.
Didn`t found statistical correlation between SNP and homocisteine level. Its level in children with C677T genotype was 13 ± 4.3 mmol/l vs 12.01 ± 3.62 mmol/l in C667Cgenotype.
Conclusion: determination of C677T polymorphisms of MTHFR is important genetic test for early diagnostic process in children with JIA.

I. Romankevych: None.

J01.38
Mutation in ST6GALNAC5 identified in family with coronary artery disease

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Coronary artery disease (CAD) is a leading cause of death worldwide and atherosclerosis which results in decreased supply of blood to the heart is its major cause. Myocardial infarction is the most severe outcome of CAD. Despite extensive efforts, the genetics of CAD is poorly understood. We aimed to identify the genetic cause of CAD in a pedigree containing several affected individuals. Genetic analysis including linkage analysis and exome sequencing was performed on the pedigree. Functional analysis was done to access the effect of the identified causative mutation on protein localization and enzyme activity. The identified gene was screened in 100 additional unrelated patients. A missense mutation in ST6GALNAC5 that encodes Sialyltransferase 7e was identified as the cause of CAD in the pedigree. Changed intracellular localization of the mutated enzyme was not observed, however the mutation was shown to cause increased sialyltransferase activity. Another mutation in ST6GALNAC5 was found in two unrelated CAD patients while screening only 100 additional cases. In conclusion mutations in ST6GALNAC5 can cause CAD. Mutations in the gene may contribute to CAD in not an insignificant fraction of patients. Although sialyltransferase and sialic acid levels are not among the commonly recognized risk factors for CAD, there is substantial literature that suggests a relation between these molecules and coronary disease. Our findings provide strong evidence for the existence of this relation. There are implications of our findings for diagnosis and prevention of CAD.

K. Inanloo Rahatloo: None. **A.F. Zand Parsa:** None. **K. Huse:** None. **P. Rasooli:** None. **S. Davaran:** None. **M. Platzer:** None. **M. Kramer:** None. **J. Fan:** None. **E. Elahi:** None.

J01.39
Identification of FBN1 gene mutation in Ukrainian patients with Marfan syndrome

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Background. Marfan syndrome (MFS) is an autosomal dominant disorder of the connective tissue characterized by early development of thoracic aortic aneurysms/dissections together with symptoms of the ocular and skeletal systems. The main pathogenesis of MFS is currently thought to be driven by mechanisms due to haploinsufficiency of wild-type fibrillin-1
Purpose. To identify a mutation in the fibrillin-1 (FBN1) gene in Ukrainian patients with Marfan syndrome.
Methods. We examined 36 patients (24 male and 12 female) with MFS after aortic valve-replacing root operations. We have used an Haloplex (Agilent) assay for enrichment of 12 TAA genes and sequenced the samples with next generation sequencing on a Miseq (Illumina). After that we have confirmed the putative mutations with Sanger sequencing on an ABI sequencer (Life Technologies).
Results. We detected FBN1 mutation in 24 patients (66.7%). Patients with FBN1 mutation had heavier clinical indicators than patients without mutations. The average age in patients with FBN1 mutation during the surgery was 35.2±11.8 year, and in patients without FBN1 mutation - 47.4±13.3 year. The average Z-score in patients with FBN1 mutation was 8.2±2.9, and in patients without FBN1 mutation - 7.4±4.6. Ectopia lentis was present in 6 MFS patients with FBN1 mutation and has not been in any patient without FBN1 mutation.
Conclusions. This is a first report of FBN1 mutation in Ukrainian MFS patients. We found that patients with FBN1 have younger age at diagnosis and a higher probability of developing ectopia lentis, ascending aortic dilatation, aortic surgery, mitral valve abnormalities, scoliosis.

R. Zhuraev: None.

J02.01
Ofd1 controls dorso-ventral patterning and axoneme elongation during embryonic brain development

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Oral-facial-digital type I syndrome (OFDI) is a human X-linked dominant-male-lethal developmental disorder caused by mutations in the OFD1 gene. Similar to other inherited disorders associated to ciliary dysfunction OFD type I patients display neurological abnormalities. We characterized the neuronal phenotype that results from Ofd1 inactivation in early phases of mouse embryonic development and at post-natal stages. We determined that Ofd1 plays a crucial role in forebrain development, and in particular, in the control of dorso-ventral patterning and early corticogenesis. We observed abnormal activation of Sonic hedgehog (Shh), a major pathway modulating brain development. Ultrastructural studies demonstrated that early Ofd1 inactivation results in the absence of ciliary axonemes despite the presence of mature basal bodies that are correctly orientated and docked. Ofd1 inducible-mediated inactivation at birth does not affect ciliogenesis in the cortex, suggesting a developmental stage-dependent role for a basal body protein in ciliogenesis. Moreover, we showed defects in cytoskeletal

organization and apical-basal polarity in Ofd1 mutant embryos, most likely due to lack of ciliary axonemes. Thus, the present study identifies Ofd1 as a developmental disease gene that is critical for forebrain development and ciliogenesis in embryonic life, and indicates that Ofd1 functions after docking and before elaboration of the axoneme in vivo.

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J02.02

Genomic Variation in Congenital Heart Disease

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Congenital heart disease (CHD) constitutes by far the major cause of infant morbidity and mortality. Most CHD cases present concomitant non-cardiac anomalies such as facial dysmorphisms and/or developmental delay. And most of patients with conotruncal defects carry a hemizygous microdeletion of chromosome 22q11 justifying the use of cytogenomic analysis. In this sense, we prospectively studied a cohort of 107 patients with CHD with or without dysmorphic features or additional congenital abnormalities. We use distinct MLPA (Holland) kits (P064, P036, P070, P250, P356, P095, P029) and arrays (HumanCytoSNP-12, Illumina). Unexpected our analysis identified duplications (8,4%) as well as deletions (17,8%) associated with 22q11.2 region, and smaller atypical CNVs (19,6%) that implicated IL17-RA, CECR1, PEX26, PRODH, CLDN5, SHANK3, ACR deletions. We also found genomic changes involving 1p36, 4p16.3, 4q35.2, 5q35.3, 7p21.3, 7q36.3, 8p23.3, 9q34, 10p12.3, 11q13, 11q24, 15q11.2, 16p13.3, 17p11, 19p13.3 and 20q13.3 regions. All of these alterations were already reported in patients with multiple congenital anomalies suggesting that these CNVs are pathogenic and consistent with a model where imbalance of multiple genes associated with contributes to congenital heart disease.

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J02.03

A rare case of a complete 9p trisomy without autistic features and resulting from a de novo unbalanced t(9;15) translocation

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Trisomy 9p is one of the most frequent autosomal anomalies compatible with long survival rate. The phenotype of the 9 p duplication syndrome is clinically recognizable, and phenotype/genotype correlations suggested that the critical region is located on 9p22. We report on a rare case of a trisomy for the whole short arm of chromosome 9 resulting from a de novo unbalanced t(9;15) translocation. A 3-year-old girl was referred to genetic counselling because of facial dysmorphism, seizures, and moderate mental retardation. MRI (Magnetic resonance imaging) was interpreted as normal. Chromosome analysis revealed a 46,XX,der(15)add(15)(p11) karyotype. Parental karyotypes were normal. Thus, we conclude that this chromosomal rearrangement occurred de novo. In order to identify this additional material on chromosome 15, we performed array CGH (Nimbelgen Whole genome tiling135K HG18) that showed a duplication of the whole short arm of chromosome 9. No other imbalances were found elsewhere in the genome. FISH studies performed with whole chromosome 15 and chromosome 9 painting probes confirmed that the chromosomal material added on chromosome 15 derived from 9p. Our observation is unique because the duplication of the whole short arm of chromosome 9 without monosomy of another chromosome and resulting from a de novo unbalanced translocation was never reported. Our patient had the most common clinical findings of the trisomy 9p syndrome but neither visceral anomalies, nor autistic features. Our report doesn't strengthen the hypothesis that a critical region for autism or autism spectrum disorders (ASD) may be located on the area of 9p23-24.3.

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J02.04

Novel mutations involved in Bardet-Biedl syndrome

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Bardet-Biedl syndrome (BBS, #209900) is a rare, human genetic disorder with an estimated prevalence in Europe of 1:160 000 live births. This syndrome shows early-onset retinitis pigmentosa, obesity, polydactyly, hypogonadism, intellectual disabilities and renal abnormalities as primary features. BBS is inherited in an autosomic recessive manner, although some authors suggest that triallelic inheritance could explain some family pedigrees. To date 17 BBS genes (BBS1-17) are involved in the development of BBS phenotype. Pathogenic mutations in these genes are responsible for 70-80% of the cases. Therefore, strong efforts are needed to find new genes and mutations related with BBS.
The purpose of this work was to analyse two BBS genes with a high prevalence, BBS1 and BBS12, to identify pathogenic mutations.
We chose patients with a negative result for BBS/AS Asper Ophthalmics genotyping microarray (Asper Biotech), among a set of 90 Spanish, Indian and Arab affected families. We studied the coding region of BBS1 and BBS12 genes, 27 and 39 patients respectively, by direct sequencing and analysis with specific software.
We found two pathogenic mutations in coding region of 10 patients, confirming their clinical diagnosis. Four of these pathogenic changes are novel mutations: c.68G>A / p.W23X (BBS1) in an Indian patient and c.1510_1520delCACCTGCAGAA / p.H504fsX552 (BBS1), c.1082delG / p.G361fsX382 (BBS12) and c.1140delA / p.T380fsX382 (BBS12) in Spanish families.
Classical molecular approach by direct sequencing remains a good strategy to discover new pathogenic changes in clinically diagnosed BBS patients.

S. Castro-Sánchez: None. **M. Álvarez-Satta:** None. **D. Valverde:** None.

J02.05

Phenotype and genotype of Polish patients with Mowat-Wilson syndrome.

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Introduction:
Mowat-Wilson syndrome (MWS, OMIM #235730) is a rare congenital disorder characterized by distinctive facial features, intellectual disability and characteristic congenital anomalies. Estimated birth incidence is 50000-100000 life births. The only known causes of MWS are mutations in ZFHX1B gene or rarely micoredeletions in region 2q22-23.
The aim of the work was to evaluate the phenotype of patients with MWS confirmed by molecular analysis of ZFHX1B gene and to assess the differences between them and in whom any genetic alterations were detected.
Material and Methods:
Clinical diagnosis of MWS based on distinctive facial dysmorphy, developmental delay or intellectual disability and at least one congenital malformation were established in 10 patients. Patients were not consanguineous. They came from various regions of Poland. All patients were performed cytogenetic and subtelomeric MLPA test, which did not reveal any abnormalities. Molecular tests of ZFHX1B gene were performed including direct bidirectional sequencing of ZFHX1B gene and MLPA reaction with P169 KIT by MRC Holland.
Results:
Diagnosis of Mowat-Wilson syndrome was confirmed in five cases - two females and three males. Patients has typical facial dysmorphy with uplifted earlobes with a central depression visible irrespective of patient's age. Among congenital malformations the most common were heart defects, agenesis of corpus callosum and kidney defects. Psychomotor or intellectual development were severely retarded in all cases. Especially verbal language abilities were impaired.
Conclusion:
Mowat-Wilson syndrome is still underestimated entity. The further examinations and analysis are needed to study the phenotype of patients with MWS and establish clinical criteria.

A. Jakubiak: None. **K. Szczaluba:** None. **M. Kugaudo:** None. **J. Pilch:** None. **A. Rauch:** None. **R. Smigiel:** None.

J02.06

Position effect leading to haploinsufficiency in a ring chromosome 14 in a boy with mental retardation, epilepsy and cryptorchidism

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Ring chromosome 14 is a rare cytogenetic disorder, associated with mental retardation, epilepsy, retinal anomalies, and cranio-facial dysmorphisms. Most of these features are also found in patients with linear terminal deletions of chromosome 14, except for seizures and retinal abnormalities. We report a patient with cryptorchidism and a ring chromosome 14 with a small terminal deletion that was characterized by array-CGH. The patient was referred to genetic counseling at 04 years because of facial dysmorphism, microcephaly, hypotonia, drug-resistant epilepsy, and cryptorchidism. MRI noted cortical atrophy, while ophthalmologic examination detected an abnormal macula. Chromosome analysis showed a ring chromosome 14 with a 46,XY,r(14) karyotype. Parental karyotypes were normal and confirmed that the structural abnormality occured de novo. FISH studies with 14 q telomeric probe demonstrated that subtelomeric region was not deleted in the ring chromosome 14. Array-CGH performed with Nimbelgen Whole genome tiling135K, showed a small terminal deletion with a size of 1.03 Mb within the 14q32.33 region, which was confirmed by FISH analysis using a BAC clone : RP11-5F6. Analysis of informative microsatellite markers on chromosome 14 showed the absence of uniparental disomy (UPD). Our report is very intersting because genital anomalies such as cryptorchidism was never reported in ring 14 patients. We could also hypothesize that a candidate gene called ADAM6 located nearly from the breakpoint on 14q, and expressed in testis, could explain cryptorchidism in our patient. Therefore, we suggest that position effects need to be taken into account, when analyzing genotype-phenotype correlations based on chromosomal imbalances.

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J02.07

Cytogenetic microarray in characterization of unexplained mental retardation or developmental delay with dysmorphism

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Mental retardation (MR) is a common problem encountered in clinical practice. Down syndrome and Fragile X syndrome are two common genetic causes. In some cases, metabolic disorders are detected especially mucopolysaccharidosis. Syndrome diagnosis in MR cases is facilitated by use of databases. In some cases, even after detailed evaluation, the underlying cause is not identified. We did chromosomal microarray analysis (CMA) in seven cases of MR with dysmorphism attending the Genetic Clinic of a tertiary care centre, after a detailed initial evaluation, including biochemical and radiological tests. An underlying chromosomal defect was identified in at least 3 cases. One child showed deletion of 9.4 megabases at cytogenetic band 7p21p15.3, including the TWIST gene. Another child showed abnormal marker on routine karyotyping and the origin of the marker was ascertained on microarray analysis. One boy showed duplication of 2p25.3. Two others showed duplication of chromosomal regions with doubtful clinical significance. Copy neutral LOH on chromosome 15q was found in one case. Prenatal diagnosis was done in one family using karyotyping, CMA and MLPA analysis, and the fetus was found to be normal. Thus, CMA can have a high yield if done in carefully selected cases of MR with dysmorphism, after initial screening tests. Limitations of CMA analysis include inability to detect balanced chromosomal rearrangements, inversions, balanced insertions, and some cases of polyploidy and mosaicism.

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J02.08

15 Mb tandem microduplication in chromosome 11q22.3-q24.1 associated with developmental delay, dysmorphic features and overweight. Further characterization of duplication distal 11q region.

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Isolated duplications of the distal 11q region are rare with only a few cases reported to date in the literature. All patients presented with intellectual impairment and dysmorphic features. Hypotonia, short stature and different congenital anomalies were also described.
Here we report the molecular karyotyping and phenotypic description of a new patient with a 15.1 Mb de novo tandem microduplication of 11q22.3-q24.1 region. Our patient presents with moderate developmental delay, severe verbal impairment, dysmorphic features, cryptorchidism and overweight.
Our observation contributes to the further characterization and dissection of a distal region 11q duplication. The possible role of genes within the duplicated region is discussed. Some genes located in the critical region, e.g. SORL1 and ASAM, may play an essential role in development of intellectual impairment and obesity, respectively. A comparison with previous case reports is provided.
Description of additional patients in the future will help to refine detailed genotype-phenotype correlation associated with this chromosomal region.
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J02.09

16p12.1 microdeletion and developmental delay: a case report

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We describe the case of a boy that was born to non consanguineous parents at 38 weeks of gestation by cesarean section performed for podalic presentation. Reported poor fetal movements. Birth weight was 2.900 kg. Perinatal history was normal. Phenotypic examination: failure to thrive, dystrophic appearance, axial hypotonia, muscolar hypotrophia, body asymmetry, microcephaly, hypotelorism, micrognatia, pectus carenatum, joint stiffness, hypertonia with difficulty to the extension of the lower limbs, kyphosis. Array-CGH showed a microdeletion in the 16p12.1 region of paternal origin, extending aproximately 651 kb. Also the father showed poor growth in infancy and psychomotor delay (walking and first words at the age of 2 years) but with actual performance within normal limits.
Specific human chromosomes are enriched for interspersed segmental duplications and multiple genomic disorders have already been assigned to these "hotspot" regions of the genome. The short arm of human chromosome 16 is enriched for large segmental duplications that have arisen specifically during human-great ape evolution. The 16p12.1 microdeletion is a risk factor for intellectual disability and developmental delay, and also acts in concert with other large copy-number variants (CNVs) to modify neuropsychiatric phenotypes, thereby supporting a "two-hit" model for the generation of severe cognitive deficits involving this region. Analysis of other microdeletions suggests that this model may help to explain the variability in expressivity of recurrent CNVs associated with neuropsychiatric phenotypes. However, variable phenotypes were associated with the 16p12.1 microdeletion, such as congenital heart defects, seizures and severe growth abnormalities.

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J02.10

A case of early diagnosis of 1p36 deletion in a girl with multiple congenital abnormalities including pulmonary lobulation defect, tetralogy Fallot, and early onset of focal epilepsy

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We present a case of early diagnosis of 1p36 in a child with multiple congenital abnormalities including tetralogy of Fallot, pulmonary lobulation anomaly, and focal epilepsy. A 4-month-old girl was the product of the first pregnancy of healthy non-consanguineous parents. Prenatal ultrasound examination revealed tetralogy of Fallot and intrauterine growth retardation.

Delivery was complicated by long first period with intrauterine hypoxia. Apgar score was 5/6. There was a significant muscular hypotonia with inability to suck. In the second month of life, focal seizures of right arm or right side of the body began. Craniofacial features included microbrachycephaly with wide forehead and frontal bossing, mild bitemporal narrowing, hypoplasia of supraorbital ridges, absence of eyebrows, deep-set eyes with short palpebral fissures, wide and plant nasal root, long and fine formed philtrum, Pierre Robin sequence. Thin upper lip covered lower lip. The patient was also characterized by short neck and limbs. Karyotyping at 450 band level did not reveal any chromosome anomalies. Aminoacid, organic acid, and acylcarnitine spectrum was normal. Deletion in the 1p36.33 region was detected by SALSA MLPA kit P290. Further examination revealed pulmonary lobulation defect with two lobes in the right lung. There were hypertrophic pyloric stenosis and hypoplasia of left kidney. In the fourth month of life microphthalmia was diagnosed. Absence of eyebrows with dysplastic but long supraorbital ridges, relative or real microphthalmia, Pierre Robin anomaly were noted in publications on early diagnosis of 1p36 monosomy. We suggest these findings may be helpful for early diagnostics of 1p36 syndrome.

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J02.11

Haploinsufficiency of genes involved in the synaptic pathway and located on chromosome 5q33q34 leads to pharmaco-resistant epilepsy, ataxia, and severe mental retardation.

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Deletions of the long arm of chromosome 5 could vary considerably, and involvement of all bands between 5q11 and 5q35 have been described (Royer-Pokora et al, 2006; Schoch et al, 2005). Constitutional interstitial deletions of 5q are less common, and very few cases were described in the literature. The deleted segments vary in region and size, making it difficult to delineate a typical syndrome. To our knowledge, very few cases of *de novo* 5q33q34 deletions were previously cytogenetically described (Giltay et al, 1997; Spranger et al, 2000). Due to the large number of genes involved in these deletions, and the lack of precisely defined boundaries, genotype-phenotype correlations are not easy to evidence. We describe here a young female patient involving a proximal 5q deletion, mapping to region 5q33.3-q34. The deletion appeared *de novo*. Our patient shows the following features: microcephaly, strabism, psychomotor development retardation, stereotypes, and hypotony. A pharmaco-resistant epilepsy appeared at the age of 18 months. She then displayed great ataxia when walking, and, at the age of fourteen, she was severely mentally delayed. We depict a distinct phenotype associated to this region, and delineate a minimal critical region involved in pharmaco-resistant epilepsy, ataxia and mental retardation. We further highlight the involvement of the deleted genes in the synaptic transmission network.

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J02.12

Analysis of deletion size in regions 7q11.23 and 22q11.21 and their correlation to the clinical phenotype in patients with Williams-Beuren (WBS) and Di George (DGS) syndromes

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Background: WBS is caused by hemizygous deletion (1.5-1.8 Mb) in 7q11.23. Molecular defects in DGS include 1.5-3 Mb deletion in 22q11.2. Both syndromes are relatively common in clinical practice and show wide clinical variability.
Aim: The aim of this study was to clarify differences in phenotype of patients with WBS and DGS according to affected chromosome regions.
Materials and Methods: DNAs from 150 patients, with mental retardation and/or multiple congenital abnormalities were examined. Initially patients were tested by MLPA using P245-A2 kit. Deletions/duplications have been confirmed by MLPA P064- B2 kit. ArrayCGH was used to determine breakpoints and the size of deletions. We paid special attention to patients with atypical clinical presentation and typical deletion size.
Results: We detected 7q11.23 deletion (1.413 Mb, 1.434 Mb and 1.918Mb) in three patients with clinical picture, relevant to WBS. The severe phenotype found in patients with DGS is result of haploinsufficiency of more genes than WBS. Five patients have 22q11.21 deletion (2.023 Mb, 2.438 Mb, 2.471Mb, 2.471 Mb, 2.522 Mb). The complexity of the clinical picture is likely to depend not only on the deletion size, but also to be a result of influence of molecular changes outside the causing deletion.
Conclusion: The application of high resolution molecular tests could provide more detailed insight into the relationships between the deletion size and clinical phenotype. This is important for making correct genetic diagnosis, genetic counseling and management of the affected individuals, but also from the point of view of molecular pathogenesis of the disease.

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J02.13

Hypomandibular faciocranial dysostosis: Two new cases in the same family without mutations in PRRX1, PRRX2 and OTX2 genes

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Hypomandibular faciocranial dysostosis syndrome, HFD (OMIM #241310), is a very rare condition with typical craniosynostosis, prominent eyes, deficient midface and zygomatic arches, short nose with anteverted nares, protruding lower face, minute oral aperture, persistent buccopharyngeal membrane, severe mandibular hypoplasia, and various extracephalic anomalies. The most typical features are abnormalities in the first branchial arch and derivatives.
Case report: we present genetic study of two 20 week old foetus with HFD syndrome in two consecutive and spontaneous miscarriages in a Spanish woman. Both parents were young and healthy, non-consanguineous. These two foetus were males with multiple abnormalities: malar hypoplasia, prominent eyes, short and flat nose, microstomy, protruding lips, tongue present and a skull with normal structure. In the first foetus we could appreciate in the intestinal cavity a diaphragmatic hernia, intestinal hypoplasia, duodenal stenosis, intestinal malrotation, absence of duodenum and anus atresia. In the second foetus we could appreciate esophageal atresia, intestinal hypoplasia, duodenal atresia and intestinal malrotation. Both foetus present normal karyotypes 46, XY. Mutations of PRRX1, PRRX2 and OTX2 genes have been recently associated with abnormal development of mandible, so we have performed genetic studies of DNA from foetal tissues. We screened for mutations the whole coding region of the three genes, including intron-exon junctions. First, amplification of all exons was performed followed by Sanger sequencing using ABI3100XL but we did not identify mutations in these genes. Probably another genes would be implicated in the HFD syndrome. Intestinal malformations have not been described in HFD syndrome previously.

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J02.14

Allgrove (triple A) syndrome with *AAAS* common Slavic mutation in Russian families

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Allgrove (triple A) syndrome is a rare autosomal recessive disorder caused by mutations of *AAAS* gene. Along with "triple A" (alacrima, achalasia of cardia and addisonism) numerous neurological disorders which may appear much later are typical. We diagnosed Allgrove syndrome in three non-consanguineous Russian families. 1. A 47-year-old female had congenital asymptomatic alacrima and achalasia. After esophagocardiomyotomy in childhood she was free of symptoms up to 4th-5th decades, when dysphonia, dysphagia, ataxia, tremor, general weakness and weight loss developed; initial diagnosis was motor neuron disease. On examination bulbospinal amyotrophy, polyneuropathy with foot deformity, ataxia, autonomic disorders and mild adrenal insufficiency were found. Case is familial: patient's brother had achalasia and died in 6 yrs of unrecognized severe adrenal insufficiency which developed acutely after esophagal surgery. 2. A 17-year-old male had moderate dysphagia since 7-8 yrs and slowly progressing leg spasticity since 9-10 yrs, initial diagnosis was Strumpell's disease. On examination in 15 yrs spastic paraparesis, axonal polyneuropathy with foot deformity and severe adrenal insufficiency were found. Adrenomyeloneuropathy was excluded, and signs of achalasia were ignored. We suspected Allgrove syndrome 1.5 yrs later, and only then achalasia and alacrima were recognized. 3. A 5-year-old boy had alacrima and achalasia. In all patients

homozygosity for most common in other Slavic populations *AAAS* mutation c.787T>C (p.Ser263Pro) was detected. Ours are first findings of the mutation in Russians, an only previously reported Russian family had compound heterozygosity for novel mutations. Delayed diagnosis in cases 1 and 2 points to Allgrove syndrome underestimation in practice.

G.E. Rudenskaya: None. **E.Y. Zakharova:** None.

J02.15
Thoracic syrinx and ventriculomegaly in a girl with alpha-thalassemia mental retardation syndrome, chromosome 16 related

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We describe a 5-year-old girl with dysmorphic features, developmental delay, alpha-thalassemia trait and hypotonia who, at 3 years old, was discovered to have ventriculomegaly and a thoracic syrinx on MRI. A genomic microarray identified a 1.819 Mb isolated monosomy of 16p13.3. Fluorescence in situ hybridization of the proband and her parents confirmed that this deletion was de novo. Alpha-thalassemia mental retardation chromosome 16 related syndrome (ATR-16) is a contiguous gene syndrome due to the deletion of α-1, α-2 and a variable number of surrounding genes. A literature review revealed only 23 reported cases of pure monosomy of 16p13.3. Although several of these cases have described neurologic abnormalities, such as hypotonia, impaired coordination or seizures, only two patients have reported radiologic imaging which were both abnormal: one patient had asymmetry of the periventricular white matter volume with thinning of the corpus callosum on CT, while the other patient had an arachnoidal cyst in the right temporal lobe on MRI. Syrinx are rare with an estimated 8.4 new cases of syrinx per year per 100 000 people. Our patient is the first reported case of a syrinx in a child with ATR-16 syndrome. It remains unclear whether a thoracic syrinx is a more frequent finding in ATR-16 than in the general population. However, based on the clinical presentation of our patient, we advocate for early imaging of the entire spine in children with ATR-16 and focal neurologic symptoms.

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J02.16
Anal Atresia, Coloboma, Microphthalmia, and Nasal Skin Tag in a Female Patient with 3.5 Mb Deletion of 3q26 encompassing SOX2

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A full term female newborn presented with prominent forehead, bilateral microphthalmia, iris coloboma and cataract, wide intercanthal distance, large, low set and protruding ears, skin tag at the left nasal nostril, imperforate anus with rectovestibular fistula, and postnatal growth delay with brachymicrocephaly. A marker chromosome was not detectable and the copy number of 22q11 was normal. However, array CGH revealed a 3.5 Mb microdeletion of chromosome region 3q26.32-3q26.33 (chr.3:178,598,162-182,114,483; hg19) which comprised the SOX2 gene. While SOX2 haploinsufficiency is known to cause microphthalmia and coloboma, it has not been described before in patients with anal atresia. Possible relations to the Notch and sonic hedgehog pathways will be evaluated.

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J02.17
CNV analysis in monozygotic twin pairs discordant for uro-rectal malformations

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Monozygotic (MZ) twins are expected to be identical for their genome and phenotype. A large number of reports on discordant MZ twin pairs suggest that this phenomenon might be explained by very early post-twinning mutational events. Uro-rectal malformations, especially anorectal malformations (ARM) and the bladder-exstrophy-epispadias complex (BEEC) occur mostly sporadic and are associated with reduced reproduction. We hypothesized that early post-twinning CNVs might contribute to the discordance in MZ twin pairs with ARM or the BEEC and this may help in identifying further chromosomal regions involved in the development of these malformations.
We investigated four discordant MZ twin pairs (3 ARM, 1 BEEC) using Illuminas' HumanOmni1-Quad Chip. CNVs were predicted using the program QuantiSNP (v2.2) that used an Objective-Bayes Hidden-Marcov model for the estimation. In order to identify possible disease causing de novo CNVs we searched for CNVs present (i) only in the affected twin but not in the unaffected twin or the parents and (ii) in both twins but not in their unaffected parents (resembling incomplete penetrance). The remaining CNVs from each patient were also filtered against an internal control cohort as well as publicly available databases.
After filtering no potentially causative CNV remained. Our results suggest that post-twinning CNV events in the human coding regions have not contributed to the discordant phenotypes in the investigated MZ twin pairs. Possible causes for the discordant phenotypes include changes in regulatory elements or smaller genetic changes within coding regions, which may be detectable with whole genome sequencing.

M. Draaken: None. **F. Baudisch:** None. **E. Bartels:** None. **E. Schmiedeke:** None. **S. Bagci:** None. **P. Bartmann:** None. **M.M. Nöthen:** None. **M. Ludwig:** None. **H. Reutter:** None.

J02.18
A sporadic Tunisian Apert syndrome case resulting of Pro253Arg mutation in fibroblast growth factor receptor 2

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Unregulated fibroblast growth factor 2 (FGF2) signaling caused by mutations in the fibroblast growth factor receptor (FGFR2) leads to human craniosynostosis such as the Apert syndrome.
We report on a sporadic Tunisian Apert syndrome case for who the genetic evaluation revealed heterozygous C-G transversion in exon 7 of FGFR2 gene [dbSNP:rs77543610], resulting in a pro253-to-arg (P253R) gain-of-function substitution within a highly conserved linker region between the second and third extracellular immunoglobulin domains of the protein.
A 6-month-old girl, born of healthy distant consanginous parents, was referred for our genetic consultation because of severe cranial and limbs skull malformations, facial dysmorphic features and congenital heart defect (atrial septal defect). She died few months before diagnosis confirmation of Apert syndrome which was made in the basis of dysmorphic evaluation. In fact, the patient presented cranial skull malformations with brachycephaly, high and prominent forehead and a flat posterior cranium resulting from premature fusion or synostisis of bilateral coronal sutures demonstrated by radiography. Moreover, she had short neck, a concave face due to midfacial hypoplasia, short nose, low set ears and bulging and broadly spaced eyes. She had particularly the uncommon severe form of limbs abnormalities (rosebud hands and feet) with bilateral complex syndactly of hands and feet.
Molecular investigation of hot spot mutations by direct sequencing and detection of P253R mutation was helpful in confirmation of the diagnosis. Additionally, syndactyly in both the hands and the feet which was reported as more severe in patients with the P253R mutation was elucidated.

R. Louati: None. **N.B. Abdelmoula:** None. **W. Ben Romdhane:** None. **M. Zenker:** None. **T. Rebai:** None.

J02.19
Acute lymphocytic leukaemia in a child with Beckwith-Wiedemann syndrome

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Beckwith-Wiedemann syndrome (BWS) is a rare overgrowth syndrome associated with cancer predisposition. BWS is phenotypically variable, but it is usually recognisable at birth based on clinical features. It may include macrosomia, macroglossia, abdominal wall defects and visceromegaly. Between 2% and 30% of BWS patients (depending of the molecular anomaly identified) develop cancer, most commonly of embryologic origin.There has been three reports of BWS patients who develop acute leukemia, but little information about the molecular analysis was available for most of the patients. Herein we report the case of a 5-year-old female patient diagnosed with BWS who developed a B-type acute lymphoblastic leukaemia (ALL).

She is the first child of non consanguineous parents diagnosed with BWS during the neonatal period, after an unenventful pregnancy. Triad of macrosomia, posterior helical ear pits and macroglossia led to the diagnosis of BWS. Follow-up of this patient was carried out as recommended. At 5, she felt fatigue and suffered from shortness of breath. Upon physical examination she exhibited pale skin and hepatosplenomegaly. Laboratory studies were performed and the diagnosis of ALL was made. Cytogenetic analysis of peripheral blood was previously normal and further molecular testing to identify epigenetic and genomic alterations of chromosome 11p15 is being performed. Different genetic disorders, such as trisomy 21 and ataxia-telangiectasia, have been associated with ALL. Further investigation is necessary, but it is conceivable that a causal relationship exists between BWS and ALL.

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J02.20

First familial case of inherited 2q37.3 deletion with isolated skeletal abnormalities including brachydactyly type E and short stature.

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Albright hereditary osteodystrophy-like syndrome is also known as Brachydactyly-mental retardation syndrome (BDMR) (OMIM 60040). This complex disorder includes hypotonia, intellectual deficiency, seizures, sleep disturbances, obesity, short stature, craniofacial abnormalities and skeletal abnormalities including brachydactyly type E in approximately half of cases. Patients with 2q37 microdeletions or HDAC4 mutations are defined as having an AHO-like phenotype with normal stimulatory G (Gs) function. We report on the first family case of 2q37.3 deletion with brachydactyly E and short stature without intellectual deficiency. The proband is a 11 year-old female, the first child of healthy unrelated parents. She first consults at age of 8 for bilateral shortening of the IVth, Vth metacarpals and of the III, IV, V metatarsals. She had mild dysmorphic features, a normal psychomotor development. At the age of 11, she measures 131 cm (-1,8DS). Her father has similar dysmorphic features. He has normal intellect. His skeletal manifestations comprised bilateral shortening of the IV, Vth metacarpals and bilateral shortening of the III, IV, V metatarsals and relative short stature. The sisters, mother and grand-mother of the father have a short stature and brachymetarcarpy /brachymetatarsy. DNA microarray analysis revealed a small deletion at 2q37.3 inherited from proband's father. This deletion includes HDAC4, interrupted in intron 2 and TWIST2, MGC16025 included in HDAC4, FLJ43879 and the 3' telomeric part of LOC151171. To conclude, we suggest that haploinsufficiency of HDAC4 causes a variety of phenotypes going from isolated brachydactylie E to brachydactyly-mental retardation syndrome.

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J02.21

Abnormal skull shape, bowing of the femora and tetralogy of Fallot as prenatal presentation of Carpenter syndrome.

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Carpenter syndrome, also known as 'acrocephalopolysyndactyly type II', is a rare autosomal recessive disorder characterized by multiple sutures craniosynostosis, preaxial polydactyly of the feet, post-axial polydactyly of the hands and soft-tissue syndactylies. Six different mutations in the *RAB23* gene have been identified in 21 patients from 16 independent families until now.

We report here the first prenatal description of Carpenter syndrome with a *RAB23* mutation. It was the fifth pregnancy of healthy consanguineous Turkish parents. Cystic hygroma and bowed femora were seen on ultrasound scan since 14-15 weeks of gestation. In addition, at 22 weeks a complex heart defect (double outlet right ventricle of Fallot type) was detected. At 28 weeks abnormal skull shape with irregular outlines and too easily visible brain structures, associated with bowing of the femora raised the question of osteogenesis imperfecta. This was reinforced by the fetal bone CT scan which showed the same skeletal anomalies and suggested a depressed fracture of the skull. However, physical examination and skeletal X-rays of the newborn patient lead to the diagnosis of Carpenter syndrome. Direct sequencing of the *RAB23* gene identified a novel homozygous frameshift mutation c.481G>C (p.Val161Leu).

This observation illustrates the difficulty of prenatal diagnosis of Carpenter syndrome. Tetralogy of Fallot and bowing of the femora are rare postnatal findings which were reported each in only one patient among the 21 cases of Carpenter syndrome with *RAB23* mutations. Carpenter syndrome should therefore be considered in case of abnormal skull shape with bowed femora, and cardiac defect.

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J02.22

Autosomal structural changes detected after birth,Genetic Center experience

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Introduction

Chromosome abnormalities account for 50% of all spontaneous miscarriages and are present in 0,5-1% of all newborn infants. On an individual basis, most of these are very rare, but together they make a major contribution to human morbidity and mortality.

Material and Methods

Were cytogenetically investigated a total of 927 patients between the years 2010 - 2012 using G banding technique for identifying and characterizing the chromosomes of lymphocytes.

Results

We identified a total of 121 chromosomal aberrations (13%), of which 83 were number abnormalities (8.9%) and 38 were structural abnormalities (4.1%). Of the 38 structural abnormalities, 28 were autosomal chromosomal abnormalities. Changes in chromosome structure encountered were: different translocations-11 cases, deletions-7 cases, inversions-5 cases, 3 cases chromosomal polymorphisms and 2 cases of marker chromosomes.

Conclusions

Detection of structural chromosomal abnormalities is a key element for both correct etiologic diagnosis and genetic counseling. The results are comparable to those of similar studies.

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J02.23

CNVs and complex rearrangements: Investigation in patients with congenital anomalies and development delay

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Introduction: The genomic imbalances are the most common cause of miscarriage, congenital anomalies and developmental delay, however the etiology of these imbalances are not well understood, making difficult the counseling genetics and the treatment. In recent years the improvement of cytogenomics diagnostic techniques, such as the MLPA (Multiplex Ligation-dependent Probe Amplification) and the screening by arrays showed that changes in gene copy numbers (CNVs) influence the pathogenic variability of phenotypes in different syndromes. Patients and methods: Thus, the present studied the presence of CNVs in 278 patients with congenital anomalies and developmental delay, by MLPA technique using specific kits for microdeletions syndromes (P064) and subtelomeric regions (P036 e P070). Results: We identified changes in the gene copy numbers (CNVs) in 89/ 278 patients (32%), 49 patients (17.6%) by P064 kit and 40 patients (14.4%) by P036 and P070 kits. Complex rearrangements were observed in seven patients, including three patients with normal gene copy numbers interspaced with genomic duplications and/or deletions, confirmed by oligo-array technique (CytoScan™ Affymetrix®). Conclusion: Although array is the first-tier cytogenomic test for detection of CNVs, the MLPA technique can be used like an alternative screening test, mainly in the cases with multiple congenital anomalies and developmental delay. In addition, the recognition that the

human genome contains a large number of CNVs associated with the origin of chromosomal rearrangements improved our understanding of genetic variation and the genotype-phenotype correlation.

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J02.24

A coalescence of two syndromes in a girl with a terminal deletion and inverted duplication of chromosome 5

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Rearrangements involving chromosome 5p often result in two syndromes, Cri-du-chat (CdCS) and trisomy 5p, caused by a deletion and duplication, respectively. A cat-like cry is considered to be a hallmark observed in CdC patients. The 5p15.2 has been define as critical region of CdCS, however genotype-phenotype studies allowed isolation of particular characteristics such as speech delay, cat-like cry or mental retardation, caused by distinct deletions of 5p. A varius clinical outcome was also observed in patient with trisomy 5p. A duplication of 5p10-p13.1 manifest in more severe phenotype, while trisomy of regions distal to 5p13 mainly causes mild and indistinct features. Combination of terminal deletion and inverted duplication of 5p are infrequent in literature. Consequences of these chromosomal rearrangements differ, depending on size of deletion and duplication in particular cases, although authors mainly describe deletion as causative for clinical picture observed. Here we present a newborn girl with de novo terminal deletion and inverted duplication of chromosome 5p. Our patient presents a cat-like cry, characteristic of CdCS, but features like dolichocephaly, macrocephaly and ear malformations, observed in trisomy 5p, are also present. We assume that deletion will probably have minor impact in clinical outcome, involving just a region responsible for speech delay as described in CdCS. A duplication on contrary will probably result in more severe phenotype, especially considering that some characteristics are still not fully revealed. Interestingly, a cat-like cry was noted in our patient, despite the fact that deletion is not fully consistent with previously defined critical region.

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J02.25

Contribution of 7q33 deletion in a portuguese family with intellectual disability and multiple dysmorphic features

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Intellectual disability (ID) is one of the most frequent/ disabling neurological impairments in adolescents. aCGH in routine diagnostic may reveal undetermined genomic imbalances and ID associated genes.
A maternally inherited 7q33 deletion is reported (19-year-old male, mild ID, IQ= 53). Clinical evaluation: short stature, dysmorphisms (frontal cowlick-two whirlwind at forehead, spiky hair, bulbous/snub nose, downslanting palpebral fissures, epicanthic folds, deep-set eyes, bushy eyebrows, thin upper lip, poor dental implantation, narrow cleft palate, dysplastic ears and prognatism, aggressiveness and hyperactivity. His sister (IQ=62, short stature, facial dysmorphisms) has the same alteration. Their mother has mild MR and similar dysmorphisms.
The deletion was determined by aCGH analysis (human genome CGH Agilent 180K custom array, mean resolution of 17Kb) (Agilent, Santa Clara-CA). Confirmation studies and inheritance analysis were done by qPCR (fragment designed inside altered region).
aCGH revealed a 2Mb deletion (15 genes: *AGBL3, AKR1B1, AKR1B10, AKR1B15, BPGM, C7orf49, CALD1, CNOT4, EXOC4, LRGUK, NUP205, SLC35B4, STRA8, TMEM140, WDR91*) in 7q33. One of the most interesting deleted genes is the *CNOT4* (CCR4-NOT transcription factor complex, subunit 4) gene: *CNOT4* protein plays both positive and negative roles in transcriptional regulation and positive role in transcriptional elongation. In yeast, the ortholog of *CNOT4* (Not4) regulates the expression of Jhd2 (the yeast ortholog of *JARID1C*, for which mutations have been described in a X-linked ID patient). aCGH was essential for detecting this imbalance and this family's diagnosis. Comparison with similar cases, expression and functional studies may help us clarify the relevance of the deleted genes for ID.

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J02.26

Down Syndrome and the spectrum of associated malformations

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Aim: To present the spectrum of associated malformations in Down syndrome patients from our clinic, the largest in the west part of Romania.
Material and methods: Between 2010-2012, a number of 29 patients confirmed with Down Syndrome, were admitted in our clinic. The age was between 1 mo and 15 yo. Sex ratio: 52% female, 48% male. 68 % were from urban area and 32% from rural. All of them performed clinical examination, lab tests, ECG, Echocardiography, abdominal ultrasound and selective cardiopulmonary X ray and angio CT.
Results: Congenital heart defect(CHD) was present in 75% of patients. Only 5 cases presented cyanotic CHD: 75% Fallot Tetralogy, 25% double outlet right ventricle. 24 cases presented noncyantic CHD: 39% atrial septal defect(ASD), 33% complex heart malformations, 22% ventricular septal defect(VSD), 6% complete atrioventricular canal defect. Two patients with heart defect associated renal malformations: unilateral hydronephrosis and ureteral hypoplasia or stenosis, severe in one case, due to gr. III/IV of hydronephrosis. The patient with severe hydronephrosis had to perform kidney surgery, prior to heart intervention. One case associated to heart malformation, congenital ectropion and cataract.
Conclusions:
In our group, ASD was dominant. Lower than in literature was VSD and atrioventricular canal defect. Severe renal malformation was discovered late in life and was a priority to surgery. Not all parents with Down Syndrome children are directed to a pediatric screening in the first year of life. To screen for malformations of all Down syndrome patients is mandatory at discharge from the newborn unit.

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J02.27

Down syndrome with Type 2 Abernethy malformation, porto-systemic shunt and hepato-pulmonary hypertension

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Introduction: The type II Abernethy malformation is an extremely rare abnormality of the splahnic venous system, where portal venous blood is diverted away from the liver through latero-lateral shunts. Associations between Abernethey malformation and portal aneurysm are rare; few cases have been described in the literature until now.
Material and Methods: To report a one month-old male, diagnosed with Down syndrome, who was admitted in our clinic for complex investigations, to detect associated pathology.
Results: Atrial septal defect (ASD), with platypnea and increased vascular markings on X ray were found. The ASD was too small to explain the increased pulmonary circulation. No pulmonary artery hypertension was found. Pulmonary artery and inferior vena cava were dilated. Additional investigations were performed: abdominal ultrasound and angio CT scan. Type II Abernethey malformation and portal vein aneurysm, with porto-systemic shunt were the diagnose that stated to produce hepato-pulmonary hypertension. The O2 Sat vary from orto to clino position.
Conclusions: It is the first case described in the literature of Down syndrome with congenital heart defect (ASD) associated with type 2 Abernethy malformation and portal aneurysm with porto-systemic shunt. Our patient is at high risk of developing hepato-pulmonary syndrome due to angiogenic factors from spleen, that bypass the liver, hepatic encephalopathy and long term metabolic complications due to liver dysfunction. He was addressed to the hepatic surgery department, to decide the closure of the porto-systemic shunt or to wait for hepatic transplant.

R. Stroescu: None. **T. Bizerea:** None. **O. Marginean:** None. **G. Doros:** None.

J02.28

Ellis-Van Creveld syndrome with preauricular sinus: report of two Turkish female patients

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Ellis-Van Creveld(EVC) Syndrome(OMIM 225500) is a rare chondro-ectodermal dysplasia characterized by short ribs, polydactyly, growth retardation, ectodermal and heart defects. The birth prevalence in non-Amish population is estimated as 7/1,000,000. The phenotype is variable and is inherited in an autosomal recessive pattern with parental consanguinity has been reported in about 30% of cases. Mutations in EVC and EVC2 genes, located in a head-to-head configuration on chromosome 4p16, have been associated with this syndrome. We report two Turkish female patients with EVC syndrome born to consanguineous parents.

Patient 1: A 8-year-old female patient presented with short stature, short limbs, sparse hair, blue sclera, broad base to nose, simple philtrum, high palate, hypodontia, prominent and simple ears, pectus carinatum, increased lumbar lordosis, sacral dimple, lateral deviation of the toes, dysplastic nails. She has undergone surgical correction of postaxial polydactyly of all four limbs.

Patient 2: A 2-year-old female patient presented with short stature, short limbs, prominent occiput, sparse hair, epicanthus, depressed nasal bridge, high palate, short and deep philtrum, hypodontia, preauricular sinus and skin tag behind the right ear, deep plantar creases, hemangioma located on neck(2x0.5cm), dysplastic nails. She had a history of neonatal teeth and surgical correction of bilateral postaxial polydactyly of hands.

In conclusion, we present the first report, to our knowledge, of EVC syndrome with preauricular sinus in the literature and also discuss the variable expression, management and genetic counseling.

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J02.29

Interstitial dup(6)(q22.3q24) characterized by cCGH resulting from familial inv ins(6)(p11.2q25.3q22.3): case report

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We report on a female child aged 2 months, referred for complex cardiac anomalies and Down-*like* facial dysmorphisms, strongly suggesting a possible chromosomal abnormality. Chromosomal studies with high-resolution GTG banding showed an abnormal chromosome 6. The parents were investigated and their karyotypes revealed a normal chromosomal constitution in the father and an inv ins(6)(p11.2q25.3q22.3) in the mother; the latter was also present in the maternal grandmother, allowing us to conclude that the child's abnormality was a recombinant of the apparently balanced familial inverted insertion. Comparative genomic hybridization (cCGH) techniques proved that the abnormal chromosome 6 found in the proband has a duplication of the segment 6q22.3→6q24, with an extension of 24 Mb, resulting in the partial trisomy of that segment. The final karyotype of the child was thus: 46,XX,rec(6)dup(6q)inv ins(6)(p11.2q25.3q22.3)mat.ish cgh dup(6)(q22.3q24).

The patient's follow-up studies were only possible until she was 5 months and her main clinical features included dysmorphic facial features with plagiocephaly, membranous auricular and ventricular septal defects and developmental delay.

This is the first presentation of a "pure" interstitial duplication of bands 6q22.3 to 6q24. The authors enhance the importance of an adequate use of molecular cytogenetic techniques such as the use of oligonucleotide-based array-CGH in a clinical diagnostic laboratory for detecting subtle chromosome imbalances and accurately define the breakpoints in patients with atypical phenotypic characteristics, while assuring a good cost-efficiency diagnostic rate. The cytogenetic and clinical findings in this newly reported duplication are also compared with previously published similar data.

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J02.30

A Novel *MEK1* Mutation in a Patient with LEOPARD Syndrome

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LEOPARD syndrome (LS) is characterized by lentigines, ECG conduction abnormalities, ocular hypertelorism, pulmonary stenosis, abnormal genitalia, growth retardation, and sensorineural deafness. *PTPN11*, *RAF1,* and *BRAF* have been reported to be causal for LS [Digilio et al. 2002, Pandit et al. 2007, Sarkozy et al. 2009]. Here, we report a patient with LS who has been found to have a novel *MEK1* mutation. The patient, a Japanese 13-years-old boy, was born at 41 weeks and 4 days of gestation with the birth weight as 4350g. He showed hypotonia and sucked poorly in the neonatal period. His psychomotor development was delayed with DQ as 55 at age 19 months. Around the age 3 years, lentigines appeared on his face and limbs. He had flexion deformity of bilateral knees. His linear growth was retarded but showed spurt from age 9 years because of precocious puberty. When seen by us at age 10 years, he weighs 22.1kg (−1.5SD), height 130cm (−1.2SD), and OFC 51.8cm (−1SD). He had multiple lentigines, ocular hypertelorism, and sensorineural hearing impairment, but showed no ECG abnormalities or hypertrophic cardiomyopathy (HCM). He was clinically diagnosed as LS according to the criteria by Voron [1976]. Molecular investigation demonstrated a *de novo* heterozygous missense mutation in *MEK1* (c.305A>G; p.E103G). To date, heterozygous missense *MEK1* mutations have been reported in four patients clinically diagnosed as cardiofacialcutaneous (CFC) syndrome without HCM in all four and with nevi in one [Dentici et al. 2009]. Heterozygous missense *MEK1* mutations could cause phenotypic spectrum including CFC and LS.

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J02.31

Molecular findings of three different male under- virilization cases with 47, XXY karyotype.

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Introduction: Male under virilization is a rare condition mostly due to the mutations of hormone genes that effect male reproductive tract. One of the most important gene mutations that effect that pathway is the androgen receptor gene (AR) mutations, located at Xq12 in individuals with 46, XX. In this report, we present the *AR* and *SRD5A2* gene analysis of three different under- virilized patients with 47, XXY karyotype.

Materials and Methods: Chromosome analysis of the patients were assessed by standard lymphocyte karyotype, with Giemsa staining. PCRs were carried out by amplifying all the exons of related genes, and direct sequencing protocol was applied for mutation detection.

Results: One of the patients had no mutation in *AR* and *SRD5A2* genes, but had a 23 repeat polymorphism on exon 1 of AR gene. The second had no mutation in AR gene, had a 22 and 23 repeats polymorphism, but had a homozygous p.G196S mutation in *SRD5A2* gene. The third had a heterozygous mutation in p.F891L in *AR* gene, with a 16 and 21 repeat polymorphism, and had no mutation in *SRD5A2* gene.

Discussion: 47, XXY karyotype is a very rare condition in male virilization cases. Mutations in *AR* gene, in addition with *SRD5A2* gene, are thought to be the common reasons of this condition. According to our results, we suggest that not only the *AR* gene analysis, but also *SRD5A2* gene analysis and poly-Gln polymorphism of the exon 1 of *AR* gene have important impacts for the diagnosis of male under virilization.

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J02.32

Diagnostic pitfalls and mosaic unbalanced translocations: a case of 18q- deletion syndrome

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We report a male patient with intellectual disability, club feet, growth hormone deficiency and diffuse white matter hypomyelination.

The patient was first seen at 4 years. On physical examination we noticed: height 91 cm (3-10° cnt), weight 14 kg (3-10° cnt); microcephaly (head circumference 47.5 cm, <3°cnt), brachycephaly, frontal bossing, blue sclerae, sparse eyebrows, deep-set eyes, mild strabismus, tented upper lip and single palmar crease were noted. Stereotypical behaviours and bruxism were present.
Due to neuroradiological and clinical findings, a deletion of the long arm of chromosome 18 was hypothesized, but karyotype and subtelomeres FISH analysis were normal.
The follow-up evaluation at 10 years of age confirmed the clinical signs and symptoms. Array-CGH analysis was then performed and showed two rearrangements: a 26 Mb deletion of 18q21.2q23 region (52258392-77982126; UCSC hg 19) and a 37 Mb duplication of 9p24.3p13.2 region (271257-37246576; UCSC hg 19). FISH analysis demonstrated a mosaicism involving two cell lines: one showing normal karyotype and one with an unbalanced autosomal translocation involving the short arm of chromosome 9 and the long arm of chromosome 18. Parental karyotype and subtelomeres FISH analysis were normal, indicating a de novo postzygotic rearrangement in the proband.
In this case a correct clinical diagnostic hypothesis had been ruled out by citogenetic testing and lately confirmed by means of Array-CGH.

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J02.33
Noonan-like Syndrome with Loose Anagen Hair in a Girl Presenting with Perinatal Distress and Coarctation of Aorta

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Noonan syndrome belongs together with cardiofaciocutaneous, Costello, LEOPARD, neurofibromatosis type I, Legius and CBL-mutation associated syndrome into a group of developmental disorders termed RASopathies. These conditions are caused by germ line mutations in genes encoding for proteins involved in the RAS/MAPK signalling cascade and share many phenotypic features (facial dysmorphism, heart defects, short stature, developmental impairment and other anomalies). Previously, Noonan-like syndrome with loose anagen hair (NS/LAH) with facial features reminiscent of NS, reduced growth, neurocognitive impairment, cardiac anomalies and easily pluckable, slow growing, thin and sparse hair was described. Recently, a missense mutation c.4A>G in SHOC2 resulting in the Ser2Gly amino acid substitution in the encoded protein was documented. We report on a patient with molecularly confirmed NS/LAH exhibiting perinatal distress and coarctation of the aorta. The girl was rapidly born at 37 weeks gestation at home, 3-minute CPR must have been performed. Increased nuchal translucency and aortic coarctation with small VSD was described prenatally, hypertrophic cardiomyopathy and tricuspidal valve dysplasia were detected after delivery. The proband presented with facial dysmorphism typical of NS with very thin, sparse, slow growing hair, relative macrocephaly, redundant skin at the back and neck and abnormal sweating. Reduced growth, considerable dystrophic appearance and failure to thrive together with hypotonia and developmental delay mainly in motor milestones occured. Endocrinological evaluation revealed very low IGF-1 levels. To our knowledge, coarctation of the aorta has not been described in this condition yet, so we extend phenotypic spectrum of NS/LAH. Supported by the research organisation project 00064203.

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J02.34
Looking like Oculo-auriculo-vertebral spectrum, but different diagnosis - clinical study of two cases

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Epibulbar dermoids and preauricular tags are clinical features very suggestive for the diagnosis of Oculo-auriculo-vertebral (OAV) spectrum. However, associated features may change the final diagnosis. We present two cases that looked like OAV at the first glance, but received a different final diagnosis due to the associated features, to illustrate two rare disorders and to discuss particularities and patient management.
Case 1: 6 years old female, 5th child of a healthy, young, unrelated couple; pregnancy and birth uneventful; normal growth and psycho-motor development; physical examination revealed scalp defects and areas of different hair, cleft upper right eyelid, marked bilateral epibulbar dermoids and inverted nipples; normal spine radiography, cardiac investigations (ECG, echocardiography) and abdominal ultrasound scan. Diagnosis: Oculo-ectodermal syndrome;
Case 2: 8 years old female, raised in an institution (no family, pregnancy or birth history); moderate/severe growth and psychomotor retardation; physical examination revealed proportionate dwarfism, marked facial asymmetry, downslanting palpebral fissures, nasal tag, mouth deviated to the right, median upper alveolar incisure, asymmetric mandible, abnormal right ear, moderate/severe intellectual disability; spine radiography: cervical vertebral block; echocardiography: ASD; normal renal ultrasound. Diagnosis: Pai syndrome.
In conclusion, we present two cases that look like Oculo-auriculo-vertebral spectrum (but have a different diagnosis) in order to discuss differential diagnosis, but in the same time to present a comprehensive literature review, to illustrate two rare disorders and to discuss patient management.

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J02.35
Case report: a novel mutation in *GJA1* gene in a family with oculodentodigital syndrome

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Introduction: Oculodentodigital syndrome is a rare, congenital autosomal dominant disorder that is characterized by abnormalities of the face, eyes, teeth, and limbs. The most common clinical findings include a thin nose, short palpebral fissures, type III syndactyly, and dental abnormalities including generalized microdontia and enamel hypoplasia. The syndrome is caused by *GJA1* mutations (6q22-q23), which encodes the gap junction protein connexin 43.
Clinical case: A two year old girl was referred for genetic evaluation because of short stature (3-10c), craniofacial dysmorphic features (dysplastic ears, microphtalmia, microcornea, palpebral fissure hypoplasia, epicanthal folds, thin nose, hypoplastic alae nasi with small nares, prominent columella, cleft soft palate, microdontia, enamel hypoplasia, fine and sparse hair) and syndactyly of third and fourth fingers of the right foot, syndactyly with camptodactyly of fourth and fifth fingers of the hands. Neither neurological symptoms nor congenital defects of internal organs were present in the girl. The same facial features and syndactyly of the fingers of the hands in 11 year old sister of the proband was observed. The mother of the girls presented similar facial features with additional unilateral decreased visual acuity and brachydactyly but not syndactyly.
Results: In three family members (mother and two daughters) a novel mutation c.G139C (p.D47H) in *GJA1* gene was detected.
Dissusion: Oculodentodigital syndrome is characterized by intra- and interfamilial phenotypic variability. The affected family members presented striking similar facial features, but variable skeletal manifestations. In prevention and treatment of the variety of clinical manifestations of oculodentodigital syndrome an early diagnosis is important.

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J02.36
Severe Pitt-Hopkins-like syndrome caused by a pericentric inversion resulting in a 6.7 Mb loss at 18q21.2q21.32 and a deletion at 2q24.2

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Pitt-Hopkins syndrome (PHS) is caused by mutations in TCF4. Additionally, significant proportion of PHS cases occurs due to both submicroscopic and microscopically visible chromosome imbalances. Karyotyping of a girl with severe congenital malformations (renal, ocular and neurological abnormalities; scoliosis; anal atresia; microcephaly) and intellectual disability sho-

wing facial dismorphisms similar to PHS has suggested a rearrangement of chromosome 18. Molecular cytogenetic analysis by FISH has shown that a de novo inversion of chromosome 18, i.e. inv(18)(p11.2p21.3), has occurred. To gain further insights into genomic basis of the phenotypic outcome, we performed array CGH using NimleGen 135K oligonucleotide array. We have detected a 6.7 MB loss at 18q21.2q21.32 (50,396,511-57,063,895), which has resulted in a loss of 21 OMIM genes including TCF4. Therefore, a genomic basis for PHS-like phenotype was provided. However, some phenotypic features could not be attributed to this imbalance. We also detected a de novo 2 Mb deletion at 2q24.2 (160,185,503-162,242,742), which has resulted in a loss of 9 OMIM genes. Taking into account the phenotype observed in the index case and bioinformatic analysis performed for evaluation of clinical relevance of genomic rearrangements, we concluded that a cumulative effect of detected genome rearrangements is the most likely explanation of the phenotypic outcome. To our knowledge, this is the first chromosome 18 inversion associated with PHS-like syndrome. Our report demonstrates that array CGH with bioinformatic analysis allow the identification of intrinsic causes of clinical conditions regardless of their erroneously evident phenotypic manifestations.

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J02.37

The craniometric evaluation on a group of patients with Prader-Willi phenotype

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Through availability, facility as technique, craniometry is an investigation that allows evaluation, monitoring and description of the subject and three-dimensional quantification of craniofacial morphology. The aim of the study was to assess dynamic changes in morphometric analysis in a well defined Romanian group of subjects (26 patients aged 4 to 33 years old) presenting clinical signs for Prader-Willi syndrome.
For the study group were registered cephalic, facial and nasal heterogeneous-types and the craniometric typologies were studied within the 2 groups defined by genetic investigations, one with Prader-Willi syndrome (18 patients) and the other (8 patients) with varied etiopathogenic substratum, integrated as Prader-like.
Within the study group, the mesocephalic-pattern was the most frequent (36,46%), then dolichocephalic (26,92%) and brachycephalic (23,08%), only three hyperbrachicephalic cases (11,54%). Regarding the patients' ages in the study group, the dolichocephalic-pattern was specific in childhood. The euryprosope-type had the greatest incidence (50%), followed by leptoprosope-type (30,77%). As nose model, the 2 Caucasian-characteristic types, leptorrhine and mesorrhine, had comparable incidences 38,46%, respectively 34,61%.
For the patients with Prader-like phenotype, heterogeneous data was observed regarding cranial-type, facial-type and nose-type.
The mesocephalic pattern was the greatest within the Prader-group (55,56%) followed by dolichocephalic (27,78%), the brachycephalic-type being the lowest (16,67%). Regarding the patients' age, dolichocephalic-type was specific in children.
For the Prader-group, the greatest incidence of facial-type was euryprosope (50%). The increased frequency of facial euryprosope-type is correlated with the cephalometric parameters which, within this casuistry presented reduced size values of inferior-anterior facial floor and mandibular plane angle.

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J02.38

Mutational Spectrum of Smith-Lemli-Opitz Syndrome Patients in Hungary

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Smith-Lemli-Opitz (SLO) syndrome is an autosomal recessive disorder characterized by multiple congenital abnormalities and mental retardation. The disease is caused by the deficiency of 7-dehydrocholesterol reductase (DHCR7) which catalyzes the final step in cholesterol biosynthesis. The prevalence of SLO syndrome is between 1:20,000 and 1:40,000 among Caucasians. Although the mutational spectrum of the disease is wide, approximately 10 mutations are responsible for more than 80% of the cases. These mutations show a large interethnic variability. Diagnosis of SLO is based on the measurement of 7-dehydrocholesterol (7-DHC) in the patient serum and on the mutation analysis of DHCR7 gene. In order to establish the mutation distribution data from Hungary, thirteen patients were tested with suspected SLO syndrome in our laboratory. First, serum 7-DHC and total cholesterol were measured and molecular genetic analysis of the DHCR7 gene was performed. Complete genetic background of the disease could be identified in 12 cases. In 1 case only 1 mutation was detected in a heterozygote form. One patient was homozygous for the common splice site mutation c.964-1G>C, while all other patients were compound heterozygotes. One novel missense mutation, c.374A>G (p.Tyr125Cys) was identified.

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J02.39

Smith-Magenis syndrome in three infants with congenital heart defects

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We report on clinical, cytogenetic and molecular-cytogenetic findings in 3 infant girls with Smith-Magenis syndrome (SMS). Case 1. The 19-day-old girl with the ventricular septal defect had displastic, low-set ears with the thick helixes; short nose; up-slanted palpebral fissures; prominent upper lip, long philtrum; micrognathia and short neck. At the age of 2 years she had a typical neurobehavioral pattern: sleep disturbance, self-injurious and aggressive behavior. Case 2. The 28-day-old girl with Tetralogy of Fallot had low-set ears; narrow palpebral fissures; long nose; micrognathia; long, thin fingers and clubfoot. Case 3. The 12-day-old premature girl with Tetralogy of Fallot; displasia of corpus callosum and congenital hydrocephaly had low-set ears with the displastic helixes; cleft of soft palate; narrow palpebral fissures; long nose and micrognathia. She had the respiratory distress and died on the 17th day of her life. The standard cytogenetic analyses revealed the interstitial deletion of chromosome 17p11.2 in all 3 patients, which was confirmed by FISH-analyses, karyotype: 46,XX,del(17)(p11.2p11.2).ish del(17)(p11.2p11.2)(RAI1-). Many features of SMS are subtle in infancy and become more recognizable with advancing age. This report represents new data in SMS manifestations which is significant for its clinical polymorphism exploration.

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J02.40

Clinical Findings of Stuve-Wiedemann Syndrome from Turkey: Case Report

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Stuve-Wiedemann syndrome is a congenital bone dysplasia characterized by small stature, congenital bowing of the long bones and other skeletal anomalies and is inherited as an autosomal recessive trait. This syndrome is caused by mutations in the leukemia inhibitory factor receptor (LIFR) gene on chromosome 5p13. Patients present with serious complications including respiratory and feeding distress and recurrent episodes of unexplained hyperthermia. Survivors have progressive scoliosis due to severe spinal deformities, prominent joints, osteoporosis, and spontaneous fractures. We confirm that survival in this syndrome is possible and that the prognosis improves after the first year of life. This should be taken into consideration when counselling parents of affected children. We describe a 5-years-old-

female patient presented with the phenotypic and the radiographic features consistent with the diagnosis of Stuve-Wiedemann syndrome. She came us for growth retardation. She had also motor-mental retardation, short stature, severe scoliosis and bilateral cataract. Her parents had consanguineous marriage (mother 27-years-old and father 35-years-old) and she was one of their four daughters, her sisters were healthy. We report what might be the first clinical report of Stuve-Wiedemann syndrome from Turkey.

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J02.41
A genotype-phenotype correlation of two cases with terminal 4q deletion

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Deletion of the terminal region of the long arm of chromosome 4 is a rare, well-recognized syndrome. The patients' clinical findings include developmental delay, craniofacial, digital, skeletal and cardiac anomalies. Distinctive phenotype of distal 4q deletion depends on size of the deletion and ranges from only minor physical findings to more severe abnormalities. The 4q31-q34 region was suggested as a critical region for most of the features of clinical phenotype.
Hereby we present molecular and clinical findings of two patients with de novo distal deletion of chromosome 4q. The sizes of the deletions were estimated by aCGH which revealed a 25.92 Mb distal deletion in 4q32.3 in the first patient, whereas in the other one, apart from 37.92 Mb distal deletion in 4q31.3, also 1.45 Mb distal duplication of 6p25.3 was found.
Both of our patients apart from characteristic facial dysmorphism had digital stigmata typical of 4q deletion syndrome. Patient 1, who died at 4 months, had a complex cardiac defect. Besides two right, three left renal arteries and inguinal hernia were noted.
Patient 2, mildly mentally retarded, presented with delayed speech development and hearing loss diagnosed at 6 years. His height at 23 years of age was 182 cm. Inguinal hernia and bicuspid aortic valve were also noted.
We would like to present detailed genotype-phenotype correlation of two cases with terminal 4q deletion with review of the literature.
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J02.42
Clinical and molecular cytogenetic characterization of two patients with a *de novo* trisomy 9p

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Trisomy 9p is the fourth most frequent chromosome anomaly in liveborns after trisomy 21, 18 and 13. A possible explanation might be that these chromosomes as well as 9p are relatively gene poor. The first case of trisomy 9p was described in 1970 by Rethore *et al*. Since then, more than 150 patients with partial or complete trisomy 9p have been reported and this kind of chromosomal imbalance was characterized as a clinically recognizable syndrome.
The phenotype of the syndrome is characterized by variable degrees of mental retardation, mild downslanting of palpebral fissures, hypertelorism, prominent or bulbous nose, downturned corners of the mouth, hand-foot anomalies, and abnormal dermatoglyphic patterns.
We present two cases of a *de novo* trisomy 9p syndrome detected using G-banding, fluorescent *in situ* hybridization, spectral karyotyping, and microarray based comparative genomic hybridization (array CGH). The first case represent a 4-year-old girl with partial trisomy 9p with trisomic segments extending from 9p22 to 9p13. In second case we present a 15-month-old girl with duplication involving the whole short arm of chromosome 9. Using array CGH analysis we estimated the size of the duplication about 38.6 Mb. Clinical findings common to both cases were craniofacial dysmorphism, developmental delay, and hand-foot anomalies. The karyotypes of the girls´ parents were normal.
The spectrum of clinical severity in trisomy 9p roughly correlates with the extent of trisomic chromosome material. Previously reported phenotype genotype correlation studies proposed that the critical region for phenotype in trisomy 9p is located in 9p22.

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J02.43
A case report of mosaicism in trisomy 8-Turner syndrome (45,X/47,XX,+8) in a patient with aortic dissection

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Chromosomal aneuploidy is quite frequent and may involve either autosomes or sex chromosomes. While double aneuploidy involving both autosomal and sex chromosomes is rare, there are dozens of reported cases of sex chromosomal aneuploidies in combination with trisomy 21, such as Down-Klinefelter and Down-Turner syndrome. In contrast, trisomy 8-Turner syndrome has been rarely described to date. Here we report a case of a 28-year-old female with mosaicism in trisomy 8-Turner syndrome. The patient was referred to our hospital for operation of aortic dissection. On physical evaluation, her phenotype, including short stature, webbed neck and cubitus valgus, suggested congenital anomalies such as Turner syndrome. Chest CT showed aortic dissection with coarctation. Cytogenetic analysis of peripheral blood by G-banding revealed mosaicism with 2 cell lines (45,X[17]/47,XX,+8[33]). FISH analysis showed that 15% of the cells had monosomy X karyotype and 85% of the cells had XX karyotype; trisomy 8 was only detected in the XX cells. While the patient showed clinical features of Turner syndrome, such somatic stigmas are not clearly distinguishable from those of trisomy 8, such as short stature, skeletal and cardiac abnormalities. Most double aneuploidy cases showed that the patient's phenotype did not have a significant correlation to the ratio of autosomal and sex chromosomal aberrations. The patient deceased from septic shock 36 days after surgery. Mosaicism in trisomy 8-Turner syndrome has rarely been documented and we believe this is the first reported case of mosaicism in trisomy 8-Turner syndrome presenting with aortic dissection and surviving into adulthood.

M. Lee: None. **K. Choi:** None. **D. Kim:** None. **S. Lee:** None. **S. Kim:** None.

J02.44
Three new Omani cases of Warburg micro syndrome with two different *RAB3GAP1* novel mutations

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Warburg Micro Syndrome is a rare autosomal recessive disorder in which less than 60 cases have been reported. The condition is characterised by ocular and neurodevelopmental findings including microophthalmia, microcornea, optic nerve atrophy, congenital cataract, microcephaly, corpus callosum hypoplasia, spastic quadriparesis, hypogenitalism and intellectual disability. We hereby report three new Omani cases from families of Arab origin. Although the families came from the same village, two different novel homozygous mutations were identified in the *RAB3GAP1* gene. The first patient is a four year old girl from a family with multiple loops of consanguinity. Clinical features include: bilateral congenital cataract, microcornea, microcephaly, deep-set eyes, prominent nasal bridge, overriding toes and spastic quadriparesis. Brain MRI had been reported as normal. The second patient is a 10-month-old baby boy from a non-consanguineous family. His main features were bilateral microphthalmia, microcornea, congenital cataract, microcephaly, deep-set eyes, bilateral cryptorchidism, overriding toes, and spasticity. Brain MRI revealed thin corpus callosum with mild increase in extra axial CSF spaces. Recently we identified a third patient who is a double cousin of the first patient. Diagnosis has been confirmed by full sequencing *of RAB3GAP1* gene. Two unreported mutations were identified [p.E83100 (c.2491G>T) and IVS 13-2 A>G (c.1235-2A>G)]. *RAB3GAP1* is a regulator for synaptic vesicle exocytosis with predominant brain expression. The finding of two different novel mutations from two families that are habituating the same geographical area was unexpected. Incidence of this syndrome may be underestimated in our area and other mechanisms like carrier selective advantage may be present.

K.S. Al-Kharusi: None. **Z. Bruwer:** None. **A. Al Kindy:** None. **F. Al Murshedi:** None.

J02.45

Overgrowth and intellectual deficiency in a female with an 6,8 Mb Xp21-p22 duplication

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We report on the case of a 29 year-old woman presenting moderate intellectual disability (ID) specially affecting language and an overgrowth phenotype (over 3 S.D.), macrocephaly and increasing overweight. At the endocrinal level, hirsutism was associated with type B insulin resistance. Coarse facial features, almond shaped eyes, bowed forehead, prognathism and multiple naevi were noticed. Axial hypotonia and hand tremor were observed during childhood. Many cases of diabetes in her family (paternal and maternal branches) are reported.
A *de novo* interstitial duplication of 6.8 Mb in the chromosomal region Xp22.11p21.2 was discovered using the Affymetrix CytoScan HD SNP array. Furthermore, no skewed X chromosome inactivation was found.
This duplication, involving the *ARX* and *IL1RAPL1* genes, is well known to be associated with intellectual disability (1). Both male and female patients have been reported with a huge expression variability.
Our observation extends the clinical spectrum of the Xp21-p22 duplication syndrome to overgrowth and endocrine manifestations.
(1) Clinical and molecular characterization of overlapping interstitial Xp21-p22 duplications in two unrelated individuals.Thorson L, Bryke C, Rice G, Artzer A, Schilz C, Israel J, Huber S, Laffin J, Raca G.Am J Med Genet A. 2010 Apr;152A(4):904-15.

M. Miguet: None. **Y. Alembik:** None. **V. Pelletier:** None. **E. Flori:** None. **V. Kremer:** None. **F. Girard-lemaire:** None. **H. Dollfus:** None. **N. Calmels:** None.

J02.46

A Complex Phenotype-Patient with de novo 16q21-q24.3 Duplication and 15q11.1-q11.2 Deletion

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Duplication of the entire long arm of chromosome 16 causes a malformation syndrome with early postnatal lethality, a phenotype resembling trisomy 18. In 18.03.2011, the boy was born with vaginal delivery at 34th weeks of gestation.Broad and flat nasal bridge, telecanthus, hypertelorism, long eyelashes, optical ocular coloboma and ptosis in the right eye, bilateral low-set ears, hearing test is negative in both ears, preauricular pit on the left ear, long philtrum, frenulum of the tongue is short, no high-arched palate, micrognathia, inguinal hernia, bilateral ureteropelvic obstruction. *Transthoracic Echocardiography*: Partial anomalous pulmonary venous turn, right ventricular dilatation, mild pulmonary hypertension. *Cranial MRI:* Hypoplasia of the corpus callosum. *Cranial BT:* Bifrontal subarachnoid benign enlargement. **Laboratory Findings:** G-banding and high-resolution chromosome analysis showed **46,XY,16q+.** Karyotypes of his parents are **46,XY and 46,XX,16qh+**. Spectral karyotyping analysis elicited that extra-chromosomal structure observed in chromosomal analysis originated from chromosome 16. 2,407,775 bp heterozygous deletion on 15q11.1-q11.2 region and 25,832,063 bp heterozygote duplication on 16q21-q24.3 region were detected and also partial trisomy of 16q21-q24.3 was showed with aCGH analysis.15q11.1-q11.2 is reported as the classic PWS / AS deletion is flanked by either of the proximal BP1-BP2 region spans approximately 500 kb and contains four evolutionarily conserved genes (NIPA1, NIPA2, CYFIP1, and TUBGCP5) that are not imprinted in previous studies. Based on the literature data, intermediate-distal 16q duplications are assosiated with growth retardation, developmental, mental and speech delay, learning difficulties, behavioral problems, dysmorphic facial features, congenital anomalies in skeletal, genitourinary, and central nervous system and epilepsy.

H. Gurkan: None. **H. Tozkır:** None. **Ü. Vatansever-Ozbek:** None. **G. Sarı:** None. **M. Yazar:** None.

J02.47

22q13.3 deletion syndrome in a newborn with ring chromosome 22 presenting with congenital hypotonia

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The 22q13.3 deletion syndrome or Phelan-McDermid syndrome is characterized by neonatal hypotonia, global developmental delay, absent to severely delayed speech, normal to accelerated growth with minor dismorphic features such as dolichocephaly, large or unusual ears, long eye lashes, bulbous nose, pointed chin, large hands and dysplastic toe nails. Most individuals have moderate to profound intellectual disability with autistic like behaviour and decreased pain perception. Frequency of the syndrome is equal in males and females while incidence is still unknown. Beside the simple deletion, loss of 22q13.3 can also result from translocation, ring chromosome formation and other less common structural changes affecting the region containing SHANK3 gene on long arm of chromosome 22. The most features of the syndrome thought to be the consequence of loss that specific gene.
We present a case of female newborn with Phelan-McDermid syndrome. The child was born on term, from first, uncomplicated pregnancy with unremarkable family history. She was initially treated in NICU because of early neonatal infection. Because of congenital hypotonia with mild facial dysmorphic features genetic analysis were performed. The karyotype revealed ring chromosome (22): 46,XX,r(22)(p12q13.3). A fluorescent in situ hybridisation analysis was performed, indicating that the deletion encompassed the critical 22q13.3 region. After good clinical evaluation, congenital hypotonia especially with dysmorphic features may be the only indication for genetic evaluation, so we suggest importance of genetic review in cases like this.

D. Begovic: None. **S. Huljev Frković:** None. **R. Lasan Trčić:** None. **J. Stipanović Kastelić:** None. **L. Letica:** None. **M. Šalamon Janečić:** None.

J02.48

2q11.2-12.2 de novo deletion - report of a patient

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Introduction: genomic imbalances cause a significant proportion of MR/MCA syndromes; array-CGH has uncovered many rare imbalances associated with MR/MCA. We report a patient with a deletion in chromosome 2q11.2-12.2 and review patients with similar anomalies.
Clinical case: female, second child of non-consanguineous couple; mother had learning difficulties, healthy older. Neonatal asphyxia occurred with good recovery. She had global developmental delay with hypotonia, behavior problems (hyperactivity, aggression, tantrums), growth retardation, macrocephaly, facial dysmorphisms and no major malformations or other health problems.
Molecular analysis (aCGH): genomic DNA was extracted from blood using the Citogene® DNA isolation kit (Citomed, Portugal). The aCGH analysis was performed on a human genome CGH Agilent 180K custom array (AMADID: 023363) designed by Dr. Klass Kok for use in the Low Lands Consortium. Hybridization and image analysis were performed using the across-array methodology described previously (Buffart T et al, 2008). aCGH data was analyzed using Nexus Copy Number 5.0 software with FASST Segmentation algorithm and a minimum of three probes in a region required to be considered an copy number alteration.
Molecular analysis (qPCR): the qPCR fragments were designed in exon 1 for the GPR45 gene. qPCR run was carried out in a 7500 FAST equipment (Applied Biosystems) and relative quantification analysis was performed using Data Assist™ Software (Applied Biosystems).
Discussion: genomic analysis is a powerful tool to uncover imbalances associated with developmental problems; detailed description of phenotypes associated with those imbalances allows clinicians to more accurately and rapidly make diagnosis and correctly interpret results obtained with array-CGH

G. Soares: None. **M. Reis-Lima:** None. **A. Fortuna:** None. **F. Lopes:** None. **P. Maciel:** None.

J02.49

Novel NHS gene mutation in a Turkish family with Nance-Horan syndrome

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Nance-Horan syndrome (NHS) is a rare X-linked syndrome characterized by congenital cataract which leads to profound vision loss, characteristic dysmorphic features and specific dental anomalies. Microcornea, microphthalmia and mild or moderate mental retardation may accompany these features. Heterozygous females often manifest similarly but with less severe features than affected males. We describe two brothers who have the NHS phenotype and their carrier mother who had microcornea but not cataract. We identified a previously unreported frameshift mutation (c.558insA) in

exon 1 of the NHS gene in these patients and their mother which is predicted to result in the incorporation of 11 aberrant amino acids prior to a stop codon (p.E186Efs11X). We also discussed genotype phenotype correlation according to relevant literature.

F.E. Percin: None. **E. Tug:** None. **N.F. Dilek:** None. **S. Javadiyan:** None. **K.P. Burdon:** None.

J02.50
Chromosome markers charazterization by Array-CGH. Case report.

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Microarray-based comparative genomic hybridation (array CGH) has provide a relatively quick method to scan the genome for gains and loses of chromosomal material with significantly higher resolution and greater clinical yield than was previously possible. This new methodologies have led to identification of genomic material from chromosome marker detected by karyotyping and determine the possible pathological effect on patients.
A ten years old girl who was noticed to have growth and psicomotor delay, microcephaly and dismorfic features was referred for genetics studies. She was the third baby born to a healthy non-consanguineos couple. The examination findings were: small frontal, hypertelorism, flat nasal bridge, peripheral mild hypotonic, microcephaly and growth and psicomotor delay.
Chromosome culture and karyotyping were realized by standard thecnics. The array-CGH was performed at NimbleGen CGX Cytogenetics Microarrays Platform.
The karyotype result was 47,XX + mar (47 %); 48,XX + mar + mar (53 %) and then the array-CGX for chromosome marker genomic material detection was performed.
The array-CGX detected a pericentric duplication of 7,47 Mb with 22 genes implicated on chromosome 8.
With this results is possible to think that the second chromosome marker detected en part of the metaphases is a duplication del original marker, and the presence of this marker can be de responsible for the patient pathology. Previously a girl with similar pathology and with a chromosome marker also originated on pericentic region of chromosome 8 has been described.
As a conclusion, array CGH actually is the election method for chromosome marker genomic characterization.

M. Perez Sanchez: None. **A. Mora.:** None. **J.L. Barrionuevo:** None. **A.R. González.:** None.

J02.51
Currarino syndrome with a terminal deletion of long arm of chromosome 7 (7q)

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We describe a 7q terminal deletion in a 15 year-old girl with partial Currarino syndrome, born to non consanguineous parents from Mali.
Fetal karyotype, prompted by detection of microcephaly and IUGR detected with second trimester echography revealed a 7q deletion. At birth at 41 GW, head circumference was 29cm (-5 SD), weight was 2500g (-2SD), length 42,5 cm (<-3SD). Pregnancy was normal (no drug nor alcohol). She stayed 2 months in neonatology and required feeding by naso-gastric tube. She was lost to follow up afterwards till 14 year-old. The anomaly was recently reassessed by SNP array.
The patient had moderate developmental delay, she started walking at 27 month-old, she is toilet trained during the day. She makes simple sentences. She has followed special schooling until 14 year-old, but remained unable to read or write.
At the last assessment, at age age 15, OFC was 46cm (-6SD), weight 31kg (-3,5SD), and length 137cm (-3,5DS). She has scoliosis, short fifth metacarpals, and no sign of puberty. She has facial dysmorphism with prognathism. Echocardiography was normal, MRI revealed a small brain with normal gyration. She had coccyx and sacral defects (S3-5), tethered spinal cord and presacral mass (13x10mm), but no anorectal malformation. Neurosurgical consultation is pending.
The malformations of this patient are explained by HLXB9 haploinsufficiency: Currarino syndrome is an autosomal dominant disorder with reduced penetrance. Mutations in a homeobox gene, HLXB9, localised in 7q36, were identified in several affected patients.DI and microcephaly are caused by deletion of other genes within 7q36 region.

J. Lanneaux: None. **A. Lavillaureix:** None. **J. Fabre-Teste:** None. **J. Masliah-Planchon:** None. **C. Dupont:** None. **S. Passemard:** None. **S. Drunat:** None. **A. Tabet:** None. **A. Verloes:** None.

J02.52
A Case of dup(3q) Syndrome

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Duplication of 3q is an extremely rare disorder characterized by "mental retardation, deficiency of growth, broad nasal root and hypertrichosis". Which had similar clinical findings with Cornelia de Lange syndrome. Although it is generally accepted that the duplication of the 3q25-qter region is sufficient to generate characteristic face, there is a debate about critical region. An interesting point of view, the dup(3q) cases also had additional chromosomal anomalies as in our presented case. The patient had copy gain at 21q22.12 (partial trisomy of Down syndrome critical region). In this report, we present the clinical and molecular findings of a case with dup(3q) syndrome.

A. Koc: None. **O. Ozer:** None. **A. Yilmaz Ekmekci:** None.

J02.53
A case report of newborn girl with interstitial deletion of chromosome 1: del(1)(p22;p31)

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An interstitial deletion means that the chromosome had broken in two places and the broken ends fused, leaving out the deleted segment. We report a case of newborn girl with interstitial deletion of the short arm of chromosome 1. Given the newborn's dysmorphic appearance, cytogenetic analyses were indicated for the baby girl and her parents. Karyotype was analyzed from cultured peripheral blood lymphocytes, using G-banding method. Parental chromosome analysis showed a normal chromosome constitution with normal banding patterns, while karyotype of the newborn revealed "de novo" interstitial deletion of the short arm of chromosome 1, with deleted segment: p22 - p31. No pregnancy or delivery problems have been reported. A female newborn was born at term, and birth measures were within normal range. Specific dysmorfic features were present at birth: dolychocephaly, low forehead and neck hairline, prominent eyebrows, broad nasal bridge, down slant eyelids and narrow eye opening, microphtalmia, cataract, anteversion of nostrils, macrostoma, high-arched plate, micrognathia, short neck, overlapping fingers and toes. No internal system/organ major anomaly was found. Contrary to the fact that this deletion is reported with incidence approximately 1:5000 liveborns, this was the single one diagnosed in our centre, which is the only laboratory that conducted cytogenetic diagnostics in Montenegro since 2000 (approximately 8000 liveborns per year).

S.D. Teofilova: None. **M. Bulatovic:** None. **T. Ostojic:** None. **O. Miljanovic:** None.

J02.54
Ring chromosome 6 in an infant with multiple congenital anomalies

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Ring chromosome 6 has been reported in the literature as a clinically recognizable syndrome, which occurs rarely and manifests as various phenotypes. We present clinical and cytogenetic findings on a four-week-old female infant.
The girl was born of a second pregnancy to a 23 year-old mother at 39 gestation week by vaginal delivery. The couple had previously had one first trimester spontaneous pregnancy loss. Birth weight was 3550 g (50% centile), height 50 cm (25% centile); head circumference was 31 cm (<3% centile). At the immediate neonatal period, she failed at all attempts to breastfeed and required a nasogastric feeding tube. Dysmorphic findings on clinical genetics evaluation showed ocular hypertelorism, flat nasal bridge with anteverted nares, short grooved philtrum, open triangular-shaped mouth, high arched palate, bifid tongue, retrognathia, large low-set ears, short neck, widely spaced nipples, tapering fingers, camptodactyly of the left 5th finger and overlapping toes. Hirsutism was presented on the back, upper arms and lower legs. She had ectopic vaginal anus with stenosis and congenital bilateral hip dislocation. An echocardiogram revealed a moderate patent ductus arteriosus and secondary atrial septal defect. Ophthalmologic examination

has revealed bilateral chorioretinitis. Hearing screening was negative. High resolution chromosome analysis revealed a ring chromosome 6, designated by the karyotype, 46,XX,r(6)(p23q27). Parental chromosome analyses were normal.
Comparison with the previously reported cases of ring chromosome 6 illustrates the phenotypic variability of this syndrome. To our knowledge anorectal malformations have not been previously reported. Further investigation is needed to ascertain the genes that were deleted.

B. Burnyte: None. **G. Brusokiene:** None. **Ž. Čiuladaite:** None. **N. Drazdiene:** None. **A. Utkus:** None.

J02.55

Saethre-Chotzen syndrome with unusual cranial features and intrafamilial phenotypic variability.

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Saethre-Chotzen Syndrome (SCS; OMIM #101400) is a dominantly inherited disorder characterized by broad spectrum of dysmorphic features: craniosynostosis, facial asymmetry, ptosis, partial cutaneous 2 / 3 syndactyly and prominent pineal crura. Less common manifestations include short stature, ocular hypertelorism parietal foramina and hearing loss.
SCS is caused by mutation in TWIST1 gene leading to haploinsufficiency of the protein product, Twist-related protein 1. In some cases there is an underlying chromosomal aberration involving 7p21.
We report on a four generation family with 6 members affected with Saethre Chotzen syndrome presenting with significant intrafamilial phenotypic variability. Our proband, the youngest affected individual in a family, has been evaluated in neonatal period by medical geneticist because of dysmorphic features: facial asymmetry, high forehead, bilateral ptosis, symmetrical prominent ear crus, broad haluces and significantly dilated parietal foramina. Examination of the mother revealed similar dysmorphic features with additional partial cutaneous 2 and 3 syndactyly. She also reported the presence of unusual cranial feature in infancy: asymmetric "lack" of frontal bones. She has been unaware of dysmorhic features being the part of inherited disorder. Detailed family history identified 4 more individuals with similar phenotypic expression. The unusual frontal bone malformations were present in all of them with a varied degree.
The clinical diagnosis of Saethre-Chotzen syndrome has been confirmed by sequence analysis of TWIST1 leading to identification of mutation c.421dup21.
This report underlines the intrafamilial phenotypic variability and adds an unusual feature: frontal bone anomaly to the broad spectrum of phenotypic expression in Saethre-Chotzen syndrome.

A. Poluha: None. **A. Sobczyńska-Tomaszewska:** None. **I. Jaszczuk:** None. **D. Winnicka:** None. **M. Lejman:** None. **B. Styka:** None. **M. Babicz:** None. **J.R. Kowalczyk:** None.

J02.56

A patient with SMC(22) due to the maternal translocation t(16;22)) (p13.1;q11.2)

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Partial trisomy 16p is a rare chromosomal abnormality, associated with microcephaly, distinct facial features, psychomotor and growth retardation. 22pter-q11 trsisomy or tetrasomy, cat eye syndrome, is characterised with mild mental retardation, ocular coloboma, cardiac, kidney, and skeletal malformations. Our patient, 1 months old girl, refered to the department of medical genetics because of congenital heart defects. She was born via spontaneous vaginal way at a gestational age of 36. She required neonatal intensive care for 35 days. She has microcephaly, microretrognati, dysplastic low set ears, pitosis, wide spaced nipples. Echocardiografic examination revealed ASD, VSD, and pulmoner hypertension. Cytogenetic analysis from patient's peripheral blood showed a supernumerary derivative chromosome 22. FISH analysis confirmed partial trisomy 22q11. The chromosome analyses of father was normal. Mother has balanced translocations between chromosome 16 and 22 [46,XX,t(16;22)(p13.1;q11.2)]. We decided that supernumerary der(22) is originated from mother's balanced translocation, t(16;22), due to the error in maternal meiosis and yielding partial trisomy 16p13.1-pter and partial trisomy 22pter-q11.2.

M.Y. Alp: None. **T. Tos:** None. **N. Okumus:** None.

J02.57

A recurrent mutation of TCOF1 gene in a Moroccan patient with Treacher-Collins syndrome: report of a new case and review

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Treacher Collins syndrome (TCS, OMIM # 154500) also known as mandibulofacial dysostosis and Franceschetti-Zwahlen-Klein syndrome is a rare congenital disorder of craniofacial morphogenesis, its prevalence ***is*** 6 per 100000 persons. Most TCS is inherited in an autosomal dominant manner, a small portion (~1%) is inherited in an autosomal recessive manner. Typical cases of TCS are ***characterized by*** four ***major clinical manifestations,*** hypoplasia of the zygomatic bones and mandible, external ear abnormalities, lower eyelid abnormalities and family history consistent with autosomal dominant inheritance. Mutation of one of three genes is known to be causative, *TCOF1* (78%-93% of individuals with TCS) and *POLR1C* or *POLR1D* (8%). The TCOF1 gene located at ***5q32-q33.1*** encodes a nucleolar phosphoprotein known as Treacle, which is an important spatiotemporal regulator of ribosome biogenesis. More than 130 disease-causing mutations in *TCOF1* have been documented in individuals with TCS, only one mutation c. 4369_4373delAAGAA, p.Lys1457GlufsX12, has been identified as commonly recurrent; it is present in 16% of individuals with an identifiable mutation. Mutations in *TCOF1* lead to treacle haploinsufficiency which disrupts neural crest cell formation and proliferation, causing the hypoplasia characteristic of TCS craniofacial anomalies.
We report here a 7-year-old Moroccan boy with typical features of TCS. The molecular studies showed the recurrent homozygous mutation at c. 4369_4373delAAGAA of *TCOF1* gene.

A. Lamzouri: None. **I. Ratbi:** None. **S. Chafai Elalaoui:** None. **C. Collet:** None. **I. Turquois:** None. **A. Sefiani:** None.

J02.58

A unique case of intrachromosomal triplication of 2q13q21.1 and contiguous small duplication in a 13 years old boy

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Cytogenetically visible intrachromosomal triplication producing partial tetrasomies are rare and have been reported for few different chromosomes. Regarding to proximal 2q intrachromosomal triplication, only three cases have previously been described. Two of them with more proximal 2q triplication did not survive. The third with a smaller triplication segment was an infertile 33-year-old female. The chromosomal breakpoints with duplication or triplication of proximal 2q are varied, as well as the clinical features. We describe a 13-year-old boy with mild intellectual disability and dysmorphic craniofacial features with a large face, deep set eyes, small nose, prominent cheeks and retracted malar region, prognathism, webbed neck and posterior low hairline. Cleft palate and bilateral cryptorchidism were surgically corrected; diabetes mellitus was diagnosed at 6-year-old and renal failure at 10-year-old. The karyotype was 46,XY,trp(2)(q13q21.1) and arrayCGH analysis (Affymetrix CytoScan HD microarray) showed a complex rearrangements consisting of triplication at 2q13q21.1 (minimum of 20,207 kbp) and duplication at 2q21.1 (minimum of 2,759 kbp). Some genes within this region as *NPH1* and *IL1* could play role to patient´s features. Mutations in the *NPH1* have been related to most patients with juvenile nephronophthisis, however our patient presenting triplication shows similar effect to loss of gene function. Polymorphisms in *IL1* were associated with diabetic nephropathy and it may play role in the patient´s deterioration of renal function. To our knowledge, the present patient has the biggest triplication 2q proximal segment, and he is alive once the triplication of this region is not as harmful to vital functions.

K.T. Abe: None. **M.F. Pereira:** None. **D.R. Carvalho:** None. **M. Schneider:** None. **L.L. Roese:** None. **L.M. Formigli:** None. **M.V. Oliveira:** None. **N. Sakai Jr:** None. **A.V. Coelho:** None. **I.M.P.O. Rizzo:** None. **C. Speck-Martins:** None.

J02.59
A case of de novo deletion 9q33

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Partial deletions of 9q are associated with mental retardation, distinct facial features, developmental delay and congenital heart defects. Common deletions involving 9q are subtelomeric deletions of 9q34. Terminal deletion 9q33 is very rare.We report a 10 years old girl with de novo partial deletion 9q33. She referred to the department of medical genetics because of mental retardation and developmental delay. She has short stature (<3th percentile), delayed developmental milestones, bushy eyebrows, thick lips, and simian variant in left hand. She is also suffering from epilepsy. MRI findings revealed cortical dysplasia localised to the left temporal lobe. Cytogenetics analysis from peripheral blood showed distal partial deletion of the long arm of chromosome 9. Parental karyotype examinations were normal.

T. Tos: None. **M.Y. Alp:** None. **N. Andıran:** None. **E. Yağlı:** None.

J02.60
Newborn with agenesis of right lower limb and right renal agenesis case presentation

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I will present the case of a female newborn from a monitored pregnancy, gestational age=41 weeks, birth weight=2600gr, Apgar score=5; her father, suffering from chronic renal failure, is enrolled in a dialysis programme. According to anamnesis no teratogen factors that could act during pregnancy are revealed.
At hospital admission she has poor health status general health status, dysmorphic skull, cardiopulmonary functions within normal limits, systolic breath grade III/VI, agenesis of right lower limb - absence of thigh and shank, abnormal foot size that is attached to the hip through a cord.
Radiography of lower limbs shows the absence of right lower limb bones, while the radiography of lumbosacral head shows no structural changes.
Ultrasonography of the heart indicates tricuspid insufficiency grade I. Abdominal ultrasonography: normal structure liver, left kidney in the renal lodge.
CT scan with contrast substance has been performed, revealing right hemiabdomen and pelvis reduced in size and hypoplastic.
Right kidney is evidenced neither in the right renal lodge, nor in other abdominal-pelvic section. Right renal artery emergent from aorta is not evidenced; left kidney of normal size; spleen of normal size; thoracolumbar scoliosis with axial rotation of vertebral bodies; right coccyx bone is hypoplastic, and the right femur cannot be evidenced.
Case particularity:
Newborn with right hemi-abdomen and pelvis hypoplasia associated with right renal agenesis and right lower limb agenesis, in which no teratogen factors that could have been act during pregnancy are revealed.

D. Iacob: None. **M. Boia:** None. **A. Manea:** None. **R. Iacob:** None.

J02.61
The clinical phenotype in a case with 49,XXXXY

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The 49, XXXXY syndrome is a rare sex chromosomal aneuploidy syndrome characterized by the presence of three extra X chromosomes in males and was first reported in 1960 by Fraccaro et al. It has an annual incidence of 1/85,000 to 1/100,000 male births.
We report here, a male child with mental retardation and impairment of language abilities, remarkable facial dysmorphism (microcephaly, hypertelorism, large flat nose with a depressed nasal curvature, upslanting palpebral fissures, epicanthus, prognathism, folded-over ears, short neck), important anemia and other associated congenital defects (cubitus valgus, flat feet, clinodactyly of the fifth finger, joint laxity, radio-ulnar synostosis). The cytogenetic study showed the constitution to be 49, XXXXY in all cells. The parent's of the patient has solicited genetics counseling to know the risk of having another child with a chromosome disorder.
Management needs to be handled by a multidisciplinary team and includes the treatment of skeletal defects, the monitoring of psychomotor development with physiotherapy, psychomotricity, speech therapy, orthopedic and sensory management (ophthalmological examination), neurological, hormonal (if necessary) and psychological care and regular dental follow-up. Patients have an essentially normal life expectancy but will need to attend regular medical visits.

G.C. Cozaru: None. **A.F. Mitroi:** None. **M. Aschie:** None. **I. Poinareanu:** None.

J02.62
Additional genomic imbalance detected by arrayCGH in a prenatal case of Down syndrome

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Down syndrome is the most common chromosomal aneuploydy and several screening and diagnostics tests are available for prenatal diagnosis. Most cases identified have free trisomy 21, but there are reports associating other aneuploydies, usually double trisomies, involving mainly sex chromosomes. Additional structural chromosomal aberrations are very rare.
We report a prenatal case of 19 weeks of gestation, referred for cytogenetics investigations due to increased risk for Down syndrome. QF-PCR and conventional cytogenetics analysis were performed. QF-PCR detected trisomy 21 in a male fetus. Chromosomal analysis revealed three copies of chromosome 21 in all metaphases evaluated. The decision of the parents was to terminate the pregnancy, but since in our center an array comparative genomic hybridization study was going on, the parents gave concern to this analysis, as they wanted to further investigate the case. ArrayCGH was carried out on a NimbleGen ISCA Plus 3x1.4M Platform (Roche) and data was analyzed using Nexus Copy Number software (BioDiscovery). ACGH detected the 21 trisomy indentified by conventional cytogenetics, but additionally detected several duplications larger than 500 kb, on subtelomeric regions of chromosomes: 2p, 4p, 7p, 7q, 8p, 8q, 9q, 10p, 10q, 11p, 12q, 13q, 16q, 17q, 18q, 19p, 20q, 22q and on centromeric regions of chromosomes 2, 3, 6, 7, 8, 12. It is well known that 1.5-fold increase in dosage of transcripts and proteins encoded by genes in a trisomic segment, directly affects the phenotype. To evaluate a putative contribution of genomic imbalances detected, aCGH analysis for both parents should be performed.

M. Stoian: None. **C. Ionescu:** None. **V. Celmare:** None. **A.F. Anca:** None. **G. Cardos:** None.

J02.63
R3321Stop Mutation in MLL2 Gene Causes Kabuki Syndrome in a Spanish Patient

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Kabuki syndrome, KS (OMIM# 147920) is a rare syndrome characterized by mental retardation and multiple congenital abnormalities. Mutations in *MLL2* gene have been recently identified in the majority of cases and rarely, mutations in *KDM6A* gene are causing the syndrome.
Case report: We report the molecular and phenotype studies of a Spanish patient who have typical features of KS. The patient is a 7 y.o. infant with mental retardation, a peculiar facies characterized by long palpebral fissures, a broad and depressed nasal tip, large prominent earlobes, a cleft palate, scoliosis, radiographic abnormalities of the vertebrae, hands, and hip joints, and recurrent otitis media. Genomic DNA was extracted from the patient and parents and mutation screening of coding exons and intron-exon junctions of the *MLL2* gene was performed by direct sequence analysis using a 3130XL Genetic Analyzer. All found changes were *in silico* analyzed (Polyphen 2.0) to estimate a possible damaging effect in the protein. We identified, for the first time in a Spanish patient, a mutation in exon 34 of *MLL2* gene causing the disease. The nucleotide change is a C>T substitution (c.9961C>T) causing a nonsense mutation in the exon 34 producing a truncated protein by the change of an Arginine by a stop codon (p.R3321X). This mutation is absent in both parents and the *in silico* analyses showed that this change is potentially damaging the protein function. This mutation has been previously described but this is the first genetic study of a patient with KS in Spanish population.

G. Pi: None. **L. Pedrola:** None. **A. Sanchis:** None. **S. Climent:** None. **A. Martinez:** None. **M. Ortiz:** None. **A. Zuñiga:** None.

J02.64

Oro-Facio-Digital syndrome in two Iranian patients

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Oro-Facio-Digital syndromes (OFDS) are a heterogeneous group of disorders characterized by abnormalities of the face, oral cavity, and digits of the upper and lower limbs. We report on two patients with OFDS type IV (OFD IV) (OMIM 258860), also known as Mohr Majewski syndrome. OFD IV is differentiated from other types of OFD by its autosomal recessive mode of inheritance and severe tibial dysplasia. OFD IV is characterized by lobulated tongue, pseudo-cleft of the lip, pre- and postaxial polydactyly, severe talipes equinovarus, mesomelic limb shortness associated with tibial hypoplasia. Both cases are the first and only child of first cousin Iranian parents. They both have multiple anomalies including lobulated and bifid tongue with nodules, gingival hypertrophy, hypertelorism, low-set ears, bilateral postaxial polydactyly of hands, brachydactyly with syndactyly of fingers, preaxial polysyndactyly of toes. In addition, Case 1 has microcephaly, hypotonia, repaired median cleft lip and palate, multiple oral frenuli, left cryptorchidism. Case 2 shows prominent forehead, micrognathia and absent frenuli, and microphallus. Recently, it has been shown that TCTN3 mutations as the cause of a severe form of Mohr-Majewski syndrome associated with bone dysplasia, tibial defect, brain anomalies and cystic kidney disease. Sequencing of TCTN3 is being performed and if negative, genome sequencing will be performed on these two families.

A. Rajaee: None. **A. Kariminejad:** None. **F. Afroozan:** None. **M. Kariminejad:** None.

J02.65

Sonic hedgehog and sexual organ development : a case report.

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The sex development anomalies are among the most frequent prenatal malformations. Multiple genes pathways are involved in the sexual organ development. With this case report, we focused on the sonic hedgehog (shh) role.
A 20 weeks pregnancy was terminated because of holoprosencephaly. At the fetus autopsy, the pathologist noted the presence of alobar holoprosencephaly, aortic stenosis, intestinal malrotation, bilateral hydroureteres and ambiguous external sexual organs with large labia and clitoris. The abdominal cavity examination revealed gonads in the pelvic region. Their microscopic analysis concluded to testicular tissues. A microarray analysis of the fetal DNA identified a male fetus with 7q deletion and a 13q trisomy: 46,XY,del(7)(q36) 13q33.2 x3. None of the13q33 genes had a known function. We assumed that shh found among the deleted 7 q genes explains the major sex differentiation anomalies.
Studying the role for cholesterol in early mouse embryonic development, the function of specific gene products, such as Shh signaling protein, was demonstrated in pathologies combining holoprosencephaly, limb and genitalia anomalies. Shh knockout mice lack external genitalia and have a persistent cloaca.
This case report of a 7q deletion including shh gene reminded us the early and crucial role of shh signaling pathway through the human sexual differentiation and a review of the scientific literature was done.

U. Ullmann: None. **B. Grisart:** None. **C. Dugauquier:** None. **K. Dahan:** None.

J02.66

A case with de novo 46,XX,add(6)(p25) karyotype.

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Chromosomal aberrations commonly cause phenotypic abnormalities. Furthermore they are associated with 50% of the spontaneous miscarriages and encountered in the 0.5% to 1% of live births. Here, we present a case with *de novo* 46,XX,add(6)(p25) karyotype.
The patient was a 9-year-old girl with suspected diagnosis of syndromic child. She was delivered by cesarean section, weighing 3300 gr. She was able to sit when she was four months old. Although she started to talk and walk around at the age of one, she occasionally had imbalance. Around five years of age, she had an ear operation because of the occurred sensorineural hearing impairment.
The parents were not related and no similar cases were seen in the family. Her height, weight and head circumference were in normal range. A small nose with a flat nasal bridge, upturned nares, and deep-set nails were found. MRI showed cerebral atrophy, a 9x31 mm arachnoid cyst in the left temporal lobe and left mastoiditis. Secundum atrial septal defect, mild mitral failure and minimal left renal pelviectasis were other findings. Ophtalmologic examination revealed bilateral embryotoxon, congenital esotropia, and a best corrected visual acuity of 0.5 bilaterally.
Cytogenetic analysis revealed a 46,XX,add(6)(p25) karyotype. As the father, mother and two sisters did not have any chromosomal abnormality, chromosal aberration of our case was regarded as *de novo*.
46,XX,add(6)(p25) karyotype of our case and the phenotypic findings may be associated with a new chromosomal syndrome. Advanced molecular and molecular cytogenetic tests have been planned to define the relevant chromosomal region.

K. Delil: None. **H. Simsek:** None. **E. Cerman:** None. **F.I. Sozen Delil:** None. **M.A. Soylemez:** None. **S. Yildirim:** None. **I. Guney:** None.

J02.67

Klippel-Feil syndrome: a case report

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Klippel-Feil syndrome (KFS) is characterized by congenital vertebral fusion of the cervical spine resulting from faulty segmentation along the embryo's developing axis. A wide spectrum of associated anomalies may be present. This heterogeneity has complicated elucidation of the genetic etiology and management of the syndrome.
It's about a 32-year- old women complained of torticollis. Her medical and family histories were unremarkable. Physical examination reveals Pain on palpation of vertebral spine apophyses and neck muscles and limited movement in the cervical spine without any associated anomalies. A radiography show a C5-C6 fusion on the cervical spine and the isolated klippel Feil syndrome was diagnosed. It's the rarest form of congenital fused cervical vertebrae which is predisposed to the risk of spinal cord injury and neurologic problems.
We insist that clinical heterogeneity and radiographic abnormalities found in Klippel-Feil syndrome may simulate acute pathology and thus require comprehensive evaluation and delineation of diagnostic and prognostic classes.

R. Frikha: None. **S. Daoud:** None. **T. Rebai:** None.

J02.68

Oro-facio-digital syndrome type 1 in child with celiac disease: coincidence or a correlation?

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Oro-facio-digital syndrome type1 (OFD1[MIM#311200]) is an ectodermal dysplasia characterized by lobulated tongue, lingual harmatomas, cleft palate, hyperplastic frenulum, polydactyly, brachydactyly and syndactyly. OFD1 is transmitted as an X-linked dominant condition with lethality in males caused by a mutation in OFD1 gene (Xp22.3-p22.2). Celiac disease (CD) is an autoimmune alteration triggered by ingestion of gluten in patients genetically predisposed and have been associated with several chromosomal syndromes such as Down syndrome, Williams's syndrome and Turner syndrome. This work reports for the first time a case of a child diagnosed with celiac disease and oro-facio-digital syndrome type1 and suggest an association between OFD1 and CD. This studie shows genetic clinical features of OFD1, and sorological tests (TtG-IgA, Anti-endomisium) and HLA typing for Celiac disease.

F.C. de Almeida: None. **R. Pratesi:** None. **P.M. Fritsch:** None. **L. Gandolfi:** None. **H.P.N. Safatler:** None.

J02.69

A novel splice site mutation in the MLL2 gene in a Moroccan patient with Kabuki syndrome

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Kabuki syndrome (also known as Niikawa-Kuroki syndrome) is a rare au-

tosomal disorder, characterized by an unusual face, short stature, skeletal, visceral and dermatoglyphic abnormalities, cardiac anomalies, mental retardation, and immunological defects. Point mutations and large intragenic deletions and duplications of the mixed lineage leukemia 2 (MLL2) and exons deletions of lysine demethylase 6A (KDM6A) genes have been identified as its underlying causes.
We report on the first description of a Moroccan Kabuki syndrome patient with typical facial features, developmental delay, finger pads, and other anomalies carrying a novel splice site mutation in the MLL2 gene that produces a truncated and likely pathogenetic form of MLL2 protein.

I. Ratbi: None. **N. Fejjal:** None. **L. Micale:** None. **B. Augello:** None. **C. Fusco:** None. **J. Lyahyai:** None. **G. Merla:** None. **A. Sefiani:** None.

J02.70
Uncommun Case of Trisomy 13

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Trisomy 13, or Patau syndrome is a rare chromosomal disorder characterized by a triad of cleft lip and palate, postaxial polydactyly and microcephaly. It was described firstly in 1960. Complete, partial, or mosaic forms of the disorder can occur.
Here, we report on a case of five months old girl with feeding difficulties, uncommon facial dysmorphy of trisomy 13: microcephaly, high forehead with facial cleft, hypertelorism, horizontal palpebral fissures, epicanthal folds, bifid nose with anteverted nares, posterior cleft palate, long philtrum, low-set and dysplastic ears. She has also short neck, overlapped fingers and toes with hypoplastic thumbs, dislocated hips, hypotonia and she has no epilepsy, nor congenital heart defect, or visceral malformation. Cytogenetic analysis demonstrated trisomy 13 with Robertsonian translocation. The karyotype of the parents was normal. There was no holoprosencephaly in Brain CTScan. Genetic counseling and prenatal diagnosis were offered to the parents.

I. Chelly: None. **K. Lilia:** None. **K. Ichrak:** None. **M. Faouzi:** None. **G. Najoua:** None. **C. Habiba:** None.

J02.71
Partial trisomy 4p due to the maternal translocation t(4;22)(p15.2;p11.2)

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We report on a 5 year and 4 months old boy with 46,XY,der(22)t(4;22)(p15.2;p11.2) due to the maternal reciprocal translocation t(4;22)(p15.2;p11.2). The patient was referred to the department of medical genetics because of mental retardation and facial dysmorphism. He had microcephaly, brachycephaly, triangular mouth, low frontal hairline, hypotelorism, strabismus, prominent simple ear. Developmental milestones were delayed. Also he had pes equinovarus of the right foot and unilateral simian crease. MRI showed partial agenesis of the corpus callosum. X-ray imaging revealed bilateral femoral coxa valga and fusion defects on the posterior arcus of the C6-C7. In our patient, balanced reciprocal translocation of mother resulted to partial trisomy 4p and partial monosomy 22p. Trisomy 4p syndrome is characterized by mental retardation, pre and postnatal growth retardation, facial dysmorphism, brain anomalies, and limb defects. It is suggested that monosomy 22p11.2 has no clinical significance.

M. Ikbal: None. **T. Tos:** None. **M.Y. Alp:** None.

J02.72
Case report of unbalanced tpanslocation involving chromosome 3 and 7.

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Background: The most common familial chromosomal rearrangements are translocations. In case of an unbalanced segregation in offsprings, the resulting imbalances consist of a combination of partial trisomy and partial monosomy. Phenotypic reports of chromosomal imbalances are an important source for genetic counselling.
Clinical case: We've performed clinical and genealogical examination of five month-old boy with primary diagnosis "bilateral ureterohydronephrosis" and unusual phenotypes: overweight (growth 60cm,weight 8,6 kg) disproportionately short limbs, microcephaly, micrognathia and short neck. At the first month of life, he had a perirectitis. During the heart examination, the foramen ovale with the extension of the right ventricle was determined. A case of the dextrocardia was reportes in the family pedigree. Moreover, the mother was treated Hodgkin's disease eight years ago.
Results: An additional material of unknown origin was detected on a distal part of a long arm (q) of the chromosome 7 in blood lymphocytes culture (analized with GTG- banding). Proband's arrangement was a result of maternal reciprocal translocation t(3;7)(p25;q36). Thus the proband's karyotype was 46,XY,der(7)t(3;7)(p25;q36)mat. So partial trisomy of the short arm chromosome3 and partial monosomy of the long arm chromosome7 were found.
Conclusion: Unbalanced tranclocation in proband chromosomes3 and 7 was cause of rare phenotype of ureterohydronephrosis. Multiply congenital malformation in family members got explanation. Clinicians should give more consideration to rare genetic chromosomal syndromes, especially in the case of unusual combinations symptoms.

V. Rumyantseva: None. **N. Oparina:** None. **Z. Sabirzyanova:** None. **A. Pavlov:** None. **E. Zaklyazminskaya:** None.

J02.73
Identification of unbalanced translocation 4;8 characterized by metaphase CGH

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We report a case of a 2-year-old child with growth retardation, physical abnormalities, hypotonia and development delay. G-banding chromosome analysis showed a normal karyotype. Molecular cytogenetic investigations were performed to revealed a chromosomal abnormalities. Metaphase comparative genomic hybridization (CGH) analysis showed loss of chromosome 4p16.1 and gain of chromosome 8p23.1. FISH with subtelomeric probes of chromosome 4 and 8 confirmed the deletion of the distal short arm of chromosome 4 and the duplication of the distal short arm of chromosome 8. This finding was found by reinspection of the G-banded karyotype at the 500 band level. GTG-banding allowed to interpret proband´s karyotype as: 46,XY, der(4)t(4;8)(p16.1;p23.1). The mother´s karyotype was normal, father was not available for karyotyping. This case of unbalanced translocation 4;8 demonstrates the usefulness of the metaphase CGH method. The aberration had previously been missed by routine analysis of G-banded karyotyping. This clearly illustrates a weakness of the GTG-banding technique in comparison to CGH. G-band analysis is dependent on the qualifications and the actual performance of the cytogeneticist and this makes the technique somewhat subjective. CGH technique has some advantages for of unbalanced chromosome aberrations: it gives a global approach which allows an objective analysis of all chromosomes in a single in situ hybridization experiment without cell culture. Metaphase CGH is non expensive and rapid technique which can be used routinely to explore patients with mental retardation and dysmorphic features.

M. Minzhenkova: None. **N. Shilova:** None. **Z. Markova:** None. **S. Korostelev:** None. **Y. Kozlova:** None. **T. Zolotukhina:** None.

J03.01
The ADAMTS18 gene is responsible for autosomal recessive early onset severe retinal dystrophy

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Background: Inherited retinal dystrophies, including Retinitis Pigmentosa and Leber Congenital Amaurosis among others, are a group of genetically heterogeneous disorders that lead to variable degrees of visual deficits. They can be caused by mutations in over 100 genes and there is evidence for the presence of as yet unidentified genes in a significant proportion of patients. We aimed at identifying a novel gene for an autosomal recessive form of early onset severe retinal dystrophy in a patient carrying no previously described mutations in known genes.
Methods: An integrated strategy including homozygosity mapping and who-

le exome sequencing was used to identify the responsible mutation. Functional tests were performed in the medaka fish (Oryzias latipes) model organism to gain further insight into the pathogenic role of the ADAMTS18 gene in eye and central nervous system (CNS) dysfunction.
Results: This study identified, in the analyzed patient, a homozygous missense mutation in the ADAMTS18 gene, which was recently linked to Knobloch syndrome, a rare developmental disorder that affects the eye and the occipital skull. In vivo gene knockdown performed in medaka fish confirmed both that the mutation has a pathogenic role and that the inactivation of this gene has a deleterious effect on photoreceptor cell function.
Conclusion: This study reveals that mutations in the ADAMTS18 gene can cause a broad phenotypic spectrum of eye disorders and contribute to shed further light on the complexity of retinal diseases.

I. Peluso: None. **I. Conte:** None. **F. Testa:** None. **M. Pizzo:** None. **R.W.J. Collin:** None. **N. Meola:** None. **S. Barbato:** None. **M. Mutarelli:** None. **F. Simonelli:** None. **S. Banfi:** None.

J03.02

A novel gross deletion in DFNB1 locus involving GJB2 and GJB6 genes is recurrent in Russian patients

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Mutations in *GJB2* gene are the most commonly identified cause of congenital, recessively inherited, sensorineural nonsyndromic hearing loss (DFNB1). More than 300 mutations in *GJB2* gene are known and were detected in the most patients with DFNB1. In several countries the gross deletions in DFNB1 locus are observed with a considerable frequency. Among four known deletions in DFNB1 only one deletion involves *GJB2* gene and was observed in single family. Here we report about a novel 101.4 kb deletion involving *GJB2* and *GJB6* genes. This deletion, NC_000013.10: g.20,757,021_20,858,394del, was revealed in three unrelated Russian patients, probably has a Ingush descent. Additional population studies are necessary to estimation of the deletion frequency. If the novel deletion is frequent, a molecular testing for it is very important to genetic counseling.

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J03.03

Examination of the genetic background of aminoglycoside induced and non-syndromic, hereditary sensorineural hearing loss

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Objectives: With the development of molecular genomics we can recognise the connection between more and more genetic variations and medications side effects and we can discover
an increasing number of genetic differences behind idiopathic symptoms. The mutations of 12S rRNA gene which encoding by mitochondrial genome are frequent causes of both aminoglycoside induced and non-syndromic sensorineural hearing impairment. The transitions make the human mitochondrial 12S ribosomes more bacteria-like and alter binding sites for aminoglycosides.
Aims: To define the frequency of occurrence in the Hungarian population of A1555G and C1494G substitutions of the mtDNA.
Methods: Participants of the research were: (1) 300 patients suffering in sensonerual syndromatic or non-syndromic hearing loss (37 patients with aminoglycoside induced, 144 with mitochondrial disease and 119 with non-syndromic) and 200 volunteering control participants. Among the patients both sporadic and familial cases were present. The mtDNA substitutions were screened by PCR-RFLP methodology.
Results: Out of examined 500 participants, the A1555G mutation appeared in 12 cases (5 patients and 7 control persons). The frequency of the mutation in the population was 2.4%. In 2 of these patients maternally inheritable hearing loss could have been discovered. The C1494G substitution was not present in these cohorts.
Conclusion: The targeted examination of mtDNA may render the screening of carriers possible in the future, which may serve as the basis for the appearance of personalized therapies. However further investigations are also necessary to detect other genetic risk factors for aminoglycosid induced ototoxicity.

G.M. Milley: None. **A. Gál:** None. **L. Noszek:** None. **P. Prekopp:** None. **L. Tamás:** None. **M.J. Molnár:** None.

J03.04

Prevalence of GJB2 (Connexin-26) and GJB6 (Connexin-30) mutations in a 123 Iranian Azeri Turkish hearing-impaired individuals: implications for diagnosis and genetic counseling

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Hearing impairment (HI) is the most common heterogeneous neurosensory disease in humans. More than 50% of cases have a genetic etiology, including syndromic and non-syndromic forms. For many populations, mutations in two connexin (Cx) genes, GJB2 (Cx26) and GJB6 (Cx30) have been identified 50 % to cause hearing impairment
In this study, we report our 4-year experience of over 123 unrelated Azeri Turkish patients with hearing impairment, who were not affected by known deafness related syndromes.
123 Azeri Turkish unrelated subjects clinically diagnosed with HI from various clinics from North West of Iran were referred by specialists to the Medical Genetic Centre of Tabriz University of Medical Sciences. A clinical diagnosis of HI was made according to published criteria. Mutation screening of the GJB2 and GJB6 genes were performed by using direct sequencing method.
54 (% 43) of these patients had one or two mutations. Of those with mutations, 22 were compound heterozygous, 19 were homozygous, and 13 had only one identifiable mutation.
The most frequent mutations were c.35delG and V153I (25% and 16% respectively) of the alleles, followed by V27I, V71K, A171T, V114G, R127H, M163V, G160S, c.119delC, c.120delA, and c.51insA mutations in GJB2 gene and no mutation in GJB6 gene was detected in our population.
The present study demonstrates that mutations in the GJB2 are important cause of hearing impairment and mutations in the GJB6 gene don't play any important role of hearing impairment in Iranian Azeri Turkish population, thus justifying their screening in a routine basis.

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J03.05

Prenatal ultrasound diagnosis of cataract congenital in two siblings with microduplication 22q11.23

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The case report presents the prenatal ultrasound findings of cataract oc. utr. in two brothers. Microduplication 22q11.23 was detected in both fetuses and their healthy mother. The beta-crystallin genes CRYBB2 (crystallin betaB2) and CRYBB3 (crystallin betaB3) lie within the region 22q11.23 and are therefore candidate genes. The relationship between the mutations of these genes and nonsyndromal cataract is known. Different beta-crystallin proteins can interact with each other to form oligomers of different sizes ranging from dimers to octamers and can also interact with other lens proteins. The protein-protein interactions are predicted to be key in maintaining the transparency of the lens.

D. Raskova: None. **M. Hynek:** None. **M. Trkova:** None. **D. Stejskal:** None.

J03.06

The analysis of the GJA8 gene in patients with isolated form of congenital cataract from Bashkortostan Republic (Russia).

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Introduction: Cataracts are one of the leading causes of blindness in humans, and mutations in the connexin 46 (GJA3) and the connexin 50 (GJA8) genes cause congenital cataract. Different mutations in these genes lead to the development of distinct cataract phenotypes. The aim of the study was to analyze the GJA8 gene in patients from Bashkortostan Republic affected with congenital cataract.
Objective: DNA samples of 40 unrelated patients with isolated form of hereditary congenital cataract from Bashkortostan Republic were analyzed.
Methodology: The analysis was performed by direct sequencing of coding regions of the GJA8 gene.
Results: Four different nucleotide alterations were detected. In one patient of Russian ethnic origin with zonular form of cataract nucleotide substitution c.179G>A (p.Gly60Asp) was detected; one patient of Bashkir ethnic origin

with anterior capsular cataract carried the deletion c.133-142delTGGGGGGATG (p.Trp45SerfsX72). Both of these changes were found in the heterozygous state, have not been described in the literature earlier, and presumably are functionally significant mutations. In one patient new nucleotide substitution c.288G>A (p.Ala96Ala) in the homozygous state was found. Two patients demonstrated nucleotide substitution c.68G> T (p.Arg23Thr), known as polymorphic variant (rs80358203).
Conclusion:Thus, three previously undescribed structural changes in the gene GJA8 were detected in patients with hereditary isolated cataract from Bashkortostan Republic. To determine their functional significance further investigation are required.

I.I. Khidiyatova: None. **I.M. Khidiyatova:** None. **T.P. Fedorova:** None. **S.R. Avkhadeeva:** None. **M.T. Aznabaev:** None. **E. Khusnutdinova:** None.

J03.07

Genetic Linkage Analysis of DFNB loci in ARNSHL pedigrees in Southern Khorasan Province in Iran

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Hearing loss is the most common sensory birth defect with an incidence of about one in 650 newborns. Approximately 70% of genetic HL cases are autosomal recessive non-syndromic (ARNSHL) and it may become even higher in countries with high rate of consanguineous marriage. Iran has a heterogeneous population due to different ethnicities and also a high rate of consanguineous marriage (38.6%). The fact that many loci are involved together with the heterogeneity of the status, necessitate studying further loci in various Iranian ethnic groups. 22 large deaf pedigrees originating from the Southern Khorasan province of Iran were selected. The families analyzed for *GJB2*, exon I & II, mutations and *GJB6* large deletions. Pedigrees negative for *GJB2* mutations were then subject to linkage analysis for loci DFNB2, DFNB3, DFNB4, DFNB7/11, DFNB9, DFNB21 & DFNB59. DFNB4, DFNB3, DFNB21 & DFNB59 have been analyzed and the project is proceeding for other loci.
Four out of the 22 families showed *GJB2* mutations. One family carried homozygous c.35delG mutation and another one carried homozygous p.W24X mutation. The third pedigree patients were compound heterozygous for p.W77X/IVS1G>A. c.380 G>A mutation. The fourth pedigree showed heterozygote mutation of p.V27I+E114G/wt. GJB6 deletions were not detected. One family showed linkage to DFNB3 and the remaining did not show linkage to the studied loci.
Our results once again emphasize the heterogeneity of HL among different Iranian ethnic groups. These results could be applied to a more efficient genetic screening and genetic counseling. This study proceeds with more loci and more families.

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J03.08

Analysis of a conserved GJB2 intronic region in nonsyndromic sensorineural hearing loss patients with only one previously identified GJB2 variant

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The DFNB1 locus (GJB2 and GJB6 genes) is a major cause of nonsyndromic sensorineural hearing loss (NSSHL), being responsible for over 1/5 of the Portuguese cases. However, some patients present only one recessive mutation. The frequency of such individuals is, in several instances, higher than the expected if they were only carriers. This fact suggests the existence of an accompanying DFNB1 mutation in some cases. We have noticed a conserved region in the GJB2 intron, which might be a potential location of pathogenic mutations.
We have thus investigated that conserved region in 27 NSSHL Portuguese patients (previously analysed regarding the basal promoter, exon 1, donor splice site, coding region and 3'UTR) harbouring one pathogenic, controversial or unclear GJB2 mutation, and none of the two common GJB6 deletions (del1830 and del1854). The control sample consisted in 42 normal-hearing individuals from the general population. The 69 subjects were analysed by direct sequencing.
A total of seven known variants in the intronic region analysed (c.-22-1207G>C, c.-22-1203A>G, c.-22-1198G>A, c.-22-1157A>T, c.-22-1153G>A, c.-22-1108G>A, c.-22-1073A>G) were identified among patients and controls, of which four were found only in controls. No variant was identified exclusively in the patients. A novel variant (c.-22-1415A>G) was found in a control individual.
The investigation here presented did not reveal, in the individuals analysed, any novel potentially pathogenic variant in the conserved intronic region. However, this region could be further investigated in other patients with only one GJB2 mutation, since it might play a role in the regulation of the gene.

T.D. Matos: None. **H. Simões-Teixeira:** None. **A.C. Gonçalves:** None. **J.R. Chora:** None. **H. Rosa:** None. **L. Monteiro:** None. **A. O'Neill:** None. **Ó.P. Dias:** None. **M. Andrea:** None. **H. Caria:** None. **G. Fialho:** None.

J03.09

Reduced expression of the OPA1 gene due to a novel nonsense mutation leads to autosomal dominant optic atrophy

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Purpose: Autosomal dominant optic atrophy (ADOA) is the most prevalent dominantly inherited optic neuropathy. The aim of the study was to identify the genetic etiology of inherited optic neuropathy in a Polish family.
Methods: We report on a 2-generation Polish family with ADOA in which nine family members are affected. MRI and detailed ophthalmological examination with visual field and electrophysiological testing were performed. DNA and protein extracts were obtained from blood samples. Linkage to OPA1, the main ADOA locus, as well as sequencing of all *OPA1* exons were conducted. Amplified fragments were analyzed on an automatic DNA sequencer. OPA1 expression was analyzed by Western blot.
Results: MRI and ophthalmological examination confirmed the diagnosis of bilateral optic neuropathy. Pattern visual evoked potentials (PVEP) presented abnormally delayed P100 wave latency. Pedigree analysis demonstrated a dominant mode of inheritance. Linkage studies revealed linkage to the major OPA1 locus in the investigated family. Sequencing of the *OPA-1* gene identified a novel C-to-T transition in exon 2 predicting a premature stop codon (*p.Q31Ter*). The mutation co-segratated with the phenotype in this family. Expression of OPA1 at the protein level was strongly reduced as compared to control samples from healthy individuals.
Conclusion: Occurrence of the premature termination codon at the beginning of the transcript, which leads to a strong reduction of OPA1 protein expression, confirms that ADOA in the investigated family is a consequence of OPA1 haploinsufficiency. The novel variant broadens the spectrum of the reported *OPA1* mutations causing ADOA.

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J03.10

Frequency of the p.Q829X *OTOF* mutation in Greek patients suffering from sensorineural, non-syndromic hearing loss

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Approximately one or two in 1,000 children suffer from deafness that appears at birth or before the age of two (prelingual deafness). About 50% of the cases presenting with non-syndromic prelingual hearing loss in the developed world are due to genetic causes, making genetic deafness one of the most frequent hereditary conditions in humans. Most mutations associated with the disease in Caucasians have been mapped in the DFNB1 locus, with the *GJB2* gene having the most important role. Several studies

have shown that the point mutation p.Q829X in the *OTOF* gene located in the DFNB9 locus is responsible for up to 3% of the cases with non-syndromic deafness in the Spanish population, while similar results have been reported in studies from South American countries and the USA. In the present study we investigated the frequency of the p.Q829X *OTOF* mutation in the Greek deafness population. A total of 100 unrelated Greek patients suffering from non-syndromic, sensorineural hearing impairment with an age of onset in childhood, were recruited. We employed the PCR-RFLP method in order to genotype our samples. Our results showed that all patients were tested negative for the p.Q829X *OTOF* mutation, indicating that the mutation is rare in the Greek population. The study is proceeding in order to explore a geographic correlation among allele frequencies in the Mediterranean region.

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J03.11

Microdeletion 2q11.1q11.2 including haploinsufficiency of SNRNP200 gene: two families report without clinical manifestations of retinitis pigmentosa

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Deletions involving the proximal region of chromosome 2q11 have been rarely reported to date. We describe two patients with overlapping microdeletions at 2q11.1q11.2 encompassing SNRNP200 gene recently associated with autosomal dominant retinitis pigmentosa (adRP).

The patient 1 is a 10 year-old boy and patient 2 is a 7 year-old girl, both referred to our department because of global psychomotor development delay. The patients exhibit minor dysmorphisms including downslating palpebral fissures and slight micrognathia. However patient 1 is macrocephalic and patient 2 has microcephaly with the stature below the 3rd centile.

Array-CGH (180K Agilent) was performed and Fluorescence in situ hybridization using specific BAC clones. A similar deletion spanning about 1,5Mb was observed in both patients, with the involvement of 12 genes described in OMIM database, three of them associated with disease: TMEM(OMIM#613403); SNRNP200 (OMIM#601664) and CNNM4 (OMIM#607805). Patient 1 deletion was inherited from his father and in patient 2 the deletion was de novo.

The patient 1 and his father were submitted to complete ophthalmological examination that failed to demonstrate pigmented changes in the retinal periphery. Patient 2 examination also revealed normal results. In the families already reported with adRP the average age of onset is different, from 7 to 17 years of age .However, the father of patient 1 has already 36 years old and to date has no clinical manifestations of RT. It is difficult to ascertain if the phenotypic effect of the missence mutations already reported could be different from the haploinsufficiency of the SNRNP200 gene.

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J03.12

Prevalence of the GJB6 gene deletion in nonsyndromic hearing loss in Mexican population

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About 1/1000 children are affected by pre-lingual deafness. Several genes have been associated with recessive hearing impairment. The GJB6 gene deletions are one of the causes of hereditary, prelingual, nonsyndromic hearing loss; they present an autosomal recessive inheritance. More than 90 GJB2 mutations affecting Cx26 expression have been reported and linked to hearing loss. Objective: To identify the prevalence of GJB6 deletion in a sample of Mexican patients. Methods The study included 246 patients from 105 non-related families with hereditary, prelingual, nonsyndromic hearing loss, all of them were analyzed through PCR and DNA sequencing from genomic DNA. Results and Discussion: We found only in one family the presence of GJB6 gene deletion in heterozygous state. We discuss the prevalence of this type of mutations in the Mexican sample and compare with the data previously reported in other populations.

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J03.13

Genetic studies in deaf individuals with inconclusive diagnostic molecular.

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Hearing impairment is the most common sensory impairment affecting approximately one in five hundred newborns. Despite the enormous heterogeneity of genetic hearing loss, up to 50% are associated with mutations in the locus DFNB1. This locus contains the *GJB2* and *GJB6* genes, which codes for the connexin 26 (Cx26) and connexin 30 (Cx30) proteins, respectively. However, a large number of affected individuals carry only a single recessive mutation in locus DFNB1, which causes problems in molecular diagnostic. The aim of this study was to elucidate the genetic cause of hearing loss in 48 monoallelic individuals for recessive mutations in the *GJB2* gene or in *GJB6* gene. Thus, mutations that are not routinely screened were investigated in these individuals. The del(CHR13 :19,837,344-19, 968698) deletion in *GJB6* gene, mutations in the cluster *miR-183* and a large number of mutations in fourteen mitochondrial genes were screened in these samples. Additionally, we investigated the presence of deletions in the DFNB1 locus, not yet described. In this study, the deletion del(CHR13 :19,837,344-19, 968698) have not been found in subjects analyzed. Five single nucleotide polymorphisms (SNP) were identified in the *miR-183* cluster. Probable deletions in the DFNB1 locus were found in 4 heterozygous individuals for mutations in *GJB2* using mass spectrometry system MassArray, Sequenom®. Besides, among the subjects studied were still found eight mitochondrial alterations detected in eighteen cases. Detection of mutations in different genes reduces the number of cases with diagnosed inconclusive. However, due to heterogeneity of genetic hearing loss many still remain without diagnosis.

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J03.14

Genetic counselling in ametropia

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Ametropia, a state where a refractive error is present, can be myopia when the eyeball is too long and a point at infinity focuses in front of the retina; hyperopia when the eyeball is too short and an image focuses behind the retina; and astigmatism when the eye has different focal points in different planes.

The purpose of this study is to present the difficulties of genetic counselling in familial nonsyndromic ametropia.

31 patients with different types of ametropia where sent to a genetic consultation between 2011 and 2013. 22 had sporadic ametropia. 30 cases had no other signs or symptoms associated. In 25.80% of cases, there was at least one more family member affected by the same or a different type of ametropia. From the four cases of myopia only one was a high grade type (-10 diopters) and had an autosomal dominant inheritance pattern. One other case diagnosed with myopia and astigmatism had the mother with multiple sclerosis. 2 more cases had hyperopia and astigmatism and one case had hyperopia at one eye and myopia at another.

We discuss the inheritance pattern in each of the familial cases, considering that genetic factors play a significant role in the development of nonsyndromic ametropia irrespective of the degree of the illness. Pedigrees are selectively analyzed, and biases (because of genetic heterogeneity, phenocopy, and environmental factors) interpreted.

In conclusion, usually a careful family history may reveal the inheritance pattern of an isolated refractive error for a given family, making counselling easier.

A. Gugu: None. **R.M. Dragotoiu:** None.

J03.15

A new mutation in*SCN9A* gene in a Moroccan patient with congenital insensitivity to pain

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Pain is a highly conserved sensory modality among all vertebrate species which confers protection against noxious and dangerous stimuli. Loss of pain sensation or hypersensitivity to pain can compromise survival.
SCN9A (Sodium channel protein type 9 subunit alpha) encodes the voltage-gated sodium channel Nav1.7, a protein highly expressed in pain-sensing neurons. Mutations in SCN9A cause three human pain disorders: bi-allelic loss of function mutations result in Channelopathy-associated Insensitivity to Pain (CIP, OMIM: 243000), whereas activating mutations cause severe episodic pain in Paroxysmal Extreme Pain Disorder (PEPD OMIM: 167400) and Primary Erythermalgia.
Congenital insensitivity to pain is an autosomal-recessive disease caused by inactivating mutations in the Nav1.7channel. To date, almost mutations in SCN9A are protein truncating and presumably lead to no protein being produced. Recently, the study proved that Nav1.7 is an essential requirement in olfactory pathway.
Here, we confirm this suggestion bye describing a 2 years old girl with CIP and a congenital general anosmia confirming bye a novel homozygous nonsense mutation in SCN9A.

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J03.16
Polymorphous ophthalmologic symptoms of patients with Down syndrome

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Aim of the work. Investigation of ophthalmic status of patients with Down syndrome (DS)
Material and methods. There were 11 patients (22 eyes, 8 males and 3 females aged 6 mo to 16 yr) with DS (full trisomy 21) under our observation. Observation period was from 2 mo to 6 yr. Routine basic ophthalmic and pediatric investigations were performed for all patients.
Results. Ophthalmic status of all patients included up-slanting palpebral fissures, bilateral medial epicanthal folds. 4 children (8 eyes) had congenital inverted eyelid with early corrective surgery (2 cases) and conservative keratoplasty therapy (2 cases). 2 patients aged 8 mo and 1 yr (4 eyes) were operated on congenital diffuse cataract without IOL and corrected with aphakic glasses. 3 patients (6 eyes) had hypermetropia of high degree. One patient (2 eyes) had myopia of high degree. 1 patient (2 eyes) had incomplete congenital central corneal opacity without surgical
intervention.
Conclusion. Early ophthalmic examination and specific treatment help to ensure adequate vision and social adaptation, including family adaptation of the patients with DS.

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J04.01
Medullary cystic kidney disease - report on two novel *UMOD* mutations and pitfalls of diagnostics

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Background: Medullary cystic kidney disease (MCKD) is a rare autosomal dominant renal disease characterized by medullary cysts, tubulointerstitial nephritis, gout and progressive chronic renal failure leading to end-stage renal disease (ESRD). Mutations in the UMOD gene (16p12.3), encoding the Tam-Horsfall glycoprotein, were found to associate with MCKD type 2. We report two families with the unusual characteristics of members affected by a nephropathy distinct from the MCKD.
Methods: Pedigrees' evaluation included renal ultra-sound and pathology with immuno-histochemical evaluation for Tam-Horsfall glycoprotein. Genetic screenings were performed by PCR, direct sequencing and MLPA.
Results: In both MCKD families, clinical findings showed intra-familial variability. Two novel UMOD mutations segregating with the phenotype were identified. However, both families included individuals presenting a non-MCKD renal phenotype: for pedigree 1, a case of polycystic kidney disease displaying a novel PKD1 mutation; for pedigree 2, an adult-onset nephrotic syndrome with a membranous pattern on the renal specimen. No UMOD mutations were found in these individuals.
Conclusions: We present two families demonstrating simultaneous occurrence of different kidney diseases, all potentially causing ESRD and with three novel mutations in total. Our results show that a) if two rather common nephropathies, like ADPKD and membranous nephropathy, can concur with MCKD, the latter is probably more prevalent than previously anticipated; b) for a given pedigree, the diagnosis of an inherited "*reno*type" should not be *a priori* extended to all affected members, particularly if the phenotype presents with variable expressivity or if ESRD is considered the sole undisputable renal phenotype characteristic.

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J04.02
VEGFA gene expression in liver cells after mesenteric ischemia and the possible affects of montelukast

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Objective: The aim of our study was to investigate the effect of mesenteric ischemia on *VEGFA* gene expression in rats liver cells, and the possible affects of montelukast on this process
Material - Method: The study protocol was approved by the Local Ethical Committee of Laboratory Animals at Abant Izzet Baysal University. A total of 24 female Wistar albino rats were used in our study. The rats were randomized into four groups as follows: group A: Sham-controls (n:6), group B: Ligation (n : 6), group C: Ligation and montelukast (n :6), group D: control and montelukast (n : 6). RNA was extracted from the liver cells. The expressions of *VEGFA* were analyzed using the Reverse Transcription Polymerase Chain Reaction. Relative quantification of mRNA was carried out using the comparative CT method.
Results: When the gene expression values of all groups were compared, a significant difference was observed at group D. It showed %100 percent increased VEGFA gene expression according to the reference group, while the expression levels of group C and group B were lower than that of group D. Also, when groups C and B were compared, the expression level in group C was found to be higher than that of group B, although the difference was not statistically significant.
Conclusion: This study demonstrates that montelukast supplementation may have a protective effect from ischemia induced liver toxicity in rat liver by causing an incremental effect on *VEGFA* gene expression in rat liver cells.

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J04.03
Hereditary Intrahepatic Cholestasis of Pregnancy and Low Phospholipids Associated Cholelithiasis: prevalence of ABCB11 variants in ABCB4 non- mutated patients.

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Inherited intrahepatic cholestasis and cholelithiasis represent a heterogeneous group of autosomal recessive liver disorders that may become symptomatic in adulthood. These disorders affect secretion of the main bile constituents and genetic studies identified sequence variations in biliary transporter genes. We aimed to 1) identify the ABCB4 and ABCB11 sequence variations in patients from Saint Antoine Hospital rare metabolic biliary diseases centre. 2) determine the prevalence of ABCB4 and/or ABCB11 sequence variations in 2 related cholestatic/cholelithiasic diseases i.e.: Low Phospholipids Associated Cholelithiasis (LPAC) or Intrahepatic cholestasis of Pregnancy (ICP). Methods: Patients were diagnosed LPAC (n=580) or ICP (n=200). DNA was extracted from blood using the Puregene kit (Qiagen). Specific PCR of 27 exons and adjacent junctional introns were run and

sequenced by Sanger technology. Whole BSEP gene was sequenced in 112 patients. Moreover, the bi-allelic status of the variant c.1331T>C of ABCB11 gene was studied in 490 patients. Fisher Test was performed for statistics. Results: ABCB4 variants were found in 50% of LPAC, and 27% of ICP. Homozygous polymorphous ABCB11 c.1331T>C was found in 94 of 290 ABCB4 non-mutated LPAC patients and 71 of 148 ABCB4 non-mutated ICP (32,4% vs. 48,0%, respectively, p<0.002). Other ABCB11 mutations were found in 3 of 60 ABCB4 non-mutated LPAC and 11 of 52 ABCB4 non-mutated ICP (5% vs. 21.2%, respectively, p<0,02). The presence of ABCB11 variants in non-mutated ABCB4 patients was found significantly more frequent in ICP than in LPAC, supporting a relationship between ABCB11 sequence variation and ICP.

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J04.04

Association study of cytokine genes polymorphisms revealed a strong association of rs1143634 of IL1B gene with peptic ulcer disease in Volga-Ural region of Russian Federation

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Ulcer disease, that is, gastric (GU) and duodenal (DU) ulcers, is a focal mucosal defect with inflammatory cell infiltration and coagulation necrosis extending through the muscularis mucosa. The genes that encode proinflammatory and anti-inflammatory cytokines are good candidate markers of host susceptibility to gastroduodenal disease.
The present study was performed to evaluate whether or not the five genetic polymorphisms of IL1B (3953C>T; rs1143634), IL1-RN (VNTR polymorphism; rs71941886), IL8 (-251T>A; rs4073), IL10 (-627C>A; rs1800872) and TNFA (-308G>A; rs1800629) genes are associated with peptic ulcer disease (PUD) in Volga-Ural region of Russian Federation.
This study enrolled 264 patients with gastric and duodenal ulcers, the control group included 282 unrelated individuals without gastro-duodenal pathology with different ethnic origins (Russians, Tatars, Bashkirs). Genotyping was performed by polymerase chain reaction - restriction fragment length polymorphism analysis.
The analysis has revealed a strong association of *C/*C genotype of the 3953C>T of the IL1B gene with PUD in common group (OR=1,7; P=0,003; $\chi2$=1,7). The control individuals had significant higher frequency of *C/*T heterozygous genotype of this SNP than patients (OR=0,6; P=0,02; $\chi2$=5,7). We have also detected in Tatars that frequency of *A/*A genotype of the -627C>A of the IL10 gene are significant more prevalent in healthy donors than in PUD-individuals (OR=0,3; P=0,05; $\chi2$=3,8). No significant difference was observed in allele or genotype frequencies of other investigated polymorphisms between PUD patients and control group (p>0,05).
Thus, we have determined statistically significant association between IL1B gene polymorphism and peptic ulcer in Volga-Ural region of Russia.

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J04.05

Relationship between ADRB2 gene polymorphism and bronchial asthma course.

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Background: functional activity of β_2-adrenoreceptor (ADRB2) can determine susceptibility of bronchial muscles to endogenous and exogenous β-agonists and influences on bronchial conductance.
Objectives: In the present study we have investigated G/R polymorphism in 16th codon of ADRB2 in bronchial asthma patients and the relationships of these polymorphic genotypes with BA course.
Methods: G/R polymorphism in 16th codon of ADRB2 was detected by RFLP in 337 children with BA. According disease course all children were divided in 3 subgroups: mild, moderate and severe.
Results: we haven't revealed significant differences in polymorphic genotypes distribution according to disease course. In analysis of genotype distribution depending disease onset we found that GG genotype was more frequent in children who developed severe BA in 1st year of life (p=0.039). Also GG genotype was associated with mild disease course in children with BA onset after the 5th year (=0.001). Prevalence of GG genotype due to disease course and onset in table is shown below.
Conclusions: GG genotype of ADRB2 associated with severe disease course in early onset BA and mild course in children who had onset after 5th year.

Table 1

BA onset, years	Mild BA course n (%)	Moderate BA course n (%)	Severe BA course n (%)
<1	2 (15.4)	3 (23.1)	8 (61.5)
1-5	23 (33.8)	26 (38.2)	19 (28.0)
>5	16 (50.0)	10 (31.3)	6 (18.8)
			p=0.039

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J04.06

The contribution of polymorphisms of ACE, SLC6A4, AGT, ANK3, APOE, COMT, LMX1A, MTHFR and NGF genes to the cognitive dysfunction (CD) in patients with metabolic syndrome (MS)

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Background. Inherited risk factors of cardiovascular and endocrinological diseases also play a role in the CD. Early diagnosis of CD in patients with MS is an important medical and economic problem.
Design. Pilot case-control study.
Aim: to determine the role of mutations in 9 genes (table 1) in the CD in patients with MS.
Materials and methods. 883 unrelated Caucasian participants (at working age) were screened for MS and CD by anthropometry, weighing, blood pressure test, blood chemistry (glucose, cholesterol, triglycerides), and neuropsychological tests. Then 30 males and 25 females (46,6±5,9 years old) with the most representative symptoms were underwent detailed examination with PCR-RFLP analysis of 9 genes (table 1). Statistical analysis was performed by Statistica 8.0.
Results. Allele *D* of *ACE* was associated with MS, but not with CD. Other eight genes demonstrated association of their polymorphisms with CD; see table 1 for unfavourable alleles.

Table 1

Gene	Polymorphism	CD-associated allele
ACE	*I/D*	*n/a*
SLC6A4	*L/S*	*S*
	rs25531	*G*
AGT	*rs699*	*C*
ANK3	*rs10994336*	*T*
APOE	*rs429358, rs7412*	*E4*
COMT	*rs4680*	*A*
LMX1A	*rs4657412*	*C*
MTHFR	*rs1801133*	*T*
NGF	*rs6330*	*C* for females, *T* for males

Furthermore, authors revealed six clinical phenotypes caused by sex and *rs6330* (table 2):

Table 2

Sex	*NGF* genotype		
	CC	*CT*	*TT*
Males	↑MS	↓ MS, ↓ CD	↑ CD
Females	↓ MS	↑MS, ↑ CD	↓ CD

Conclusion. Alleles of *SLC6A4*, *AGT*, *ANK3*, *APOE*, *COMT*, *LMX1A*, *MTHFR*, *NGF* contributed to the CD in patients with MS, and for *NGF* that association was sex-related.

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J04.07

APOE polymorphisms are not associated with the risk of coronary artery disease and in-stent restenosis in patients undergoing coronary artery stenting

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Association of *APOE* polymorphisms with coronary artery disease (CAD), the risk of in-stent restenosis and correlation with plasma apolipoprotein E levels were assessed in patients undergoing coronary artery stenting. There were 269 subjects undergoing coronary artery stenting included. The *APOE* genotype frequencies were compared to the control group including 98 normal cholesterol non-CAD subjects; correlation with in-stent restenosis occurrence was also performed. *APOE* genotype was determined using real-time PCR. In 137 CAD patients, apolipoprotein E plasma levels were measured using ELISA method. In CAD patients, the distribution of *APOE* genotypes was following: ε3/ε3, 177 (65,8%); ε3/ε4, 58 (21,6%); ε4/ε4, 3 (1,1%); ε2/ε3, 26 (9,7%); ε2/ε4, 5 (1,8%); ε2/ε2, 0. There was no statistically significant difference in genotype distribution both compared to the control group (p=0,732) and between the in-stent restenosis (n=97) and non-in-stent restenosis groups (n=134) (p = 0,432, Fisher' exact test). Apolipoprotein E levels were significantly higher in patients with ε2/ε3 and ε2/ε4 genotypes (median 29,2 μg/ml) compared to ε3/ε3 (median 18,4 μg/ml) and ε3/ε4+ε4/ε4 (median 12,5 μg/ml) ones (p=0,017 and p=0,015, respectively, Mann-Whitney U test with Bonferroni correction). In conclusion, our results do not support the hypothesis that *APOE* genotypes are associated with an elevated risk of CAD or the risk of in-stent restenosis in patients undergoing coronary artery stenting. ε2/ε3 and ε2/ε4 were associated with higher plasma apolipoprotein levels than ε3/ε3 and ε3/ε4+ ε4/ε4 genotypes.

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J04.08

Genetic basis and clinical symptoms of Azeri patients suspected with the risk of Fanconi Anemia

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Fanconi anemia is the most prevalent inherited aplastic anemia. A diagnosis based wholly on the recognition of clinical symptoms is not reliable. The aim of this study was to investigate the clinical symptoms and points of possible chromosomal breakage in people with the risk of FA in the Azerbaijan region of Iran. A cross-sectional descriptive-analytic study was conducted on 20 patients,

suspected with the risk of FA, in which were confirmed with medical examinations. The type and number of chromosomal disorders were determined. The clinical disorders of the patients were studied with reference to their medical records. The average age of the patients within the study was 9.6 years. 8 of the cases had familial relations, 7 of which were cousins. 9 patients had co-morbid anemia, 8 patients suffered from platelet deficiency and 9 patients had hand and/or finger deformities. Using cytogenetic testing, FA was diagnosed in 5 of the cases. The percentage of mitotic abnormalities in the chromosomeswithout administration of mitomycin C varied between 5-30% in the cultures of the 5 affected patients and between 0-4% in the 15 unaffected patients. With the administration of mitomycin C, these percentages increased to 35-78% and 0-20%, respectively. The percentage of mitotic abnormalities and average numbers of chromosomal breakages in patients with Fanconianemia is higher to that of unaffected patients and control samples. Considering the variety of clinical symptoms for this illness, it is of great importance to conduct cytogenetic testing in order to obtain a definitive diagnosis.

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J04.09

Blood pressure and M235T AGT gene polymorphism in children with hypertrophic cardiomyopathy

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The polymorphism of adrenoreseptors, bradycardin and renin-angiotensin systems (RAS) genes play a significant role in the pathogenesis of essential hypertension and cardiovascular diseases.
We investigated the association the genes polymorphisms with the clinical course and changes in blood pressure over time in russian children with hypertrophic cardiomyopathy (HCM).
The association study was performed in 34 children age from 1 year to 12 years with HCM and in 150 population controls. The polymorphisms of REN (-83G>A), AGT (M235T), AGTR1 (1166A>C), AGTR2 (3123C>A), BKR2 (-58T>C), ADRB2 (48A>G, 81C>G) and MTHFR (677C>T) were studied using method hybridization with oligonucleotide biochips (BIOCHIP Ltd, Russia).
As a result, TT genotype of M235T polymorphism in the AGT gene were more frequent in patients than in the population (p = 0.028). It was revealed that the presence of the 235T allele of the AGT gene was a high degree of hypertrophy of the interventricular septum.
In conclusion we suggested that M235T polymorphism of the AGT gene may contribute to the higher risk of arterial hypertension in children with HCM.

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J04.10

Human IL28B polymorphism detection allows an interferon antiviral treatment for patients with hepatitis C virus

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Patients infected with HCV genotype-1 undergoing standard of care treatment with pegylated interferon (PEG-INF) and ribavirin (RBV) achieve sustained viral response (SVR) at a rate of approximately 45% internationally and of approximately 56% in Romania. The patients who do not achieve SVR need to be identified as early as possible, in order to avoid unnecessary side effects and high costs.
Recently, it has been reported the impact of the IL28B rs12979860 polymorphism on response to therapy, the CC genotype being considered a predictive factor for achieving SVR.
The aim of our study has been to indentify the -3176C/T IL28B genotypes in order to contribute in selection of HCV infected patients for the personalized combined therapy.
The DNA was extracted from peripheral blood sampled from 22 HCV diagnosed Caucasian patients from Bucharest and nearby, with Invitrogen DNA Extraction Kit. The IL28B genotype identification was performed by Real-Time PCR method using Light Cycler 2.0 and LightMix IL28B Kit.
Our preliminary results identified the following IL28B rs12979860 genotype distribution: CC=4 patients (18,18%), CT=14 patients (63,64%) and TT=4 patients (18,18%). The IL28B rs12979860 C allele frequency observed in our patients (50%) is similar with some east European populations (Poland-46,5%), but lower than other neighboring populations (Russians -61.4-64.1%, Hungarians -65.1%).
Our study will continue with a larger HCV infected patients spectrum in order to analyze for the first time in SE Romania the correlation between the IL28B genotype of patients and their response to an interferon antiviral treatment.
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J04.11

Association of the MMP1 gene single nucleotide polymorphism with peptic ulcer in Volga-Ural region of Russia

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Peptic ulcer (PU) is a chronic disease of the gastrointestinal tract, the main

manifestation of which is the formation of a stable enough ulcer in the stomach and / or duodenum. This disease is widespread throughout the world and get 7-10% of the adult population in different countries. Inflammatory reactions of ulceration are regulated by many metalloproteinases (MMP) and MMP-1 is a well-known destructive metalloproteinase. PU has a genetic background and the aim of this study was to investigate the allele and genotype distribution of -519A>G polymorphism in the MMP-1gene in patients with PU and healthy donors from Volga-Ural region of Russia.
The patient group consisted of 248 individuals with peptic (64) and duodenal (184) ulcer (DU) with different ethnic origins (Russians, Tatars, Bashkirs), the control group included 273 unrelated subject without gastroduodenal ulcer. Genomic DNA was extracted from peripheral blood leucocytes by standard phenol/chloroform method. Genotyping was performed by polymerase chain reaction with specific primers followed by restriction digestion and gel electrophoresis.
The analysis revealed that control subjects have significant higher frequency of *A*A genotype than patients with PU and DU in common group (P=0,03; $\chi 2$=4,49; OR=0,54; 95%CI=0,30 - 0,96). It was also detected the association of *G*G genotype and *G allele with gastro-duodenal ulceration in Russians (P= 0,02;$\chi 2$=5,17; OR=2,05; 95%CI= 1,10 - 3,81 and P= 0,05;$\chi 2$=3,8; OR=1,56; 95%CI= 1,00 - 2,45, respectively).
Thus, we have determined significant association -519A>G polymorphism of MMP-1 gene and gastro-duodenal ulcer in Volga-Ural region of Russia

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J04.12
Thyroid hormone resistance in presence or absence of abnormal thyroid hormone receptor

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Background: Resistance to thyroid hormones (RTH) syndrome is characterized with decreased response to thyroid hormones at target organs and it shows autosomal dominant inheritance pattern. The syndrome is also characterized with elevated thyroid hormone and nonsuppressed TSH levels in blood. Mainly, thyroid hormones (TH) show their effect on target tissues via thyroid hormone receptor beta (TRβ) and alpha (TRα). Generally, RTH etiology is based on TRβ gene mutations. Thyroid hormones could not bind to TRβ receptors. Subsequently, thyroid hormone-activated genes are not able to be expressed.
Aim: The purpose of this study is to investigate TRβ gene mutation prevalence in RTH cases in Turkey.
Material and Methods: Members of 14 families diagnosed as RTH syndrome were enrolled to this study. TRβ gene mutations located on Exon 7-10 were screened by using PCR and automated DNA sequencing system.
Results: Of 14 families, it is detected that members of 7 families (50%) were carrier of well-known TRβ gene mutations. Only 2 cases were sporadic among these 7 families.
Conclusion: As a result, RTH etiology is based on TRβ and TRα mutations. But in 15% of the patients, who resemble each other phenotypically, TRβ and TRα mutations could not be detected. So, it might refer to mosaicism. In this circumstance, genetic analysis might be performed on skin fibroblast cells or buccal epithelial cells in these patients. If any TRβ and TRα mutations could not be found in them, MCT8 and SECISBP-2 gene mutations should be investigated.

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J04.13
Association study of Interferon Induced with Helicase C Domain1 (IFIH1) polymorphisms with type 1 diabetes in a Tunisian population

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Type 1 diabetes (DT1) is a multifactorial disease involving both genetic and environmental factors. IFIH1 senses and initiates antiviral activity against enteroviruses which are suspected in the etiology of DT1. Several polymorphisms have been studied within the IFIH1 gene such as rs1990760, rs2111485 and rs3747517. The aim of this study was to examine the association of these three polymorphisms with DT1 in the Tunisian population.
This study concerned 116 diabetic subjects and 168 controls that were recruited from Tunisia. The genotyping of rs2111485 and rs3747517 was realized by PCR followed by an enzymatic digestion and rs1990760 by MS-PCR.
The results did not show statistically significant association between the three polymorphisms and DT1 (rs1990760 p = 0,99 ; rs2111485 p = 0,44 and rs3747517 p = 0,11) although it was found that the AA genotype of rs3747517 was more frequent among controls than in diabetic subjects (p = 0,054). Furthermore the genotype GG seems to have a protective role against the diabetic retinopathy (p = 0,046). We also analyzed the association of these three polymorphisms with other clinical and biological criteria, whereas no significant association was found.
Our data indicate that the three polymorphisms of the IFIH1 gene are not associated to the DT1 and that the rs3747517 seems to influence predisposition to diabetic retinopathy in the Tunisian population.

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J04.14
Woodhouse-Sakati syndrome : a systematic review of the literature and description of two new cases

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Woodhouse-Sakati syndrome (WSS) is a rare autosomal recessive disorder, caused by mutations of the DCAF17 gene, located in 2q31.1. With the wealth of knowledge acquired since the original clinical description in 1983 and the discovery of the responsible gene in 2008, and because of the heterogeneity of the cases reported previously, it is necessary to better identify the phenotype.
The first exhaustive literature review permitted us to highlight, among the 72 patients from 29 families identified as WSS, 37 patients from 12 families whose clinical diagnosis was confirmed by the presence of one of the 9 DCAF17 mutations described. We also reported two new sibs identified with the c.127-3_127-1del(TAG)ins(AA) mutation.
WSS always encompasses (100%) hypogonadism (which is peripheral in female patients and central or mixed in male patients, with delayed puberty), low IGF1 without abnormality of GH rate and a fronto-temporal alopecia. It also combines intellectual disability of variable severity (87%), bilateral deafness (76%), cervico-facial and axial dystonia (42% of all cases, 89% after 25 years old,) and diabetes mellitus (66% of all cases, 96% after 25 years old). Symptoms occur mainly in adolescence. Patients also have discrete facial features, with a triangular elongated face, hypertelorism and a prominent nasal root.
Ubiquitous nucleolar expression of the DCAF17 protein, with overexpression in the brain, liver and skin, is compatible with the redefinition of phenotype including the 7 major signs.
Physiopathology, which may involve the role of ribosomes in the cellular activity, remains unclear.

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J04.15
New Genetic abnormalities in Mexican population affected by non-21-hydroxylase-deficiency Congenital Adrenal Hyperplasia

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Congenital Adrenal Hyperplasia (CAH) is a group of autosomal recessive disorders of sex differentiation development, resulting from deficiencies in one of five specific enzymes involved in the cortisol pathway within the adrenal gland. Deficiency in 21 α-hydroxylase, account for 90-95% of the overall CAH, the rest of these enzymatic defects is rare in most populations. The major clinical features are hypertension and virilization defects; in newborn

females, ambiguous genitalia may be seen and heterosexual precocious puberty may be a consequence. Among males, peripheral isosexual precocious puberty is frequent. Over 50 mutations in the CYP11B1 gene have been associated with this adrenal phenotype. The 17 α-hydroxylase/17, 20-lyase defect affects both the adrenal gland and the gonads, interfering with the synthesis of both cortisol (derived from adrenal 17 α-hydroxylase activity) and androgen (derived from adrenal and gonadal 17, 20-lyase activity). In female patients, the clinical picture includes hypertension, hypokalemia, lack of pubertal development and primary amenorrhea; in males, affected individuals shown impaired virilization and lack of pubertal development. More than 54 mutations have been described in CYP17A1. We described two new CYP17A1 gene mutations, in two 46, XY phenotypic females. Together with molecular findings we suggest that one the mutants could be secondary to a founder effect. We present the clinical and molecular data in new genetic abnormalities in Mexican population affected by non-21-hydroxylase-deficiency Congenital Adrenal Hyperplasia

G. Queipo: None. **M. Martin:** None. **N. Garibay:** None. **N. Najera:** None.

J04.16
Development of methodological approaches to DNA diagnostics of CAH in Ukraine

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The cause of 95% of the congenital adrenal hyperplasia (CAH) is a 21-hydroxylase deficiency. CAH is an autosomal recessive genetic disorder with a frequency of 1:5000 to 1:15000. The CYP21A2 gene, encoding 21-hydroxylase, is located on the short arm of chromosome 6 at position 21.3, there also located CYP21A1P pseudogen. Group of genes RP2-C4A-CYP21A2-TNXB has high homology with the corresponding pseudogenes, which located in the same chromosom region. It makes analysis of CYP21A2 gene mutations quite complicated.
The aim of our study was to develop methods for analysis of different mutations of the CYP21A2 gene.
Gene mutations P30L, I2G, G110_8nt, E6 cluster, Q318X, R356W, V281L were investigated by allele specific PCR and RLFP. Deletion/convertion of CYP21A2 gene was investigated with a help of long range PCR. The method was tested on control samples with known mutations in the CYP21A2.
The analysis of the CYP21A2 gene mutations was performed for five CAH patients. 4 of them have homozygous gene deletion/convertion, another patient has I2G compound with R356W.

S. Chernushyn: None. **L. Livshits:** None.

J04.17
A Case Report: Hirschsprung's Disease in three sisters

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Hirschsprung's Disease (HD) occurs in about one in 5,000 children. The affected infant frequently present such symptoms of impaired intestinal motility as constipation, emesis, abdominal pain or distention, and occasionally diarrhea in the first two months of life with typically a failure to pass meconium within the first 48 hours of life.
A healthy couple who had got two girls with HD came to us. The first daughter was diagnosed as short-segment aganglionosis and the second daughter had total colonic lesion. Both of them did not have syndrome-cognitive features. Unfortunately, their second daughter died because of severe pneumonia in spite of successful operation. The couple suspected some relation between daughters' similar conditions and sought to a medical counseling on next pregnancy. They had lost their daughter just before they came, so followed with psychological care with clinical psychologist we have talked about the cause of HD. As for genetics, we discussed sporadic cause and the possibility of single gene cause because two girls did not have any further features. They decided not to undergo any genetic test and they told us they would like to accept if the next child has HD.
About a year later, she has got pregnant naturally and came to maternity service again. Her pregnancy went well and had another daughter. The third daughter was suspected HD and needed an operation. The diagnosis was confirmed by colon biopsy. When HD was observed repeatedly in the same family, we have to consider genetic cause of HD carefully.

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J04.18
A novel *SRD5A2* gene mutation in a Turkish patient with 46,XY disorder of sex development

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Introdution: 5-alpha-reductase type 2 deficiency (264600) is a rare autosomal recessive inherited disorder. Affected 46,XY individuals usually present with ambiguous genitalia characterized by pseudovagina, microphallus, cryptorchidism and perineoscrotal hypospadias, at birth and are often raised as females. The abnormal 5-alpha-reductase type 2 as a result of mutations in the *SRD5A2* gene could lead to 46,XY disorders of sex development. Therefore, genetic testing of *SRD5A2* is a useful tool for the definitive diagnosis of 5-alpha-reductase type 2 deficiency. In this study, we report a novel mutation in *SRD5A2* gene in a Turkish patient with 5-alpha-reductase type 2 deficiency
Materials and Methods: Five of the coding regions of SRD5A2 gene, including exonic- intronic boundaries, were amplified by using spesific primers and sequenced directly.
Results: Two heterozygous mutations, c.164T>A that leads to a amino acid substitution p.Leu55Gln and c.269A>C, leading to a amino acid substitution p.His90Pro were detected in the examined patient. The latter one, c.269A>C, was a novel mutation found in the patient.
Discussion: It is important to determine the genetic factor leading to 5-alpha-reductase type 2 deficiency in patients for the proper treatment. In this report, we detected a novel mutation which can cause to the anomaly, but in order to have a proper understanding about the effect of the mutation, functional analysis should be carried out and this will be our future subject.

M. Boyraz: None. **K. Ulucan:** None. **T. Akcay:** None.

J04.19
Association of VDR (rs731236) and NFKB1 (rs28362491) gene polymorphisms with aggressive periodontitis

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Background: Aggressive periodontitis (AgP) is rare but rapidly progressive periodontal disease which affects systematically healthy individuals less the 30 years of age. It is still unclear leading etiologic and pathogenic risk factors for this disease. The aim of this study was to investigate the association between *VDR* (rs731236) and *NFKB1* (rs28362491) gene polymorphisms and AgP in Russian population.
Materials and methods: Genotyping of peripheral blood samples of 98 subjects (AgP - 43, chronic periodontitis (CP) - 20 and healthy subjects - 35) was performed with standard polymerase chain reaction-restriction fragment length polymorphism assay (PCR-RFLP). Gene-gene interaction among loci of two genes was studied by using the multifactor dimensional reduction method (MDR).
Results: There was an association between the del/del genotype of *NFKB1* gene and AgP (p=0,027). MDR test has shown a statistically significant association of combined genotypes del/del (*NFKB1*) and T/T (*VDR*) with AgP (p=0.0004) compared with CP.
Conclusion: del/del genotype of *NFKB1* (rs28362491) gene is associated with AgP and has gene-gene interaction with T/T genotype of *VDR* (rs731236) gene.

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J04.20
Gene expression profile of TGF-β Signaling and Extracellular Matrix and Adhesion Molecules pathways in human end stage Heart Failure.

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Although fibrosis was enhanced, collagen content in end-stage HF was only slightly higher than in control tissue. We hypothesis that alteration in genes expression is the major cause of interstitial fibrosis. Using the TGF-β Signaling and Extracellular Matrix PCR Array, we examined the gene expression. Methods: End -stage heart failure tissue (LV) was taken during heart transplantation. The controls were myocardial tissue from multi-organ donors. Cardiac fibrosis was evaluated by Picrosirius red staining. Total RNA was

converted into cDNA. Each PCR Array is a 96-well plate containing RT qPCR primer assays set of 84 related genes. Expression of SMADs proteins was investigated by immunohistochemistry (IHC) staining. Genes demonstrated at least 4-fold differences were taken into account. Results: Among the 84 ECM and Adhesion Molecules related genes, up-regulated was observed in 23 genes while 7 genes were down-regulated. Among the 84 TGF-β signaling related genes, up -regulated was observed in 23 genes while 4 genes was down-regulated. A subset of 8 of the 23 genes represent TGF-b and TGF-β superfamily cytokines, and 12 genes represents SMADs and SMAD target genes. All the results presented were highly significant statistically. IHC confirmed on protein level overexpression and translocation to the nucleus SMADs complexes. Although all patients received β-blockers and ACE inhibitors in all we observed interstitial fibrosis. Our results suggest that interstitial fibrosis mainly results from increased activity of TGF-β signaling pathway. Upon activation, the SMAD1 forms a complex with SMAD4 and translocates to the nucleus to regulate transcription.

M. Szperl: None. **A. Parulski:** None. **E. Stankiewicz:** None. **M. Franaszczyk:** None. **M. Roszczynko:** None. **A. Rozanski:** None. **M. Fedorowicz:** None.

J04.21

A novel homozygous mutation in the GCMB gene in a patient with hypoparathyroidism

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Mutations in the calcium-sensing receptor gene (CaSR), in the parathyroid hormone gene (PTH), or in the glial cells missing gene (GCMB) may cause familial isolated hypoparathyroidism. The condition may be inherited either autosomal dominant or autosomal recessive. Here, we present a case of autosomal recessive hypoparathyroidism with a novel mutation in the GCMB gene.

A 22 year-old male was referred to Medical Genetics Department due to mental retardation and seizures. His parents were not consanguineous and there was no family member having similar findings. He first presented at age 3 with seizures due to hypocalcemia and hypomagnesemia. On physical examination he had short stature, motor, mental, growth retardation, and chronic tetany. Brain magnetic resonance imaging (MRI) showed calcifications in basal ganglia. Ophthalmologic examination revealed bilateral cataract. Cranial computered tomography (CT) imaging showed diffuse cerebral atrophy. He had a chronic hypoparathyroidism with hypocalcemia and hyperphosphatemia. No mutation was found in 2-7 exons of the CaSR gene. A novel homozygous mutation (c.90+1 G>A) affecting intron 2 donor splice site was identified in the GCMB gene.

Therefore, we concluded that patient had autosomal recessive hypoparathyroidism due to absence of functional GCMB protein.

B. Erturk: None. **E. Karaca:** None. **C. Silve:** None. **F. Ozkinay:** None.

J04.22

Study of mutations in exons 12-15 and 19-23 MYH7 gene in HCM (Hypertrophic Cardiomyopathy) patients in Chaharmahal Va Bakhtiari province

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Background: Hypertrophic cardiomyopathy (HCM) is the most common kind of Mendelian inherited heart disease, affects 0.2% of the global population and also is the most common cause of sudden cardiac death in individuals younger than 35 years old. Approximately in %35 of cases, exons 8-24 of MYH7 gene, which encoded heavy chain of β-Myosin, are affected. The aim of present study was to investigate the possible presence of mutation in exons 12-15 and 19-23 MYH7 gene, which has already been reported to accommodate some mutations, in 30 patients with HCM in Chaharmahal Va Bakhtiyari province. Method: DNA was extracted using standard phenol-chloroform method and then was used for amplification and gel electroploresis by PCR- SSCP/HA procedure. Finally, the suspected cases containing possible mutations were selected for the direct sequencing and the results were seen by chromas software. Results: In exons 13, 14, 15, 20, 21 and 23 there was no change, but two polymorphisms including 5811 C>T and 5845 G>A were found in exon 12 of one and six patients, respectively. And two mutations including 9692 C>T and 10824 G>A were found in exons 19 and 22 of two and one patients, respectively. Conclusion: Based on present study, we've concluded that changes in exons 12, 19 and 22 are involved in HCM disease in this province. However, further study on much more cases of disease and in other exons of this gene is essential, to determine the role of this gene and its relationship with HCM in this province.

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J04.23

A case of Thalassemia major and gastric cancer

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β-Thalassemias are hereditary blood disorders characterized by abnormalities in the synthesis of the β hemoglobin chains. This disease causes excessive storage of iron in all organs. Treatment of β-thalassemia major consists of regular blood transfusions, iron chelation and management of secondary complications of iron overload. Iron and its binding proteins have immunoregulatory properties, but iron excess may produce several effects. The oxidative damage, in correlation with elevated ferritin levels, occurs in thalassemia through the production of oxygen-free radicals. In turn, oxidative damage can give rise to a neoplastic clone through genetic or epigenetic alterations. These theories could indicate a pathogenetic correlation between thalassemia and cancer. We describe the first report of a gastric cancer in thalassemic patients. A 40-year-old male with thalassemia major presented 7 Kg weight loss in a month and began to complain of intense stomach pain, nausea and bloat. The determination of CA 19.9 showed abnormal levels. Gastrointestinal endoscopy demonstrated a irregular ulcer in the pericardial gastric fundus. Biopsy of the gastric mucosa showed: "adenocarcinoma scraps, intestinal type, moderately differentiated, infiltrating with reaction desmoplastic stroma (HER score 2=1) which was associated with chronic gastritis and findings of haemosiderosis"; H. pylori was not found. CT of the chest, abdomen and pelvis showed enlarged epigastric, lymph nodes and ascites. The coexistence of malignancy and beta thalassemia is not rare. Whether there is a link between thalassemia and malignancy or whether malignancy is more frequent in thalassemic patients simply because they live longer, is still to be defined.

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J04.24

Evaluation of the Verigene warfarin genotyping kit: Buccal swab as an applicable sample

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Warfarin is the most widely used prescribed oral anti-coagulant for the treatment of thromboembolism. However, warfarin dosing is problematic because of its narrow therapeutic range and large inter-patient variability. We assessed the feasibility of the Verigene Warfarin Metabolism Nucleic Acid Test (Nanosphere, Inc., Northbrook, IL), a automated microarray-based assay, compared to bidirectional sequencing, to evaluate the analytical performance of the Verigene test. Fifty samples were included; 36 healthy persons and 14 patients with arrhythmia who treated with warfarin. The genotype of *CYP2C9* and *VKORC1* analyzed by Verigene test was completely agreeable with direct sequencing method. *CYP2C9**1*1 allele was detected in 90% (45/50) and remaining 10% (5/50) presented *CYP2C9**1*3 allele. *VKORC1* 1173TT allele and 1173CT were found in 78% (39/50) and 22% (11/50), respectively. The frequencies of combined genotype were as follows: *CYP2C9* wild type plus *VKORC1* 1173TT was 68% (34/50), *CYP2C9* *1*3 plus *VKORC1* 1173TT was 10% (5/50), and *CYP2C9* wild type plus *VKORC1* 1173CT was 22% (11/50). We also evaluated the potential of buccal swab as an applicable sample for Verigene test because peripheral blood cannot be used in some patients such as post-hematopoietic stem cell transplantation status. Genotyping was successfully performed using suspension of buccal swab when the sample contains at least 0.3×10^6 epithelial cell/mL. As conclusion, the Verigene test offers advantages for the detection of *CYP2C9* and *VKORC1* polymorphisms such as easiness of use and rapid detection time, and the buccal swab can be an excellent alternative sample of peripheral blood in case of need.

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J04.25

The Approach to the Case of Rarely Seen Adult Morque Syndrome at Emergency Service

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Morquio syndrome (MPS IV) which is a kind of mucopolysaccharidosises is a hereditary lysosomal storage disease. These patients generally pass away due to cardiopulmonary

and neurologic function disorders at young adult period. However,

medical care and close clinical observation developing recently have extended the life time of the patients until sixth decade. The adults who have congenital genetic disease apply to adult emergency clinics as a result of these developments. It is aimed in this case to present a case of morque syndrome patient who came to our emergency clinic with the complaint of abdominal distension and got the diagnosis of strangule umbilical hernia.The male patient who was 27 years old and had morquio syndrome (MPS IV) was brought to our emergency clinic by his family due to the complaints of nausea, vomit, abdominal distension existing for 4 days. As a result of general surgery consultation, etrangule umbilical hernia was detected and operation decision was taken. Necessary preparations for difficult intubation were made due to existing dysmorphologic appearance, being 4 of mallampati score, being restricted of mouth opening and being big of tongue.

Firstly, the operation of the patient was performed with successful spinal anesthesia instead of planned gegional anesthesia. The patient who did not need postoperative intensive care was sent to general surgery service without any problem. Development of early diagnosis methods, starting to treatment early, progress of rehabilition studies improve the the patients' quality of life who have severe genetic diseases.

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J04.26

NPHS2 R229Q polymorphism in children with nephrotic syndrome

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The podocin (NPHS2) gene encodes podocin protein, which has an important role in glomerular ultrafiltration and controlling slit membrane permeability. Mutations in NPHS1, which encodes nephrin, are the main causes of congenital nephrotic syndrome (CNS) in Finish patients, whereas mutations in NPHS2, which encodes podocin, are typically responsible for childhood-onset steroid-resistant nephrotic syndrome in European populations.**Material and method**: The study group consisted of 48 children diagnosed with nephrotic syndrome (NS) in a Romanian population. This cohort included 3 samples of congenital or infantile onset NS cases and 45 samples of sporadic cases of NS (10 cases with steroid-resistant nephrotic syndrome and 35 cases with steroid-sensitive nephrotic syndrome). Fifty healthy children were enrolled as the control group.
All children were genotyped for NPHS2 R229Q polymorphism. **Results**: Three cases were diagnosed with congenital nephrotic syndrome (CNS) with typical histological exam, and the others 45 had idiopathic nephrotic syndrome.
NPHS2 R229Q polymorphism was detected only in two cases of CNS and in none patient with other type of NS or in control group. **Conclusions:** This study demonstrates that mutation of NPHS2 R229Q can be responsible for CNS in this population. Further studies with a larger number of patients are needed.

C. Duicu: None. **F. Tripon:** None. **O. Marginean:** None. **C. Banescu:** None.

J04.27

Cardiovascular events in patients with thrombophilia

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Background: Cardiovascular events, particularly in young people, are part of the research areas in thrombophilia pathology. The aim of our study was to evaluate cardiovascular events in patients with thrombophilia.
Material and method: A total of 12 patients with thrombophilia (diagnosed based on a combination of up to 3 mutations) from the Oncology Clinic "Oncomed" Timisoara, Romania were consecutively enrolled in the study between 2010-2013. We excluded pregnant women and patients undergoing anticoagulation therapy.
Results: 58.3% of the patients were homozygous and 41.7% were heterozygous. By all patients, 4 patients were with cardiovascular events: 2 with myocardial infarction (EKG documented), one with ischemic stroke (MRI documented) and one with portal vein thrombosis - (CT documented). All cardiovascular events occurred in patients under 32 years old. Homozygous patients had a significantly increased number of cardiovascular events compared with the heterozygous patients ($p<0.001$).
Conclusion: Myocardial infarction, ischemic stroke, or portal vein thrombosis occured more frequently in patients with thrombophilia, especially in homozygous forms. Thrombophillia may be a cause of cardiovascular events in young patients with no cardiovascular risk factors, but with a hereditary history of thromboembolic events.

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J04.28

The effect of 17-Beta Estradiol on the male rat heart electrophysiological parameters

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Premenopausal women have a significantly lower risk of coronary artery disease than men or postmenopausal women, suggesting that estrogens have cardioprotective effects. The underlying mechanism of this protection remains unknown. The aim of this study was to investigate the effect of 17-Beta Estradiol on the male rat heart. Effect was tested by means of electrophysiological responses through the intracellular recording of action potential (AP), contraction and by the evaluation of the immunohistochemical expressions of calcium and potassium channels. Experiments were carried out on three mounts old 27 male Wistar Albino rats. In order to achieve the planed purpose, 3 experimental groups were organized: Control (Con), Castrated (E-) and 17-Beta Estradiol given to castration (EX). Immunohistochemistry was performed with L-type calcium and potassium channel antibodies. Our results have shown that; body weights of E- and EX group decreased with an increase blood glucose levels. Recorded AP of E- and EX groups have shown to decrease maximum depolarization, resting membrane potential and the amount of depolarization. Concomitantly, measurements of the repolarization phases of AP's were found to be increased in E- and EX groups. Immunohistochemical results on the experimental groups have shown that the expression of calcium channels increased in E- group, decreased in EX group. Whereas, the expression of potassium channels decreased in E- group while did not change in EX group. This prolong repolarization phase was due to increased in the expression of the calcium channels while decreased in the expression of the potassium channels.

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J04.29

Malignant infantile osteopetrosis and pericentric inversion of chromosome 9 in an infant with severe dysmorphic features

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Background: Malignant infantile osteopetrosis (MIOP) is a rare autosomal recessive bone disease, characterized by reduced or dysregulated osteoclastic activity and increased bone mass. Major consequences include bone marrow failure and nerve compression. The pericentric inversion of chromosome 9 is the most frequently seen (1-3%) in general population and has a role in abnormal phenotype development. Material and methods: Case report of a 3 months old boy admitted to the clinic for seizures due to severe hypocalcemia. A dysmorphyc phenotype was noticed. Based on the presence

of anemia, hypocalcemia, hepatosplenomegaly, failure to thrive, mental retardation, ventriculomegaly, optic nerve atrophy and the typical radiological images diagnose of MIOP complicated by rickets was established. Genetic evaluation for chromosome abnormalities revealed apericentric inversion of chromosome 9. Conclusions: Chromosome 9 inversion has no important clinical significance .The association of osteopetrosis complicated by rickets and chromosome 9 inversion led in our case to severe dysmorphic feature. The prognosis in this case remains very poor, death occurs in the first decade of life as a complication of bone marrow supression.

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J05.01

Congenital Rett syndrome: study of FoXG1 impact on adult brain.

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Mutations in the Forkhead box G1 (FOXG1) gene, a brain specific developmental transcription factor, are responsible for congenital Rett syndrome. FoxG1 plays a fundamental role in early telencephalon development by sustaining proliferation of the progenitor pool and preventing premature cortical neural differentiation. Due to perinatal lethality of Foxg1-/- mice the function of Foxg1 in adult brain has remained unexplored for many years. Recently, Tian et al. studying conditional mutant Foxg1 mice demonstrated that this factor is espressed continuously in postnatal and adult hippocampus playing an essential role in the development of the dentate gyrus. In order to characterize Foxg1 dependent signalling during neuronal maturation and activity, we firstly compared gene expression profiles of wild-type and Foxg1+/− P30 brain. Foxg1 heterozigous mice can reach adulthood though they display microcephaly, altered hippocampal neurogenesis and behavioural and cognitive deficiencies. In order to evaluate the effects of a stronger reduction of Foxg1 specifically in the hippocampus, we then silenced Foxg1 in primary hippocampal neurons by shRNA technology and evaluated the effects of Foxg1 down regulation on gene expression, neuronal morphology, differentiation and spine formation. By cDNA microarray experiments, we identified two sets of partially overlapping deregulated genes, one related to Foxg1+/− whole brain and the other to Foxg1 silenced hippocampal neurons. Possible key co-factors underlying Rett phenotype have emerged with important consequences on therapeutic strategies (Nnat, Oxt, Avp, Mef2c, Tubb2b, Marcksl1, Acot7). Interestingly, Foxg1 silenced hippocampal neurons exhibit alterations in dendrite development suggesting a function of this gene during early post-mitotic morphogenesis and wiring.

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J05.02

Functional polymorphisms in *VEGF*, *MMP-2*, and *MMP-9* genes and intraventricular hemorrhage of prematurity

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Background: Intraventricular hemorrhage (IVH) is a major complication of prematurity. It is primarily ascribed to fragility of vasculature in the germinal matrix where relative hypoxia induces expression of vascular endothelial growth factor (VEGF) promoting angiogenesis. The matrix metalloproteinase (MMP) system is also activated by hypoxia that may contribute to hemorrhage. In this study, functional polymorphisms in the *VEGF* and *MMP* genes were assessed for their correlation with IVH.
Methods: *VEGF* (-2578 C/A, -1154 G/A, -634 G/C, +936 C/T), *MMP2* (-1306 C/T, -735 C/T), and *MMP9* (-1562 C/T) polymorphisms were assayed in 189 premature newborns (62 with IVH) and 187 term newborns by RT-PCR and PCR-RFLP. Genotype distribution, clinical characteristics, and laboratory parameters were compared between newborns with and without IVH.
Results: Significant association was found in newborns of < 28 weeks gestation between IVH and TT/CT genotype of *VEGF* +936 C/T (P = 0.046), and AA/AG genotype of *VEGF* -1154 G/A (P=0.011). There was no significant association between IVH and polymorphisms in *MMP2* or *MMP9*. Patent ductus arteriosus, hypotension, and maternal history of chorioamnionitis were also significantly associated with IVH. Logistic regression analysis showed that TT/CT genotype of *VEGF* +936 C/T was the strongest predictor of IVH amongst clinical and potential genetic risk factors studied.
Conclusions: The +936 C/T polymorphism in *VEGF* is associated with higher frequencies of IVH in newborns <28 weeks gestation. This polymorphism is associated with decreased expression of VEGF and may result in delayed maturation of blood vessels as an underlying etiology.

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J05.03

Diagnostic investigations in Rwandan patients with intellectual disability and/or multiple congenital abnormalities

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Multiple congenital anomalies and Intellectual Disability (MCA/ ID) affect approximately 3% of newborns. There is no available data on genetics of ID and MCA in Central Africa.
The aims of this study was to detect chromosomal abnormalities such as submicroscopic microdeletion/microduplication and subtelomeric imbalance in this group of patients. Newborn, children and young adults were recruited from the Teaching University Hospitals of Kigali and Butare, Rwanda. All individuals were clinically examined and karyotype was done in Rwanda and a blood sample was obtained for genetic testing in Belgium (Center for human genetics, Liege). All patients with trisomy 21, 13 and 18 and were excluded from the study.
Karyotype analysis of the remaining 52 patients was normal. Fragile-X was excluded in all these patients and we found premutation in 2 male patients. Thus the MLPA was performed and identified (SALSA MPLA P245 Microdeletion and MLPA P0 36 and P070 Human Telomere, MRC Holland) one chromosome 10q duplication, one chromosome 9p duplication, one deletion of the 22q11.22 region and one 7q11.23 microdeletion (William-Beuren syndrome). For the remaining cases array-CGH was performed and seven patients showed microdeletion and microduplication such as del 8q23, duplication 1p31.1q35.2.
This study will allow to identify the etiology of ID/MCA in Rwandan patient and will show the importance of genetic testing in low income countries.

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J05.04

A Synonymous Change, p.Gly16Gly in MECP2 Exon 1, Causes a Cryptic Splice Event in an Atypical Rett Syndrome Patient

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Mutations in MECP2 are the main cause of Rett Syndrome. To date, no pathogenic synonymous MECP2 mutation has been identified. Here we investigated a de novo synonymous variant c.48C>T (p.Gly16Gly) identified in a girl displaying an atypical RTT phenotype. In silico analyses to predict effects of sequence on mRNA splicing were employed, followed by sequencing and quantification of lymphocyte mRNAs from the subject for splice variants MECP2_E1 and MECP2_E2. Analysis of mRNA confirmed predictions that this synonymous mutation activates a splice-donor site at an early position in exon 1, leading to a deletion (r.[=, 48_63del]), codon frameshift and premature stop codon (p.Glu17Lysfs*16) for MECP2_E1. For MECP2_E2, the same premature splice site is used, but as this is located in the 5'untranslated region, no effect on the amino acid sequence is predicted. Quantitative analysis specific to this cryptic splice variant also revealed a significant decrease in the quantity of the correct MECP2_E1 transcript, which therefore suggests that this is the etiologically significant mutation in this patient. These findings suggest that synonymous variants of MECP2 as well as other known disease genes_and de novo variants in particular_ should be re-evaluated for potential effects on splicing.

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J05.05

Intellectual Disability Cell Bank (Lymphoblast & Fibroblast)

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The rapid advance of genetic research and applied technologies has led to a

considerable increase in interest in collections of human biological material. Collection of tissue samples and cell lines from which additional vivo study can be performed is very essential. These biobanks represent an important resource for diagnosis and basic research. Currently, there are a large number of „culture collections and bioresource centers" that serve an individual part of the process of bioengineering.
The Genetics Research Center(GRC) Intellectual Disability Cell Bank (IDCB) of the University of Social Welfare and Rehabilitation Sciences (USWRS) in joint effort with Max Planck Institute (MPI) have constantly provided an essential internal service establishing, analyzing and banking cell cultures derived from patients affected by intellectual disability (ID). According to ethical and legal recommendations, the samples are being taken for analysis and cell banking after written donor informed consent, approved by the regional ethics committee of the USWRS for medical research. This bank provides the storage of biological samples and specimens (dermal fibroblast, lymphoblast) derived from patients with ID, for whom the causing genes and mutations have been identified as known and candidate genes. This bank also provides cell lines for functional studies, involved in transcription and translation of specific genes which expressed in brain or other genes which their proteins are involved in brain function. The cell bank website can be visited via „idcb.uswr.ac.ir"

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J05.06

A new case of 15q26.3 deletion in a patient with unbalanced translocation t(15;16)(q26.3;q23.3)

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Objective: We present a case of 15q26.3 unbalanced translocation t(15;16)(q26.3;q23.3) in a boy with a complex phenotype.
Material and methods: The patient is a 1- year -old boy, born to healthy, unrelated parents, with growth retardation, an Apgar score of 9, delayed psychomotor development. Clinical evaluation showed: growth retardation (weight 5,540 kg, height 64 cm), microcephaly, dysmorphic facial features; mild mental retardation. Heart ultrasound showed atrial septal defect. Abdominal ultrasound showed polysplenia, and transfontanelar ultrasound was normal. Wrist X ray showed normal bone age. Thyroidian and growth hormons were in normale range. Cytogenetic investigations, including karyotype, FISH and array-CGH were performed.
Results and discussions: Array CGH showed a deletion on chromosome 15 (15q26.3) and a duplication on chromosome 16 (16q23.3-q24.3). FISH testing confirmed the above-mentioned anomalies in our patient and detected a balanced translocation t(15;16)(q26.3;q23.3) in his mother. Terminal deletions of chromosome 15q represent a rare condition, only several cases with or without ring chromosome being described. The phenotype is heterogeneous and includes growth retardation, microcephaly, dysmorphic features, renal abnormalities, lung hypoplasia, failure to thrive, developmental delay, mental retardation, and skeletal abnormalities. The IGF1R gene was considered as responsible for the phenotype.
Conclusions: Our report brings new insights in the phenotype of this rare syndrome. The phenotype of our patient will be compared with those of other patients with the same deletion previously described to further delineate the 15q26 deletion syndrome. Also, the role of 16q23 duplication on the phenotype will be discussed.
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J05.07

Identification of MECP2 gene duplications in female patients with intellectual disability and speech delay

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Intellectual disability (ID) is substantial limitations in cognitive functioning that manifests before the age of 18. The worldwide prevalence of ID (IQ < 70) is 1.5-2%, and 0.3-0.5% are severely impaired, with IQs of <50. Severe ID has a strong genetic component; increasing numbers of single genes are being discovered in which mutations are associated with both syndromal as well as non-syndromal forms : ARX, MECP2 and FMR1.
During the participation in the FP7 CHERISH project (grant agreement N223692), we have collected the cohort of the 96 patients with ID. CNV analysis using array CGH and MLPA analysis for known microdeletion syndromes was conducted. Pathogenic rearrangements, revealed during this investigation, were confirmed using qPCR.
In the analyzed cohort three female patients with mild ID and speech delay carrying de novo duplications in Xq28 chromosomal region including MECP2 gene were identified using SALSA MLPA P245-A2 Microdeletion-1 kit. Female patients with Xq28 duplications are rare and usually asymptomatic, due to skewed X-chromosome inactivation (XCI) pattern with preferential inactivation of the rearranged chromosome. X inactivation test showed random XCI in all cases, therefore we suggest that the chromosomal region with duplication is functional and causes the phenotype. Further studies, including the confirmation of the presence of mutations and their borders identification using array CGH will be conducted. In order to confirm the hypothesis, that the association of duplication found in this study with phenotypic manifestations of ID, it is necessary to study the MECP2 gene expression level in our patients.

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J05.08

Mowat-Wilson syndrome: report on a patient with marfanoid habitus and arachnodactyly

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Mowat-Wilson syndrome (MWS) is a rare genetic disorder of unknown incidence, consequence of loss-of-function mutations of *ZEB2* (*ZFHX1B, SIP1*) homeobox zinc-finger transcriptional repressor. Recent studies indicate that Zeb1 protein is required to generate cortical interneurons in embryos; in its absence, cells appear to transform toward a subtype of GABAergic striatal interneurons.
Here, we report on a 10 year old male patient with partial deletion of *ZEB2* and phenotype suggestive of MWS. The patient presented psychomotor retardation (he sat at 18 months, walked alone at 6 years, and uttered first syllables at 7 years) and dysmorphic features: large ears, up-slanted palpebral fissures, thick eyebrows, long eyelashes, large nose, short philtrum, thick lips, micrognathia, marfanoid habitus, and arachnodactyly. Additionally, he was diagnosed with pyramidal syndrome, ventricular septal defect and epilepsy and had frequent episodes of respiratory infections. His cerebral CT scan was normal. He was severely intellectually disabled (IQ 32), not sociable, and exhibited hyperkinesia with aggressivity. aCGH on an 105K Agilent platform resulted in the following molecular karyotype: arr 2q22.2(144,909,193-145,157,644)x1,4q13.2(69,431,473-69,483,227)x1,5p15.33(733,455-795,803)x1,6p21.32(32,487,424-32,552,156)x1,8p11.22(39,237,438-39,380,654)x3,15q11.2(20,849,110-22,304,655)x1. Deletion at 2q22.2 included the 5'terminus of *ZEB2*, while the only other OMIM gene affected by the above-mentioned anomalies is *BCL8*, suggested to be linked to diffuse large cell lymphoma.
The spectrum of MWS features is relatively narrow and well characterized. Here, we first report on a patient that associates MWS with marfanoid habitus and arachnodactyly, thus adding new insights to the tableau of this disease.

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J05.09

Three-way unbalanced translocation leading to 1q duplication and 10q deletion in a child with severe psychomotor retardation, dysmorphic features and congenital malformations

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Complex chromosomal anomalies, involving more than two breakpoints on two different chromosomes are rare events. Balanced rearrangements in healthy carriers are associated with reproductive failures, while the unbalanced anomalies present with psychomotor retardation, dysmorphic features and/or congenital malformations, in most cases.
We present the clinical and cytogenetic findings in a 4-year-old girl referred for genetic investigations due to dysmorphic features and psychomotor re-

tardation.
The clinical evaluation revealed: height 85 cm (<2DS), weight 8,2 kg (<2DS), occipitofrontal circumference 44 cm (<2DS); dysmorphic features (hypertelorism, down-slanting palpebral fissures, long eyelashes, synophrys; big, malformed ears; anteverted nostrils; short philtrum, thin upper lip, high-arched palate, micrognathia; single palmar crease, congenital talipes equinovarus; pectus excavatum; external genital organs hypoplasia). Neurological examination showed hypotonia, severe psychomotor retardation. Heart ultrasound showed atrial septal defect. Cerebral computed tomography showed cerebral and cerebellar atrophy. Abdominal ultrasound was normal.
Classical cytogenetic investigation was performed on peripheral blood lymphocytes by GTG-banded karyotyping, for the child and her parents. Array-CGH studies (Agilent platform) for molecular characterization are on-going. Chromosomal studies revealed a complex unbalanced translocation in the proband, leading to 1q32-qter duplication and 10q26-qter deletion. A balanced three-way translocation t(1;8,10)(q32;q13;q26) was detected in the proband's healthy mother.
In this paper we report on a new case with an unbalanced, three-way translocation, harboring partial trisomy 1q32-qter and partial monosomy of 10q26-qter. The correlations of the clinical findings with the genomic imbalances in our case will be discussed; we will also compare our case with others reported by date.
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J05.10
The absence of polymorphic variants associations of catalase (CAT), catechol-O-methyltransferase (COMT), and serotonin transporter (SLC6A4, 5HTT) genes with the risk of alcoholism in the Russian population of the West Siberian region

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Alcoholism is a severe multifactorial mental disorder. At the present time genetics of alcoholism remains an actual problem for predictive (personalized) medicine, because of uncertainty of risk assessment of multifactorial diseases. The purpose of this study was to analyze the associations of ethanol-metabolizing and antioxidant enzyme CAT and two enzymes of the serotonergic system (COMT and SLC6A4) with the alcoholism risk at the allele and haplotype levels in Russians of the West Siberian region. Polymorphisms rs11032700, rs34964953, rs10836244, rs769217, rs2420388 in the gene CAT, rs174695, rs35617967, rs6267, rs4986871, rs165774 in the gene COMT, and rs3794808, rs140700, rs2228673, rs6354, 5HTTLPR in the gene SLC6A4 have been studied by the Real Time PCR. Alcoholism was diagnosed according to the International Classification of Diseases Tenth Revision (ICD-10). Russian cohorts of alcoholics (n=96) and controls (n=95) did not differ either in allele frequencies or the haplotype frequencies of all three genes. Therefore, these two cohorts were combined for further analysis of the haplotype structure. It was shown that the first common haplotype AGCG (66.5%) is separated from the second most frequent haplotype CTTA (20.2%) by four mutations (recombinant steps). The third haplotype CGCG (11.3%) is separated from the first one by one mutation step. All haplotypes are in a single block, therefore, they can recombine with a very low frequency only. So the revealed fact of the presence of four hypothetical mutational steps between haplotypes AGCG and CTTA is surprising. This work was supported by RFBR grant #09-04-99083-r_ofi and #12-04-00595-a.

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J05.11
A regulatory path associated with X-Linked Intellectual Disability and Epilepsy links the histone demethylase KDM5C to the Polyalanine expansions in the transcription factor ARX

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Polyalanine expansion mutations of Aristaless-related homeobox gene (ARX) cause a spectrum of X-chromosome phenotypes with Intellectual Disabilily (ID) and various forms of malignant paediatric epilepsy. We have established that ARX regulates and binds a CNE element in the 5' region of the lysine (K)-specific demethylase 5C (KDM5C), a known XLID gene involved in chromatin remodeling and neuronal maturation. By in vitro studies, we have analyzed five Polyalanine mutants establishing a decreased trans-activating activity and a reduced, but not abolished, binding to the KDM5C regulatory region. By quantitative RT-PCR, we have showed in murine Arx-KO ES cells and neurospheres a dramatic downregulation of the Kdm5C mRNA levels that leads to a decrease of the KDM5C protein. In Arx KO GABA-oriented model, which presents severe abnormalities in dendrite formation and GABAergic maturation, we have found a KDM5C reduction in coupling with a global increase of H3K4me3 signalling, potentially due to a compromised KDM5C activity. Since H3K4me3 is the hallmark of open chromatin, ARX-dependent KDM5C defects could compromise cyclical rounds of methylation-demethylation and consequently the chromatin remodelling. Starting from these data, we are now able to break up a unique ARX-dependent epigenetic road involved in epileptogenesis, suggesting that the molecular pathogenesis of ARX Polyalanine mutations may be in part caused by aberrant histone demethylation as a result of KDM5C defect. As chromatin modifications are reversible, it is possible that epigenetic drugs could compensate KDM5C-H3K4me3 deregulation opening further studies to cure or ameliorate ARX Polyalanine-related epilepsy.

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J06.01
Allele-specific programming of Npy and epigenetic effects of physical activity in a genetic model of depression

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Neuropeptide Y (NPY) has been implicated in depression, emotional processing and stress response. Part of this evidence originates from human single nucleotide polymorphism (SNP) studies. In the present study, we report that a SNP in the rat Npy promoter (C/T; rs105431668) affects in vitro transcription and DNA-protein interactions. Genotyping studies showed that the C-allele of rs105431668 is present in a genetic rat model of depression (Flinders Sensitive Line; FSL), while the SNP's T-allele is present in its controls (Flinders Resistant Line; FRL). In vivo experiments revealed binding of a transcription factor (CREB2) and a histone acetyltransferase (Ep300) only at the SNP locus of the FRL. Accordingly, the FRL had increased hippocampal levels of Npy mRNA and H3K18 acetylation; a gene-activating histone modification maintained by Ep300. Next, based on previous studies showing antidepressant-like effects of physical activity in the FSL, we hypothesized that physical activity may affect Npy's epigenetic status. In line with this assumption, physical activity was associated with increased levels of Npy mRNA and H3K18 acetylation. Physical activity was also associated with reduced mRNA levels of a histone deacetylase (Hdac5). Conclusively, the rat rs105431668 appears to be a functional Npy SNP that may underlie depression-like characteristics. In addition, the achieved epigenetic reprogramming of Npy provides molecular support for the putative effectiveness of physical activity as a non-pharmacological antidepressant.

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J06.02
Association analysis of YWHAE gene polymorphic loci and suicide in two ethnic groups of Bashkortostan (Russia).

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Recently, M.Yanagi et al. (2005) genome-wide study identified YWHAE gene as susceptibility gene for suicidal ideation and completed suicide. To examine whether the YWHAE gene plays a significant role in pathogenesis of suicide, we conducted a replicative study to investigate the association between three SNPs (rs1532976, rs3752826, rs9393) of this gene and attempted suicide in Russian and Tatar patients from Bashkortostan (Russia). We genotyped DNA samples of 236 cases (Russians - 127, Tatar -109) who had suicide attempts and 362 control subjects (Russians - 134, Tatar - 228) using PCR-RFLP technique. For pairwise linkage disequilibrium and haplotype analysis, the Haploview 4.1 program was used. Odds ratios (OR) with

95% confident intervals (CI) were calculated.
We found a strong allele association between YWHAE rs3752826 and suicide: C was significantly overrepresented in patients with suicide as compared to controls (P=0,02, OR=1,91, 95%CI 1,09-3,36) regardless of the ethnicity. Moreover we observed in Russian ethnicity group YWHAE rs9393 allele T (P=0,002, OR=2,21, 95%CI 1,32-3,57), YWHAE rs1532976 genotype T/T (P=0,018; OR=2,73, 95%CI 1,17-6,50) and YWHAE rs1532976 allele T (P=0,025; OR=1,52, 95%CI 1,037-2,22) were significantly overrepresented in patients with SB as compared to controls. Haplotype analysis showed a significant overrepresentation of the YWHAE (rs1532976 and rs9393) A-T haplotype (P=0,025, OR=1,54; 95%CI 1,06-2,24) in suicide attempters as compared to controls. This result suggests YWHAE as a potential suicide-susceptibility gene, and confirms ethnic specificity of this association.
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J06.03

Frequency of the polymorphisms of the genes of the enzymes alcohol dehydrogenase (ADH1B and ADH1C) and aldehyde dehydrogenase (ALDH2) in Brazilian alcohol abusers woman.

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Introduction: The influence of polymorphisms in loci encoding the enzyme alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) is known to be associated with alcohol dependence, however this influence is not yet characterized for the Brazilian population. Objectives: Determine the frequency of the polymorphisms ADH1B (ADH1B*2 - Arg47His and ADH1B*3 - Arg369Cys), ADH1C (ADH1C*2 - Ile349Val), and ALDH2 (ALDH2*2) in Brazilian alcohol abusers woman. Methods: 56 alcohol abusers woman were screened for alcohol dependence by CAGE, T-ACE, TWEAK and Short Alcohol Dependence Data - SAAD. The region of interest of the genes were amplified by PCR and analyzed by electrophoresis gel.Results: According to the CAGE, T-ACE and TWEAK evaluation 66,67% of the woman were classified as severe alcohol depedence; 45% as moderate and 5,88% as mild. The polymorphisms distribution were: ADH1B*2 : ADH1B*1/1 94,34%; ADH1B*1/2 5,66%; ADH1B*3: 94,23% ADH1B*1/1; 3,85% ADH1B*1/3 and 1,92% ADH1B*3/3; ADH1C*2: 64,15% ADH1C*1/1; 32,08%; ADH1C*1/2 and 3,77% ADH1C*2/2. ALDH2* :100% ALDH2*1/1. There were no significant differences between the distribution of the genotypes ADH1B*2, ADH1B*3 and ALDH2*2 between the alcohol abusers to the healthy Brazilian population. The ADH1C * 2 allele was significantly less frequent among alcohol abuser as compared to the healthy Brazilian population (p=0,0058). Conclusions: Although it is still a small sample, we can suggest that the presence of ADH1C * 2 allele might be a protective factor to alcohol dependence among women in Brazil.Sponsor: FAPESP 2011/08960-8.

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J06.04

Analysis of polymorphisms in GABRA2 and CHRM2 genes in patients with alcoholism from Russia

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Several recent studies have provided strong evidence that gamma-aminobutyric acid (GABA) A receptor subunit genes clustered on chromosome 4 are implicated in alcoholism in humans. The cholinergic muscarinic 2 receptor (CHRM2) gene has been considered a candidate gene for the alcohol dependence in that it might underpin certain risk factors for this condition. We designed a classical case-control association study for polymorphisms in GABRA2 gene (rs279858, rs495818) and CHRM2 gene (rs1824024, rs1455858). Population stratification and ancestry differences within populations may compromise the success of association studies. Therefore we performed analysis in three homogeneous ethnic groups from Russia.
333 men with diagnosis of alcoholism (122 Russians, 101 Tatars, 110 Bashkirs) and matched control groups were typed for the above-mentioned gene variants using PCR-RFLP and TaqMan assay techniques.
Analysis of the rs1455858 polymorphism in the CHRM2 gene showed significant association of CHRM2*T allele with acute alcoholic psychosis in Russian population (p<0.05;OR= 1.95). In total sample the frequency of individuals carrying the CHRM2*G/*G genotype of the rs1824024 was significantly higher in the group of alcoholics compared to the healthy controls (p<0.05;OR=2.35). Analysis of the rs279858 in the GABRA2 gene revealed that GABRA2*G allele was the marker of high alcoholism risk in young adults (p<0.05; OR=1.40).
Our findings are in line with a number of recent studies questioning the association between alcoholism and polymorphisms in GABRA2 and CHRM2 genes. rs1455858, rs1824024 and rs279858 SNPs are probably involved in the development of alcoholism in some populations from Russia. This work was supported by RFBR (grant#11-04-97032-r_povolzhye_a).

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J06.05

Pharmacogenetic Study of Second Generation Antipsychotic Therapy in Autism Spectrum Disorders

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Background: Autism spectrum disorders (ASD) are among the most common children neurodevelopment disorders. ASD patients often have co-morbid conditions, such us obsessive-compulsive, sleep disorders, self-destructive behavior, increased irritability, aggression and self-injury. One third of ASD patients take an antipsychotic medication. One of the most popular second-generation antipsychotic is risperidone. Two cytochrome P450 genes, CYP2D6 and CYP2C19, are involved in metabolization of psychotropic medications.
The aim of the study was to determine the incidence of CYP2D6*4, CYP2D6*41, CYP2C19*2 alleles in the case and control groups, and prognostic assessment of risperidone therapy in association with defined alleles.
Methods: Ninety-five patients with ASD participated in the study. The control group consisted of 190 healthy, non-related individuals without ASD. CYP2C19*2, CYP2D6*4, CYP2D6*41 were genotyped by the TaqMan method. Alleles' and haplotype frequency differences in case and control groups were compared by chi-square (χ^2) test. Frequency of adverse reactions and therapy corrections were evaluated in the patient group.
Results: Association with ASD pharmacotherapy was not found for the haplotypes CYP2D6*41C/T – CYP2D6*4C/T (p=0.4), therefore these cannot be safely used in the drug therapy prognostics. In the case group CYP2D6*41 allele T was observed more frequently than in the control group (p=0.01).
The most common adverse drug reaction was hyperprolactinemia, the cause of which may be associated with ASD neurotransmitter defects, and not with the primary phase of xenobiotic metabolism.

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J06.06

Abnormal growth and dysmorphic features in children with autism spectrum disorders

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Autism spectrum disorders (ASDs) are very heterogeneous neurodevelopmental disorders of children diagnosed solely on the basis of the triad of persistent social and language deficits and stereotypic behaviors. Several lines of evidence have indicated the strong role of genetics in the etiology of ASDs. Progress in understanding the genetic and biological basis of autism has been impeded by the variability among individuals with autism. Clinical morphology might be used as a biomarker for ASDs to clarify the complexity of the disorder.
In an effort to delineate more homogeneous autism subgroups for genetic study, we evaluated 83 autistic individuals. The clinical examination consisted of standard morphological measurements and comprised a broad range of qualitative and quantitative physical measurements. Our findings show that morphological features are associated with autism and a comprehensive clinical morphology examination that classifies autistic children as either phenotypically normal or abnormal is the first step needed for separating autism into causally distinct subgroups. Exploring potential underlying genetic mechanisms of this association might lead to a better understanding of autism. Identifiable endophenotypes and reliable biomarkers within ASDs would help to focus molecular research and uncover genetic causes and developmental mechanisms.

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J06.07

Cytogenetic analysis in autistic disorder

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Autistic disorder (AD) is a very complex and severe neuropsychiatric condition, increasingly frequent among children, with intricate and yet uncertain etiology, defined by impairments in communication and social skills, with onset prior to age 3 years. Genetic and cytogenetic studies revealed that most of the chromosomes are associated with autism, abnormalities of chromosomes 15 and X being most frequently documented.
AIM OF THE STUDY: Cytogenetic analysis on peripheral blood samples from children with autism was performed to identify possible structural or numerical chromosomal abnormalities by GTG-banding.
RESULTS: The study revealed normal karyotypes in most cases (47 out of 50 individuals). Two of the male patients had modified karyotypes (2% mosaicism): chromosome 9qh+ polymorphism and terminal deletion on chromosome 15q, respectively. One of the female patients had an extra chromosome 8 (2% mosaicism).
CONCLUSION: This is the first cytogenetic testing performed specifically on autistic children in Romania.
Our results show that karyotyping analysis is not conclusive in most cases with autism. Nevertheless, the test should be proposed and performed for every autistic patient, in order to eliminate possible numerical or structural chromosomal abnormalities as the cause for AD.

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J06.08

Marfan syndrome with Asperger disorder and schizophrenia in an adolescent boy

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Aim: To present the rare association of 2 psychiatric disorders in an adolescent with Marfan syndrome. Methods: A 17 years old boy was evaluated in our neuropsychiatry department for a psychotic episode. Results: The adolescent was in a state of extreme agitation and presented auditory and visual hallucinations. Furthermore, he had persecution delirium, was highly suspicious and anxious and feared for his life. The symptoms appeared gradually during the 3 weeks prior to admission. His medical history revealed he was previously diagnosed with Marfan syndrome and Asperger disorder. The physical examination was suggestive for the connective tissue disorder and otherwise unremarkable. The EEG didn't show abnormal electrical activity, while a heart ultrasound confirmed a mildly dilated aortic bulb. He received antipsychotic and sedative treatment with a favorable outcome and remains in psychiatric follow-up. Conclusions: Although, several cases with Marfan syndrome and different psychiatric disorders are reported in the literature, to our knowledge this is the first time both schizophrenia and Asperger disorder are found in such a young patient with Marfan syndrome

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J06.09

Exceptional case of a partial deletion of a long shoulder of a chromosome 18

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Carrying out classical cytogenetic and molecular and genetic research is of great importance for identification of the reasons of emergence of many hereditary diseases at patients, especially in children's psychoneurological practice. Even small chromosomal reorganization can have the expressed clinical manifestations.We give diagnostics case at the girl of nine years of an exceptional case of a partial monosomiya of a long shoulder of a chromosome 18. The child is observed at the psychiatrist with the diagnosis: easy intellectual backwardness (F70), IQ 67, syndrome of a motive hyperactivity with deficiency of attention, a delay of speech development. At survey small anomalies of development were noted: mikrotsefaliya, high forehead, gipertelorizm, epikant, large antihelix and antitrestle, the long and flat filter, makrostomiya, the high sky, the low growth, the reduced mass of a body, partial skin sindaktiliya of fingers of hands.
At cytogenetic research at the girl the partial deletion of a long shoulder of a chromosome 18 is revealed. Karyotype of the patient
46,XX, del(18)(q22). Parents of the child are inaccessible to cytogenetic research.
Generally the phenotype of our patient corresponds
to clinical descriptions of patients available in literature with a deletion of a long shoulder of the chromosome 18. However the patient has only small anomalies of development and intellectual backwardness while accompanying somatic pathology described in literature and rough developmental anomalies are
absent.

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J06.10

Replicative association analysis of schizophrenia in Kazakh population

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Ethnic background is an important factor in personalized health care and predictive medicine directed to prevention of common diseases. Common psychiatric and neurological disorders including schizophrenia are the subject of intensive genetic research based on genome-wide association studies (GWAS) and targeted resequencing of genomic regions of interest. Genetic variants associated with cognitive impairments, which are an important endophenotypes for schizophrenia, also have been revealed by GWAS. The aim of this study was to replicate the GWAS findings in European and Chinese populations in ethnic populations of Kazakhs. Kazakhs, the Mongoloid Turkic-speaking population, represent the major ethnic group of Republic of Kazakhstan and also a significant ethnic minority in neighboring Russia, Uzbekistan, Mongolia and China.
15 SNPs strongly associated with schizophrenia and cognitive performance according to recent GWA studies were genotyped by real-time PCR in Kazakh patients with paranoid schizophrenia and in healthy control group (N= 302). The association of rs2312147 located in the region of vaccinia-related kinase 2 (VRK2) gene, previously reported in GWAS by Stefansson et al. (2009), was confirmed in Kazakhs (OR = 1.72, p = 0.008). Rs2247572 located in the vicinity of KCNB2 (potassium voltage-gated channel) gene found in genome-wide association with cognitive performance traits (Need et al, 2009) was also associated with schizophrenia in Kazakhs (OR = 1.64, p = 0.016).
Association of genetic markers in loci for TLR4, SLCO6A1, NOTCH4, TCF4, ZNF804A, AGBL1, RELN, ZFP64P1, CSMD1, CPVL, NRG and NRIP1 was not replicated in Kazakh population.
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J07.01

Muscle-specific FXR1P isoforms regulate p21 levels: Implications for the pathophysiology of Facio-Scapulo Humeral Dystrophy

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The Fragile X-Related 1 gene (FXR1) is a paralog of the Fragile X Mental Retardation 1 gene (FMR1), whose absence causes the Fragile X syndrome, the most common form of inherited intellectual disability. FXR1P plays an important role in normal muscle development and its absence causes muscular abnormalities in mice, frog and zebrafish. A reduction of muscle-specific isoforms is found in myoblasts from Facio-Scapulo Humeral Dystrophy (FSHD) patients. FXR1P is an RNA-binding protein involved in translational control. In the present study, gene expression profiling of C2C12 myoblasts reveals that transcripts involved in cell cycle and muscular development pathways are modulated by Fxr1-depletion. We observed an increase of p21 - a regulator of cell-cycle progression - in Fxr1-knocked-down mouse C2C12 and FSHD human myoblast. This phenotype is rescued by re-expressing human FXR1P muscle-isoforms. FXR1P muscle-specific isoforms bind p21 mRNA via direct interaction with a conserved G-quadruplex located in its 3' untranslated region. The FXR1P/G-quadruplex complex reduces the half-life of p21 mRNA. In the absence of FXR1P the upregulation of p21 mRNA determines the elevated level of its protein product that affects cell-cycle progression inducing a premature cell-cycle exit and generating a pool of cells blocked at G0. Our study describes a novel role of FXR1P that has crucial

implications for the understanding of its role during myogenesis and muscle development since we show here that in its absence a reduced number of myoblasts will be available for muscle formation/regeneration, shedding new light into the pathophysiology of FSHD.
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J07.02
Molecular-genetics research of the common form of sarcoglycanopathies in Russia

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Sarcoglycanopathies - diseases in a group of lap-limb muscular dystrophies with autosomal recessive inheritance. There are four forms of sarcoglycanopathies (LGMD2C-2F), depending on the type of the affected protein. The most common of them is LGMD2D due to mutations in the SGCA gene. Mutations in the genes of sarcoglicans lead to the clinical picture, similar to Duchenne myodystrophy (DMD).
The aim of the work: study of the coding sequence of SGCA gene in the samples of some girls, whose guide diagnosis was DMD. All samples from the base of the Laboratory of DNA-diagnostics RCMG RAMS.
By direct automated DNA sequencing we studied 34 probands. The study identified previously described mutations (c.271G> A, c.229C> T, c.518T> C) in 14.7% of cases (5 probands). We detected two „common" mutations (c.271G> A, c.229C> T). Replacing c.229C> T met at 2 of the probands in the homozygous and 1 proband in the heterozygous state, replacing c.271G> A met at one of the proband in the homozygous and 1 proband in the heterozygous state. Theese two „common" mutations encountered in 4 of 5 patients (80%), 8 of the 10 affected chromosomes (80%).
This research shows the need of study SGCA gene for girls with DMD clinics. The first step in the molecular genetics diagnosis of the gene should be a study of two „common" mutations, and only in the case of absence of them, study whole the coding sequence.

D. Poliakova: None.

J07.03
Frequency of non-distrophic myotonias in RF

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The cohort of 84 unrelated patients with different non-distrophic myotonias was formed in laboratory of DNA diagnosis of MGC RAMS. And for all of them direct sequencing of CLCN1 gene or SCN4A gene in tandem was performed depending of diagnosis. Tomsen's (TM) and Becker's (BM) myotonias with mutations in CLCN1 gene were revealed in 66 patients. Paramyotonia of Eulenburg, hyper- and hypokalemic paralisys and other sodium channel myotonias were revealed in 14 cases. So fractions chloride channel and sodium channel myotonias were 82% and 18%. There were only 5 cases of AD TM (8%) and 61 cases of AR BM (92%) in group of chloride channel myotonias. It is interesting that the most prevalence mutation in CLCN1 gene was recessive nonsense-mutation c.2680C>T (p.Arg894*) - 36 chromosomes out of 118 chromosomes with all revealed CLCN1 gene mutations. There were 112 chromosomes with recessive CLCN1 gene mutations, so fraction of p.Arg894* in all BM patients is 32% (95%Confidence Interval (CI) = 24% - 42%). Allelic frequency of p.Arg894* in population RF is 1,2%, so frequency of all carriers of recessive CLCN1 mutations is 1/13 (95%CI = 1/35 - 1/6). In result of this investigation we calculated the frequencies of AD and AR forms of myotonias and in RF: TM - 1:710 (95%CI = 1/5000 - 1/145) and BM - 1:8165 (95%CI = 1/31746 - 1/26). Summarized frequency of chloride channel myotonia in RF estimated as 1: 653 (95%CI = 1/4320 - 1/22).

E. Ivanova: None. **A. Polyakov:** None.

J07.04
Frequent mutations and new allelic variants of congenital merosin-deficient muscular dystrophy in Russia

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Congenital muscular dystrophy, merosin-deficient (MDCMD) is a severe form of CMD with absence merosin around muscle fibers, specific MRI features, elevated serum creatine kinase in early months of life. Common clinical features are severe hypotonia, weakness and contractures, respiratory insufficiency.
Eighteen patients with clinical features of MDCMD, MRI and immunohistochemistry data were investigated for LAMA2 gene sequence analysis. Mutations were found in 72% of cases (13 patients of 18). Seven new allelic variants of MDCMD were found in this investigation. Interesting, that three mutations occurred more, than one time. Mutations c.2049_2050delAG and c.7732C>T were found on two chromosomes (8% each of confirm cases either). This mutations continually were described in literature. Mutation c.7536delC was found on seven chromosomes (27% of confirm cases) including one monozigotes stage, that allows to suppose existence of founder effect for Russian patients. Signally, that the mutation was described in literature only once. Also we separatet "hot" exons for top-priority search of mutations for Russian patients, that is important for time and cost saving of research. On findings and literary data the system of identification most frequent mutations in LAMA2 gene was created by us for time and cost saving of research too.
At present huplotype analysis for three frequent mutations and calculation of population carrier state of MDCMD are realizing by us.

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J07.05
Role of thrombophilic genetic markers in Ischemic Stroke patients from North India

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Background:Very few attempts have been made to study the role of genetic variation in development of stroke in Indian population. This study looked at correlations, if any, between the thrombophilic genetic markers and severity of the stroke.Methods:This study was conducted on 120 adult patients of ischaemic stroke underwent gene mutation analysis for FactorVLeiden ,MTHFR(C677T),Prothrombin(G20210A),GPIIb/IIIa(PLA1/A2) and PAI-1-(4G/5G) polymorphisms by PCR RFLP.Severity of stroke was assessed on admission based on National Institute of Health Stroke Scale (NIHSS).Multiple risk factors for ischemic stroke were also included.Results:The mean age of the patients was 52 years(range 20-92 years)included 77 males and 53 females.As per NIHSS scoring the severity of stroke was mild in 24.1%(29), moderate in 63.3%(76), moderately severe in 5%(6) and severe in 7.5%(9). The T allele frequency of MTHFR among mild, moderate, moderately severe and severe groups were 17.4,17.7,33.3 & 33.3% respectively which was not significantly distributed. A2 allele of PLA1/A2 was significantly associated with moderately severe and severe phenotype(p=0.02).Frequencies of 4G allele of PAI-1 were 50% in mild, 62.5% in moderate,91.6% in moderately severe and 72% in severe phenotype, was not quite significant(p=0.06).FVL mutation was present in only two patients each from mild and moderate group.Whereas,prothrombin mutation was completely absent in our patients.Among all the risk factors chronic alcoholism, obesity and vitamin B12 levels were significantly associated with the high severity (p=0.03,0.04 & 0.03 respectively).Conclusion:A2 allele of PlA1/A2 polymorphism is associated with moderately severe and severe group of patients.Chronic alcoholism, Obesity and Vitamin B12 levels are associated with the severity of the stroke.

V. Arya: None. **P. Correia:** None. **C.S. Agarwal:** None. **M. Bhargava:** None.

J07.06
Mutational analysis of NEFL gene in hereditary motor and sensory neuropathy patients from Bashkortostan Republic (Russia)

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Hereditary motor and sensory neuropathy (HMSN) is a clinically and genetically heterogeneous disorder of peripheral nervous system. The HMSN frequency in Bashkortostan Republic (BR) is 10,3 :100000. We examined HMSN patients from BR and detected spectrum and frequency of specific mutations in the NEFL gene using direct sequencing of its coding regions. The NEFL gene cause the demyelinating (1F) and axonal (2E) subtypes of HMSN. The NEFL gene (8p21) codes neurofilament light protein - an important factor in determination of the axonal diameter, axonal transport, regeneration of axons and conduction velocity of peripheral nerves. Molecular-genetic investigation of HMSN was conducted in 125 of the 197 unrelated families with previously excluded mutations in genes PMP22, GJB2, MPZ,

EGR2, GDAP1 and MFN2. We detected 4 different nucleotide changes in the NEFL gene. One nucleotide substitution c.488A>T (p.Glu163Val) which hadn't been described previously was found in the heterozygous state in the HMSN I patient and was not detected among healthy family members and controls (n=100). This change is supposed to be the disease causing mutation. Three nucleotide changes revealed in HMSN patients appeared to be polymorphic variants: c.639C>G (rs35575466), c.1402G>A (rs57153321), and 1168-11T>C (rs76347846) (5% among all HMSN types in total of patient sample). Thus, NEFL gene alterations are rare causes (<1%) of HMSN. The received data will contribute to optimization of medical and genetic consulting of HMSN families in our region.

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J07.07

Point mutation analysis of SMN1 gene in patients with spinal muscular atrophy in four unrelated Russian families.

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INTRODUCTION: Most patients with the clinical diagnosis of SMA (95%) have homozygous deletion of *SMN1* gene. The remaining 5% are compound heterozygous with a deletion of the *SMN1* gene on one of their alleles and a point mutation on the other allele.
OBJECTIVE: To identify the point mutations in *SMN1* gene and confirm the diagnosis SMA in the patients with heterozygous deletion of *SMN1* gene.
METHODS: MLPA was carried out to measure the copy number of *SMN1* and *SMN2* genes in the patients. The point mutation analysis of *SMN1* gene was performed by direct sequencing.
RESULTS: (Tab. 1).
CONCLUSION: The mutations IVS6-2A>T and p.Gln15X caused severe clinical phenotype SMA I. This study suggested that it is necessary to detect the point mutations in *SMN1* gene in the patients with heterozygous deletion of *SMN1*. It would be beneficial for prenatal diagnosis and genetic counseling in such families.
Table 1. The results of current study.

Patient	Identified mutations in *SMN1* gene	Parents data	Copy number of *SMN2* gene	Type SMA
P.6805	IVS6-2A>T + 7-8 exons deletion	father: IVS6-2A>T / N mother: 7-8 exons deletion / N	2	I
P.7413	p.Gly275Ala + 7-8 exons deletion	father: IVS6-2A>T / N mother: 7-8 exons deletion / N	3	II
P.7830	p.Gln15X (de novo) + 7-8 exons deletion	father: N / N mother: 7-8 exons deletion / N	2	I
P.7864	p.Thr274Ile + 7-8 exons deletion	father: p.Thr274Ile / N mother: 7-8 exons deletion / N	3	III

V.V. Zabnenkova: None. **G.E. Rudenskaya:** None. **E.L. Dadali:** None. **A.V. Polyakov:** None.

J07.08

SMN1, SMN2 and NAIP gene deletions in Romanian patients with spinal muscular atrophy

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Spinal muscular atrophy (SMA) is characterized by degeneration of motor neurons that cause progressive muscle weakness and muscle atrophy. SMN1 homogizot gene deletion is present in most patients with SMA (~95%). The objective of this study was to evaluate the SMN1, SMN2 and NAIP gene mutational status in SMA romanian patients. In this study, we analyzed by PCR-RFLP 53 patients with suspected SMA and 32 relatives. We also performed a prenatal test fetal with DNA obtained by amniocentesis from mother, who had prior history of another child diagnosed with SMA.
We identified homozygous deletion of exons 7 and 8 of SMN1 and SMN2 genes in 23 patients, and only deletion of exon 7 at 2 patients. Homozygous deletion of exon 7 and 8 of SMN2 gene was identified in 2 patients and one relative of a patient. NAIP gene deletion was identified in 8 patients and 3 relatives. For prenatal testing, we identified homozygous deletion of exon 7 and 8 of SMN1 gene.
Molecular diagnosis of SMA by identifying of SMN1 gene deletion is a useful tool for the diagnosis of SMA in Romania, being necessary deermination of SMN copies for a better matching phenotype - genotype.
This work was supported by CNCSIS - UEFISCDI, project number PN II - IDEI 2152/2008 and Program 8, the project „Intervention for diagnosis and management of spinal amiotrophy and muscular dystrophy type Duchenne and Baker, and the prevention of their hereditary transmission" 2011.

M. Stavarachi: None. **M. Toma:** None. **P. Apostol:** None. **S. Spandole:** None. **N. Butoianu:** None. **C. Iliescu:** None. **C. Burloiu:** None. **M. Budisteanu:** None. **D. Craiu:** None. **D. Iancu:** None. **L. Bărbării:** None. **D. Cimponeriu:** None.

J07.09

Molecular and prenatal diagnosis of spinal muscular dystrophy in the Iranian population

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Spinal muscular atrophy (SMA) is an autosomal recessive disease, caused mutations in several genes named SMN1, SMN2 and NAIP. Here we report the results of our study on the frequency of mutations in SMN1 and NAIP genes in the Iranian patients with different types of SMA. A total of 126 patients with clinical diagnosis of SMA (36 patients type I, 7 type II, 7 type III and 7 older than 20 years patients). Also, prenatal diagnosis was performed on 57 CVS samples form carrier parents with an affected child. Our results on patients with type I SMA showed deletion in NAIP4, NAIP5, Exon7, Exon8 with frequency of 80%,20%, 80%, 80% and 20% , respectively. In patients with type II SMA, the results were 86%, 28%, 28%, 43% and 14% deletion/mutation in NAIP4, NAIP5, Exon7, Exon8 (SMNI), respectively. The data of our prenatal diagnosis indicated five one out of 57 has deletions in NAIP4 and Exon8 , two fetuses only in NAIP4 and the rest showed no deletion. Together, our data show that deletion in NAIP5 always was associated with other deletions in the exon 7 and 8 of the SMNI gene, which could be used as a molecular marker in diagnosis of the Iranian SAM patients.

R. Valian Boroujeni: None. **S. Vallian Broojeni:** None.

J07.10

A novel mutation in RYR1 causes severe congenital form of central core disease

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Central core disease (CCD) is the first described congenital myopathy. It is characterized by central cores in most type 1 muscle fibres on muscle biopsy. A large phenotypic variability has been demonstrated including almost asymptomatic and severe congenital forms. CCD is caused by mutations in *RYR1* gene, which are inherited in autosomal dominant fashion, but among more severe also autosomal recessive forms have been reported. Here we report a patient with a congenital form of CCD and a *de novo* mutation in *RYR1*, which has not been previously reported.
The patient was an eight months old girl, born to unrelated Caucasian parents after uneventful term pregnancy with a negative family history. At birth the child was hypotonic, had reduced movements, contractures of wrists, bilateral hip dislocation, and scoliosis, and she was oxygen dependent. The girl received invasive ventilation briefly, now replaced for non-invasive respiratory support during sleep or illness. She received partial nasogastric tube feeding for four months. By age 8 months she had not yet achieved head control and had no antigravity movements.
Among other investigations a muscle biopsy was done, which showed a pattern characteristic for CCD. The DNA sample was obtained for sequencing of *RYR1*. By sequencing four exons of *RYR1* in the mutational hotspot a novel missense mutation c.14759C>T (T4920I) was found, located in a highly conservative region for vertebrates. The mutation was not detected in parents showing a *de novo* event. Autosomal recessive inheritance cannot however be fully excluded before full gene sequencing is performed.

I. Micule: None. **J. Strautmanis:** None. **N. Pronina:** None. **B. Lace:** None.

J07.11

Duplication of 17p12 region (PMP22 duplication) in demyelinating CMT neuropathy in very early infancy

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CASE REPORT: We are reporting on a female infant (2yrs) with early onset out developmental delay, hypotonia and without dysmorphism. In her 24 months of age she cannot independently sit, walk and speak. She is only able to crawl on her back. Distal weakness of lower legs is dominating. Her karyotype is 46,XX and mutations in SHOX and SMA1 genes have been excluded. Inborn errors of metabolism investigations were negative. Family history was negative. Congenital CMV infection was suggested in different diagnosis. Brain MRI has showed diffuse CNS hypomyelination. The clinical features called for more precise genetic testing.
We provided the whole-genome screening by SNP array analysis (Illumina HumanCytoSNP-12v2.1) as the most recent effective method. We identified a 1.4Mb duplication in 17p12 region covering 16 RefSeq genes, which includes the PMP22 gene. From the clinical point of view is the duplication of the gene PMP22 the most important. The finding of the PMP22 duplication is consistent with a diagnosis of Charcot-Marie-Tooth disease type 1A (CMT1A, OMIM 118220) or subset 1E with deafness (CMT1E, OMIM 118300) or hypertrophic neuropathy of Dejerine Sottas (DSS, OMIM 145900), which are supported by clinical features in this patient. The parents of child are being tested.
Establishing the diagnosis of a very early demyelinating neuropathy with sensory impairment will enable clinicians to evaluate and manage more specific examination and health supervision by a multi-disciplinary team. However this hereditary CMT form is non-treatable.

M. Hejtmankova: None. **M. Trkova:** None. **V. Becvarova:** None. **L. Hnykova:** None. **H. Machkova:** None. **D. Stejskal:** None.

J07.12

Duchenne/Becker muscular dystrophy: First report on clinical, biochemical and genetic study in Gujarat population, India

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Background: Duchenne muscular dystrophy (DMD) is one of the most common inherited neuromuscular diseases, affecting 1 in 3500 males. It is an X-linked disorder caused by mutations in the dystrophin gene. Mutations leading to a truncated protein cause the severe phenotype of DMD, whereas mutations retaining the mRNA reading frame cause the more benign phenotype of Becker muscular dystrophy (BMD).
Material and Methods: In this study 60 clinically suspected D/BMD patients were tested by M-PCR (26 exons) for genetic analysis whereas biochemical indices were measured by using the COBAS 400 analyzer.
Results: Of the 60 suspected D/BMD patients. The diagnosis of D/BMD was confirmed by M-PCR in 57 patients. The mean age of onset was 4.6±0.19 years and the mean age at presentation was 10.6±0.43 years. The mean CK, myoglobin, LDH and calcium level were 7934.5±822.82 u/l, 875.55±47.14 ng/ml, 852.78±60.89 u/l and 9.15±0.12 mg/dl respectively. Of 57 cases, 8.8% were found deletion in proximal hotspot while remaining in distal hotspot. Most frequent deletion was found in exons 47, 48, 49, 50, and 51. The deletion rates were 36.84%, 47.36%, 54.38%, 57.89% and 42.1% respectively. Out of these, 7% had in-frame mutations.
Conclusions: This significant analysis has been carried out first time for D/BMD patients particularly from Gujarat, India. This study also emphasizes the need for further investigation into the genotype/phenotype aspects of the Gujarat D/BMD population.

G.M. Sindhav: None. **M.V. Rao:** None. **J.J. Mehta:** None.

J07.13

Hereditary motor and sensory neuropathy type 1B and multiple exostoses type II of Monozygotic twins - a family case report

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Hereditary motor and sensory neuropathy type 1B (HMSN 1B) is a rare autosomal dominant disease with mutations in the *MPZ* gene and dramatically disrupted of myelin compaction (MIM#118200), with late onset of independent walk, uncertain shaky gate, ataxia, distal paresis, sensory loss, generalized tendon areflexia of clinical phenotypes and very low nerve conduction velocity (NCV) of Electrophysiology. Multiple exostoses type II (EM II) is a rare autosomal dominant disease (MIM#133701) with mutations in *EXT2* gene and multiple bony spurs/lumps and deformities of long bones of patients.
We present a family (father aged 32 and his two daughters monozygotic twins aged 6) with two diseases HMSN 1B and EM II. The clinical phenotypes of are the same in all the patients they all have sensory ataxia, sensory loss, generalized tendon areflexia and distal paresis. Superficial palpation and X-ray examination revealed bilateral multiple exostoses shoulders, femurs and ribs. Median motor NCV was less than 6 m/s and sensor NCV was absent in all the patients.
In three DNA samples direct sequencing detected missense mutation c.389A>G (Lys130Arg) in exon 3 of *MPZ* gene (locus 1q23) and missense mutation c.678C>A (Tyr226Stop) in exon 4 of *EXT2* gene (locus 11p11) all of them in heterozygous state.
The HMSN 1B and EM II, occur independently of one another, however, their coexistence in the same patient are not described before.

S.A. Kurbatov: None. **V.P. Fedotov:** None. **N.M. Galeeva:** None. **T.B. Milovidova:** None. **A.V. Polyakov:** None.

J07.14

Implementation of triplet repeat primed PCR (TP PCR) in the molecular diagnosis of myotonic dystrophy type 1 (DM1)

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Background: Myotonic Dystrophy type I (DM1) is a dominantly inherited disorder with a multisystemic pattern affecting skeletal muscle, heart, eye, endocrine and central nervous system. DM1 is associated with the expansion and instability of CTG repeat in the 3' untranslated region of the DMPK gene located on chromosome 19q13.3. Unequivocal molecular characterization of the DM1 triplet expansion requires the use of different PCR protocols to amplify normal and mutated alleles combined with Southern blotting analysis to accurately size the expansion. Nevertheless, expansion detection by PCR may be somewhat problematic in homozygous individuals. The purpose of this study was to evaluate triplet repeat primed PCR (TP-PCR) as a screening method for DM1 diagnosis in the diagnostic laboratory.
Aim: implementation of routine method for diagnosis of DM1 patients in Bulgaria.
Materials and methods: Our cohort consists of 120 patients from 63 families with clinical symptoms referred to those of the disease. We used short range PCR and TP- PCR to identify CTG expansion. Expansion was confirmed by Southern blot.
Results: From 120 analyzed patients TP-PCR assay identified the DM1 expansion in 66 individuals form 28 families, with one normal allele and one expanded allele. We used Southern blot to confirm CTG expansion in 32 of the positive cases.
Conclusion: TP- PCR proved to be reliable method for routine diagnosis of DM patients. However it is not able to estimate the exact number of CTG repeats it is able to detect large expansions which are related with manifestation of DM symptoms.

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J07.15

Rigid spine congenital muscular dystrophy produced by selenoprotein N, 1 (*SEPN1*) mutations (RSMD1): long-time course in a young adult patient

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RSMD1 is a rare autosomal recessive disorder with several distinctive features. Unlike many congenital muscular dystrophies, early motor improvement and normal CPK are typical, while in contrast to structural myopathies there is no specific muscle morphology. Rigid spine, early scoliosis and joint contractures are characteristic. We diagnosed RSMD1 in a 27-year-old Russian female with previous diagnosis of unspecified myopathy. She had severe congenital muscle weakness with no head control in 12 months, and motor prognosis was considered poor. However, she walked independently in 15 months (though typical 'dropped head' persisted for some time), and her motor abilities were relatively satisfactory later on. Since 7-8 years scoliosis rapidly progressed. Spine surgery at the age of 15 was complicated by

cardiac arrest, while scoliosis did not improve. Soon persistent nocturnal hypoventilation with necessity of device support developed (one more RSMD1 typical feature). The patient has higher education, works part-timely in office, exercises regularly in gym. Lately she noticed some increase of weakness but walks independently. Apart from proximal myopathy and severe scoliosis her signs are: short stature (147 cm corrected for scoliosis), small feet 32 cm, long face, high palate, nasal speech, moderate dysphagia, stiff neck and forced abnormal head position, mild elbow contractures. CPK is normal. DNA test detected compound heterozygosity for two earlier described *SEPN1* mutations: c.683_689dup7 and c.1397G>A (p.Arg466Gln). This is our second RSMD1 case. In first patient, a 4-year-old boy from Uzbek (Central Asia) consanguineous family, homozygosity for *SEPN1* novel mutation c.998delC (p.Arg330GlyfsX71) was found out.

V.A. Kadnikova: None. **G.E. Rudenskaya:** None. **A.V. Polyakov:** None.

J07.16

Novel p.Gly484Asp mutation in *SPAST* gene is associated with infantile-onset autosomal dominant spastic paraplegia

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Hereditary spastic paraplegia (HSP) is a disorder with heterogeneous genetic etiology and variable phenotypic expression, typically presenting with progressive lower extremity spasticity and weakness. Autosomal-dominant (AD), autosomal-recessive, and X-linked inheritance has been reported and symptoms can occur in isolation or with other neurologic abnormalities. Spastic paraplegia type4 (SPG4) due to mutations in the *SPAST* gene is the most common AD form of HSP.
We report a 6 year old male with lower leg stiffness/spasticity, in whom symptoms developed in early infancy. Torso and upper extremity are unaffected at this time; growth and cognitive development is age appropriate. Family history analysis revealed similar symptoms in the patient's biological father and paternal grandfather, which were contributed to other disease processes (i.e. cerebral palsy, multiple sclerosis). The father's symptoms also started in infancy and were obvious at onset of walking.
Genetic work-up included sequencing of genes commonly associated with AD-HSP, including *SPAST* (Spastin), *ATL1* (Atlastin), *NIPA1* (SPG6), and *REEP1* (SPG31). No mutations were found in the latter 3 genes; while a novel heterozygous sequence variant (SNV) was detected in *SPAST*. This SNV (c.1451G>A, p.G484D) affects a highly conserved amino acid, was not found in HGMD, or the Exome Variant Server, and is predicted to be damaging/not tolerated by POLYPHEN/SIFT. Family studies are ongoing to link the SNV to the affected individuals in 3 generations. This case indicates a new pathogenic mutation in *SPAST* associated with infantile-onset autosomal-dominant spastic paraplegia.

G. Scharer: None. **A. Pickart:** None. **D. Helbling:** None.

J07.17

Molecular genetic testing of SMA in the Republic of Bashkortostan, Russia

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Spinal muscular atrophy is a severe autosomal recessive disease of the nervous system. SMA is caused by a progressive loss of motor neurons of the anterior horns of the spinal cord that occurs due to deletions 7 and/or 8 exons SMNt.
For 10 years (2003-2012) we studied 114 patients with a clinical diagnosis of SMA and 178 family members. The survey revealed that one-third of families (50 families out of 134) the disease is associated with the presence of 55-probands homozygous deletion of 7-8 exons SMNt. In 58 patients with suspected spinal muscular atrophy SMNt gene mutations not found, but this does not exclude the presence of mutations in other genes that cause SMA.
We have carried out 22 prenatal diagnostics performed in families where one child has spinal muscular atrophy. In four cases identified homozygous deletions. In other families found heterozygous deletions. Search for mutations was performed using the restriction fragment length polymorphism according to the protocol „Center for Molecular Genetics", Moscow.
Thus, the combination of clinical and molecular-genetic research methods helps the correct diagnosis of spinal muscular atrophy and allows medical genetic counseling families, including prenatal diagnosis.

A. Aupova: None. **H. Karavanova:** None. **H. Gysina:** None. **A. Frolov:** None.

J07.18

Establishing a Registry of Inherited Neuromuscular Disorders in Kuwait:A Preliminary Report

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Inherited neuromuscular diseases are broad category of disorders that affect all age groups worldwide. They, as a group, are relatively common with a prevalence of one in 3000. Many of these disorders were described since the nineteenth century but the advances in the imaging and other diagnostic techniques improved our understanding and classification of them. Broadly, these disorders can be classified into three categories. These categories are that affects peripheral nerve (neuropathy), diseases of muscle (myopathy and dystrophy) and diseases of spinal cord motorneurons (motor neuron disease). The prevalence of these disorders is not well characterized in Kuwaiti population. Kuwait Medical Genetic Center is the only medical genetic center in Kuwait which serves all Kuwaiti districts. I will present our experience in establishing the first registry for inherited neuromuscular disorders in Kuwait to know the burden of the disorder in our community as a step towards improving the quality of life for such patients.

L.A. Bastaki: None.

J07.19

Decade of raised awareness of neuromuscular diseases in Latvia

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Numbers of neuromuscular diseases (NMD) are inherited conditions affecting muscle, nerve and neuromuscular junction, and caused by mutations in the nuclear and mitochondrial genes. Every year expanding knowledge of these conditions add new genes to this list. In 2002, there was established a strategy in Latvia, to increase availability for diagnostics in patients with NMD, which included new diagnostic techniques, open NMD clinic for all patients and education for medical professionals.
Objective of the study was to evaluate diagnostic efficiency of NMD patients in Latvia in the past ten years.
Patient information was obtained from Medical Genetics clinic register, Molecular Biology laboratory and Latvian Biomedical Research and Study centre data in the two time periods: 2002-2008 and 2010-2012. Incidence, standard deviation and confidence interval at 95% calculated.
Results. Steady 6% increase was observed each year since 2002, due to the introduction of the new molecular biology techniques for DMD, CMT1a and SMA. In 2010 added new tests for MD, mtDNA sequencing and LGMD, which increased clinically or molecularly confirmed diagnosis up to 70% since 2002. The most common diagnosis were CMT (CI95%-0.07, SD-15), SMA (CI95%-0.06, SD-14) and FSHD (CI95%-0.01, SD-3.5), not DMD.
Conclusions. New diagnostic techniques complimented with education of medical professionals are the most effective instrument to increase diagnostic availability for the patients in the long term.

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J07.20

Management and molecular diagnosis in romanian patients with Duchenne/Becker muscular dystrophy

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Duchenne and Becker muscular dystrophy (DMD/BMD) are the most common muscular dystrophies with X-linked inheritance caused by mutations in the DMD gene. DMD is the most severe form with a complete absence of dystrophin synthesis. The most frequent DMD gene mutations are large deletions and duplications of one or more exons.
Method. For molecular diagnosis of patients diagnosed with DMD/BMD only on the base of clinical symptoms, family history and creatine kinase levels we used MLPA (Multiplex Ligation-dependent Probe Amplification) technique. Genomic DNA was extracted from blood samples and 35 unrelated patients and 20 relatives were analyzed. The MLPA products were quantified by capillary electrophoresis and interpreted using Coffalyser software.
Results. Large deletions were identified in 24 patients (68,57%) and large duplications in four patients (11,42%). 14 female patients with heterozy-

gous genotypes confirmed previously identified mutations in male patients and in 6 subjects, relatives of DMD patients, no mutations were found. In one case prenatal diagnosis was performed on a patient having a child with DMD, confirmed by molecular diagnosis. In 7 patients (20%) no deletions or duplications were identified and they were further investigated by sequencing.
Conclusions. Our results showed an overall frequency of large deletions and duplications of 80%. MLPA technique is particularly effective if Duchenne disease, allowing the detection of mutations in most cases and both prenatal diagnosis and diagnosis in children with specific symptoms of disease. A larger group of patients needs to be investigated for a more accurate evaluation on DMD gene mutations spectrum.

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J07.21
Role of medical rehabilitation program in chromosome 2 deletion with hypotonia - case study

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Many genetic conditions are related to changes in different genes on chromosome 2. Recent studies suggest that genes on chromosome 2 may be involved in intelligence determination. Partial deletion or loss of genes from specific regions of chromosome 2 is characterized by intellectual disability, epilepsy and autism spectrum disorder, which may be associated with syndromes. In this paper we report the case of a young girl, admitted in our hospital for severe retardation in motor acquisitions, at 1 year just active rolling, with severe hypotonia, specific symptoms and signs related to autism, severe mental retardation, microcephaly and mild facial dysmorphism, who was found to have a rare genetic condition represented by a small deletion of the long arms of chromosome 2. We included her in a complex rehabilitation program from the age of fourteen months. The objective was obtaining age appropriate functional independence. At the first admission she had a severe hypotonic syndrome, severe retardation in the acquisition of motor skills and mental retardation. After 7 hospitalizations of 21 days each, during 2 years, with complex rehabilitation programs-pool with thermal water and hydrokinetotherapy, individual kinetotherapy, electrotherapy, general massage, occupational therapy, the evolution was favorable with motor improvement. Independent standing, alternating steps, wider support base, ability to rise after falling and walking on short distances were achieved.
Rehabilitation treatment is effective in improving motor skills and must be an early intervention. Early diagnosis and improvements in management of such patients by a multidisciplinary team will improve prognosis and quality of life.

F. Cioara: None. **M.I. Cevei:** None. **C. Avram:** None. **D. Stoicanescu:** None.

J07.22
Novel mutation in IGHMBP2 gene causing spinal muscular atrophy with respiratory distress, type 1

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Spinal muscular atrophy with respiratory distress, type 1 (SMARD1, MIM# 604320) is an inherited condition that causes muscle weakness and respiratory failure, typically beginning in infancy. Early features of this condition are difficult and noisy breathing, especially when inhaling; a weak cry; problems feeding; and recurrent episodes of pneumonia. Typically, between the ages of 6 weeks and 6 months, infants with this condition will experience a sudden inability to breath due to paralysis of the the diaphragm. The muscle weakness is progressive and predominately affects the lower distal extremities. Other symptoms, although not always presented include intrauterine growth retardation, seizures, autonomic nervous system dysfunction, decreased pain perception, excessive sweating, constipation, and bladder incontinence. The genetic basis of SMARD1 is unrelated to the 5q-linked SMN-related classical SMA, and is due to mutations in another gene called IGHMBP2 (MIM*600502). Mutations in this gene occur in approximately 1% of patients with diaphragmatic SMA and the disease is characterized by autosomal recessive inheritance. Here we report on a family with two severely affected SMARD1 sibs. Both patients developed progressive muscular weakness and respiratory distress and died before 6 months of age. SMN gene was negative, but two mutation were detected in IGHMBP2 gene: one novel deletion c.780delG; p.(Gln260Hisfs*24), inherited from the father and a nonsense mutation c.1488C>A; p.(Cys496*) inherited from the mother.
To the best of our knowledge, this case might be considered as the most severe form of SMARD1, reported so far, presumably due to total absence of IGHMBP2 enzyme activity.

T. Todorov: None. **I. Litvinenko:** None. **A. Kirov:** None. **A. Todorova:** None. **V. Mitev:** None.

J07.23
Detecting exon mutations of the DMD gene using Multiplex Ligation-dependent Probe Amplification (MLPA)

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In order to explore the impact distinct genetic polymorphisms implicated in Duchenne myodystrophy on disease severity and age of onset were carried out molecular genetic tests of 33 patients with clinical manifestations of disease.
Material for the study was DNA samples extracted of standard protocol using «Qiagen» (USA). Detecting exon mutations of the DMD gene using Multiplex Ligation-dependent Probe Amplification (MLPA; P-034 and P-035 kits from MRC Holland) and capillary electrophoresis. Results of study were interpreted using the program «Coffalyser.Net».
Analysis of results of MLPA showed that from 9 (33%) patients had diagnosed the deletions of exons DMD gene. The detecting deletions were from 41 to 60 exons of DMD gene and it had the length from one to four exons. 11 % patients had the deletions the 13 exons of DMD gene with early debut and severity of disease.
In conclusion, the using of MLPA allows to determine the bigger length of deletions and duplications of exons of DMD gene, which in turn is the important stage of differentiated diagnosis and the prognosis of the treatment.

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J07.24
Rare Duplications in the DMD gene: Report of two Cases from Southwest Iran

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Duchene muscular dystrophy (DMD) is a common X-linked recessive neuromuscular disorder, affecting 1 in 3,500 live male births. About 65% of cases are caused by deletions; ~5% to 8%, by duplication; and the remaining, by point mutations of the dystrophin gene. The frequency of complex rearrangements (double-deletion and non-contiguous duplications) is reported to be 4%. Becker muscular dystrophy (BMD) is a milder allelic form, with a lower prevalence. DNA deletion and duplication were determined as the major mutation underlying DMD and BMD.
DMD gene, located at Xp21, is the largest identified gene in the human genome, spanning 2.4 Mb and 79 exons. It has a high mutation rate, with approximately one-third of all cases resulting from a spontane[[Unsupported Character - Codename]]ous mutation. Its transcript is 14 kb long and is expressed in skeletal muscle and brain.
Applying multiplex ligation-dependent probe amplification (MLPA), we have analyzed 2 unrelated DMD subjects from Iran.
Genomic DNA was extracted using standard procedures from the peripheral blood leukocytes, and MLPA was applied to detect DMD gene to identify genetic mutation.
using MLPA technique, in the first case were seen a duplication spanning from exon 2 to exon 9 of the dystrophin gene. In the second we observed a duplication of exons 10 and 11. The important point in this report is that none of the two duplications observed in this patients have been reported in Iran as yet, and these duplications are nearly rare cases in the world.

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J07.25

Variable expression of myotonic dystrophy type 1 in a three-generation family

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Myotonic dystrophy type1 (MD1), is a common dominantly inherited neuromuscular disorder, involving muscle wasting, myotonia, cataracts, hypogonadism. MD1 is result of a tandemly repeated expansion of trinucleotide CTG in the 3'UTR of DMPK gene (19q13.3). Normal alleles usually less than 35 repeats, can expand from 50 up to several thousand, showing variable clinical manifestation, from adult-mild to severe congenital form. We present a three generation affected family with MD1 with clinical symptoms indicating consequtively worsening of the disease. Index case was a newborn approached to clinic due to the poor sucking and hypotonia. He had all signs of congenital MD1- muscle weakness, dysmorphic face, relative macrocephaly, talipes equinovarus, hypogonadism. He had frequent respiratory infections. MRI showed global cerebral atrophy and severe developmental delay. The mother had mild symptoms: mild mental retardation, expressionless long face, ptosis, nasal speech, frontal balding, open mouth appearance. EMG confirmed myotonia. Both eyes have lenses opacities. The grandmother had normal intelligence, long face, ptosis and minor muscular weakness on arms after her 40's. Cataract was operated at 58. The diagnosis was confirmed in all three cases using TP-PCR method. PCR and Long range PCR using flanking primers of the repeated sequence gave only normal fragment of 13 repeats in propositus and 14 in mother/grandmother. TP-PCR method gives a characteristic ladder on the fluorescence enabling identification of large pathogenetic repeats that cannot be amplified using normal PCR procedure. Southern blot analysis should be performed in order to determine the exact expansion of pathogenic allele.

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J07.26

Study of relation between NAIP gene deletion, TTV infections and spinal muscular atrophy in Romanian patients

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Proximal spinal muscular atrophy (SMA) is a genetically heterogeneous disease with paresis and muscle atrophy due to loss of anterior horn cell function. The survival of motor neuron gene (SMN) and neuronal apoptosis inhibitory protein (NAIP) play a primary role. Torque teno virus (TTV) seems to be able to interfere with production of some proinflammatory cytokines and was associated with some neurodegenrative diseases. The objective of this study was to evaluate the relation between NAIP deletion, TTV infections and SMA in Romanian patients. In this study, we included 25 SMA patients and analyzed by PCR the NAIP deletion and TTV infection status. We identified the deletion of NAIP gene in 8 patients (32%), and TTV infections in 21 patients (84%). We didn't find any relation between distribution of TTV infections and NAIP deletion ($p > 0.05$).
This work was supported by CNCSIS - UEFISCDI, project number PN II - IDEI 2152/2008 and Program 8, the project „Intervention for diagnosis and management of spinal amiotrophy and muscular dystrophy type Duchenne and Baker, and the prevention of their hereditary transmission" 2011.

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J08.01

Astrocyte dysfunction triggers neurodegeneration in a lysosomal storage disorder

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The role of astrocytes in neurodegenerative processes is becoming increasingly appreciated. Here we investigated the contribution of astrocytes to neurodegeneration in Multiple Sulfatase Deficiency (MSD), a severe Lysosomal Storage Disorder (LSD) caused by mutations in the *Sulfatase Modifying Factor 1 (SUMF1)* gene. Using Cre/Lox mouse models, we found that astrocyte-specific deletion of *Sumf1 in vivo* induced severe lysosomal storage and autophagy dysfunction with consequential cytoplasmic accumulation of autophagic substrates. Lysosomal storage in astrocytes was sufficient to induce degeneration of cortical neurons *in vivo*. Furthermore, in an *ex vivo* co-culture assay, we observed that *Sumf1*$^{-/-}$ astrocytes failed to support the survival and function of wild type cortical neurons, suggesting a non-cell autonomous mechanism for neurodegeneration. Compared to the astrocyte-specific deletion of *Sumf1*, the concomitant removal of *Sumf1* in both neurons and glia *in vivo* induced a widespread neuronal loss and robust neuroinflammation. Finally, behavioural analysis of mice with astrocyte-specific deletion of *Sumf1* compared to mice with *Sumf1* deletion in both astrocytes and neurons allowed us to link a subset of neurological manifestations of MSD to astrocyte dysfunction. This study indicates that astrocytes are integral components of the neuropathology in MSD and that modulation of astrocyte function may impact disease course.

C. Di Malta: None. **J.D. Fryer:** None. **C. Settembre:** None. **A. Ballabio:** None.

J08.02

Whole exome sequencing combined with linkage analysis in an Iranian family with recessive dystonia-parkinsonism

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In addition to pure Parkinson disease and pure dystonic syndromes, there are a group of disorders with overlapping features. The differential diagnosis of these dystonia-parkinsonism syndromes can be complex. Various genes have been identified which may cause recessive dystonia-parkinsonism. In this study we sought to use exome sequencing in conjunction with linkage information to identify candidate causative gene in an Iranian family with dystonia-parkinsonism. Linkage analysis of this family comprising two affected and 8 unaffected individuals revealed linkage signals at 2 loci on chromosomes one and two with maximum LOD scores of 2.4 and 2.35, respectively. We captured exomes of one affected individuals from the family and performed sequencing analysis by a second-generation sequencer. The genetic variants of the affected individual in the linked loci were filtered against the 1000 Genomes Project, HapMap and the dbSNP131 database. After annotation and functional expectation, two novel genes were found to be candidates for dystonia-parkinsonism syndrome in this family. Functional studies are being performed for these two candidate genes to unravel the causative gene in this family.

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J08.03

Mutations in the CDKL5 and MECP2 genes, causing an atypical form of Rett syndrome

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Rett syndrome (RTT) is a severe neurodegenerative disease that affects approximately 1 in 10000 female births. Classic RTT is diagnosed based on a set of main clinical criteria. Atypical RTT has a milder form and more severe clinical form and diagnosed when only some of criteria are present.
Milder form is presented by the preserved speech variant caused by point mutations and large deletions with phenomenon of nonrandom X-inactivation (NXI) in MECP2 gene (Xq28).
Severe form of atypical RTT include the early-onset seizure type caused by mutations in the X-linked CDKL5 gene (Xp22).
Mutation analysis of MeCP2 and of CDKL5 genes was performed in 20 patients with clinical pictures for atypical RTT.
MECP2 mutations were detected by PCR following by sequencing and by real-time PCR. We identified two pathogenic mutations in the MECP2 gene: a deletion of 45 nucleotides (p.L386fs) and a missense mutation (p.R133C). The patient with R133C mutation has NXI.
Mutations in CDKL5 have been associated with West syndrome and atypical RTT with infantile spasms. Analysis of CDKL5 mutations was conducted by PCR following by sequencing and by using SSCP analysis. We identified two pathogenic mutations in the CDKL5 gene: a frameshit mutation of exon 15

(p.H728fs) and a splice site mutation of intron 7 (c.463+1G).
Both patients demonstrated clinical pictures for atypical RTT with early-onset seizures: infantile epilepsy with onset before 6 months, stereotypical hand movements, psychomotor regression; clinical features similar to those of patients with a severe case of Rett syndrome.

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J08.04

Early onset Parkinson's disease caused by a novel *ATP13A2* truncating mutation

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Parkinson's disease (PD) is the most common neurodegenerative movement disorder characterized by tremor at rest, rigidity , bradykinesia and postural instability whose average age of onset is 60 years. 10% of patients in whom symptoms first manifest before age of 40 years are classified as early onset PD (EOPD) cases. Mutations in *parkin*/PARK2, *PINK1*/PARK6, *DJ1*/PARK7 have been found in EOPD. A recent gene *ATP13A2*/PARK9 is related to early-onset Parkinson's disease (PD). Mutations in *ATP13A2* are the least common. *ATP13A2* is ubiquitously expressed with the highest levels in the brain. While wild-type ATP13A2 is found in the lysosome, mutant forms are retained in the endoplasmic reticulum and degrade by the proteasome. There is evidence that this protein transports multiple cations and clears α-synuclein aggregates. We screened 14 Iranian EOPD patients with age at onset of 20 years in whom mutations in *PRKN, DJ1, and PINK1* were previously ruled out. Twelve cases were sporadic and two of them were familial. In addition to several polymorphisms, *ATP13A2* sequencing revealed a novel homozygous truncating mutation in exon 23 in one familial case. The mutation created a stop codon, and is expected to result in deletion of 323 amino acids at the C-terminus of the protein. The patient has a younger brother with PD, who carries the same mutation.This mutation were not found in 100 Iranian control chromosomes.The novel truncating mutation in *ATP13A2* was associated with an increased risk of Parkinson's disease. Further studies are needed to clarify the functional role of this mutation.

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J08.05

Genetic basis and phenotype of early onset and familial Parkinson's disease in the Hungarian population

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OBJECTIVES: To evaluate phenotype and genetic alterations of known Parkinson's disease (PD) genes in Hungarian PD patients.
METHODS: Detailed anamnestic data, neurological status and biological materials of 144 PD patients (mean age at onset: 46 years) with early onset/familial PD were collected in a biobank. At 118 patients the LRRK2 gene p.G2019S mutation hotspot has been investigated using PCR-RFLP. In 50 patients PARK2, LRRK2, PINK1, SNCA genes were sequenced by Sanger's method. The CNVs of these genes were assessed at 49 patient by MLPA.
RESULTS: Family anamnesis was positive at 31 patients. In the LRRK2 the p.G2019S substitution in 118 patients was not present. Sequence analysis of LRRK2 detected one likely pathogenic splice site mutation. In this gene a high number of intronic and exonic SNPs were present. The homozygous rs11564148 SNP was present in 4 patients. Previously it showed associaton with increased risk of PD in Asian population. In the PRKN gene a substitution (p.R402C), with a high SIFT score, but unclear pathogenic nature was found. CNVs were present in 3 patients (SNCA: exon5, exon6, and PARK2: exon2-4 heterozygous deletion).
CONCLUSIONS: In our cohort 5 pathogenic mutations were found in the genes, most commonly altered in PD. In the Jewish population common LRRK2 p.G2019S mutation was not present in our cohort. Some of the detected SNPs in the LRRK2 in our patients, previously were associated with PD in Asian population. The genetic background of Hungarian PD need to be further studied to evaluate the genetic risk factors.

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J08.06

Molecular genetic analysis of a large consanguineous Iranian family with Wolfram syndrome reveals a novel WFS1 gene mutation

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Wolfram syndrome (WS) is a rare autosomal recessive neurodegenerative disorder that might represent a considerable fraction of diabetes in childhood especially among countries in the consanguinity belt. The main responsible gene is WFS1 for which over one hundred mutations have been reported from different ethnic groups. The aim of this study was to identify the molecular etiology of WS and to perform a possible genotype-phenotype correlation in large Iranian kindred, composed of three nuclear families, from Yazd province, center of Iran.
Three alive patients from the extended family were clinically studied and WS was suspected. Genetic linkage analysis via 5 STR markers was carried out. For identification of mutations, DNA sequencing of WFS1 including all the exons, exon-intron boundaries and the promoter was performed.
Linkage analysis indicated linkage to the WFS1 region. After DNA sequencing of WFS1, one novel pathogenic mutation which causes frameshift alteration in exon 8 (c.1228-1229 del CT) was found. The genotype-phenotype correlation analysis suggests that the presence of the homozygous mutation may be associated with an early onset of disease symptoms especially urological disease. This study stresses the necessity of considering the molecular analysis of WFS1 in childhood diabetes with some symptoms of WS.

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J08.07

Epidemiology of hereditary spastic paraplegias in Bashkortostan Republic

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Hereditary spastic paraplegias (SPG) - genetically heterogeneous group of neurodegenerative diseases, with preferentially lesion of the pyramidal tract. Prevalence of the disease varies from 1.0 to 4.0 cases per 100 000 persons in different populations. Our goal was to analyze the prevalence of SPG among citizens of Bashkortostan Republic of various ethnic origins and area of residence. According to National Genetic automated Register „hereditary spastic paraplegias" by January 1, 2013 there are 144 SPG patients in genetic counseling, and 23 possible carriers from 97 families. We have also registered 25 cases of deaths by SPG. The prevalence of SPG in Bashkortostan is 3.5 per 100,000 persons that is significantly higher than in the previous study (2,00 per 100,000 people). This is caused by improvements in diagnosis and registration of SPG. The highest prevalence rate was among Tatars (5,3 per 100,000 people), less among Russians - 2.4 per 100 000 people and Bashkirs- 0.8 per 100 000 people.
Prevalence among rural residents was 2.7 per 100 000 people, among urban residents - 3.7 per 100,000. A growth of SPG prevalence rate and its geographical expansion in Bashkortostan Republic emphasize the importance of further analyzing information of SPG patients and potential mutation carriers in National Automated Genetic Register.

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J08.08

Identification of new modifier genes at DMD/BMD and patient lifespan

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Duchenne/Becker Muscular Dystrophy, monogenic disease with modifier genes influence, could represent a good model to describe phenotype variations which determine lifespan of the patient. It requires large data analysis, such as expression profiling.
GEO GSE465 microarray expression set was used, including data from Affymetrix Human Genome U95, A to E arrays, Affymetrix GeneChip Custom-Made Muscle Chip and Affymetrix Human Genome U95, version 2 chip, to ensure expression measure reliability (Children's National Medical Center, Washington, USA). Expression variations were estimated by index of expression (IF) as logarithmic ratio between probe signal in DMD and normal patients. Genes with IF<1 were filtered out, in several steps, followed by cross comparison between the gene lists obtained from each of the arrays. Only genes which manifested similar IE level or trend between the arrays were finally selected.
As the result, 17 genes with high positive index of expression (between 2.02 to 6.15) in male and various one (-1.59 to 5.78) in female patients were identified. The genes were grouped by their function, revealing at least five clusters, the largest being "Transcription regulation" and "Signal transduction", each comprising five genes. Four of the selected genes, were also identified in other surveys.
Thus, these genes shared similar variation of their expression, regardless to the array or chip. The genes suppose to be tested in DMD patients to understand their modifier effects, while the results will help in elaboration of the concept related to the impact of new modifier genes on the DMD patient lifespan.

A. Levitchi: None. **V. Sacara:** None.

J08.09

Polymorphisms in *CLU*, *CR1*, *ACE* and *catD* genes and Alzheimer's disease: a case-control study in Brazil

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Genome-Wide Association Studies (GWAS) have identified many genes related with increased risk to Alzheimer's disease (AD). The *ApoE-e4* allele, a known risk factor for the disease, as well as, variants in *CLU* (8p21.1) and *CR1* (1q32) genes encoding, respectively, clusterin and complement receptor 1, were reported in these studies. Moreover, variants in genes encoding angiotensin-converting enzyme (*ACE,* 17q23) and cathepsin D (*catD,* 11p15.5) were also suggested to be involved with risk to AD. In the Brazilian population ApoE-*e4* allele showed positive association with the disease. In order to research genetic variations causing susceptibility for AD in Brazil we investigated polymorphisms in *CLU* (rs11136000), *CR1* (rs6701713), *ACE* (I/D) and *catD* (rs17571) genes through PCR-FRLP in samples from Vitória (Southeast region). A total of 243 individuals were selected through clinical parameters, including 81 patients and 162 controls matched by gender, age and ethnicity. We found no statistic differences in allelic and genotypic frequencies between the groups (p>0.05), even after stratification by *ApoE-e4* status. The lack of significance in *CLU* and *CR1* genes could be due to the small sample size, since these variations only showed association with AD in GWAS studies that analysed thousand of individuals. On the other hand, variations in *ACE* and *catD* genes, even using small samples, showed controversy results between distinct populations. These results revealed the necessity of validation of genetic variations for the risk of AD among populations with distinct ethnic profile. Supported by FAPES, FACITEC and MCTI/CNPQ/MEC/CAPES.

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J08.10

Searching for a novel gene causing infantile neuroaxonal dystrophy

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Infantile neuroaxonal dystrophy (INAD) is a rare neurodegenerative disease characterized by motor, mental and visual deterioration leading to death in childhood. Axonal swelling and spheroid bodies are observed in tissue biopsy samples. Recessive mutations in PLA2G6 were shown to underlie the disease pathogenesis. Even though PLA2G6 is the only gene associated with INAD so far, rare genetic heterogeneity has been demonstrated by linkage analysis.
We investigated five consanguineous families afflicted with INAD. PLA2G6 was screened for mutations and novel homozygous mutations were identified in four families. The remaining family has two affected sibs, who are adults at present. Their longevity is unusual for INAD phenotype, albeit they both have total neurodegeneration. Another atypical feature they share is facial dysmorphism. The patients, parents and the unaffected sibling were genotyped using SNP markers, and the data were used in linkage analysis. Two candidate loci totalling 10.6 Mb were found at 9p24.1 and at 13q32.3, and none harboured a gene associated with a disease with similar characteristics as that in our patients. We launched exome sequencing on DNA sample of one of the patients to find the variants at those loci. We anticipate that exome sequencing will reveal a novel gene that is responsible for the disease in our study family and be another example that confirms that new generation genetic technologies enable discovery of new disease genes in even small families.

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J08.11

Association of Interleukin (IL)-4 gene intron 3 VNTR polymorphism with multiple sclerosis in Turkish population

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Objective: Genetic risk factors are known to contribute to the etiology of multiple sclerosis (MS). Interleukin (*IL)-4* gene polymorphisms have been associated with immune-mediated diseases. The aim of this study was to explore the frequency of *IL-4* gene intron 3 VNTR (variable number tandem repeat) polymorphism in a cohort of Turkish patients with MS.
Methods: The study included 105 patients with MS and 160 healthy controls. Genomic DNA was isolated and genotyped using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analyses for the *IL-4* gene intron 3 VNTR polymorphism.
Results: The distribution of genotype and allele frequencies of *IL-4* gene intron 3 VNTR polymorphism was statistically different between MS patients and control group (p=0.005 and p=0.002, respectively). There were statistically significant associations between EDSS score, MS types and IL-4 VNTR polymorphism in MS patients (p=0.034 and p=0.000, respectively).
Conclusion: The results of this study suggest that intron 3 VNTR polymorphism of the *IL-4* gene was positively associated with predisposition to develop MS in Turkish population.

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J08.12

Analysis of association of Parkinson's disease with polymorphic variants of MART-region (17q21.31) in three ethnic groups of the Republic of Bashkortostan (GWAS replication results)

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Replication of genome-wide association analysis (GWAs) of Parkinson's disease (PD) in three ethnic groups from Bashkortostan Republic (BR) was performed using DNA collection of 550 PD patients (Russians - 215, Tatars - 243, Bashkirs - 90) and 622 control individuals (Russians - 190, Tatars - 338, Bashkirs - 94). The study included analysis of 4 polymorphic loci of chromosomal region of gene MAPT (17q21.31) - rs11012, rs2942168, rs393152 and rs1724425. The locus rs11012 was associated with PD only in Russians: genotype *G*A (p = 0,06; OR = 2,09) and allele *A (p = 0,007; OR = 2,12) were risk markers for PD development. However, this result is contrary to the GWAs result, according to which allele *A is protective for PD development in Europeans (Pankratz et al., 2009). As for the rest three loci - rs2942168, rs393152 and rs1724425, - the association with PD was found only in Tatars: genotype *C*C (p= 0,01; OR = 1,75) and *C allele (p = 0,02; OR = 1,60) of rs2942168, genotype *A*A (p = 0,001; OR = 2,06 ;) and *A allele (p = 0,01; OR = 1,92) of rs393152 and genotype *C*C (p = 0,01; OR = 1,57) and *C allele (p = 0,049; OR = 1,3) of rs1724425 were markers of the increased risk for PD development. No associations were detected in Bashkirs, that may be explained by a significant proportion of Mongoloid component in their genepool.

I. Khidiyatova: None. **K. Putenikhina:** None. **G. Akhmadeeva:** None. **I. Gilyzova:** None. **A. Baytimerov:** None. **R. Magjzanov:** None. **E. Khusnutdinova:** None.

J08.13

Methylation profile of the PARKIN/PARK2 gene in brain tissues of Parkinson´s disease patients and healthy controls.

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PARK2 gene mutations have been linked to Parkinson´s disease (PD). Abnormal expression of *PARK2* has been found in pathological tissues and could contribute to the origin and progression of PD. We hypothesised that *PARK2* silencing through promoter methylation could contribute to the pathogenesis of PD. To address this issue, we studied a CpG island in three different brain areas (occipital cortex, cerebellum, and substantia nigra) from five PARK2 non-mutated PD patients and two healthy controls. We also measured *PARK2*-transcrip levels in the same tissues. Our study revealed no difference in methylation between the brain regions or between PD patients and healthy controls. The presence of similar amounts of the transcript in all the brain regions of patients and controls agreed with this result. In conclusion, our results suggested that methylation of *PARK2* was not a hallmark of brain tissue from PD, and would thus not contribute to the pathogenesis of this common neurodegenerative disease

L. De Mena: None. **L.F. Cardo:** None. **E. Coto:** None. **V. Alvarez:** None.

J08.14

Identification of one novel causative mutation in exon 4 of WFS1 gene in two Italian siblings with classical DIDMOAD syndrome phenotype.

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Wolfram syndrome (WS), also known by the acronym DIDMOAD (diabetes insipidus DI, diabetes mellitus DM, optic atrophy OA and deafness D), is an autosomal-recessive disorder usually diagnosed in childhood when non-autoimmune, insulin-dependent DM is associated with OA. Additional characteristics include ureterohydronephrosis, neuropsychiatric and endocrinological impairment and cataract.
The gene involved (WFS1), which was identified on chromosome 4p, spans 33.4 kb of genomic DNA and includes eight exons: the 1st is non-coding, 2-7 are coding and the 8th is 2.6 kb long. WFS1 mRNA encodes an 890-amino acid polypeptide with nine putative transmembrane domains and a 100-kDa molecular mass (wolframin).
The aim of the present paper is to describe a novel missense mutation (G107R) of WFS1 gene that was unexpectedly detected, in two siblings from Southern Italy, outside exon 8; a very unusual finding which has previously been reported only twice in Italian patients with Wolfram syndrome (WS). Although in Spanish pedigrees' WFS1 mutations are frequently located in exon 4, this finding is very infrequent in other pedigrees, particularly in Italian patients. Conclusions: a) our report of two siblings with one novel WSF1 mutation (G107R) expands the molecular spectrum of WS; b) this is the 3rd report of Italian patients harbouring one mutation outside exon 8 and the 2nd with one mutation in exon 4; c) on the basis of the present observations, and literature data we can infer that mutation locations outside exon 8 do not seem to be clearly associated with peculiar phenotype expressions of WFS1 gene.

L. Rigoli: None. **C. Di Bella:** None. **F. Pugliatti:** None. **S. Cara:** None. **V. Salpietro:** None. **F. Lombardo:** None. **F. Lombardo:** None. **G. Salzano:** None. **D. Iafusco:** None. **C. Salpietro:** None. **F. De Luca:** None.

J08.15

Oligomeric alpha-synuclein levels in blood plasma in LRRK2 - linked Parkinson's disease

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Parkinson's disease (PD) is the second of most common neurodegenerative disorders. The aggregation of alpha-synuclein in dopaminergic neurons is a critical factor PD etiology of. The increased level of oligomeric alpa-synuclein has been found not only in brain tissues of PD patients but also in extracellular fluids, such as cerebrospinal fluid. The identification of causative mutations in several genes has demonstrated the existence of monogenic forms of the disease. PD caused by mutations in the leucine-rich repeat kinase 2 (LRRK2) gene is the most frequent.
The aim of our study was to estimate the level of oligomeric forms of alpha-synuclein in blood plasma in PD patients. The dataset was composed of 13 drug-naïve PD patients (the mean age 61.3±8.8, 7 females, 6 males) and 5 patients with LRRK2-associated PD (mean age 67±5,26, 2 females, 3 males) (all cases with mutation G2019S). In all cases the blood plasma was obtained using standard method. All subjects were residents of the North-Western region of Russia. The oligomeric forms of alpha-synuclein were estimated using enzyme-linked immunosorbent assay ELISA (Human Synuclein OLIGO kit aj Roboscreen, Germany).
There were no significant differences in the levels of oligomeric alpha-synuclein between PD patients (median 0,3, range 0 - 4,8, ng/ml), patients with LRRK2 G2019S mutation (median 0,06 range 0-0,38, ng/ml) and controls (median 0,3, range 0-12,5, ng/ml) (p=0,728, p=0,156, respectively). Therefore our data suggest that the oligomeric alpha-synuclein could not be used as a suitable marker of PD.

P.A. Andoskin: None. **A.K. Emelyanov:** None. **A.F. Yakimovsky:** None. **A.A. Timofeeva:** None. **S.N. Pchelina:** None.

J08.16

Novel missense ASPA mutation in a compound heterozygous state in a patient with Canavan disesase

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Canavan disease is a rare autosomal recessive leukodystrophy caused by mutations in the aspartoacylase (*ASPA*) gene which has been mapped to chromosome 17 pter-p13. It is more prevalent among children of Ashkenazi Jewish descent but has been diagnosed in different ethnic groups from all over the world. We report on a 12 year old Turkish boy having typical clinical manifestations of Canavan disease. He presented first symptoms at the age of 6 months. Brain magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) revealed white matter degeneration specific to Canavan disease. Molecular analysis of the ASPA gene showed compound heterozygosity for a known mutation p.G27R (c.79G<A) and for the mutation p.H244Y(c.730C>T), which has not been described in Canavan disease. Since different mutations involving the same codon and causing different amino acid changes have ben described in previous Canavan cases, thisnovel mutation is considered to be deletorius and disease causing mutation.

F. Ozkinay: None. **H. Onay:** None. **T. Atik:** None. **A. Durmaz:** None. **M. Karakoyun:** None. **O. Çogulu:** None.

J08.17

Molecular-genetic diagnostics of Huntington's disease patients from Eastern Slovakia

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Huntington´s disease (HD) is a progressive neurodegenerative disorder, which is clinically characterized by motor dysfunction, cognitive impairments and psychiatric disturbances. Gross pathology of HD is limited to the brain, with atrophy most prominent in the caudate, putamen, and cerebral cortex. Huntington´s disease is generally accepted that few mutational events account for the origin of the pathogenic CAG expansion. The prevalence rate of HD in the most of Europe is 5 cases per 100 000 individuals. The molecular basis of the disease is the expansion of the trinucleotide CAG in the first exon of the huntingtin gene in chromosome 4p16.3. Diagnosis of HD has been greatly simplified by the direct triplet repeat gene test. In Eastern Slovakia (2011-2012) CAG repeat lenghts in the HD gene were tested in seven patients with suspected diagnosis of HD. PCR products were resolved used genetic analyzer ABI 3500xl. In affected patients clinical diagnosis of HD was confirmed by molecular-genetic methods. The sex ratio in the cases, in which the diagnosis of HD has been confirmed was 4:3 in favor of female individuals. The mean age of patients in the time of diagnosis Huntington disease was 48.85 and ranged from 37 to 59 years. This confirm the affirmation of onset of symptoms typically in the third and fourth decade of life. Mapping the occurance of HD in Eastern Slovakia is a part of population-genetic analyses and monitoring of population health status.

J. Bernasovska: None. **I. Boronova:** None. **I. Bernasovsky:** None.

J08.18

Improvement of neurologic symptoms in a heterozygote case with Wilson disease after complex medical rehabilitation

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Wilson disease is a rare disease, characterized by disturbance of copper metabolism. We present the case of a 30 years old patient hospitalized for treatment and functional rehabilitation in Medical Rehabilitation Clinical Hospital Baile Felix, Romania. At admission she had rigidity and weakness of trunk, upper and lower limb muscles, deglutition and psycho-emotional problems, ROM limitation of the hip joint, presence of Kayser-Fleischer ring. The onset of the disease was at 7 years old, with hepatic dysfunction as initial clinical manifestation, fatigue and pain in the limbs, but the diagnosis was overlooked and established later, at 23, when the symptoms were severe pain in lower limbs, difficulty in gait, abnormal synergies, hypersalivation with deglutition disorder, change of voice, psychoemotional lability. The most significant laboratory findings were very low level of serum copper and ceruloplasmin and high urine copper levels. Molecular analysis of exon 14 of ATP7B gene revealed the presence of a C3207A replacement in heterozygote state. In exon 15 no mutations were found. Main goals of rehabilitation were maintaining and increasing the range of motion in all activities, preventing joint ROM limitations and correcting the existing ones, decreasing rigidity and preventing development of muscular atrophies, improving coordination, speech, maintaining and increasing the functionality in activities of daily life, psychological adaptation to the disease. The therapeutic plan included medication, physical therapy, hydro-thermo-therapy, occupational, speech therapy, psychotherapy. Even if the diagnosis was late, extrapyramidal neuromotor syndrome remarkably improved, gaining of independence in daily activities have led to improvement of psychic state.

M. Cevei: None. **D. Stoicanescu:** None.

J08.19

Screening of c.2363C>T Mutation in ATP7B Gene by Direct Sequencing in a Family with Wilson disease

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Wilson disease (WD, MIM#277900) is an autosomal recessive disorder of copper metabolism characterized by decreased biliary excretion of copper first described as hepatolenticular degeneration. The excess copper accumulates in various organs and tissues predominantly in the liver, brain, cornea and kidney. The disease is diagnosed on the basis of typical symptoms and conventional biochemical indicator, which include low serum concentration of ceruloplasmine and elevated excretion of urinary copper. The disease has a worldwide frequency of 1/30,000 to 1/100,000 live births and a carrier frequency of 1 in 90. To protect the patients for fatal liver cirrhosis and neurological damage, it is necessary to treat with zinc salts and copper chelating agent such as penicillamine. Prompt and appropriate treatment depends on correctly diagnosed WD in the patient and any affected siblings. Early diagnosis is critical and prevents lifelong neurological disability and liver cirrhosis. In our study DNA was isolated from index patient of Wilson disease and his family members. ATP7B gene was amplified by PCR. Mutation was detected by DNA sequencing. We found c.2363C>T mutation in both alleles (homozygous) of ATP7B gene of index patient while his father, mother and a sister have heterozygous of mutant alles. One sister has no mutation. The index patient has severe neurological abnormalities and mental retardation. He has kayser fleischer ring, low serum seruloplasmin and high urinery copper. He has score 9 of Wilson disease according to international criteria.

Ö. Şimşek Papur: None. **S. Aşık Akman:** None. **O. Terzioglu:** None.

J08.20

hnRNPA2/B1 and nELAVs regulate CDK5R1 by interecting with a poly-U stretch within the 3'-UTR

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CDK5R1 encodes p35, an activator of CDK5, a proline-directed serine/threonine kinase that phosphorylates proteins involved in CNS development and maintenance. CDK5 and p35 were found to have an important role in neuronal migration and differentiation during CNS development and were implicated in some neurodegenerative and cognitive disorders. Both the CDK5R1 3'-UTR remarkable size and its conservation are indicative of an important function in post-transcriptional regulation. We showed that CDK5R1 3'-UTR affects transcript stability and translational efficiency. We identified within the 3'-UTR by luciferase assays a 138 bp region with a high destabilizing activity. To assess if this region interacts with RBPs and to identify possible binding site/s, we performed UV cross-linking and site directed mutagenesis assays. Following the identification of a poly-U stretch binding site, we demonstrated that nELAVs interact with this motif by UV CLIP assays. Since the over-expression and silencing of nELAVs showed a stabilizing activity on p35 expression, we search for destabilizing factors by means of pull-down experiments, allowing to detect an interaction with the hnRNPA2/B1. Through RNA immunoprecipitation experiments we confirmed hnRNPA2/B1 binding to CDK5R1 mRNA. hnRNPA2/B1 over-expression studies showed a destabilizing activity reducing p35 expression. Silencing experiments together with assays aimed to verify the mutual action of the identified RBPs, will shed light on their role on post-transcriptional regulation of CDK5R1. This study besides providing new insights in functional role of hnRNPA2/B1 at now little known, will address studies on the CDK5R1 pathogenetic implications in CNS diseases such as neurodegenerative and cognitive disorders.

P. Zuccotti: None. **A. Barbieri:** None. **C. Colombrita:** None. **S. De Palma:** None. **S. Moncini:** None. **C. Gelfi:** None. **A. Ratti:** None. **M. Venturin:** None. **P. Riva:** None.

J09.01

Next-generation sequencing of complete mitochondrial genomes of Slovenian Lebers's Hereditary Optic Neuropathy patients revealed one novel mutation and several probable synergistic variants

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Purpose. Leber hereditary optic neuropathy (LHON) is maternally inherited eye disorder. It results from point mutations in highly polymorphic mitochondrial DNA (mtDNA). Although the LHON in the majority of the patients is a result of one of the three most common mutations, in some cases the genetic background is not clear. We investigated nine Slovenian patients diagnosed with LHON, from which only two were positive for one of the most common mutations. In order to find novel pathogenic variants, we performed deep sequencing of whole mtDNA.
Methods. DNA of nine LHON patients and 2 controls was extracted from whole blood samples. Patients were first screened for most common LHON mutations using Sanger sequencing. In addition, for 6 patients and 2 controls we performed whole mtDNA amplification and deep sequencing using Ion Torrent technology.
Results. Two patients were positive for T14484C and G3460A mutations. In the other patients, whole mtDNA deep sequencing detected novel homo and heteroplasmic variations. We identified 25 non-synonymous and 36 synonymous substitutions in mtDNA protein-coding regions. Their impact on protein structure and function was determined using bioinformatic prediction tools. We found 16 novel non-synonymous LHON-associated variants, from which 11 were homoplasmic and 5 were heteroplasmic.
Conclusions. The prevalence of most common LHON mutations in the Slovenian patients is lower than in other parts in Europe. Using deep sequencing approach, we identified new potentially pathogenic mtDNA variations in Slovenian LHON patients, which do not harbour one of the three most common mutations.

D. Glavac: None. **M. Tajnik:** None. **M. Jarc-Vidmar:** None. **M. Hawlina:** None.

J09.02

Genetic polymorphisms of warfarin metabolizing enzymes VKORC1 and CYP2C9 in the Greek-Cypriot population

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Two variants in the gene encoding the cytochrome P450 2C9 enzyme (*CYP2C9*) and one variant in the vitamin K epoxide reductase (*VKORC1*) are the most significant genetic risk factors associated with bleeding after warfarin prescription. Since the prevalence of these three variants in the Hellenic population of Cyprus (Greek-Cypriots) has not yet been studied, we aimed to determine the genotypic and allelic frequencies and to compare allele frequencies with those in other major ethnic groups. All four variants

were genotyped by PCR-RFLP assays and statistically analyzed in 148 unrelated healthy Greek-Cypriot volunteers. The allele frequencies of *CYP2C9*2* and *CYP2C9*3* were 0.162 and 0.112 respectively, whereas *VKORC1*–1639A was 0.534. The latter frequency differs significantly when compared with Caucasians, Asians and Africans and is still significant when compared with the geographically and culturally closely related to Greek-Cypriots, Hellenes of Greece. Interestingly ~18% of our population are carriers of four or three risk alleles regarding warfarin metabolism, therefore they have a high predisposition for bleeding after taking high or even normal warfarin doses. Generally, our data show no significant difference in the frequency of *CYP2C9* allelic variants when compared to the Caucasian population, but differ significantly when compared with Africans and Asians. Also, the frequency of variant *VKORC1*–1639A differs between Greek-Cypriots and every other population we compared. Finally, about 1/5 Greek-Cypriots carry three or four risk alleles and ~50% of them carry at least two independent risk alleles regarding warfarin metabolism, a potentially high risk for over-anticoagulation.

K. Voskarides: None. **D. Hadjipanagi:** None. **S. Chrysanthou:** None. **C. Deltas:** None.

J09.03

Studying the functions of heterologous intron-derived fragments within and outside the coding region on the expression of the human coagulation factor IX in cultured mammalian cells

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Hemophilia B is attractive candidate for gene therapy. In order to produce functional protein an efficient expression vector is required. In this study, eight CMV-regulated hFIX-expressing plasmids with different combinations of two human beta-globin (*hBG*) introns (in their corresponding locations inside the *hFIX*-cDNA) and liver-specific rat aldolase B intronic enhancer (*rABE*; upstream the CMV promoter) were constructed and used for the hFIX expression analysis in cultured HepG2 and Hek-293T .
In HepG2, *rABE* increased the hFIX expression levels when it was combined with the CMVp in different clones. Potential of the *rABE* in the intron-less construct was evidenced by 3 to 32.5-fold increase of the hFIX expression levels in comparison with other constructs.
In Hek-293T, the highest hFIX expression level was obtained from the intron-less construct.
Evaluation of the intron removal from pre-mRNAs of different transfected cells revealed that the *hBG* introns are spliced either partially or improperly in both of the examined hosts which could explain the reduced hFIX expression levels from intron- containing plasmids.
Interestingly, mature hFIX mRNA was generated from the *hBG* intron-II containing construct in Hek-293T, whereas no detectable product of proper splicing was evidenced from the same construct in HepG2. Our results support the gene- and host-dependence of fate and function of heterologous introns in a protein expression cassette. Based on data, the *rABE* is attractive element to achieve a higher expression level of therapeutic proteins in hepatocytes. Such a hybrid regulatory element might be able to prevent the inactivation of the CMVp *in vivo*.

M. Sam: None. **A. Zomorodipour:** None. **G. Kardar:** None. **M. Ghorbani:** None. **M. Shokrgozar:** None. **A. Amanzadeh:** None.

J09.04

The HMGCL gene mutation c.504_505delCT causes two transcripts that are subject to Nonsense Mediated mRNA Decay

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Eukaryotic cells can be protected against mutations that generate stop codons by nonsense-mediated mRNA decay (NMD) which eliminates the affected transcript and decreases the mRNA levels. Here, we study these phenomena in the stop codon mutation c.504_505delCT, the third most frequent mutation in HMG-CoA lyase deficiency (MIM #246450). This mutation determines the occurrence of three mature transcripts: the mRNA transcript corresponding to the CT deletion itself, another containing a frameshift deletion of exon 6 and the physiological transcript with exon 5 plus 6 deletion.
As a model we used fibroblasts from a patient homozygous for the mutation c.504_505delCT which were treated with puromycin, a NMD mechanism inhibitor. After 6 hours of incubation with 100 µg/ml of puromycin, fibroblasts were harvested and total RNA extraction and quantitative real-time PCR experiments were performed to analyze the level of three alternative mRNA transcripts before and after puromycine treatment.
The results showed that two mRNA transcripts which gave rise to a stop codon, one with CT deletion and other with exon 6 deletion, were affected by this process, increasing their levels by 10-fold after treatment. This is surprising because the mRNA transcript bearing the CT deletion generates a stop codon 31 nucleotides upstream the 3' exon-exon junction and does not follow the rule that the stop codon must be located further than 50 nucleotides to be affected by NMD. This is, according to a new model of regulation of NMD, more complex and takes into consideration other sequence signals or distance parameters.

B. Puisac: None. **E. Teresa-Rodrigo:** None. **M. Gil-Rodríguez:** None. **M. Hernández:** None. **C. Baquero:** None. **A. Pié:** None. **G. Bueno-Lozano:** None. **F.J. Ramos:** None. **J. Pié:** None.

J09.05

Evaluation of 454 Patients referred for Molecular Analysis of Alpha1-antitrypsin Deficiency

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Alpha1–antitrypsin (AAT) is a serin proteinase inhibitor that protects the connective tissue of the lungs from the elastase released from leucocytes. The AAT enzyme deficiency is a common autosomal codominant disorder characterized by a predisposition to emphysema and cirrhosis. The cause of the liver damage is not the deficiency of the protease inhibitor. Liver damage arises from the pathological polymerization of the AAT variant before its secretion from hepatocytes. AAT is encoded by the SERPINA1 gene on chromosome 14. The variants within the AAT gene are classified according to the protease inhibitor (PI) system in which M is the normal allel and the inheritance of S and Z alleles is associated with decreased levels of the protein.
The objective of this study is to investigate the presence of S and Z alleles in the patients tested for AAT mutations and having various indications. Between the period of 2006-2013, 454 patients were tested for SERPINA1 gene using reverse hybridization assay in the molecular genetics laboratory at the Depertmant of Medical Genetics, Ege University.
Among 454 patients, 433 patients (95,3%) were found to be normal MM genotype, whereas 8 patients (1,76%) were PIMZ, 7 patients (1,54%) were PIMS, 6 patients (1,3%) carried homozygous mutations with PIZZ genotype. Mutation detection rate was found to be low in our study group. It is considered that careful examination and laboratory work of the patients are needed before the molecular genetic analysis for alpha1–antitrypsin deficiency.

S. Tunc: None. **H. Onay:** None. **E. Karaca:** None. **A. Durmaz:** None. **A. Aykut:** None. **B. Durmaz:** None. **F. Ozkinay:** None.

J09.06

Association of fibroblast growth factor (FGF-21) as a biomarker with primary mitochondrial disorders, but not with secondary mitochondrial disorders (Friedreich Ataxia) in human patients

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Mitochondrial respiratory chain deficiencies (RCD) are a group of more than 100 disorders of adults and children, with highly variable phenotypes. The high prevalence of mitochondrial disorders (MIDs) urges the clinician to diagnose these disorders accurately, which is difficult in the light of highly variable and overlapping phenotypes, transmission patterns and molecular backgrounds.
Fibroblast growth factor 21 (FGF-21) is an important endocrine and paracrine regulator of metabolic homeostasis. FGF-21 could play a role in the metabolic alterations that often are associated with mitochondrial diseases. The aim of this study was to define the association of FGF-21 biomarker with human primary MIDs and secondary MIDs in the suspected patients in Iran.
Serum FGF-21 levels were determined using ELISA in 47 mitochondrial pati-

ents including 32 primary MIDs, 15 secondary MIDs and 30 control subjects. We choose the Friedreich Ataxia (FA) as a model of secondary MIDs in this study. Serum FGF-21 levels were significantly ($p < 0.05$) higher in subjects with the primary MIDs compared to subjects without the MIDs. However, serum FGF-21 level did not show significantly increase in secondary MIDs with FA subjects. In conclusion, there is an association between increasing concentrations of FGF-21 with mitochondrial diseases and FGF-21 can be used as a biomarker for diagnosis of primary MIDs in human. This biomarker is not appropriate choosing for diagnosis of FA patients.

Group	Control	Primary MIDs	FA
Number of subjects	30	32	15
Gender (M/F)	17/13	17/15	9/6
FGF-21 level in male (pg/ml)	86.6	261.4	69.2
FGF-21 level in female (pg/ml)	88.7	422.4	75.1
FGF-21 level in all (pg/ml)	87.1	346.2	71.6

M. Salehi: None. **M. Houshmand:** None. **M. Sadeghizadeh:** None. **O. Aryani:** None.

J09.07

Course and outcomes of Hurler syndrome in patients with later start of enzyme replacement therapy with human recombinant alpha-L-iduronodase (ERT).

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Background: Mucopolysaccharidosis type I (MPS) is a genetic disease with a severe progressive multi-organic involvement and fatal prognosis. Early diagnostics and treatment, particularly ERT and hematopoietic stem cell transplantation improve quality and duration of life by delay of organ damage.
Objective: to evaluate the course of the disease in cases of later start of ERT.
Materials and methods: 3 children aged 69,3 ±6,4 months at the beginning of ERT with the same mutation Q70X/ Q70X (2 girls - identical twins and 1 boy) were monitored .
Results: the total number of infusions was 55 and 181 in twins and 127 in boy. Urine glycosaminoglycan during ERT decreased to 33%, 56% and 71%, respectively and reductions of hepatosplenomegaly, limitation of motion in affected joints, frequency of sleep apnea as well as absence of hydrocephalus progress and hypertrophic cardiomyopathy were observed. In the end stage of the disease all children had respiratory failure (obstructive in boy, chronic restrictive in one girl and due to acute pulmonary oedema in the other), hypertrophic cardiomyopathy, relapse of hepatosplenomegaly, hypocalcaemia and seizures. Few side effects, including low-grade fever and urticarial rashes were observed after the infusions 6-7.
Conclusions: the main cause of death in MPS I (Hurler) with later start of ERT was heart or respiratory failure after 15 - 45 months of ERT, growing steadily during the last 3 - 6 months of life despite the efficacy of ERT during the first period of treatment.

N.V. Buchinskaya: None. **O.V. Kalashnikova:** None. **M.M. Kostik:** None. **V.G. Chasnyk:** None.

J09.08

Exome sequencing revealed a NPC2 mutation in an Iranian Niemann Pick C-type 2 disease family

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Niemann-Pick type C (NPC) disease is a severe autosomal recessive neurovisceral disease that cause of mutation of the NPC1 or NPC2 gene resulting in accumulation of unesterified cholesterol in late endosomes and lysosomes. NPC disease is difficult to be diagnosed and treated. We found an Iranian consanguineous family with two affected individuals with a recessively inherited juvenile Niemman-Pick C-type 2 disease. Exome sequencing of these two affected siblings led us to identify a homozygous mutation in the NPC2 gene. The confirmation of NPC disease was assessed by filipin staining. Fibroblasts from the two NPC siblings showed a massive accumulation of unesterified cholesterol in the lysosomal compartment. MRI of patients showed white matter signal change and frontal lobe atrophy.

A. Alavi: None. **E. Elahi:** None. **S. Nafissi:** None. **H. Shamshiri:** None. **M. Houshmand:** None. **M. Malakooti Nejad:** None.

J09.09

Non-classical PKU: report of a known missense mutation for the first time in iran

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PTPS deficiency is caused by mutations in PTS gene that encodes the 6-pyruvoyl-tetrahydropterin synthase, which is required for the second step of the de novo biosynthesis of BH4 starting from GTP. It is inherited as an autosomal recessive trait. We described here a germ line homozygous missense mutation in exon 6 of PTS gene that determines an amino acid substitution (Gly-> Arg) at codon 125. The index case was 4- year- old patient with an apparent deficiency of tetrahydrobiopterin (BH4), microcephally, psychomotor retardation, seizure and swallowing difficulties. PTS gene mutations screening was performed in exons 1-6, and the corresponding intro/exon boundaries by sequencing analysis. A rare homozygous GGA->AGA point mutation was found at codon 125 in exon 6. As expected on the basis of the homozygosity of the index case, the parents were consanguineous (second-degree cousins). His mother and father were positive for heterozygous PTS mutation.
In conclusion, we identified a known germ line PTS gene mutation responsible for non-classical phenylketonuria (PKU) during a routine screening of a patient referred for classical PKU.This is the first report of a nonclassical PKU due to PTS gene mutation in Iran.

A. Saberi: None. **G. shariati:** None. **M. Hamid:** None. **H. Galehdari:** None.

J09.10

Biotinidase deficiency: Novel mutations in Algerian patients.

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Biotinidase deficiency (BD) is an autosomal recessive disorder of biotin metabolism leading to varying degrees of neurologic and cutaneous symptoms when untreated. The disorder is characterized by mutational heterogeneity; indeed more than 100 mutations causing profound BD have been described. In the present study, we report the clinical and molecular investigation of BD in Algerian population.
We investigated four unrelated consanguineous Algerian families including five patients with profound BD and one child characterized as partial BD. Molecular investigation was performed by direct sequencing of BTD gene. Mutation analysis revealed three novel mutations, c.del631C and c.1557T>G within exon 4 and c.324-325insTA in exon 3 of BTD gene.
To our knowledge, the present study is the first reporting clinical and molecular investigation of BD in Algeria. Since newborn screening is not available, cascade screening in affected families would be very helpful to identify at risk individuals.

A. Tiar: None. **A. Mekki:** None. **M. Nagara:** None. **F. Ben Rhouma:** None. **O. Messaoud:** None. **N. Ben Halim:** None. **R. Kefi:** None. **M. Hamlaoui:** None. **A. Lebied:** None. **S. Abdelhak:** None.

J09.11

The association between Factor V G1691A mutation and obstructive sleep apnoea syndrome: Preliminary results from Turkey

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Obstructive sleep apnoea syndrome (OSAS) is a chronic complex disorder and characterized by recurrent episodes of complete or partial collapse of the upper airway during sleep, resulting in apneas or hypopneas, respectively. Factor V Leiden G1691A is an autosomal dominant nucleotide transition resulting in an R506Q amino acid missense mutation that causes activated protein C resistance. In the present study, we aimed to investigate the association between Factor V Leiden G1691A mutation and the susceptibility and clinicopathological development of obstructive sleep apnoea syndrome.
For diagnosing OSAS, at least one full-night polysomnography was performed in the patients. Factor V Leiden G1691A mutation was genotyped among 28 patients in comparison with 100 healthy individuals in case-con-

trol study. The mutation analysis was performed by StripAssay technique using the ViennaLab CVD StripAssay.
There were 22 (78,6%) subjects with GG genotype and 6 (21.4%) subjects with GA genotype in patients and there were 93 (93%) subjects with GG and 7 (7%) subjects with GA genotype in healthy controls. We did not detect any homozygote for AA genotype in 128 subjects. There were 50 (89.2%) G allele and 6 (10.8%) A allele in OSAS Patients and 193 (96.5%) G allele and 7 (3.5%) A allele in healthy controls. (p: 0.036 OR: 0.276 95% CI 0.084-0.9030 for genotype distribution and (p: 0.041 OR: 0.302 95% CI 0.086-1.070) for allele distribution.
There were statistically significant difference between patients and controls in terms of FV Leiden genotype and allele distributions in the preliminary results of our study.

G. Bagci: None. **H.K. Kurtulgan:** None. **O.T. Dogan:** None. **O. Ozdemir:** None.

J09.12

The potential association between IL6 -174G>C polymorphism and obesity

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Polymorphisms in IL6, ACE and ATR genes are associated with obesity. Torque teno virus (TTV) seems to be able to interfere with production of some proinflammatory cytokines associated with obesity and related phenotypes.
The aim of this study was to test the potential association between obesity, TTV infection and the IL6 -174G>C (rs1800795), ACE I/D (rs4646994), AT1R A1166C (rs5186) polymorphisms.
The polymorphisms and the presence of TTV were detected in blood samples from 150 obeses and 150 normoponderal, healthy subjects using PCR based methods.
IL6 -174 CC genotype was more frequent in all obese patients (P=0.02) and in patients without TTV infections (P=0.03) than in controls. Obese women had more frequent TTV infections compared with normoponderal women (P=0.046). Obese subjects, regardless of gender (women P=0.03, men P=0.04), and healthy men (P<0.01) carriers of AT1R C allele had higher triglycerides levels compared with non-carriers. The frequency of TTV in control group (70.67%) was similar with data reported in other populations.
The present study indicated that IL6 -174 CC genotype and TTV infections in women can be associated with common form of obesity.
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J09.13

The protective effect of MCP-1 -2518 A>G promoter polymorphism in chronic renal failure patients requiring long-term hemodialysis in the central region of Turkey

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Objective: Monocyte chemoattractant protein-1 (MCP-1), is a member of the CC chemokine family and responsible for monocyte and T lymphocytes recruitment in acute inflammation conditions. MCP-1 plays a major role in the pathogenesis and progression of different types of human renal disease. -2518 A>G polymorphism of the MCP-1 enhances expression of MCP-1 gene. In this study, we aimed to investigate the frequencies of MCP-1 gene -2518 A>G promoter polymorphism among chronic renal failure (CRF) patients requiring long-term hemodialysis.
Patients and methods: Study population consisted of 201 unrelated, consecutive adult chronic renal failure patients requiring long-term hemodialysis and 194 healthy controls living in city of Sivas in Turkey. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used for genotyping of MCP-1 -2518 A>G polymorphism. Differences between genotype distribution of two groups were compared by using χ^2 test.
Results: Genotype frequencies of the MCP-1 -2518 A>G were 103 for AA, 79 for AG, and 19 for GG in the chronic renal failure patients and 67 for AA, 110 for AG, and 17 for GG in healthy controls. There were statistically significant difference in terms of genotypic (χ^2=12.69, p=0.02) and allelic (χ^2= 5.36, p: 0.02) frequencies of MCP-1 -2518 A>G between CRF patients and control subjects.
Conclusions: According to our results, in the patients group MCP-1 -2518 AA genotype frequency was significantly higher than that of control group. Current data shows that AA genotype may cause susceptibility to CRF while G allele may have a protective effect on CRF patients.

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J09.14

The common polymorphism Val109Asp in the omentin gene is associated with daily energy intake in the Central-European population

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Background: Omentin was originally described in 2003 and was reported to be expressed specifically in human omental adipose tissue. Later on, it was reported that omentin is predominantly expressed in visceral adipose tissue, with adipose tissue stromal cells being the main source. Very little is known about the relationship between the genetic variability of the omentin gene and pathophysiology of obesity, although omentin is believed to play an important role in visceral obesity development. The aim of the study was to investigate two common polymorphisms in the omentin gene (rs2274908 and rs2274907) and dietary composition and anthropometric parameters of obesity in the Central European population.
Material and methods: The total of 495 subjects were included into the study (F/M 368/127, 46.0+-13.6y) that were further divided into the obese and non-obese cohorts (F/M 224/79, 50.3+-11.7y and F/M 144/48, 39.3+-13 .7y, respectively). Dietary habits were established using the 7-d food records and anthropometric parameters were measured.
Results: In the multivariate modelling, the rs2274907 polymorphism expressed independent prediction role for the daily energy intake, independently on the age and gender distribution (p = 0.03), whereat the TT genotype was associated with the lowest average energy intake (7877+- 2780 J/day) and the AA genotype with the highest daily intake of energy (8764+-2467 J/day).
Conclusion: This is so far the first study to investigate the polymorphisms in the omentin gene in a large population cohort of obese and non-obese individuals. Based on our results, the rs2274907 polymorphism could be associated with the daily energy intake.

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J09.15

MTHFR, MTR and MTRR polymorphism in Moldavian patients with phenylketonuria

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In a previous study on PKU Moldavian children the high level of Homocystheine and low levels of Methionine and Taurine have been appreciated. The aim of our research is to appreciate the frequency of folate metabolizing genes in PKU and in control groups.
Materials and Methods: 80 Moldavian PKU patients and 165 healthy children as a control group have been tested. The PCR-RFLP assay was used to indentify the *MTHFR* C677T, *MTR* A2756G, *MTRR* A66G polymorphisms with HinfI, HaeIII and NdeI enzymes respectively. The distribution of genotypes in case and control groups was tested for deviation from Hardy-Weinberg equilibrium (HWE).
The results: Genotype frequencies in case group was conformed to HWE

expectation for *MTHFR, MTR* and *MTRR*. There was identified no significant differences in frequency in case and control groups in heterozygous state of *MTHFR* (47.5% vs 37.6%) and of *MTRR* (56.3% vs 63.0%) genes. The emphatic differences of heterozygous frequency there was determined in *MTR* gene (28.2% vs 42.4%). Patients with the *MTR* AG genotype may be show a lower risk of severe manifestation of disease (OR 0.53, 95% CI 0.29, 0.95) and these data were statistically significant (p=0.031). Individuals with *MTRR* GG genotype were suspected to have an increased risk of severe PKU manifestations (OR 4.62, 95% CI 0.4, 53.2) compared to individuals with AA (Ref) or AG genotypes (OR 0.80, 95% CI 0.4, 1.45), but this data were not significant.**Conclusion**: Methionine cycles genes may be as a genetic modifier of PKU severity.

K.R. Boiciuc: None. **N.M. Usurelu:** None. **V.C. Sacara:** None.

J09.16
Mass-specrometry and fluorimetry in diagnostics lysosomal storage diseases

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In order to explore the activity of the enzymes α-galactosidase and α - iduronidase in patients with deficiency of lysosomal enzymes, the diagnosis from dry blood spot was introduced.
The dry blood spots we investigated using the method of mass spectrometry so to determine the activity of the enzymes α-galactosidase (GLA), α-iduronidase (IDUA) by mass spectrometer ABI. The following pathologies were diagnosed: MPS I type in 43%, MPS II type in 14%, MPS IV type in 14%, MPS VI type in 29%. The external quality control samples were from the Center for Disease Control and Prevention.
The selective screening in the dry blood spots of healthy newborns using fluorimetry was conducted to identify the activity of the enzyme α-galactosidase and α - iduronidase. The optical density of the studied enzyme extracts and standards were measured using the immunofluorescence with wavelength of excitation 365 mm, 450 mm emission.
The standard calibration curves were constructed, thus the regression equation was obtained: y = 22385x +80,679; R2 = 0, 9975 for measuring the activity of the enzyme α - galactosidase and y = 0, 0064 x +0, 5179; R2 = 0, 9821 for measuring the activity of the enzyme α - iduronidase. The preliminary reference values for the activity of the enzyme α - iduronidase consisted in range 0, 1-2,47 pmol were obtained.

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J09.17
Genetic screening of Iranian families with phenylketonuria

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Abstract
Phenylketonuria (PKU) is the most prevalent inborn error of metabolism an autosomal recessive disorder resulting from a deficiency of phenylalanine hydroxylase. PKU and non-PKU mild hyperphenylalaninemia (HPA) caused by mutations in the PAH gene. Untreated PKU can lead to mental retardation, so early diagnosis is very important to prevent severe mental illness. About 1–2% of cases of HPA are due to mutation in gene coding for tetrahydrobiopterin (BH4) which is PAH cofactor.
The incidence of PKU varies in different population. PKU occurs in about 1 in 10,000 births in Caucasians. The incidence of the disease varies between 1.7:10000 and 1:4000 in Iranian population. Due to relatively high prevalence of PKU in Iranian population of which the most severe forms are preventable by prenatal diagnosis, PKU carrier detection and PND has become part of national health program since September 2012. Among 20 families who referred to our laboratory since then, 15 known mutations including 10 homozygote and 5 compound heterozygote mutations have detected using indirect RFLP analysis and direct DNA sequencing. A total of 5 families with incomplete genotype findings from both methods were further analyzed for existence of mutations in BH4 coding genes in non-classic PKU.
Molecular analysis for the remaining families is underway in order to determine the prevalence of the most frequent mutations in PAH gene in Iranian population.

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J09.18
Genetic association studies between known PTPN22 and SUMO4 gene variants and Type 1 diabetes (T1D) in Tamil, Indian population

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Type 1 diabetes (T1D), also known as 'insulin-dependent diabetes mellitus (IDDM)' results from a cellular-mediated autoimmune destruction of the β cells of the pancreas. Recently an increased incidence of gene polymorphisms such as PTPN22, SUMO4, CTLA4 and IL2RA associated with T1D was reported, however no common gene mutations or pathogenic causative genomic variations have been identified until. We have recruited 800 sporadic cases with T1D patients, and an equal number of age-matched controls from south India. Recently we completed an initial targeted two known and well-associated their polymorphism in its involvement in the pathogenesis of Type-1 diabetes (i.e. PTPN22 and SUMO4) in 100 selected T1D individuals and equal controls using SNP array. The data showed PTPN22 polymorphism (SNP rs2476601, 1858C>T) was not associated with in any of the TD1 subjects of Indian origin as the SNP marker rs2476601 was monomorphic (G/G) in both T1D subjects and controls. The data of SUMO4 gene variation (rs237025, 163A>G, M55V) which has been shown to be a susceptibility marker in type-1 diabetes showed a higher frequency of combined AG and GG genotypes (64%) in the affected individuals than in matched controls (56%) of Indian origin. However, the "G" allele frequency between cases and controls are not statistically significant (40% in cases vs. 35% in controls, OR (C.I.) = 1.23 (0.82-1.85), chi2=1.07, p=0.30), probably due to small sample sizes. To the best of our knowledge, this is the first targeted genetic study on the association of genetic loci in Type 1 diabetic patients in India.

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J09.19
11 β-hydroxylase deficiency: identification of two novel CYP11B1 mutations in a Tunisian family

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Steroid eleven beta hydroxylase deficiency (11β-OHD) is the second most common form of congenital adrenal hyperplasia, accounting for 5-8% of all cases. It is an autosomal recessive enzyme defect impairing the biosynthesis of cortisol. Virilization and hypertension are the main clinical characteristics of this disease.

In Tunisia, the incidence of 11β-OHD appears higher due to a high rate of consanguinity (17.5% of congenital adrenal hyperplasia). The identical presentation of genital ambiguity (females) and pseudo-precocious puberty (males) can lead to misdiagnosis with 21 hydroxylase deficiency. The clinical hallmark of 11β-hydroxylase deficiency is variable, and biochemical identification of elevated precursor metabolites is not usually available.

In order to clarify the underlying mechanism causing 11β-OHD, we performed the molecular genetic analysis of the *CYP11B1* gene in a female patient diagnosed as classical 11β-OHD. The nucleotide sequence of the patient's *CYP11B1* revealed two novel homozygous mutations in exon 4: a missense mutation c.650G>T (p.S217I) combined with an insertion of a thymine at the c.652-653 position (c.652_653insT), leading to a reading frame shift, multiple incorrect codons, and a premature stop codon, that drastically affects normal protein function leading to a severe phenotype with ambiguous genitalia and hypertension characterizing the congenital adrenal hyperplasia disease due to 11β-hydroxylase deficiency.

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J09.20

Cystic fibrosis and severe liver disease

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Background: Liver cirrhosis is the end stage of cystic fibrosis liver disease (CFLD) with an important implication on disease's outcome. Vascular complication, with occurrence of portal hypertension is an alarming stage considering the risk of variceal bleeding.
Objective: Evaluation of severe liver disease with portal hypertension (PH) in CF patients and identification of its prevalence and risk factors.
Methods: Study evaluated prospectively, 159 patients for five years. They were routinely followed-up by clinical assessment, liver biochemical tests, ultrasound examinations (US); transient elastography , magnetic resonance MRI and endoscopy in some cases.
Results: Fifty five patients, with the median age at diagnosis 12.4 years were diagnosed with cystic fibrosis associated liver disease, with slight predominance of boys. Severe liver disease occurred in 11 patients (20%) of which 5 with oesophageal varices. All of them had history of meconium ileus and homozygous for F508 del; with an important boys predominance in patients with portal hypertension (80%). CF associated liver cirrhosis occurred more frequently in patients aged over 10 yrs. Pancreatic insufficiency and severe mutations was strongly associated with portal hypertension.
Conclusion: Children with cystic fibrosis older than 10 year, with history of meconium ileus, pancreatic insufficiency and severe mutation were more likely predisposed to liver cirrhosis with portal hypertension. Further studies should follow risk factor for development of cystic fibrosis liver disease and how they can be influenced, in order to prevent the development of liver cirrhosis.

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J09.21

Bulgarian newborn screening program and selective screening for inherited metabolic diseases - improvements by tandem mass spectrometry

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Introduction: National Genetic Laboratory offers modern and widely applicable genetic services for diagnosis and prevention of Inherited Metabolic Diseases in Bulgaria. The Laboratory performs: mass neonatal screening for Phenylketonuria (up to now more than 2 million newborns were screened); selective screening for over 70 Inherited Metabolic Diseases; Prenatal metabolic and enzymatic diagnosis. Aims: improvement of newborn screening program in Bulgaria, while preparing to expand the program. And development of selective screening program for kids and adults in risk, using the method for analysis of Amino Acids and Acylcarnitines by Tandem mass spectrometry. Results: more than 40% recall rate reduction for neonatal screening program. The cut-offs for Bulgarian population for 11 amino acids and 29 acylcarnitines were found. Samples from more than 400 patients with clinical symptoms for Inherited Metabolic Diseases were tested. Two cases of Medium-Chain Acyl-CoA Dehydrogenase Deficiency were found positive and confirmed on DNA level. The samples from the newborn cards of the patients were analyzed retrospectively and the specific profile of acylcarnitines was found, which prove the reliability of the method for newborn screening in asymptomatic phase of the disease. Conclusions: a reliable MS-MS method, which covers up to 30 disorders, has been developed. Currently the method is used for selective screening of patients with clinical symptoms. And as second tier analysis in the newborn screening program.

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J09.22

Differential diagnosis of MODY diabetes in patient population from Bosnia-Herzegovina

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MODY (maturity-onset diabetes of the young) is an autosomal dominant form of diabetes that is usually manifested before the 25-year of life. This type of diabetes is caused by defects in the primary insulin secretion. There are several types of MODY, which could be described as „monogenic" diseases, where mutations in a single gene are responsible for a particular type of MODY. Currently, there are eleven types of MODY, from which the most common types are MODY 2 and MODY 3 (with mutations on GCK and HNF1A genes, respectively).
There is no known incidence of MODY in diabetic population in Bosnia and Herzegovina, since until recently genetic testing for this type of diabetes was not available in this country. Goal of this ongoing study was to determine incidence of MODY and most common type of MODY in population of diabetics in Bosnia and Herzegovina.
As the material for this study 3 ml of whole blood from 20 diabetic subjects is used. Isolation of DNA was performed by salting-out method. Identification of mutations was made with MLPA (multiplex ligase-dependent probe amplification) technique, with MLPA kits P24 and P357 (Mrc-Holland). Statistical analyses were performed with Coffalyser software (Mrc-Holland).
All samples were typed successfully. Of the twenty subjects analyzed, mutations are identified in only two subjects, which correspond to 10% incidence of MODY in Bosnia and Herzegovina. Both subjects had mutation in the HNF1A gene, so they were diagnosed with MODY 3 type.

N. Lojo - Kadric: None. **Z. Velija - Asimi:** None. **S. Hasanbegovic:** None. **J. Ramic:** None. **K. Radic:** None. **L. Kapur - Pojskic:** None.

J09.23

A rare R151S missense mutation in MEFV gene in a Turkish FMF patient

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Familial Mediterranean fever (FMF) is an autosomal recessive autoinflammatory disease and characterized by recurrent fever and inflammation of peritoneum, synovium and pleura. The disease primarily affects populations surrounding the Mediterranean basin: Jews, Armenians, Arabs, Turks and Greeks. The responsible gene, MEFV located chromosome 16p13.3 and consists of 10 exons and encodes a protein of 781 amino acids called pyrin or marenostrin.
Up to date, 250 sequence variants in MEFV gene have been reported in Infevers database (accessed 12 Feb. 2013). The most frequent mutation M694V followed by E148Q, M680I (G/C) and V726A in Turkish FMF patients. During MEFV gene mutation screening among FMF patients for molecular diagnosis we identified a rare R151S (c.453G>C; p.Arg151Ser) missense mutation in 2th exon of MEFV gene which shown compound heterozygosity with E148Q mutation in a 18 years-old Turkish male patient with bilateral wrist arthritis and who lived in Sivas, the central region of Turkey. According to Infevers database, our case is the second R151S mutation, other case have been reported on 2008 by Berdeli et al. in Turkey.
We used direct DNA sequencing method for sequencing in two exons (2,10) of MEFV gene. SeqScape 2.0 sequence analysis software was employed for evaluation of two exon's sequences.
It is important to identify missense mutations in order to clarify their importance in FMF pathogenesis. Detailed clinical and molecular analyses are helpful for providing data to be used in genetic counseling for FMF patients.

H.K. Kurtulgan: None. **G. Bagci:** None. **G.E. Goktolga:** None.

J09.24

Analysis of mitochondrial DNA polymorphism in Tunisian Type 2 Diabetes

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Background: Type 2 diabetes (T2D) is a complex metabolic disorder characterized by hyperglycemia as a result of impaired insulin secretion and/or insulin resistance. The phenotypic expression of the disease may be influenced by ethnic differences, environmental and genetic factors. Genetic variations in the non-coding region of mitochondrial DNA may play an important role in the pathogenesis of T2D and its complications. Nevertheless, the implication of certain mitochondrial variants in T2D is still controversial.
We aimed to explore whether mitochondrial DNA variants contribute to the susceptibility to T2D in Tunisian population.
Patients and methods: A case control association study was performed on 64 T2D patients and 77 controls. The hypervariable region 1 (HVS1) from np16069 to np16400 of the mitochondrial DNA was amplified and sequenced. Statistical analysis was carried out using STATA program.

Results: Analysis of the T16189C variant showed that this SNP is common in Tunisia with a frequency of (~30%) in both T2D patients and controls. Statistical analysis showed that T16189C variant was unlikely to be associated with T2D in Tunisians. In addition, the remaining studied SNPs (87) from the HVS1 region showed that only the distribution of the G16390A variant was significantly different between T2D and controls (p = 0.04). Multivariate logistic-regression analysis with adjustment for age, sex and BMI revealed that G16390A variant was not associated with susceptibility to T2D.
Conclusion: We found no statistical evidence to support an association between T2D and HVS1 polymorphism in the studied Tunisian population.

S. Hsouna: None. **N. Ben Halim:** None. **K. Lasram:** None. **I. Arfa:** None. **A. Abid:** None. **S. Abdelhak:** None. **R. Kefi:** None.

J09.25
A rare case of Hunter syndrome

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We present a rare case of mucopolysaccharidosis (MPS) with an atypical presentation of high stature, mental retardation and absence of corneal clouding.This report is of a 2.7 year-old boy who presented to hospital with a history of recurrent respiratory tract infections and frequent ear infections. On examination he was high statured (SDS + 3.46), moderately overweight (+ 25.94% on his height). His neck was short, he had coarse facial features, a depressed nasal bridge and small stubby fingers with flexion of distal interphalangeal joints, joint stiffness, protruding abdomen with umbilical hernia, hepatomegaly and splenomegaly. There was mild mental retardation. The clinical appearance was suggestive of a mucopolysaccharidosis. Dosage of enzymes playing role in mucopolysaccharide lysosomal metabolism revealed low levels of iduronate-2-sulfatase (IDS), changes suggestive of type II mucopolysaccharidosis. Enzymatic diagnosis was confirmed by molecular DNA analysis that revealed that mutation IDS gene in intron 3 (c.419-2A> G) in homozygous form. Enzyme replacement therapy with recombinant human iduronate-2-sulfatase (Elaprase®) was started.

O. Marginean: None. **C. Banescu:** None. **C. Duicu:** None.

J09.26
Case of Hutchinson-Gilford Progeria syndrome from Russia

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Hutchinson-Gilford Progeria syndrome (HGPS) is a rare sporadic autosomal-dominant disorder (MIM 176670) an incidence of ~1 in 4000000 live births characterized by short stature, low body weight, early loss of hair, lipodystrophy, scleroderma, and facial features that resemble aged persons. The leading cause of death is cardio-/cerebro-vascular accidents associated with atherosclerosis. The genetic basis in most cases is a mutation at the nucleotide position 1824 of the lamin A gene.
We herein report a girl aged 2 years 8 months with a weight of 10.5 kg and a length of 76 cm. She was a child of the second pregnancy, 34 weeks of gestation with a mass of 2600 g and a length of 46 cm from young and healthy parents. Physical development of the girl proceeded to a year delay, no backlog of psychomotor development. Proband had typical phenotypic features: alopecia (loss of hair including scalp and eyebrows), prominent scalp veins and forehead, micrognathia, significant loss of subcutaneous fat, a violation of depigmentation in patches of irregular shape, camptodactyly hands and short stature. Cardiovascular disease during the examination of the child has not been identified.
The genetic study of peripheral blood-derived DNA of patient with classical progeria showed a typical de novo single-base pair substitution c.1824C>T (GGC>GGT, p.Gly608Gly) within the exon 11. This mutation creates an abnormal splice donor site, leading to the formation of a truncated lamin A protein progerin.
Future research into HGPS could also provide important clues about the general process of aging and aging-related diseases.

N.V. Komarova: None. **T. Adyan:** None. **V.P. Fedotov:** None. **A.V. Polyakov:** None.

J09.27
Familial case of c.413T>C *PHEX* gene mutation leading to X-linked hypophosphatemic rickets

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We report on a 3 years old girl referred for evaluation because of familial skeletal abnormalities (short stature, lower extremity bowing). The proband is the first child of non-consanguineous parents, born after uncomplicated pregnancy with birth weight 3600 g, height 57 cm. Her psychomotor development was normal; she has got prophylactic doses of vitamin D. At the 5 months old varum deformity of legs was observed. Hypophosphatemia, increased alkaline phosphatase level, lower level of 25-OH vitamin D and normal level of calcium were detected in serum; parathyroid hormone level was slightly increased. Proband`s hearing and dentition were normal, her stature was short (3-10‰).
Skeletal abnormalities were observed in proband`s father`s side of the pedigree: genu varum and short stature (proband`s father height is 162 cm, his brother - 149 cm, their mother (proband`s grandmother) - 149 cm). The extremity bowing was more severe in males. The teeth of proband`s father were irregular size and position.
Clinical diagnosis of X-linked hypophosphatemic rickets (XLHR) was formulated. The sequencing of all exons and exon-intron boundaries of *PHEX* gene detected c.413T>C missense mutation, leading to p.Leu138Pro substitution, which is predicted to cause changes in three-dimensional protein structure. Substituted leucine is strictly conserved not only human, but also in mammals' endopeptidases. c.413T>C mutation was first published by Rowe et al. in 1997, since then it was described as pathogenic in two more papers. To our knowledge, it is the second reported case of familial c.413T>C *PHEX* gene mutation. The clinical diagnosis of XLHR was confirmed.

L. Cimbalistiene: None. **O. Liaugaudiene:** None. **A. Utkus:** None.

J09.28
Gonadal mosaicism with a New Mutation in the Wilson Disease Gene, ATP7B in a Family

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OBJECTIVE: Wilson disease (WND), an autosomal recessive disorder of copper transport with a broad range of genotypic and phenotypic characteristics, results from mutations in the ATP7B gene. We report a new mutation in the Wilson disease gene ATP7B in a family.
CASE PRESENTATION: Mutation analysis was carried out on 5 subjects in one family and new mutation not previously reported was identified: c.3106G>A. This new mutation was carrying by mother and two siblings. However, father was carrying heterozygous c.2128G>A mutation with two siblings. Interestingly, one of the siblings was not carrying c.3106G>A mutation, but was carrying c.2128G>A and c.3007G>A mutations, and showed clinical and biochemical characteristics of the Wilson disease. The mutation c.3007G>A was related with gonadal mosaicism. There was no clinical findings with youngest sister but molecular genetics and biochemical results showed concordance with Wilson disease. The oldest sister (proband) did not have clinical findings and biochemical results with Wilson disease, but had compound heterozygosity, one of the mutation was new, and the other mutation was related with gonadal mosaicism. The proband was 27 years old, and new mutation was not showing biochemical and clinical findings of the Wilson disease, however it must not exclude the age-dependent effect.
CONCLUSIONS: We do not know genetic effects of the new mutation c.3106G>A and clinical future of patients, but we must monitor carefully the biochemical abnormalities and clinical picture of the Wilson disease. The discovery of the new Wilson disease gene could open up a new molecular diagnostic approach.

M.A. Soylemez: None. **A.I. Guney:** None.

J10.01
Exploring the role of microRNAs in multiple sclerosis: an unexpected anti-inflammatory potential?

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Abstract

In the past years, microRNAs (miRNAs) have arisen as novel regulators of most cellular processes in homeostasis and disease. Lately, their involvement in autoimmune diseases has become of interest, particularly in mul-

tiple sclerosis (MS). To investigate the contribution of miRNAs to this heterogeneous disease and study their role as potential therapeutic targets, we have conducted an in-depth analysis of the cellular pathways regulated by the potentially most relevant miRNAs in MS.
For this purpose, we performed an extensive and stepwise search on published data in order to identify miRNAs that, on one hand, were known to play key roles in naïve T cells and differentiated pathogenic, memory and regulatory CD4+ T cell subsets (Th1 / Th2 / Th17/ Tregs) and, on the other hand, were found to be dysregulated in MS patients, obtaining a shortlist of 13 candidate miRNAs. In a second step, by using online databases and restrictive filtering we generated a set of 903 validated and highly predicted gene targets from these miRNAs. Functional annotation studies conducted with Ingenuity Pathway Analysis led to the identification of a number of cellular pathways regulated by candidate miRNAs that are known to play important roles in MS etiopathogenesis. Overall, our results point to an immune-suppressive role of miRNAs in T cells during MS and provide a rationale for future research studies of selected candidate miRNAs in the disease.

J. Perez: None.

J10.02
Protective effects of Interleukin (IL)-6 gene polymorphisms against recurrent aphthous stomatitis

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Recurrent aphthous stomatitis (RAS) is a common ulcerative disease of the oral mucosa. The aim of the present study was to examine the possible role of Interleukin (IL)-6 gene polymorphisms in the development of RAS. This study comprised 184 RAS patients, 118 females and 66 males, and 150 healthy controls, 88 females and 62 males. Peripheral blood samples were genotyped using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analyses for the IL-6 gene -174G/C and -572G/C polymorphisms.
The distributions of genotype and allele frequencies of IL-6 gene -174G/C and -572G/C polymorphisms were statistically different between RAS patients and control group (genotype p=0.000 and p=0.001, allele p=0.000 and p=0.000, respectively). The genotype and allele frequencies of variant type of both the polymorphisms were higher in control group. So it seems that these polymorphisms had protective effect against RAS. After stratifying RAS patients according to clinical and demographical characteristics, no significant association was observed.
In conclusion we demonstrated that genotype and allele frequencies of IL-6 gene -174G/C and -572G/C polymorphisms were statistically different between RAS patients and controls and were protective against RAS. Further studies with larger number sizes of patients are needed.

N. Karakus: None. **S. Yigit:** None. **A. Rustemoglu:** None. **G. Kalkan:** None. **N. Bozkurt:** None.

J10.03
Circulating levels of RANTES and -403G/A promoter polymorphism in association to acute heart failure after STEMI and to cardiogenic shock

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Chemokines, including RANTES, play crucial role in processes of inflammation during cardiovascular disorders. They have been associated with increased risk of atherosclerotic progression and therefore an increased risk of acute myocardial infarction and subsequent complication - cardiogenic shock, heart failure. This study aimed to evaluate the role of RANTES -403G/A polymorphism and levels in circulation in processes of development and progression of myocardial infarction and cardiogenic shock. A total of 609 patients with ST segment elevation myocardial infarction, 43 patients with cardiogenic shock and 130 control subjects were enrolled in the study. RANTES -403G/A promoter polymorphism and baseline serum RANTES levels were analyzed. In present study, we associated RANTES -403 G/A promoter polymorphism with acute heart failure and ejection fraction in patients with myocardial infarction (p = 0.006; p = 0.02). A difference in circulating RANTES levels was observed between controls and STEMI subjects (p = 0.03). We found a decreasing tendency of serum RANTES levels with the severity of myocardial infarction and progression, with the lowest levels in patients with cardiogenic shock. Further, in STEMI patients the serum RANTES levels were correlated to levels of BNP and NTproBNP (p = 0.003; p = 0.03), and to ejection fraction (p = 0.05). Our results suggest the role of RANTES in cardiogenic shock and acute heart failure in hospital phase after myocardial infarction. RANTES might be considered as a marker of cardiogenic shock and acute heart failure in hospital phase after myocardial infarction at the time of admission.

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J10.04
Is individual susceptibility to periprosthetic osteolysis associated with differences in cytokine expression-response of peripheral mononuclear cells (PBMC) to pro-inflammatory stimulus?

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Individual susceptibility to periprosthetic osteolysis around total hip arthroplasty (THA) may be associated with genetic variations in cytokine genes. Here, we investigated whether cytokine expression-response of PBMC to pro-inflammatory stimulus differ between patients with severe/mild osteolysis and in subgroups according to the presence of TNF-238*A, an allele associated with severe osteolysis.
PBMC obtained from 32 THA patients (severe osteolysis, n=23; mild osteolysis, n=9) were stimulated with lipopolysaccharide; further subgroups were formed according to the TNF-238 genotype (11 carriers/21 non-carriers of TNF-238*A allele). Protein concentrations of IL-2, IL-5, IL-10, IFN-γ, TNF-α, VEGF, OPG and RANKL in supernatants were measured using Luminex and ELISA.
Of studied cytokines, elevated expression of IL-2, IL-5, IFN-γ, VEGF, OPG, RANKL ($p<0.05$) was observed in supernatants from patients with severe osteolysis compared to those with mild osteolysis, irrespective of TNF-238 genotype. Patients with TNF-238 GG genotype with severe osteolysis showed up-regulated expression of all studied cytokines, except IL-10 and VEGF, comparing to those with mild osteolysis. In severe osteolysis, the carriers of rare TNF-238*A allele showed lower expression of IL-2, IL-5, IFN-γ, TNF-α, RANKL ($p<0.05$) when comparing to non-carriers.
In conclusion, carriers of TNF-238*A allele with severe osteolysis showed different cytokine expression pattern in response to pro-inflammatory stimulus comparing to non-carriers of TNF-238*A allele with severe osteolysis as well as THA patients with mild osteolysis. Molecular mechanism by which TNF-238*A allele increases a risk of severe osteolysis should be further investigated.
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J10.05
Expression profiling revealed similar cytokine-expression profiles in pseudosynovial tissues retrieved from aseptically failed hip and knee arthroplasties

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Aseptic loosening accompanied by osteolysis is the most frequent long-term complication of total hip (THA) and knee (TKA) arthroplasties. Despite anatomic, kinematic and biomechanical distinctions between THA/TKA, there is no information on differences in cytokine-expression profiles between periprosthetic tissues retrieved from aseptically failed THA and TKA.
We therefore investigated mRNA expression profiles of candidate cytokines and their receptors involved in the inflammation (TNF-α, IL-2, IL-2R, IL-6, IL-10, IL-10R, TGF-β) and osteoclastogenesis (RANKL, OPG, SOCS3, DC-STAMP) in pseudosynovial tissues obtained from 36 patients with aseptically failed THA/TKA and 31 control patients with hip/knee primary osteoarthritis using quantitative RT-PCR.
When compared to controls, the expression of OPG, TNF-α, IL-10, IL-10R was lower in failed arthroplasties, however the difference reached significance only in TKA ($p\leq0.05$). IL-2, IL-2R, IL-6 and TGF-β mRNA expression did not

differ between patients and controls (p≥0.05). DC-STAMP was upregulated only in THA (p=0.0008), while reduced expression of RANKL (p<0.005) and elevated expression of SOCS3 (p<0.01) was detected in both THA and TKA tissues comparing to controls. Higher number of mRNA transcripts for all investigated cytokines and receptors was detected in THA comparing to TKA tissues. Although similar cytokine-expression profiles were associated with aseptically failed TKA and THA, higher cytokine-gene expression was detected in tissues from failed THA comparing to TKA. In general, our data support the hypothesis about the "inactive inflammatory response" in periprosthetic tissues during the later stages of disease leading to the reoperation. Grant support: IGA MZ CR NT/11049 and IGA LF_2013_13.

T. Tomankova: None. **E. Kriegova:** None. **J. Gallo:** None.

J10.06

The investigation of Killer cell immunoglobulin like receptor (KIR) genotyping in patients with Systemic Lupus Erytematosus and Systemic Sclerosis.

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterised by the production of autoantibodies and the involvement of multiple organ systems. The etiopathogenesis of SLE is unknown. Systemic sclerosis (SSc) is another autoimmune disease that effects mainly connective tissue. Its pathogenesis is unknown too. We will aim to analyse the role of killer cell immunoglobulin-like receptors (KIRs) genotypes and their existence with the respective HLA ligands in the pathogenesis of SLE and SSc. We examined the presence/absence of KIR2DL1, 2DL2, 2DL3, 2DL4, 2DL5A, 2DL5B, 2DS1, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1, 3DL2, 3DL3, 3DS1, 2DP1, 3DP1 and their known HLA ligands in 45 SLE, 25 SSc and 40 healhty controls. The KIR2DL5B (46.8% vs 25%, P = 0.036; OR = 2.6, 95% CI 1.05-6.6) and KIR2DS3 (42.6% vs 22.5%, P = 0.048; OR = 2.5, 95% CI 0.99-6.5) gene phenotype frequencies were found to be significantly increased in SLE patients compared to healthy controls. KIR2DS3 gene frequency was observed significantly increased in SSc patients too (48% vs 22.5%, P = 0.032; OR = 3.1, 95% CI 1.08-9.36).We did not find any associations of other observed KIR genes between the patients groups and controls. No significant difference was observed for KIR ligand between the patient groups and controls as well. KIR2DL5B and KIR2DS3 genes may have an role in autoimmune mechanisms. We are investigating this KIR genes epigenetic variations between the study groups. *This study was supported by TUBITAK (111S153).*

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J10.07

Genetic variation in L-selectin might prone the individuals to atopic dermatitis

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Background and aim: Atopic dermatitis (AD) is a common chronic or relapsing inflammatory skin disease, which often precedes asthma and allergic disorders. Chronic inflammation is the main pathogonomic feature of AD. Selectin adhesion molecules participate in the interaction between leukocytes and the endothelium, as well as in inflammatory cell recruitment. Genetic factors have an important influence on the risk of developing atopic disease. The aim of this study was to evaluate the association between genetic variants of L-selectin and susceptibility of AD.
Materials and Methods: This case-control study recruited 122 patients with AD; aged 8.92±5.07 years old and 151 age, sex, and ethnic background matched healthy controls. Genomic DNA was isolated, and amplification of L-selectin 206 Phe/Leu polymorphic region was performed by PCR incorporating sequence-specific primers (PCR-SSCP) to distinguish the genotypes.
Results: The frequency of the 206 Phe/Leu polymorphism was significantly more prominent in AD patients compared to the controls (27.9% 15.2%, p=0.003). Logistic regression analysis when fixed for covariates sex, age, self and familial histories of atopy revealed that the presence of Leu/Leu genotype increased the disease risk up to 2.75 (95% CI; 1.4-5.4).
Conclusion: The higher frequency of L-selectin 206 Leu genetic variant in patients with AD than in control individuals suggests that the F206L polymorphism could make individuals more vulnerable to atopic dermatitis.

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J10.08

Interferon induced helicase (IFIH1) rs1990760 polymorphism with Systemic lupus erythematosus in Tunisian population

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Systemic lupus erythematosus (SLE) is a complex autoimmune disease, characterized by the production of a wide range of autoantibodies and a broad spectrum of clinical presentation encompassing almost all organs and tissues. The etiology of this disease includes genetic and environmental factors. The aim of this study was to examine the association of a single-nucleotide polymorphism, rs1990760, of the IFIH1 gene in a Tunisian population.
The study population consisted of 87 SLE patient and 126 healthy controls from Tunisia. The polymorphism of rs1990760 was detected with the Ms PCR. The genotype and allele frequencies were calculated and analyzed.
Our study showed a statistically significant association between IFIH1 rs1990760 and SLE (p = 0.002). It was also found that the TT genotype was significantly more frequent in SLE patients than in healthy controls [odds ratio, 3.17 (95% confidence interval, 1.61-6.26); P = 0.001]. No statistical significance was observed in the comparisons amongst SLE patients with and without the main clinical and biologogical characteristics.
The results suggested that the rs1990760 TT genotype was associated with susceptibility to SLE and that the IFIH1 polymorphism analyzed did not seem to be implicated in the pathology and clinical manifestation of the lupus in the Tunisian population.

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J10.09

Sequencing 306 bp region of ANXA11 gene including rs1049550 polymorphism for sarcoidosis susceptibility in Turkish Patients

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Sarcoidosis is a multisystemic immune disorder with unknown etiology. The disease characterized by noncaseating epithelioid granulomas across body invasion especially lungs, skin and eyes. Recently, genetic susceptibility to sarcoidosis counted as a important factor to disease progression. Latest GWAS datas pointed out a new sarcoidosis susceptibility gene, ANXA11. According to these studies, a nonsynonymous SNP (rs1049550) in this gene is strongly associated with sarcoidosis. Our aim was to determine the association between rs1049550 SNP and sarcoidosis in Turkish patients and to scan other variations associated with sarcoidosis within 306bp region of ANXA11. Genomic DNA isolated from peripheral leukocytes of the 53 sarcoidosis patients and 52 controls. 306bp region of ANXA11, including rs1049550 amplified with PCR. Amplicons sequenced with Sanger method (Applied Biosystems 3110). The sequence data of both patients and controls analysed in BLAST database for identification of variations. Allele frequencies and genotype distribution of the groups were analyzed with the chi-square test. No other genetic variation was observed except rs1049550 polymorphism in the 306bp region. There was no statistically difference (x2=2.689 p=0.273) when the frequencies of CC, CT and TT genotypes in sarcoidosis group (58.5%, 30,2%, 11.3%, respectively) were compared with genotypes of control group (65.4%, 17.3%, 17.3%, respectively). When allele frequencies of sarcoidosis patients (C= 73.6%, T = 26.4 %) are compared with controls (C = 74.0 %, T=26.0 %,), no statistically significant difference (X2=0.006, p=0.940) observed for the rs1049550 polymorphism. According to our results ANXA11 rs1049550 polymorphism is not a genetic predisposition marker for Turkish population.

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J10.10

MTNR1B polymorphism rs10830962 influences the reproductive outcomes in women with SLE

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Background: Systemic lupus erymathosus (SLE) is an autoimmune disease that affects predominantly females of child-bearing age. SLE increases the risk for pregnancy complications and is important to find possible genetic factors, affecting the reproductive outcomes. Recent studies have shown the important role of melatonin receptor polymorphism in reproductive disorders. However, the impact of these polymorphisms on the reproduction in young women with autoimmune diseases is unclear.

Methods: A total of 101 SLE patients were genotyped for MTNR1B rs10830962C/G gene polymorphism. The reproductive history of all patients concerning menstrual, infertility, number of pregnancies, miscarriages and live-births was obtained, and genotype-phenotype relationships were determined.

Results: 85.1% of the patients with MTNR1B rs10830962 genotypes GC or GG had at least one pregnancy in comparison to 67.6% of CC genotype carriers (p=0.067). Accordingly, the G-allele-carriers with at least one live-birth were significantly more than the CC women (83.6 vs. 55.9%, p=0.004). The presence of GG/GC genotypes was related to significantly more live-births even after adjustment for age and disease activity (OR 3.209, 95%CI 1.034-9.959, p=0.044). However, the rs10830962G allele did not influence the development of menstrual disorders, self reported infertility or miscarriages.

Conclusions: Our preliminary results showed that MTNR1B rs10830962C/G polymorphism could influence the reproductive outcomes in lupus patients. The beneficial effect of G allele on the reproductive success could be related to greater ovarian reserve in the patients or to other biological/psychological features that remain to be established.

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J10.11

Quantitative analysis of GSDMB and ORMDL3 gene expression in peripheral blood leukocytes of asthma patients

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The genome-wide association study of bronchial asthma in the Volga-Urals region of Russia discovered a significant association with SNPs located in gasdermin B (GSDMB) and orosomucoid 1-like 3 (ORMDL3) genes (Karunas A.S. et al., 2011). Using RT-PCR, we performed gene expression analysis of GSDMB and ORMDL3 in peripheral blood leukocytes of 13 patients with asthma and 13 healthy control subjects. Nine patients had a severe form of the disease. A highly sensitive quantitative real-time PCR method for GSDMB and ORMDL3 mRNA quantification was performed using the SYBR Green. In accordance with «delta-delta Ct» method, the mRNA amounts of a target gene were normalized to an endogenous control (GAPDH). Normality of quantitative data was assessed using the Kolmogorov-Smirnov test. T tests were used when normality assumptions were satisfied, otherwise the Mann-Whitney test was utilised. The statistical analysis was done using the GraphPad Software and statistical significance was obtained with $p<0.05$. Specimens from peripheral blood leukocytes of patients with asthma showed higher level of GSDMB mRNA expression (M±SEM: 1,76±0,32) than those from healthy patients (M±SEM: 1,16±0,17). Expression analysis demonstrated a significant increase in the expression of GSDMB in peripheral blood leukocytes of patients with severe asthma (M±SEM: 2,10±0,44) as compared with controls. ORMDL3 transcript levels did not differ between patients with asthma (M±SEM: 1,59±0,72) and controls (M±SEM: 1,84±0,73). In sum, these results support an important role for the GSDMB in susceptibility to bronchial asthma in the Volga-Urals region of Russia. Supported by the Russian Foundation for Basic Research, RFBR №11-04-97063.

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J10.12

Hemochromatosis revealing severe combined immunodeficiency

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Severe combined immunodeficiency (SCID) is a life-threatening syndrome of recurrent infections and failure to thrive, caused by numerous molecular defects that lead to severe compromise in the number and function of T cells, B cells, and occasionally natural killer cells.

We will present the case of a three months old infant who was admitted for poor weight gain and repeated fever in the last four weeks. Family history identified two cases of infant deaths caused by infections (the mother's brothers). Physical examination showed a good general condition, normal weight for age, mild jaundice and generalized muscle hypotonia. Routine analysis showed increased transaminase level, so the next step of investigation was directed towards four main entities: hepatitis, sepsis, hemolysis, myolysis. The following laboratory tests were modified: moderate cholestasis, extremely high serum ferritin and rapidly increasing transaminases, while no evidence of hemolysis, anemia or any hepatic viral infection occurred. Since repeated episodes of fever were the main complain, a more profound examination of the immunity was realized, revealing severe combined immunodeficiency.

The particularity of the case rises from the early diagnosis, determined by the investigation of a chronic liver disorder with hemochromatosis.

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J10.13

Analysis of cytokines in the serum of women with breast cancer

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In the study, were determined by ELISA concentration IL-1β, IL1RA, IL2, IL4, IL-6, IL10, TNF- α and CRP (C-reactive protein) in the sera of 44 women with breast cancer, tumor stage T2-3N0-2M0 (serum taken before surgery to remove the tumor) and 56 healthy women. In women, breast cancer patients showed a significant decrease in IL-2 (p <0,0001), decreased production of IL2 is associated with the suppression of growth of natural killer cells and weakening of their cytolytic function, which ultimately leads to a lack of anti-tumor defense. Defined enhancement products IL6 (p <0,0001), which is an unfavorable factor and inducer of tumor growth. Also, we observed a significant increase in the concentration of IL6 (p <0,0001). According to the literature elevated concentrations IL6 considered as an adverse factor and inducer of tumor growth. In breast cancer patients showed a significant increase in the production of anti-inflammatory cytokines - IL10, which, according to some authors, leads to a weakening of the effector responses of T-cell immunity.

Cytokine status in women with breast cancer before surgery and healthy women, pg/ml (CRP-mg/L)

indicators	n	breast cancer patients	breast cancer patients	n	healthy women	healthy women	p
		χ±m	δ		χ±m	δ	
IL-1β (0-11)	44	2,5±0,21	1,4	56	3,8±0,28	2,1	<0,0001
IL1RA (50-1000)	44	1031,43±75,71	502	56	215±23,8	178	0,01
IL2 (0-10)	44	1,26±0,06	0,4	56	9,6±0,6	4,5	<0,0001
IL4 (0-4)	44	1,3±0,09	0,6	56	1,8±0,06	0,5	<0,0001
IL6 (0-10)	44	25±1,96	13	56	11,3±0,52	3,9	<0,0001
IL10 (0-20)	44	22,8±1,85	12,3	56	10,3±0,6	4,5	<0,0001
TNF -α (0-6)	44	3,62±0,22	1,5	56	1,86±0,09	0,7	<0,0001
CRP (0-5)	44	1,2±0,18	0,3	56	2,6±0,12	0,9	0,01

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J10.14

Cytokines gene expression in human lung epithelial A549 cells induced by non-structural NS1 protein of influenza A viruses

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NS1 protein of influenza A virus is a multi-functional protein that participates in suppression of host immune response via dysregulation of cytokine expression. In the present study we investigated relative levels of mRNA expression of IL1B, IL2, IL4, IL6, IL10, IL12B, IL18, IFNG and TNF by quantitative RT-PCR. All reactions were run in duplicate, relative quantification analysis was done using comparative Ct (ΔΔCt method) with GAPDH as a reference gene.

To evaluate NS1 induced cytokines mRNAs production, A549 cells were infected with wild type recombinant H5N1 A/Kurgan/5/05 (wtNS) and A/Kurgan/5/05 with deletion of NS1 gene open reading frame (delNS1) vi-

ruses. Cytokine response was measured after 8 hours since stimulation.
Earlier it has been demonstrated that NS1 protein inhibits pro-inflammatory cytokine and chemokine responses. In our research we revealed approximately ten-fold increase in gene expression of IL1B, IL6 and TNF for cells stimulated with delNS1 in comparison to wtNS viruses. The mRNA levels of IL12B and IL18 were comparable to all wtNS-, delNS1- and mock-infected cells. Interestingly, delNS1 virus infection induced IL10 expression, which was not detected in wtNS- and mock-infected cells. In contrast, expression of IL4 was observed only in wtNS-infected cells. IL2 and IFNG were not detected in either infected or mock epithelial cells.
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J10.15

Paradoxical reaction during antituberculosis treatment in a patient with Down syndrome. A case report

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Introduction: The paradoxical reaction (RP) is characterized by the deterioration of clinical and radiological signs of pre-existing tuberculous lesions or by development of new lesions in patients who initially responded to tuberculosis treatment.
Case-Report: We describe a case of a 7 year old girl with Down Syndrome with recurrent fever and cough for a month. The patients presented fever (39°C), inspiratory triage and dyspnea. Laboratory tests showed neutrophilia, increase in CRP and ESR. Chest X-Ray and CT-scan showed bilateral interstitial thickening with reticulo-nodular appearance. Gastric aspirated and acid-fast bacilli culture were positive. The patient started standard antituberculosis therapy and methylprednisolone. We have seen a slow improvement in respiratory symptoms on the 15th day of therapy. In the 21th day there was a worsening of clinical (fever and dyspnea) and radiological signs. The second gastric aspirates was negative.
The study of lymphocyte subsets showed initially CD4+ 13% (n.v. by age 33-41%), after 15 days 29%. This condition is described as a Paradoxical Reaction; it was observed in patients with HIV-TB co-infection as an immune reconstitution syndrome and is characterized by worsening of the general clinical condition with exacerbation of symptoms. The dose of methylprednisolone was increased, with gradual improvement in symptoms.
Conclusion: The Paradoxical Reaction can be suspected in patients with Down syndrome if there is worsening of general clinical condition during tuberculosis treatment. In this patients the corticosteroid therapy was essential.

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J11.01

BRCA mutation carriers do not have compromised ovarian reserve

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Background: Controversy exists about impact of BRCA1/2 mutations on female fertility, an issue of great interest to mutation carriers and their caregivers. Previous studies are small, or based on indirect parameters (e.g., self-reported infertility), which depend on additional factors unrelated to true fertility potential. None of the previous studies used strict fertility markers.
Objective: To evaluate the relation between carrying BRCA mutations and fertility, using AMH level, which has been previously shown to be one of the most accurate markers of fertility potential.
Patients, Methods: 40 healthy BRCA1/2 mutation carriers, aged 26-40 years, attending a multidisciplinary breast & ovarian cancer surveillance clinic, were tested for AMH levels, using a two-site-ELISA. Levels were compared to well-established normograms of the general fertile population.
Results: Mean age and parity of carriers was 33.2years (26-40, SD3.99) and 1.97 (0-7, SD1.49), respectively. All carried at least 1 Ashkenazi-Jewish founder mutation. AMH levels for most carriers were in normal range (mean2.6ng/ ml; SD3.73ng/ml, approximately 50th percentile).
Conclusions: AMH levels of healthy BRCA1/2 mutation carriers are similar to that of non-carrier women matched for age; therefore we conclude that BRCA mutation carriers do not have compromised ovarian reserve. This is the only study, to the best of our knowledge, which directly examines ovarian reserve in carriers, by an accurate marker. This study has important clinical applications, as it provides reassurance for female carriers of these mutations, regarding their fertility potential.

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J11.02

RB1 mutation influence on Superselective Ophtalmic Artery Infusion of Melphalan in Retinoblastoma patients

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Retinoblastoma (RB) is the most common primary intraocular malignancy in children. Two-hit inactivation of RB1 gene is necessary for tumor development. Sixty percent of RB cases show two somatic mutations, while 40% are hereditary with patients carrying one germline mutation and one somatic mutation. In order to improve life quality of RB patients, worldwide tendency is to develop different kind of targeted therapies. Since 2008 the Superselective Ophtalmic Artey Infusion of Melphalan (SOAIM) was introduced in Siena for RB treatment. Our aim is to evaluate the correlation between SOAIM therapy outcome and RB1 germline mutation. Among 35 RB patients under SOAIM treatment, 18 had complete remission, 8 underwent enucleation, 4 had relapse, 4 had discordant outcomes in the 2 eyes, and 1 had technical failure. Next Generation Sequencing and Multiplex Ligation-dependent Probe Amplification were performed to detect point mutations and deletions/duplications of the RB1 gene, respectively. The analysis reveals one RB1 germline mutation in 17 out of 35 patients. Considering remission versus enucleation and RB1 mutation presence versus absence and using Fisher exact test, a significant P value (0.03) is obtained. These results suggest that having a germinal mutation predisposes to a better outcome of Melphalan therapy. Since RB samples without a germline mutation have been shown to harbor an higher number of genomic somatic rearrangements (P value 0.002), we could hypothesize that this genomic mutational burden might influence Melphalan metabolism resulting in a lower response to drug therapy.

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J11.03

Genetic abnormalities in diagnostics of BCR-ABL-negative myeloproliferative neoplasms

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The goal of the study was to assess frequencies of JAK2, MPL mutations and cytogenetic aberrations in group of 567 patients with BCR-ABL-negative MPNs. The investigated group included 224 cases of Polycythaemia vera, 102 cases of Essential thrombocythemia, 102 cases of Primary myelofibrosis and 140 cases of Chronic myeloproliferative disease, unclassified (CMPD-U). Polymorphisms of JAK2 and MPL (W515L/K) were detected by realtime PCR and the sequence analysis. Conventional cytogenetics of bone marrow with chromosome banding analyses were performed for 129 patients.
The frequencies of JAK2 617F allele have been detected as follows: 89,7% in PV, 56,4% in ET, 49,7% in PMF, 8,6% in CMPD-U that confirmed MPN diagnosis for 12 patients. The mutations rate of JAK2 exon 12 was 2,9% in PV. The frequencies of MPL 515L allele were 2,3% in ET, 2% in PMF. Normal karyotypes were defined in 85,3% cases. Aberrant karyotypes were defined in 14,7% cases and included 3,9% with isolated chromosomal aberrations (favourable prognosis), 6,2% karyotypes (intermediate prognosis), 4,7% with complex abnormalities (unfavourable prognosis).

Isolated chromosomal aberrations were defined reliably more often in PV than in PMF (p<0,0000). The frequency of the complex karyotypes was statistically higher in PMF as compared to PV and ET (p<0,0000). 2 of 6 patients carrying karyotype with complex abnormalities had transformation from MPN to AML.
Point mutations in JAK2 and MPL genes are specific markers for the patients with BCR-ABL-negative MPNs. The integration of molecular genetics with cytogenetics helps to stratify patients into different prognostic groups and optimize treatment strategy.

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J11.04

Allelic imbalance on 1p36 (RUNX3), 16p22 (CDH1) and 17p13 (TP53) and microsatellite instability in gastric tumor.

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Gastric cancer is one of the most common cancers in the world and the second leading cause of global cancer mortality. Two major gastric cancer histological subtypes are recognized with distinct morphology, epidemiology, pathogenesis and clinical behavior, the intestinal and diffuse subtypes.
We investigated the clinical and prognostic importance of allelic imbalance (AI) of tumor-related genes CDH1, RUNX3, TP53 and microsatellite instability (MSI) for gastric cancer patients.
We examined allelic imbalance on 1p36 (RUNX3), 16p22.1 (CDH1) and 17p13.1 (TP53) and microsatellite instability (BAT26) in 100 paired samples of gastric tumor and morphologically normal gastric mucosa, 57/100 of which have been identified as diffuse type of gastric cancer, 43/100 tumors belonged to the intestinal type gastric cancer. Frequency of allelic imbalance was: 26% for 1p36 (RUNX3), 20% for 16p22.1 (CDH1) and 31% for 17p13.1 (TP53). We have not detected allelic imbalance in 38/100 (38%) of the tumor samples.
We have shown a significant difference in the frequency of allelic imbalance of the studied loci among different types of gastric cancer. Frequency of allelic imbalance in patients with intestinal gastric cancer was significantly higher than in patients diffuse type GC (p=0.0265). Frequency of AI in 16p22.1 (CDH1), 17p13.1 (TP53) and microsatellite instability (BAT 26) was significantly higher in intestinal type of gastric cancer than in diffuse type (p = 0.04, p = 0.016 and p = 0.01, respectively).

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J11.05

Progressive cavernous hemangiomatosis in a patient with neurofibromatosis type 1

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Apart from typical diagnostic criteria of neurofibromatosis type 1 (NF1, OMIM # 162200), some minor NF1 signs such as cavernous hemangiomas may cause severe complications. We present a case report of a male patient with NF1 and a severe haematological complication as a result of progressive cervical hemangiomatosis. A male with an uneventful pre- and perinatal history, presented at the age of three years with numberous café-au-lait spots, several neurofibromas and visible cavernous hemangiomas in the area of the scalp and axilla. From the age of 7 years until now these painless lessions progressed, reached the thoracic area and infiltrated or suppressed several organs including cervical intervertebral spaces, submandibular salivary glands and the thyroid. Oncomarker testing showed no malignancy. The extensive growth of hemangiomas recently led to severe life-threatning anemia and consumptive thrombocytopenia. Treatment with cyclophosphamide has been initiated. Genetic testing revealed the presence of NF1 gene mutation in exon 24 c.4226_4227delAGinsT NF1 gene, leading to a shift of the reading frame and premature stop codon after 10 amino acids (p.Lys1409Ile fsX10). Hemangiomas in patients with neurofibromatosis type 1 occur in about 5-6% of patients. This is a rare case with an extremely progressive hemangiomatosis with the novel mutation leading to life-threatning consumptive thrombocytopenia.

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J11.06

Diagnostic value of molecular cytogenetics and multiplex RT-PCR for risk stratification in acute myeloid leukemia

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Introduction
In addition to conventional cytogenetics, the European Leukemia Network recommends mutation analysis of *NPM1*, *CEBPA* and *FLT3* genes for genetic risk stratification in those AML cases with normal karyotype. However, due to technical limitations in cytogenetics this strategy cannot be realized in about 20% of cases. In this study, we evaluated whether Interphase-FISH and RT-PCR are of additional benefit.
Materials and Methods
In 131 AML patients an RT-PCR panel with 28 leukemia specific translocations (HemaVision®) as well as FISH analyses with selected probes were performed in addition to conventional cytogenetics. Only diagnostic material from peripheral blood or bone marrow was used that contained a minimum of 20% leukemic cells.
Results
Whilst FISH analysis was throughout feasible, cytogenetics failed in 13% due to an insufficient number of metaphases and HemaVision® in 3% due to poor RNA quality. Chromosomal aberrations were detected in a total of 71/131 cases (54%). Of the latter, cytogenetic, FISH and multiplex RT-PCR analyses were concordant in 25/71 cases (35%), whereas in 45/71 patients (64%), only cytogenetics and FISH showed a positive, concordant result. In one case a translocation was detected by HemaVision® only, since no metaphases were obtained at cytogenetic analysis and the FISH panel did not cover a probe for this specific translocation.
Conclusion
These data show that risk stratification by conventional cytogenetics in addition to a well defined FISH panel is successful in almost all AML patients. HemaVision® should be performed in a second step when initial cytogenetic/FISH analyses were uninformative.

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J11.07

CDKN2A germline mutations detected in Czech melanoma cancer families

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CDKN2A gene, Cyklin-dependent kinase inhibitor type 2A, has been identified as major melanoma susceptibility gene based on the presence of germline mutations in high-risk melanoma-prone families. The lifetime risk of melanoma in CDKN2A mutation carriers is ranging from 58% in Europe to 91% in Australia.
By the direct sequencing of CDKN2A gene, 73 melanoma families indicated by clinical geneticists were analysed.
Three germline mutations were detected. A novel germline frameshift mutation c.15_20del6insC in exon 1α of the p16/INK4a transcript variant was detected in a family with mother and daughter diagnosed with primary melanoma at age of 31 and 38, respectively. Both were mutation carriers.
A novel germline splice site mutation c.457+4delAG was detected; it is predicted to result in a cryptic splice site 88bp downstream in exon 2 of the p16/INK4a transcript variant. Other transcript variant p14/ARF might not be influenced because of ARF termination codon located 27bp upstream from the predicted cryptic splice site. cDNA analysis is pending. The c.457+4delAG mutation was detected in woman with multiple primary melanoma at age of 29 and 34.
A worldwide spread missense mutation c.71G>C, p.Arg24Pro in exon 1α of the p16/INK4a transcript variant was detected in family with father diagnosed with melanoma at age of 42 and his daughter diagnosed with multiple primary melanoma at age of 24 and 35. Both were mutation carriers.
In three families frequent polymorphism p.Ala148Thr was detected; in some studies it is presented as low penetrance susceptibility allele.Supported by OP VaVpI - RECAMO, CZ.1.05/2.1.00/03.0101.

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J11.08

Investigation of OCT1 mRNA expression in colorectal cancer

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Colorectal cancer is an important public health problem. There are over one million new cases of colorectal cancer diagnosed worldwide each year and half a million deaths. It accounts for over 9% of all cancer incidences. It is the third most common cancer among men (after lung and prostate), and the second amongst women (after breast) worldwide and the fourth most common cause of death.
The OCT1 transcription factor is a member of POU-domain containing family of homeodomain proteins whose expressions have been implicated in normal and abnormal development. POU proteins are expressed in early developmental procedures as transcriptional regulators. This family comprises six classes which OCT1 belongs to the POU protein class II and identifies its target sequences by the octamer-binding site. This study aimed to detect the expression of OCT1 mRNA in clinical samples of colorectal cancer and analyze the correlations of its expression with the clinicopathological features of colorectal cancer.Twenty-six pairs of colorectal cancer tissue and adjacent nontumoral tissue were obtained at the time of surgery from patients diagnosed with colorectal cancer. The expression of *OCT1* gene was detected by real time reverse transcriptase polymerase chain reaction (RT-PCR).
The results showed that *OCT1* was significantly downregulated in colorectal carcinoma samples in both males (58.33%) and females (57.14%). *OCT1* expression was not significantly associated with the gender and site of primary tumor (P>0.05).
The conclusion was dysregulation of *OCT1* gene might be a novel prognostic biomarker for patients with colorectal cancer.

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J11.09

Aberrant DNA methylation of VHL, P16, GSTP1 genes in renal cell carcinoma patients from Bashkortostan Republic of Russia.

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Renal cell carcinoma (RCC) is most common neoplasm affecting the adult kidney. Renal carcinoma develops as a consequence of accumulation of several genetic aberrations. The frequent molecular abnormality in RCC is inactivation of tumour suppressor genes by methylation of CpG islands of promoters. Since RCC is curable when it is confined to therenal capsule, early diagnosis is extremely important. Promoter hypermethylation is the most common mechanism for the inactivation of the tumor suppressor genes (TSG) in the development of human cancer. The aim of this study was to analyze CpG methylation status of promoter regions of VHL, p16 and GSTP1 genes in 110 renal cell carcinoma patients from Bashkortostan Republic. Then, we evaluated the association between methylation at the promoter regions of these genes and the clinicopathological parameters of the RCCs. The tumours were predominantly low stage and low grade.
Gene promoter methylation was determined by methyl-sensitive PCR. As template for polymerase chain reaction genomic DNA extracted from tumor and normal tissues, pre-hydrolyzed methyl-sensitive restriction enzyme HpaII (CCGG) was used. Verification of PCR was performed in 7% polyacrylamide gel electrophoresis using 30% acrylamide. Documentation of electrophoresis was performed using video Geldoculant. Hypermethylation of the VHL gene was detected in 3,8% of the patients. Methylation of promoters in CpG islands p16 gene found in 18% and in GSTP1 gene in 13,6% cases. Of the genes tested, not a single gene showed age- or outcome-related differences. Future studies of epigenetic modifications are needed to establish diagnostic panels for early diagnosis of RCC.

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J11.10

Epidermal growth factor receptor (EGFR) mutations identified in Romanian patients with non-small cell lung cancer

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People harboring mutant EGFR alleles are prone to develop lung and anal cancers. The last years witnessed a crescent interest in identifying EGFR mutations that could function as molecular predictors of clinical outcomes when experimental EGFR-tyrosine kinase inhibitors (TKI) are used in cancer therapy. We screened for such mutations in 114 Romanian patients with non-small cell lung cancer (NSCLC).
We performed two distinct genomic DNA extractions from each paraffin embedded lung tissues collected from our NSCLC Romanian patients. Using two different sequencing systems, we searched for variations within exons 19 and 21 of EGFR gene.
Our study revealed 25 mutations associated with a good response to TKI based cancer therapy in 24 patients with a median age of 61 years. About 80 percent of this mutations are located in exon 19, the most preeminent being delE746-A750. Four of the other mutations located in exon 19 (i.e. K739Q, L747S, E749* and K757N) are not indexed in EGFR Mutation Database. The majority of the mutations residing in exon 21 fall within the L858 somatic mutations category.
This screening represents one of the first compelling studies that aim to extensively map EGFR mutations in Romanian patients suffering of NSCLC. The most frequent mutations that we identified are also reported to be worldwide most frequent, according to EGFR Mutation Database. The fact that by far the most prevalent mutations are located in exon 19 of EGFR gene could represent a characteristic of the Romanian patients, but further investigations are required in this direction.

A.C. Ratiu: A. Employment (full or part-time); Modest; University of Bucharest. **A.M. Stan:** A. Employment (full or part-time); Modest; Genetic Lab. **I.A. Visanoiu:** A. Employment (full or part-time); Modest; Genetic Lab. **L. Savu:** A. Employment (full or part-time); Modest; Genetic Lab.

J11.11

Methylation profiles of group of miRNA genes in lung, renal and colorectal tumor.

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Methylation profiles of CpG-islands of seven miRNAs genes (*miR-9-1*, *miR-9-3*, *miR-34b/c*, *miR-129-2*, *miR-193a*, *miR-212*, *miR-203*) in non-small cell lung cancer (NSCLC), clear cell renal cell carcinoma (ccRCC) and colorectal cancer (CRC) were defined using methylation specific PCR. Significant (P≤0.05 by Fisher) increase of methylation frequency of four genes (*miR-9-1, miR-9-3, miR-34b/c, miR-129-2*) in NSCLC, ccRCC and CRC was shown, and data on methylation of *miR-129-2 in these tumors was not reported earlier. For the first time, tumor-specific differences in methylation of miR-212* and *miR-203* genes were revealed, for example, significant increase of methylation frequency of *miR-212 in NSCLC and significant decrease in ccRCC were found. It was shown that methylation frequencies of miR-9-1* and *miR-34b/c* were significantly increased in NSCLC of different histological types - squamous cell carcinoma (SCC) and adenocarcinoma (AD). Significant increase of methylation frequency of *miR-129-2*, *miR-193a* and *miR-212* was also shown in SCC, what was reported here firstly and may be useful in the differential diagnosis of SCC and AD.

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J11.12

MEN1 gene mutation in a family with ectopic cushing syndrome associated with thymic carcinoid tumor

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Subject: Multiple Endocrine Neoplasia type 1(MEN1) is an autosomal dominant syndrome. Although thymic carcinoid tumor is recognized as part of MEN1 but functioning thymic carcinoid tumor in MEN1 has not been reported as the first presentation of the MEN1. In this report, we present mutation screening in a 29 year-old male who developed ectopic cushing syndrome secondary to thymic carcinoid tumor and also further genetic

evaluation in his family.
Methods: A large family was evaluated for routine biochemical tests, calcium profile, parathyroid hormone (PTH), hypothalamus-pituitary axis and brain magnetic resonance imaging (MRI) besides abdominal computed tomography (CT scan) was performed.The coding region of MEN1 gene from exon 1 to 10 was screened for the presence of mutations using PCR amplification followed by direct sequencing. For protein modeling the sequence of menin (NP_570711.1) was submitted to the I-TASSER server. The best computed model (based on the 3U84.pdb file) was used consecutively.
Results: A single nucleotide substitution in exon 10 of MEN-1 gene at position 1689 C/T (rs104894261) was found. The same mutation was detected in three other family members. On the protein level, this substitution leads to a deletion of Arginine 527 (R527X), which would ultimately result in a truncated protein.
Conclusion: This presentation showed that thymic neuroendocrine tumor can be the first manifestation of the MEN1 syndrome and it could be diagnosed as a dominant manifestation of this syndrome in family members. We suggest biochemical or genetic screening for MEN-1 syndrome in patients with thymic carcinoid tumor.

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J11.13
Allelic imbalance and microsatellite instability in papillary renal cancer

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Renal cancer is one of 10 most common adult malignancies and is an actual problem in oncourology. There currently exists no panel of molecular-genetic markers for renal cancer. Data about genetic alterations mainly refer to the most frequent form of renal cancer - clear cell carcinoma that includes 80% of cases. Papillary carcinoma (second major type: 15% of cases) is studied less than clear cell tumor, therefore search of genetic abnormalities with diagnostic and prognostic characteristics in papillary carcinoma is particularly actual. The purpose of our work is analysis of allelic imbalance in locus 1q32 and microsatellite instability by using STR-markers in different groups of patients with papillary renal cancer to assess the prognostic relevance those alterations. We have selected 48 paired samples of papillary carcinomas (fragments of tumor and adjacent morphologically intact tissue), then analyzed these using D1S2142 and D1S3465 polymorphic repeats (locus 1q32) by PCR with fluorescent labeled primers and fragment analysis in sequencer ABI3100 „Applied Biosistems". Allelic imbalance was observed in 31.9% of informative cases, also correlation of allelic imbalance and tumor grade was obtained for the first time (Spearman's coefficient r = -0,304, p = 0,047). Analysis of paired samples using D1S2142 and D1S3465 (tetra-), CGG-GIPC1 (three-) and D9S168 (dinucleotide) repeats revealed microsatellite instability in 54.2% of tumor samples. As a whole, it was demonstrated that allelic imbalance in 1q32 and microsatellite instability are frequent events in the papillary renal cell carcinomas, and allelic imbalance in 1q32 could be considered as a prognostic marker.

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J11.14
Methylation profile of group of miRNA genes in clear cell renal cell carcinoma; involvement in cancer progression.

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MicroRNA regulates gene expression, is involved in many cellular processes, and plays an important role in the development of cancer. The regulation of the expression of miRNA genes can be achieved by methylating their CpG islands, which is shown in different types of tumors. The methylation of miRNA genes in clear cell renal cell carcinoma (CCRCC) has mainly been studied for the *miR-9* and *miR-34* families. The methylation of six miRNA genes (*miR-124a-2, miR-124a-3, miR-9-1, miR-9-3, miR-34b/c, miR-129-2*) was investigated with the use of representative set of CCRCC samples (46 cases). Methylation of three genes *miR124a-2*, *miR-124a-3* and *miR-129-2* was studied in kidney tumors for the first time. Methylation analysis was performed using methyl specific PCR. It is shown that the frequency of methylation of six genes (*miR-124a-2, miR-l24a-3, miR-9-1, miR-9-3, miR-34b/c* and *miR-129-2*) was significantly higher in tumor samples than in samples of histologically normal tissue ($P<3x10^{-5}$ by Fisher's exact test). These results suggest the properties of tumor suppressors for the six miRNA genes indicated in CCRCC. We also found correlations between the methylation frequency of some miRNA genes and signs of the progression of CCRCC (tumor size, clinical stage, loss of differentiation, and metastasis).

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J11.15
Novel translocation t(10;14) in adult acute lymphoblastic leukemia: a case report

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Acute lymphoblastic leukemias (ALLs) are characterized by clonal proliferation of lymphoid progenitor cells. Besides hematologic and immunophenotypic data, essential for assigning the blast lineage, the cytogenetic findings provide valuable diagnostic and prognostic information.
We report on an adult patient with B ALL and monosomal karyotype (loss of chromosome 7 and t(10;14)(p13;q21)), at presentation.
Bone marrow aspirate was used for morphological, cytochemical, flow cytometry and chromosomal studies. Cytogenetic investigations were performed on GTG-banded slides. FISH with BAC FISH probes, centromeric, locus specific and painting probes were applied for molecular characterization.
Hematological investigations at diagnosis showed severe anemia (haemoglobin 6.3 g/dL), moderate leucocytosis (20x109/L) and decreased platelet count (63x109/L). Bone marrow analysis confirmed the diagnosis of ALL, and immunophenotyping revealed positivity for common precursor B cell markers.
The chromosomal studies detected a single malignant clone bearing a reciprocal translocation t(10;14)(p13;q21) and chromosome 7 monosomy. Normal cells were also detected (5 out of 29 analyzed). BCR/ABL1 fusion and MLL rearrangements were ruled out by FISH studies.
To the best of our knowledge, this is the first reported adult ALL case with a translocation t(10;14)(p13;q21), as part of a monosomal karyotype. Adding new cases with well characterized, rare chromosomal abnormalities might contribute to a better understanding of disease biology and underlying pathomechanisms. Also, the cytogenetic data contributed, in our case, to prognosis evaluation, monosomal karyotype being associated with a poor prognosis.

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J11.16
Frequency of BCR-ABL Fusion Transcript Variants in Iranian Patients with Chronic Myeloid Leukemia (CML)

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Chronic myeloid leukemia (CML) is a chronic myeloproliferative disease, represented in 1 - 2 per 100,000 of the population and about 15-20% of all cases of adult leukemia. 95% cases of CML are caused by a t(9;22)(q34;q11) chromosome reciprocal translocation which fuses the ABL proto-oncogene with the BCR gene, resulting in the *BCR-ABL* fusion gene (Philadelphia chromosome) which acts as an oncogene.
The fusion gene is associated with two major forms of fusion transcripts including b2a2 (e13a2) and b3a2 (e14a2). Both fusion mRNAs are translated into the p210 BCR-ABL protein which enhances tyrosine kinase activity, allowing the cell to become cancerous. The incidence of one or the other rearrangements in CML patients differs according to varying series.
Detection of mRNA chimeric transcript can be used as a marker in molecular monitoring of patients and in the selection of the appropriate management. The aim of this study was to determine the frequency of these various types of *BCR-ABL* transcripts in Iranian patients with CML. RNA was extracted using Qiagen kit and BCR-ABL transcript assessed by RT-PCR. Out of 172

patients, 77 (44.76%) were female, and 95 (55.23%) were male. Among this patients, b3a2 was the most commonly detected variant (41.86%), followed by b2a2 (13.95%) and 38.37 % of the cases negative for BCR-ABL rearrangement. In contrast to other reports, we did not see any co-expression of the b3a2 and b2a2 variants. In conclusion, RT-PCR assay is a useful tool for the detection of BCR-ABL fusion transcript and helpful in clinical practice.

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J11.17

Analysis of p.R145W mutation of the CHEK2 gene in patients with breast and ovarian cancer from Bashkortostan Republic of Russia.

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CHEK2 is a tumor suppressor gene, which participate in the inhibition of cell cycle and apoptosis following DNA damage. Mutations in CHEK2 are associated with cancers at many sites, including breast and ovarian cancers. Different CHEK2 mutations have a particular range and frequency in various populations. Previously four mutations in CHEK2 (1100delC, IVS2+1G>A, del5395, I157T) were genotyped in BC and OC cases. In this study we analyzed mutation p.R145W in 977 BC cases, 243 OC cases and 689 cancer free controls. Detection of mutation was performed by high resolution melting curve analysis (HRM) and confirmed by direct sequencing. The p.R145W mutant form within the FHA domain Chk2 cannot be phosphorylated at ATM-depended phosphorylation site and cannot be activated by gamma radiation.

CHEK2 p.R145W mutation was found in two ovarian cancer patients (0,8%) from different ethnic groups, but it wasn't detected among patients with breast cancer and control. Thus, the p.R145W variant is rare in the population of the Bashkortostan Republic and determination of this variant in clinic should not be recommended in our population.

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J11.18

Alterations of expression levels of group of miRNA genes and the protein-coding genes RAR-beta2, and NKIRAS1 in lung, kidney and breast tumors.

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The alterations of expression levels of miRNA genes: *miR-129-2*, *miR-24-2*, *miR-17*, and *miR-9-1* and protein-coding genes: *RAR-beta2*, and *NKIRAS1* (potential target genes of *miR-17* and *miR-9-1*) were determined in non-small cell lung cancer (NSCLC) including adenocarcinoma (AD) and squamous cell carcinoma (SCC), clear cell renal cell carcinoma (ccRCC) and in breast cancer (BC). Significant ($P \leq 0.05$ by Fisher) prevalence of cases with increased expression of *miR-24-2 in* AD and with reduced expression of miR-17 in SCC and BC was revealed. Significant prevalence of elevated gene expression of *NKIRAS1* was shown in NSCLC, including SCC and AD, but a reduced expression of *NKIRAS1* was shown in ccRCC. Significantly increased expression of *RAR-beta2* predominated in ccRCC and NSCLC. For the first time, statistically significant negative correlation ($P \leq 2 \times 10^{-12}$ by Spearman) between alterations of expression levels of *miR-17* and its potential target genes *RAR-beta2* and *NKIRAS1* in NSCLC, as well as between expression level alterations of *miR-17* and *NKIRAS1* in BC was found, that is consistent with the known mechanism of suppression of protein coding genes expression under the action of miRNAs. These results also argue in favor of bioinformatics data that mRNA of *RAR-beta2* and *NKIRAS1* are potential targets of *miR-17*.

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J11.19

Identification of mutations associated with increased risk of hereditary breast and/or ovarian cancer in Colombia.

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Breast cancer is the neoplasm with the highest incidence and mortality among women in Colombia. With the aim to determine the mutacional incidence in BRCA1 and BRCA2 genes we analyzed by sequencing a total of 111 high-risk breast and/or ovarian cancer families from Colombia. Seven families were found to carry BRCA1 mutations and eleven families had BRCA2 mutations. For BRCA1 we found nine variants of uncertain significance (VUS), of which we concluded, using in silico analysis (Polyphen2, FastSNP and NNsplice), that IVS2-12C>G, T790A, R959K and E1345K are probably pathogenic. In BRCA2, we found five variants of uncertain significance (VUS), four previously described and one novel mutation (6655 T>C); using in silico analysis we concluded that 1093 A>G and 6655 T>C are probably pathogenic and 3199 A>G and IVS4-89 T>C are probably neutrals. We identify also 11 polymorphisms, 2 of them associated with a moderate increase in breast cancer risk (BRCA2 N372H and IVS21-66T>C). Our results indicate that the Colombian population has a heterogeneous spectrum of prevalent BRCA mutations.

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J11.20

Mapping of the small 5q deleted segment in myeloid malignancies using molecular cytogenetic techniques

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Deletion 5q is frequently found in bone marrow cells of patients with myelodysplastic syndrome and acute myeloid leukemia. The size of deletion varies among the patients with the most frequent breakpoints in 5q13 in proximal and 5q33 in distal region. The aim of this study was the mapping of small deleted segments of chromosome 5 in five patients with myeloid malignancies, suspected by classical cytogenetics (CC). Combination of FISH with DNA LSI probes for 5q31/q33 regions (Abbott Molecular, Kreatech Diagnostics), thirteen BAC probes (BlueGnome) for 5q15-5q34 regions, multicolor banding for chromosome 5 (MetaSystems) and aCGH (Cytochip Focus Haematology, BlueGnome) revealed the small deletion in three patients (range 12 Mb to 23 Mb, located between bands 5q31.2-5q34) and a larger deletion in size of 48 Mb (bands 5q15-5q32) in one patient. In patient No. 5 the karyotype 46,XY,del(5)(q31q33) was identified by CC, but the molecular cytogenetic techniques proved the large deletion in size of 70 Mb. This discrepancy was caused by the cryptic translocation - a part of the long arm of chromosome 11 was translocated on the deleted long arm of chromosome 5 (partial trisomy of 11q). Most of the genes localized near the breakpoints are involved in the cell cycle regulation and their role in the malignant transformation will be discussed.

Our study confirmed the relevance of molecular cytogenetic techniques in detection of small deletions that can be overlooked by classical cytogenetic analysis.

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J11.21

β-adrenoreceptor antagonists reduces cancer cell proliferation, invasion, and migration in *in vitro*

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In the present study we aimed to investigate efficacy of propranolol (non-selective antagonist), atenolol (β1-AR antagonist), and ICI118,551, (β2-AR antagonist) on cell proliferation, migration and invasion of non-stimulated breast (MCF7), colon (HT-29), and hepatocellular (HepG2) cancer cells. According to real time PCR, all cell lines expressed β-ARs. Inhibitory concentration 50 (IC_{50}) values from MTT assay revealed that the ICI118,551 was the most cytotoxic, whereas atenolol was the least effective β-blocker for

24, 48, and 72 h. Efficacy of propranolol and atenolol on MCF7 and HT-29 cells were time dependent. Boyden chamber assay demonstrated that cell invasion was inhibited by ICI118,551 (45%, 46%, and 50% for MCF7, HT29, and HepG2, respectively) and propranolol (72%, 65%, and 90% for MCF7, HT29, and HepG2, respectively) applications. Results of the in vitro scratch assay demonstrated that, propranolol and atenolol reduced migration of MCF7 and HT-29 in concentration dependent manner, wehereas inhibitory effect of ICI118,551 on cell migration was time dependent. ICI118,551 had concentration dependent inhibitroy effect on HepG2 migration, wheras effects of propranolol and atenolol were time dependent. β2-blocker seems to be the most cytotoxic β-blocker on non-stimulated cancer cells. Propranolol and ICI118,551 were more effective than atenolol in inhibiting invasion and migration of non-stimulated MCF7 and HT-29 cells; ICI118,551 being the most potent. Concordantly, β2-selective blockage seems to be more effective for non-stimulated cells. Furthermore, effect of the selective antagonsits may show variation depending on the concentration and time, as well as histological origin of cells.

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J11.22
mir-140 is a Candidate Regulator of IGFBP5 Expression in Breast Cancer

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IGF binding protein 5 (IGFBP5), an IGF system member, can show activity either IGF-dependent or independent, it also can show different effects on apoptosis, cell motility and survival in breast cancer. IGFBP5 inhibits cell growth in vivo and in vitro in human breast cancer. It is also associated with metastasis, poor prognosis and clinical outcome. The cellular localization of IGFBP5 affects its function in cell.
Experimentally shown that microRNA-140 (miR-140) targets IGFBP5. MiR-140 over expression leads to suppression of IGFBP5 expression and the suppression of miR-140 leads to over expression of IGFBP5.
In this study, tissue sections of the patients (n=29) with breast cancer and pheripheral normal tissue samples (n=11) analyzed by immunohistochemical assay to define IGFBP5 expression. IHC analysis also confirmed with mRNA RT-PCR. MiR-140 expression is later analyzed with Real-Time Polymerase Chain Reaction (RT-PCR). Statistical significance analyzed with Mann Whitney U test and Fisher's exact test.
We found that all IGFBP5 negative tumors express miR-140 (10/10) ; and IGFBP5 positive tumors are negative for miR-140 expression (11/19) (p=0.0035). miR-140 expression levels of IGFBP5 negative tumors is 82 fold higher than IGFBP5 positive tumors (p=0.0434). In terms of molecular subtypes of breast cancer, in 90% of the Luminal B type tumors, miRNA-140 expression was observed to be positive.
This is the first demonstration of the association of miRNA-140 with IGFBP5 expression in breast cancer tissue samples. Larger group of samples are needed to define the association of miR-140 expression levels with clinopahtological factors.

G. Güllü: None. **I. Peker:** None. **A. Haholu:** None. **C. Erzik:** None. **Z. Küçükodacı:** None. **H. Baloğlu:** None. **A.S. Özer:** None. **M. Akkiprik:** None.

J11.23
Cancer predispositions and intellectual disability: two sides of the same coin?

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The advent of array CGH raises the possibility for identification of cancer predispositions in patients with unrelated disorders such as intellectual disability or congenital anomalies. At our department two patients were analysed because of intellectual disability and birth defects. The first patient was diagnosed with an atypical 22q11 deletion, including the *SMARCB1* gene, associated with an increased risk for malignant rhabdoid tumors in children. The second patient was diagnosed with a proximal 1p36 deletion, including the *SDHB* gene, associated with a Hereditary Paraganglioma-Pheochromocytoma Syndrome. Both patients were asymptomatic for the cancer syndrome, but had an indication for surveillance now.
The third patient was analysed because of gastric cancer. Furthermore he had a (moderate) intellectual disability. Tumor analysis showed an MSI-High phenotype and loss of MSH2 and MSH6 protein staining. Additional analysis showed a 4.5 Mb deletion on chromosome 2, including the *MSH2*, *MSH6* and *NRXN1* gene. So Lynch syndrome was diagnosed in this patient. But, co-incidentally, the cause of intellectual disability was diagnosed as well. *NRXN1* deletions are associated with intellectual disability, developmental delay and psychiatric disorders.
We demonstrate that cancer predispositions can be unexpectedly diagnosed in patients with microdeletion syndromes. On the other hand, in our cancer patient the etiology of his mental retardation was found co-incidentally. Both scenario's require careful counselling of a patient and his family and may lead to additional surveillance or treatment. As unexpected findings may be considered as undesirable, we demonstrate that they can have important consequences for the patient and family members.
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J11.24
The Percentage of Informative STR Markers in the Turkish Population

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Multiplex fluorescent short tandem repeat (STR) analysis was used for assessing the the origin of cells after allogeneic haematopoietic stem cell transplantation. DNA was extracted from whole blood or whole bone marrow using the Rosche Blood Kit (Rosche,Germany) according to the instructions presented by the manufacturer. This column exchange. Spectrophotometry was used for DNA quantification. Polymorphic STR markers were obtained using the Promega PCR amplification kit. The following sixteen STR markers were amplified: D3S1358, FGA, D5S818, D7S820, D8S1179, vWA, D13S317, D18S51, D21S11 CSF1PO, THO1, TPOX, D2S1338, D16S539, D19S433 and Amelogenin. We followed up 61 patients within three years. We found informative markers for Turkish patients. All STR markers were informative over 50%. Eleven of STR markers were informative over 60%. Nine of STR markers were informative over 65%. Four of STR markers were informative over 70%. Two of them STR markers were informative over 75%. We showed all these table1. The most informatitive STR markers are D18S51 and D5S818 for Turkish population. Chimerism testing can be cost effective if kits could be designed according to ethnic origin.

	The percantage of informatitive STR markers at Turkish population				
STR Markers	**50%**	**60%**	**65%**	**70%**	**75%**
D8S1179	+	+	-	-	-
D21S11	+	+	+	-	-
D7S820	+	-	-	-	-
CSF1PO	+	+	+	-	-
D3S1358	+	+	+	-	-
THO1	+	+	+	+	-
D13S317	+	+	-	-	-
D16S539	+	+	+	+	-
D2S1338	+	+	+	-	-
D19S433	+	-	-	-	-
vWA	+	-	-	-	-
TPOX	+	-	-	-	-
D18S51	+	+	+	+	+
AMELOGENIN	+	-	-	-	-
D5S818	+	+	+	+	+
FGA	+	+	+	-	-
Total Number	**16**	**11**	**9**	**4**	**2**

A. Ozturk Kaymak: None. **C. Sonmez:** None. **S. Oztomurcuk:** None. **E. Ozcan:** None.

J11.25
The study of K562 cells genetic instability

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Resistance to tyrosine kinase inhibitor (TKI) is a major problem in the chronic myeloid leukemia (CML) therapy. There are BCR-ABL related and unrelated mechanisms of resistance which include BCR-ABL gene amplification, additional chromosomal abnormalities, duplication of Ph-chromosome, alternative signaling pathways genes mutations that underlie failure of therapy. Widespread use of K562 as a model to study efficiency and resistance to anticancer therapy has been determined the need for studying dynamics of genetic instability K562.
The aim of this continuing investigation is the study of mutagenesis endpoints in K562 cell line with exposure to TKI. To estimate K562 mutagen sensitivity the treatment with nonspecific prooxidant mutagen drug, dioxidine, will be used before and after selection of resistant cell clones.

At present we have began the first phase of this investigation to estimate the parameters of genetic stability before TKI treatment. We have assessed baseline and dioxidine (0.1mkg/ml) induced levels of DNA damage by alkaline comet assay and frequency of micronucleus by cytohalasin B micronucleus test. The levels of baseline DNA damage in K562 were 2.71% (%tail DNA), 0.60 a.u. (tail moment), 0.70 a.u. (olive tail moment), and after dioxidine exposition these parameters were 4.35%, 1.66 and 1.33 a.u., respectively. The frequency of baseline micronucleus in the mononuclear cells was 25‰, binuclear cells – 9‰, and after dioxidine treatment – 15‰ and 7‰, respectively.
Further research parameters of K562 cells genetic instability in different conditions will be important to identify mechanisms of CML progression and development of resistance to targeted therapy.

V.A. Nikitina: None. **A.I. Chausheva:** None. **L.D. Katosova:** None. **V.I. Platonova:** None. **S.I. Kutsev:** None.

J11.26
High Resolution Copy Number Variation Analysis using Droplet Digital PCR

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Copy number variation (CNV) is a prominent source of inter-individual variability in the human genome. CNV has also been associated with neurologic disease, autoimmunity, and adverse drug response, while specific gene amplifications in cancer cells drive tumor progression and influence treatment outcomes. Current methods to analyze CNV, including SNP-based microarrays, comparative genomic hybridization, and qPCR, lack the sensitivity and fine quantitative discrimination required for advanced CNV analysis. This is particularly true in heterogeneous samples and samples with higher order copy number states.
Droplet digital PCR (ddPCR) enables accurate and reproducible copy number determination using a simple, cost-effective workflow amenable to high throughput. Using single-well ddPCR, consecutive copy number states can be distinguished between samples of 5 and 6 copy number at 95% confidence levels. Here we present use of ddPCR to discriminate copy number status of multiple genes with low to high copy number states, including CYP2D6, MRGPRX1, and CCL3L1. We also apply ddPCR to the evaluation of challenging cancer sample types, including samples with higher order amplifications of genes such as MYC and FGFR2. The high resolution of ddPCR CNV analysis will be demonstrated using mosaic or admixed samples heterogeneous for copy number status.

J.R. Berman: A. Employment (full or part-time); Significant; Bio-Rad Digital Biology Center. **J. Regan:** A. Employment (full or part-time); Significant; Bio-Rad Digital Biology Center. **N. Heredia:** A. Employment (full or part-time); Significant; Bio-Rad Digital Biology Center. **L. Montesclaros:** A. Employment (full or part-time); Significant; Bio-Rad Digital Biology Center. **S. Hodges:** A. Employment (full or part-time); Significant; Bio-Rad Digital Biology Center. **D. Skvortstov:** A. Employment (full or part-time); Significant; Bio-Rad Digital Biology Center. **S. Tzonev:** A. Employment (full or part-time); Significant; Bio-Rad Digital Biology Center. **G. Karlin-Neumann:** A. Employment (full or part-time); Significant; Bio-Rad Digital Biology Center. **E. Hefner:** A. Employment (full or part-time); Significant; Bio-Rad Digital Biology Center.

J11.27
Next generation digital PCR technology: A simple, chip-based nanofluidic system for any benchtop.

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Digital PCR enables specific nucleic acid sequences to be accurately and precisely quantified without the need for a reference. This ability for absolute quantification is key to applications such as pathogen quantification and GMO detection. With the advent of novel nanofluidic PCR technologies, running the hundreds to thousands of reactions required for digital PCR has become feasible. To this end, Life Technologies has developed a new silicon chip-based digital PCR solution that can be rapidly loaded with little to no dead volume. At the heart of the nanofluidic system is a small chip that enables 20K reactions to be run on a single sample. Working with external collaborators, we will be demonstrating the performance and utility of the system across a wide variety of applications and sample types, such as the detection and enumeration of low frequency somatic mutations from various cancers.
"For Research Use Only. Not for use in diagnostic procedures."

P. Hegerich: None. **J. Wilde:** None.

J11.28
Description of PTEN gene mutation in Saudi women with endometrial cancer

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Phosphatase and tensin homolog (PTEN) gene is tumor suppressor gene is mutated in a large number of cancers including endometrial cancer. It is located on Chromosome 10q23.3 and encodes for lipid phosphatase that involved in regulates cell cycle and apoptosis. This study was carried out to investigate the role of PTEN in Endometrial cancer (EC) that is the most abundant female gynecologic malignancy. The median age of patients is 63 years.
DNA was isolated from paraffin embedded sections for 48 Saudi women with EC followed by PCR-SSCP analysis and direct DNA sequencing was performed for all exons.
Four novel mutations in PTEN gene were detected in exons 1, 2, 3, 4, 5a, 5b, 6, 7, 8a, and 9. The majority of them (70%) were missense (c.440del A) (c.440A>G) and frameshift mutations (c.440del AA) (c.440del AG). A deletion of codon 420 (c.420del A) were observed in (62.5%) while missense mutations (c.297-298del C) (c.297-298 C>G) and frameshift mutations (c.297-298del AC) present (60.4%) of the patients. More than half of screened DNA (54.1%) revealed a stop codon at 465 site (c.465del T) in the most exons.
These results may apply as a valuable marker to correlate the disease severity and alterations in PTEN gene. Further study from new EC patients at KAMC will add more into PTEN role in this devastating disease.

W. Al harbi: None. **M. Albalwi:** None. **F. Safi:** None. **A. Kushi:** None. **I. Alabdulkareem:** None.

J11.29
Gene expression of HIF1A, HIF2A and HIF3A in laryngeal carcinoma cells

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Hypoxia-inducible factors (HIFs) are heterodimeric proteins that mediate the adaptive response of cells to the reduced oxygen level, which is a substantial feature of tumours. There are three forms of HIFs that differ in their alfa-subunits. While HIF1A is ubiquitously expressed, HIF2A and HIF3A appear to have a cell-specific expression. However, there are no clear data about the expression of the three HIF-As in laryngeal carcinoma.
In the present study we analyzed the expression of HIF1A, HIF2A and HIF3A genes in 60 tumour and their matched normal laryngeal tissues. The expression was studied by real-time PCR and beta-actin was used as a housekeeping control gene.
HIF1A was overexpressed in 41 out of the 60 (68.3%) laryngeal tumours. In the case of HIF2A higher levels of mRNA were detected in 7 (11.7%) cancer tissues, while a decrease compared to normal tissues was found in 23 (38.3%) tumours. In 45 (75%) carcinoma tissues HIF3A was underexpressed; only 5 (8.3%) tumours showed increase in HIF3A levels. When the three forms were analyzed together, it was found that the most common combinations were: high HIF1A with low HIF3A (19 cases) and high HIF1A with low HIF2A and low HIF3A expression (12 cases).
Our results showed that in laryngeal carcinoma cells the expression varied among HIF-A genes. Comparison with clinical features of the patients might elucidate these variations and their importance for tumourigenesis.

T.E. Goranova: None. **G. Stancheva:** None. **A. Mitkova:** None. **V. Mitev:** None. **R. Kaneva:** None. **I. Chalakov:** None. **T. Popov:** None.

J11.30
Evaluation of KIT gene splice variants GNNK+ and GNNK- expression in gastrointestinal stromal tumors (GISTs).

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GISTs are the most frequent mesenchymal neoplasms arising in digestive tract. 60-89% of GISTs are caused by oncogenic mutations in *KIT* gene, 5% - by mutations in *PDGFRA*. The *KIT* pre-mRNA alternative splicing site is located at the 3' end of exon 9 and results in the expression of two isoforms GNNK- and GNNK+ with different biological activities. The GNNK- isoform has a greater oncogenic potential than GNNK+.

Analysis of mutations in 64 GIST samples was conducted by PCR following by sequencing. Isoform expression analysis was perfomed by RT-PCR. The assessment of isoforms expression was conducted by allele-specific PCR following by fragment analysis.
We observed mutations in 56/64 (88%) tumors (*KIT* exon 9 – 14%, exon 11 – 56%, exon 13 - 2%, exon 17 – 2% , *PDGFRA* exon 18 – 14 %).
We estimated the expression of both alleles (wild type and mutant allele) for each splicing isoform in 9 GIST samples with mutation in *KIT* exon 9 (p.A502_Y503dup). There are 4 possible combinations of *KIT* splice variants and its alleles: GNNK+ with or without duplication in 9 exon *KIT* and GNNK- with or without duplication. GNNK + expression without duplication notably higher than GNNK + with duplication. Expression of GNNK- isoform with duplication and without duplication is approximately equal. Expression of GNNK- with duplication higher than expression GNNK + with the same mutation.
Thus mutant allele is predominantly expressed in GNNK- form and wild type allele in GNNK+ form. The meaning of this finding demands further investigations.

A. Gaponova: None. **T. Kekeeva:** None. **L. Zavalishina:** None. **Y. Andreeva:** None. **G. Frank:** None.

J11.31
GSTM1 gene deletion as potential indicator of risk of childhood ALL development in Serbian population

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The human glutathione S-transferases (GSTs), possess both enzymatic and non-enzymatic functions and are involved in many important cellular processes, such as phase II biotransformation of chemicals, stress response, cell proliferation, apoptosis, drug resistance etc. The nonenzymatic functions of GSTs involve their interactions with cellular proteins, such as, JNK,TRAF, ASK, PKC, and TGM2. In humans, 4 major subfamilies of GSTs can be distinguished and are designated as GSTa, GSTμ, GSTu, and GSTp. Within the GSTμ subfamily, the gene coding for GSTM1 exhibits a deletion polymorphism, which in the case of homozygosity (GSTM1 null genotype) leads to the absence of enzymatic activity. The aim of the present study was to determine the frequency of GSTM1 deletions and a possible association of this polymorphism with increased risk for childhood acute lymphoblastic leukemia (ALL) in Serbian population. PCR analysis of the GSTM1 gene was performed on DNA obtained from 60 ALL patients and 182 healthy individuals. The following genotype frequencies were found: 57% for the GSTM1 null genotype and 43% for GSTM1+/+ and GSTM1+/- genotypes in the group of ALL patients. Genotypes in the control group were: 34% for the GSTM1 -/- genotype and 66 % for the GSTM1+/+ and GSTM1+/- genotypes. The difference between the frequency of the "null genotype" in the ALL group and in the control group was statistically significant. The null genotype was associated with a 2.5 fold increase of the risk (OR 2.53, 95% CI 1.395-4.591, p=0.002) of developing ALL in Serbian population.

R. Milicevic: None. **B. Popovic:** None. **L. Brankovic:** None. **H. Stamenkovic:** None. **D. Jugoviovic:** None. **D. Radulovic:** None. **J. Milasin:** None.

J11.32
Two novel germline mutations of *MLH1* and IHC result in two siblings of a Thai family with young-onset nonpolyposis colorectal cancer

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Background: Hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome I (OMIM#120435) is an autosomal dominant trait and caused by a germline mutation in mismatch repair (MMR) genes**.** HNPCC is caused by mutations in the *MSH2* and *MLH1* genes which may account for 60% and 30% of HNPCC cases, respectively.
Objective:
1. To study the incidence and correlate the genotype-phenotype relationship of germline mutation in *MLH1* and *MSH2*
2. To correlate IHC result and germline mutation in *MLH1* and *MSH2*
3. To encourage genetic testing for *MLH1* and *MSH2* in at-risk pre-symptomatic patients.
Methods: Direct whole gene sequencing in *MLH1* and *MSH2* was applied in all cases of age < 50 years, nonpolyposis CRC after confirmed by tissue pathological report. Their tissues were also tested IHC. Their relatives at-risk would also be encouraged to have genetic test and an appropriate cancer surveillance program.
Results: In a family which fit to AmsterdamII criteria, we found CC deletion at c.1404 and G deletion at c.2210+1 in *MLH1* of two siblings. IHC result also showed absent of MLH1 protein expression. These are novel changes causing frameshift mutation in both MMR genes without clinical difference.
Conclusion: HNPCC is a genetically heterogeneous disease**.** Our preliminary result revealed two new disease-causing mutation in *MLH1*. Germline analysis may help us screen in pre-symptomatic cases and offer an appropriate cancer screening program. This will help the susceptible patients to receive early diagnosis and treatment before developing an advanced CRC.

A. Tunteeratum: None. **C. Kunasol:** None. **N. Jinawath:** None. **A. Jinawath:** None. **J. Eu-ahsunthornwattana:** None. **M. Busabaratana:** None. **K. Srichan:** None. **T. Sura:** None.

J11.33
Screening of the most frequent mutation of IDH1 gene in Turkish Glioblastoma Multiforme patients

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Glioblastoma Multiforme (GBM) derived from glial cells is one of the most frequent and malignant brain tumors classified as grade IV by WHO (World Health Organization) accounts for 40% of CNS tumors. Mutations of IDH1, most commonly resulting in replacement of arginine at position 132 by histidine (p.R132H) have been described in WHO grade II, grade III diffuse glioma and secondary glioblastomas. In our study, exon 4 of IDH1 gene was sequenced by direct sequencing using DNAs obtained from paraffin embedded archival materials belongs to 54 patients diagnosed as GBM in Medical Pathology. The correlation between IDH1 mutations and age of patients was evaluated. As a result of this study, IDH1 c.395G>A (p.R132H) mutations were detected in 5 of 54 glioblastomas and the mutation frequency was found as 9.3%. The average age of the patients harboring the p.R132H mutation was calculated 46 while the average age of patients without this mutation was 56. There is no statistical significance between average age of the patients and IDH1 p.R132H mutation (Mann Whitney, p=0.078). As there is not any conducted study on genetic of the GBM in the Black Sea Region of the Turkey, this study is important and it is very unique to our region. In our ongoing study, the other exons of IDH1 gene are being sequenced.

C. Sümer: None. **H. Turgutalp:** None. **E. Kalay:** None. **B. Toraman:** None. **B. Yücel:** None. **F. Celep Eyüpoğlu:** None.

J11.34
A rare case of gastric juvenile polyposis

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Introduction: Juvenile polyposis (JP) is a form of gastrointestinal polyposis characterized by the development of multiple hamartomatous non neoplastic polyps and predisposition to gastrointestinal cancer at an early age. In most cases polyps are present in the colon and less frequently, in the stomach and small intestine. Most of the cases of juvenile polyposis have mutations in one of the the genes associated with the disease BMPR1A, SMAD4 and PTEN. A porportion of patients with JP (20-50%) shows autosomal dominant heredity. We present a case of a 27 years-old male with gastric polyposis (JPS) diagnosed by endoscopic examination with many inflammatory hyperplastic polyps. These multiple polyps located only in the stomach appeared in a period of time less than six months. There was not family history of polyposis.
Materials and Methods: We studied SMAD4 gene in genomic DNA from this patient by PCR and subsequent automatic sequencing with Big Dye Terminators.
Results: We have detect a pathogenic c.538C>T mutation in exon 4 of SMAD4 gene that generate a truncated protein p.Q180STOP. This mutation has been previously reported in a case of colon polyposis. Nevertheless, SMAD4 gene mutations are not associate to gastric polyposis. To the best of our knowledge this is the second reported case of SMAD4.

V. Rivero-Perdomo: None. **J. Pérez-García:** None. **Ó. Bengoechea-Miranda:** None. **J. Blázquez-Román:** None. **R. Vidal-Tocino:** None. **R. González-Sarmiento:** None.

J11.35

The role of determining the status of KRAS gene in colorectal cancer

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Introduction and aim: Colorectal cancer (CRC) is one of the most highly malignant tumors and is among the leading causes of death in developed countries. A very complex and longlasting process, closely related to interaction of external and genetic factors, contributes to development of this malignant disease. Activation of the KRAS oncogene is of importance in colorectal carcinogenesis.
Methods: Surgically resected colorectal cancer tissue, embedded in paraffin sections, was used for molecular analysis of the KRAS gene. DNA isolation is performed using cobas® DNA Sample Preparation Kit. The status of the KRAS gene is determined by real-time polymerase chain reaction method - Real Time PCR (cobas® 4800 System) with cobas® KRAS Mutation Test CE-IVD which is used for detection of somatic mutations of the KRAS gene.
Result: Out of the total of 40 analyzed colorectal cancer tissue samples, point mutation on codons 12, 13 or 61 was detected in 18 samples (45%). On codons 12 and 13 mutation was determined in 16 patients, and two patients had mutation on codon 61.
Conclusion: The presence of point mutations on codons 12, 13 and 61 of the KRAS gene in colorectal cancer is predictive of the lack of response to therapy with new drugs, and is determined in all patients eligible for immunotherapy. Personalized approach to CRC treatment helps avoid unnecessary side-effects and toxicity, and optimize costs in healthcare system. Combination of target therapy with predictive biomarkers improves efficacy in target patient group and the patients' benefit from applied drugs.

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J11.36

XRCC1 Arg194Trp and Arg399Gln polymorphisms and malignant lymphoma risk

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X-ray repair cross-complementing group 1 (XRCC1) is a key
component of the base excision repair system. Defects in this pathways are involved in cancer pathogenesis. Therefore, XRCC1 gene polymorphism may be involved in lymphoma susceptibility. Our study aimed to evaluate the relationship between XRCC1 Arg194Trp and Arg399Gln polymorphisms and malignant
lymphoma in a Romanian population.Material and method: One hundred and nine patients with malignant lymphoma and two hundred healthy individuals were enrolled in the study as controls. The XRCC1 polymorphisms were genotyped in all patients and
controls by PCR-RFLP assays.Results: We observed that XRCC1 194Trp variant allele was more frequent in the study group with malignant lymphoma compared to controls (p = 0.003, OR = 7.78 CI = 3.85 - 18.25). Our study revealed an increased risk for lymphoma, for homozygous (Trp/Trp) variant genotypes for XRCC1 Arg194Trp
polymorphisms (p = 0.001, OR =0.23, CI = 0.11 - 0.46). Our study did not reveal an increased frequency of the XRCC1 399Gln variant allele in the malignant lymphoma group compared to controls (p =
0.082), therefore this polymorphism could not be considered a risk factor for lymphoma. Conclusions: Our study suggests that the Arg194Trp polymorphisms of the XRCC1 gene may contribute to the risk of developing malignant lymphoma.

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J11.37

Novel germline mutation in *MSH6* gene in a large Spanish family with Lynch Syndrome

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Approximately 10% of Lynch Syndrome (LS) families have a germline mutation in *MSH6* gene. We present here a LS family with a new mutation in *MSH6* gene where affected family members showed both colonic and extracolonic tumors (colon, endometrial and gastric). Tumor analyses resulting on microsatellite instability and negative immunohistochemical stain (absence) are frequent features but not always present in all patients with *MHS6* germline mutations. We performed both tumor analyses in a patient who fulfilled Bethesda criteria for Lynch syndrome to find out if could exist some germline mutation in one of the disease-causing genes. Patient's tumor showed both microsatellite instability (IMS) and no immunohistochemical (IHC) staining for *MSH2* and *MSH6* and these findings led us to look for mutations in *MSH2* and *MSH6* genes. Genetic analyses were negative for *MSH2* gene, however one new protein-truncating mutation, c.1894_1912del19 leading to p.Lys632fsX641, was identified in *MSH6* gene in the probandus patient. Related family members both affected by Lynch Syndrome and nonsymtomatic patients were screened for the new identified mutation with the aim to asses in genetic counselling. This novel mutation in *MSH6*, the less frequent LS gene related, is being reported and submitted to the current databases to provide additional information to the genotype-phenotype in LS. Finally, our results emphasize the suitability of a combined IHC and IMS screening as a pre-selection tool previous to genetic analysis in LS.

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J11.38

Allele p.Leu1007fsinsC of NOD2 gene is not correlated with the differentiated thyroid cancer among Polish population.

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Many research in the field of oncology suggests that cancer arises from chronicle inflamed tissues. The NOD2 gene variants have been associated with susceptibility to inflammatory bowel diseases, but also was correlated with increased risk of some cancer types, in particullar p.Leu1007fsinsC variant was associated with colon cancer, lung cancer and ovarian cancer, early-onset laryngeal cancer and, breast cancer in the presence of DCIS.
Papillary and follicular thyroid carcinomas are the most frequent in endocrine system with still unidentified genetic background and 7 fold higher prevalence in women considering Polish polpulation. According to literature, one of the risk factor for thyroid cancer developing (particularly papillary carcinoma) is a history of thyroid inflammation.
The aim of the study was the analysis of 3020insC (rs5743293, p.Leu1007fsinsC) mutation among Polish patients with differentiated thyroid cancer.
DNA was extracted from whole blood leukocytes of 598 patients diagnosed with differentiated thyroid cancer (521 females and 77 males). The population group included 701 subjects (435 females and 266 males). To perform genotyping we used the pyrosequencing (PSQ96). The chi-square statistic was used to evaluate differences in genotypic and allelic frequency between patients and population group.
We didn't observed significant differences in allele frequencies in patient with differentiated thyroid cancer and population group (p=0,391). Occurrence of the insertion allele did not affect the age of onset and no major differences in the statistical analysis of the prevalence of this allele in men and women separately.
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J11.39

Testing experience of a single molecular diagnosis center in Turkey: the ALK rearrangement in non-small-cell lung carcinomas

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BACKGROUND: The Anaplastic lymphoma receptor tyrosine kinase (**ALK**)

gene rearrangements represent a recently identified molecular target in a subset of patients with non-small-cell lung cancer (NSCLC). EML4-ALK fusion oncogene has been reported in 2-13% of patients with **NSCLC**.
AIMS: The aims of this study are to study the ALK rearrangement in patients with non-small-cell lung carcinomas (NSCLCs) and find the frequency of this mutation in Turkish population.
MATERİAL AND METHODS: The study included samples from a large unselected 192 patients with a referred diagnosis of non-small cell lung cancer. Median age was 58,06±11,66 years (range 17-87), and sixty-seven percent were male. ALK fluorescence in-situ hybridization (FISH) was performed using the ALK break-apart probe set (Vysis). The cases with more than 15% break-apart signals in 50 evaluated cells were defined as ALK-positive.
RESULTS: We found twenty-two (10.5%) **ALK**-positive cases, and the median age was 62,05±11,36 years (range 34-75) in this group. Among 22 abnormal samples, fourteen were male and eight were female. The ALK positivity frequencies were 10,9% in male and 12,5% in female patients. The median ages in male and female ALK positive groups were 64,57±9,17 years (range 43-75) and 57,62±13,85 years (range 34-70) respectively.
CONCLUSIONS: The frequency of ALK gene rearrangement detected by FISH was found as 11,45% in a large unselected NSCLCs series in Turkey. Our result is concordant with other publications and being the first ALK prevalence report in Turkish NSCLC patients.

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J11.40
Association of polymorphic markers of xenobiotics biotransformation genes, tp53 and mdm2 genes with nonsmall cell lung cancer in russians of moscow region

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An association of *+/-* polymorphic markers of *GSTM1* and *GSTT1* genes, polymorphic markers *Ile105Val* of *GSTP1* gene, *Arg72Pro* of *TP53* gene and *T309G* of *MDM2* gene with risk of non small cell lung cancer has been studied in Russians of Moscow region. We found a significant association of null genotype of *GSTT1* gene with non small cell lung cancer, adenocarcinoma and squamous-cell carcinoma (OR=3.28, OR=5.43 and OR=2.9, respectively). We have also showed a significant association of *Val* allele of *GSTP1* gene with adenocarcinoma (OR=1.92). We also found a strong association of the null genotype of *GSTT1* gene with non small cell lung cancer in group of smoking patients (OR=5.13) and patients with 60 and older (OR=6.03). We found an association of *Pro/Pro* genotype of polymorphic marker *Arg72Pro* (*OR* = 5.46) and *TG* genotype of polymorphic marker *T309G* (*OR* = 5.57) with non small cell lung cancer development. We have also showed a strong association of both *Pro/Pro* and *TG* genotypes with development of adenocarcinoma (*OR* = 5.32 and *OR* = 8.13) and squamous-cell lung cancer (*OR* = 4.20 and *OR* = 8.13). We have finally found highly reliable association of combined susceptible genotype *Pro/Pro* + *TG* of *TP53* and *MDM2* genes with non small cell lung cancer and both its subtypes (*OR* = 7.90, *OR* = 9.12, *OR* = 8.59, respectively).So our results show an important role of studied genes in development non small cell lung cancer and its different subtypes.

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J11.41
Adult recurrent pilocytic astrocytoma: clinical, histological and molecular study.

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Pilocytic astrocytoma (PA) is a low grade glial tumor occuring frequently in paediatric population. This predonimantly cerebellar tumor was supposed to be curative after surgical resection. However, very few adult recurrent cases were previously reported. We report here a case of a 58-year-old man presented a right with a supratentorial tumor. Two years after a first resection a recurrence was discovered in the same area. Based on histopathological and neuroimaging findings the diagnosis of pilocytic astrocytoma was suspected. Molecular investigations using multiplex ligation probe amplification (MLPA) revealed KIAA1549-BRAF fusion gene in both the primary and the recurrent tumors. Additional genetic abnormalities, that may explain the malignant transformation and bad clinical outcome, were also detected. Based on imagining, histopathological and molecular findings, we consider that our patient showed a PA with malignant transformation rather than a de novo Glioma.

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J11.42
A Primary myelofibrosis case with a unique chromosomal translocations

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Primary myelofibrosis (PMF) is a myeloproliferative neoplasm and diagnosed based on bone marrow morphology, and cytogenetic pathogenesis and abnormalities are still not well studied. In this study, we identified cytogenetic abnormality that had not been reported in PMF. A 79 years old male with optic neuritis and posterior scleritis was admitted to the neurosurgery department in 21 Jun 2011 and then he transferred hematology department for evaluation of dizziness and anemia in 25 Jun 2011. The peripheral blood count revealed that WBC, hemoglobin and platelet count were 5.52 x 10^3/uL, 7.9 g/dL and 516 x 10^3/uL, respectively. BM analysis showed hypercellular marrow (more than 70% cellularity) with megakatyocytic hyperplasia and myelofibrosis. Mild splenomegaly and high LDH level (832 IU/L) was noted. *JAK2* mutation and *Bcr/abl* gene rearrangement were not detected. In conventional chromosome study, of 14 metaphases, 9 had a reciprocal 10; 13 translocation with break and fusion points at band 10q24 and 13q14. During treated with hydrea 500mg per 8 hour, he re-admitted in 27 Sep 2012 due to pneumonia and he still have been cared in ICUs. For evaluation of break point, FISH analysis was performed for the detection of translocations involving the locus at chromosome 10q24 and 13q14. And we got only deletion sign of *RB* probe [22/201] at 13q14, but could not obtained information of counterpart gene at 10q24.
So, we will further evaluate counterpart gene of *t*(10;13)(q24;q14) and it may be help to better understand the role of involved gene in PMF.

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J11.43
Analysis of c-erbB-2 and c-myc oncogene amplification in salivary gland tumours

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Genetic studies of salivary glands tumours were mainly focused on chromosomal changes and specific patterns of chromosome translocations have been described.
However, molecular changes in these tumours are less well characterized.
Alterations in gene copy number by amplification or deletion represent a common mechanism of gene expression deregulation and play an important role in the pathogenesis of many solid tumors.
We studied the putative role of c-erbB-2 and c-myc oncogene amplification in the development and progression of 53 cases of pleomorphic adenomas (PA) and 6 cases of carcinoma ex pleomorphic adenoma (CexPA).
The levels of gene amplification were analyzed by Real Time PCR comparative Ct method.
Amplification of c-erbB-2 was identified in 7 out of 53 (13%) cases of PA and in 2 out of 6 (33%) cases of CexPA. C-myc was amplified in 5 out of 53 (9%) PAs and in 2 out of 6 (33%) CexPAs. The amplification could not be correlated with clinical characteristics in the benign tumours group, whereas in malignant tumours c-erb amplification was related to recurrences.
In conclusion, c-erbB-2 and c-myc gene amplification might play a role in a subset of malignant salivary gland tumours (to be confirmed on a larger sample). Conversly, this alteration seems to be of minor importance for benign tumour pathogenesis, as judged from its low frequency in PA. However, a longer follow-up of the patients with
PA could uncover a potential role of c-erb and c-myc amplification as prognostic tools and predictors of adenoma transformation into carcinoma.

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J11.44

Comparative analysis of the telomere length in young women with breast cancer and controls in Castilla y Leon (Spain)

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Background: Telomeres are non-coding regions of DNA at the ends of chromosomes. In humans, they consist of tandem repeats of the sequence T2AG3 and their main function is to maintain genomic stability.
It has been described that there are not significant differences in telomere shortening in patients with breast carcinoma in relation to age, although there is an association with an earlier cancer appearance in following generations, suggesting a mechanism of genetic anticipation.
Patients and Methods: With prior informed consent, we obtained genomic DNA from leucocytes of peripheral blood of 60 women under forty years old with breast cancer without mutation in BRCA1/2 genes, and 32 blood samples of young women controls. The measurement of relative telomeric length was performed by relative comparative quantitative PCR, using as endogenous control the 36b4 gene. For the statistical analysis we used the programme GenEx, suitable for qPCR data analysis.
Results: The control group showed an average telomere length of: 3.36, while women with breast cancer revealed 3.80. These results showed highly significant differences when comparing both group´s relative telomere length, presenting a p-value: 0.01.
Conclusions: Our results show a telomere shortening in peripheral blood in young women with breast cancer with respect to controls. This could be used as a prognostic marker of earlier appearance of breast cancer in women, although the explanation of these results is difficult to determine due to the big variety of molecular, physiological and biological process which are affected in cancer development.

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J11.45

Are rare germline variant in the TP53 3'UTR causal determinants of Li-Fraumeni-like syndrome?

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The rs78378222 (A>C) single nucleotide polymorphism (SNP), a rare variant located in the 3'-untranslated region and within the polyadenylation signal of *TP53*, was recently identified in a GWAS of the Icelandic population. It occurs at a frequency = 0.0192 in that population and leads to impaired 3'-end processing of mRNA, conferring susceptibility to certain types of cancer. Considering that Southern Brazil has one of highest breast cancer (BC) incidence rates in Brazil (reaching 81.07 per 100,000 women in certain areas), the purpose of this study was to evaluate the association between this functional SNP and BC risk in a case-control cohort from this geographic area. A total of 135 BC-affected women and 302 healthy controls were genotyped using a TaqMan assay method. No homozygous mutant (CC genotype) individuals were identified in this series, and although the frequency of heterozygous genotypes did not differ between groups (P = 0,65), all C allele carriers had a family history of BC and/or other tumors. In addition, one of the SNP-positive patients, a woman diagnosed with BC at age 44 years, had a cancer family history meeting Birch criteria for LFL. No coding germline *TP53* mutations were identified in this patient. This is the first description of rs78378222[C] in an LFL family. Although previous studies reported no effect of this variant on BC risk in different European populations, further studies should be undertaken in families with the LFS/LFL phenotype to investigate the role of rs78378222[C] as a gene variant associated with LFL syndrome.

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J11.46

Effects of Sulfite molecule on URG4/URGCP, Cyclin D1, and Bcl-2gene expressions in SH-SY5Y Human Neuroblastoma Cells

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Sulfite is a highly reactive molecule which is commonly used in foods, cosmetics and pharmaceuticals. Although, many studies have shown that sulfite may cause toxicity both in vivo and in vitro, there is no consensus on the mechanism by which sulfite toxicity affects cells. Up-regulated gene 4/Upregulator of cell proliferation (URG4/URGCP) is a novel gene located on 7p13. URG4/URGCP stimulates cyclin D1 (CCND1) mRNA expression, and RNAi-mediated URG4/URGCP silencing diminishes CCND1 mRNA expression in HepG2 cells. In this study, the effects of sulfite treatment on URG4/URGCP, CCND1, and Bcl-2 gene expression changes on control and dose group were analyzed. SHSY5Y cells were cultured in the appropriate conditions. Cytotoxic effects of sulfite in SHSY5Y cells were detected in time and dose dependent manner with the IC50 doses within the range of 0.5-10 mM by using XTT. Genotoxic effect of sulfite was shown by commet assay. IC50 doses in the SHSY5Y cells were detected as 5mM. Total RNA was isolated with Tri-Reagent. Expression profiles of the target genes were determined by semi quantitative RT-PCR. An URG4/URGCP protein change was determined by western blot analysis. According to results, URG4/URGCP, CCND1, and Bcl-2 gene expression levels were decreased in dose group cells compared to the control cells. Our preliminary results are suggesting that sulfite treatment inhibits the proliferation of SHSY5Y cells as chemotherapeutic agents. In according to our findings, the mechanism of this result may berelated with sulfite dependent inhibition of cell cycle at the G1 phase by downregulating URG4/URGCP or CCND1 gene expression.

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J11.47

Chromosome Imbalances and Alterations in p53 Gene in Uterine Myomas from the Same Family Members: Familial Leiomyomatosis

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Uterine leiomyomas (UL) are extremely common neoplasms in women of reproductive age, and are associated with a variety of characteristic chromosomal aberrations (CAs). The p53 gene has been reported to play a crucial role in suppressing the growth of a variety of cancer cells. Therefore, the present study investigated the effects of CAs and p53 gene on ULs. We performed cytogenetic analysis by G-banding in 10 cases undergoing myomectomy or hysterectomy. Fluorescence in situ hybridization (FISH) with p53 gene probe was also used on interphase nuclei to screen the deletions in p53 genes. In patients, CAs were found in 23.4% of 500 cells analysed. This proportion was significantly different between patients and the control group ($p<0.001$). In the patients, 76% of the abnormalities were structural aberrations (deletions, translocations and breaks), and only 24% were numerical. Deletions were the most common structural aberration observed in CAs. Among these CAs, specific changes in five loci 1q11, 1q42, 2p23, 5q31 and Xp22 have been found in our patients and these changes were not reported previously in UL. The chromosome breaks were more frequent in cases, from high to low, 1, 2, 6, 9, 3, 5, 10 and 12. Chromosome 22, X, 3, 17 and 18 aneuploidies were observed to be the most frequent among all numerical aberrations. We observed a low frequency of p53 losses (2-11%) in our cases. The increased incidence of autosomal deletions, translocations, chromatid breaks and aneuploidy, can contribute to the progression of the disease along with other chromosomal alterations.

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J11.48

V617F mutation of Jak2 gene in patients with myeloproliferatif diseases except CML in Morocco

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The identification in 2005 of the V617F mutation of the Janus kinase 2 gene (JAK2) has been a promising discovery that changed the way myeloproliferatif disorders (MPDs).The objective of our work is to determine the prevalence of the V617F mutation in Moroccans patients with SMPs. we studied 420 individuals referred by different departments of Hematology in Morocco. The V617F mutation was investigated by PCR Allele-specific.
Our results showed that the average age of patients was 46.5 years with a male predominance, and 54.5% of patients had the mutation.The incidence of the V617F mutation in Polycythemia Vera, essential thrombocythemia and idiopathic myélofibrosis are respectively 79%, 37% and 26%. We also detected the mutation in 25% of cases of essential eosinophilia and in 15 cases of unclassified SMPs, but it was absent in patients with secondary polycythemia vera or thrombocytosis. We also found that patients with this mutation have higher levels of hemoglobin and hematocrit compared with patients without this mutation.
For patients without V617F mutation (45.5%), the other mutations (MPL W515L and W515K) are studied by the HRM and sequencing.
Our study is the first to assess the status of the V617F mutation of the JAK2 gene in patients with SMPs in Morocco. Our data seem to confirm that the JAK2 V617F mutation is specific for myeloproliferatif disorders, it help to confirm the diagnosis and guidance of therapeutic choice to improve the prognosis of MPDs in Morocco.

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J11.49

The effect of silencing siRNA against b-catenin on drug sensitivity of SW480 as a model of colon cancer cells

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Colon cancers are reportedly resistant to chemotherapy. In more than 90% of cases of colorectal cancers, constitutive activation of the canonical Wnt signaling pathway is an early event in tumorigenesis. Activation of this pathway is through stabilization of β-catenin and its translocation into the nucleus.
In this study, two different shRNA plasmid vectors against β-catenin, were constructed. After cloning into pSilencer neo2.1 and integration into genome, these plasmids could persistently generate shRNA against β-catenin. Cloned shRNAs were stably transfected into SW480 cells using effectene as the transfection reagent and treating the cells with G418 for 21 days. The down-regulations of β-catenin in these two clones of cells were approved by real-time PCR.
The LC50 of 5-FU in non-transfected SW480 cells was 30 mM while the LC50s of two stably transfected cells namely SW2024 and SW978 were 42.5 micromole and 22.5 micromole respectively.
Following treatment of the cells with lethal dose of 5-FU, the percentages of apoptotic cells were 4.45, 4.66 and 31.46 in SW480, SW2024 and SW978 respectively.
Conclusively silencing β-catenin in SW480 cells render SW978 more sensitive to 5-FU induced apoptosis while in SW2024 the results were vice versa. However the LC50 of SW480 and SW978 were not different so much, the LC50 of SW2024 were significantly higher than SW480. This result in SW2024 and SW978 showed that silencing different parts of mRNA would affect the efficiency of silencing differently. This effect may be due to different effect of silencing β-catenin on proliferation and apoptosis in the cells.

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J11.50

Frequency of 5382insC mutation of BRCA1 gene among prostate cancer patients: an experience from Bashkortostan Republic of Russia.

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A family history of prostate cancer (PC) is a strong risk factor for the disease, indicating that inherited factors are important in this disease. BRCA1 and BRCA2 gene mutations cause hereditary breast and ovarian cancer syndrome and it is known BRCA mutation carriers to be predisposed to the high risk of breast, prostate, stomach cancer compared to the general population. The 5382insC mutation is the most common of BRCA1 mutations.
The study included 91 unrelated males with histologically confirmed diagnosis of PC. The mean age of PC patients was 62,1 ± 12 years. Material for the study and the results of pathologic classification of tumors were provided by urologists from Bashkir State Medical University. The control group consisted of healthy men with no signs of PC and normal PSA level. Mean age of the control group was 65,7 ± 4 years. Genomic DNA was extracted from peripheral blood lymphocytes using standard phenol-chloroform extraction. The 5382insC mutation in the 20th exon of BRCA1 gene was identified using direct sequencing. Mutation 5382insC was found in 2 cases of Russian ethnic origin (2,1%) and in 1 individual of Russian ethnic origin from the control group (1%). These data suggest that the 5382insC mutation is rare and unlikely to be pathogenic for prostate cancer in the Republic of Bashkortostan, but further investigations based on large DNA samples are necessary to confirm the results.

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J11.51

DNA repair gene and life style factors determines as biomarkers for breast cancer

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MGMT gene is the only known critical gene involved in cellular defense against alkylating agents in DNA DRR pathway. The DRR pathway in breast cancer risk and potential interaction with cigarette smoking and dietary antioxidants is genotyped [C250T (Leu84Phe), A427G (Ile143Val) and A533G (Lys178Arg)] for these variants from CONACYT project blood samples of Jalisco, Mexico, included 267 cases and 673 controls. Heavy smoking (431 pack-year) significantly increased breast cancer risk for women with codon 84 variant T-allele [odds ratio, OR ¼ 3.0, 95% confidence interval (95% CI) ¼ 1.4-6.2]. Inverse association between fruits and vegetables consumption and breast cancer risk was observed among women with wild-type genotype for codon 84 (OR ¼ 0.8, 95% CI ¼ 0.6-0.9 for _35 servings of fruits and vegetables per week and CC genotype versus those with 535 servings per week and CC genotype). The association between fruits and vegetables consumption and reduced breast cancer risk was apparent among women with at least one variant allele for codon 143 (OR ¼ 0.6, 95% CI ¼ 0.5-0.9 for _35 servings of fruits and vegetables per week and AG or GG genotype versus those with 535 servings per week and AA genotype). Similar patterns were observed for dietary a-carotene and supplemental b-carotene, but not for supplemental vitamins C and E. Polymorphisms in MGMT may modulate the inverse association previously observed between fruits and vegetables consumption, dietary antioxidants and breast cancer risk, and support the importance of fruits and vegetables on breast cancer risk reduction.

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J11.52

Familial case of Cowden syndrom and oncogenetic counselling

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A female 45-year-old patient referred to us for oncogenetic counselling after a chirurgical treatment of a renal carcinoma, with chromophobes cells began at 20-year-old for a goiter of her thyroid gland. At 43-year-old, she had a total colectomy for polyps of the colon, there were also a lipoma of the duodenum and polyps of the stomach. One of her sisters was a stillbirth and another had a cancer of the thyroid; her mother had a cancer of the brain. The clinical examination showed a tall woman with a macrocephaly, unrefined features, some oral papillomas of the tongue. She had dermal fibromas.

The clinical diagnosis of a Cowden syndrome was made and a genetic study of the PTEN gene was performed, which confirmed the diagnosis : she had a mutation of the exon 3 of the PTEN gene : c.209+1G>A. After this result, she was followed-up by a multidisciplinary staff and had a prophylactic hysterectomy with an endometrial polyp, a bilateral mastectomy revealing a bilateral and multiple intra-canalaire adenocarcinoma, and a negative bilateral annexectomy. Her son a 20-year-old was a tall boy, and was followed-up because he had the same mutation. We emphasize the interest to detect the mutation of PTEN gene, with an autosomal dominant transmission to adapt the prophylactic chirurgical treatment, permitting a longer survey for the index case ; and also the interest of the predictive test to follow up the son with the same mutation of PTEN.

R.H. Hanitra: None.

J11.53

Association between snp rs 4132601 in gene *IKZF1* region and childhood acute lymphoblastic leukemia

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Background. Acute lymphoblastic leukemia (ALL) is the most common cancer among children, with an annual incidence rate of approximately 3.9 per 100,000 children.According to literature single nucleotide polymorphism (SNP) rs4132601 in gene - *IKZF1,* which
is located on chromosome 7, in genome wide association studies analyzing cases and controls, statistically significantly increase risk of developing childhood ALL.
Aim. To detect frequency of SNP rs4132601 G allele, in case and control groups and to evaluate it as a possible risk allele.
Material and methods. We analyzed SNP rs4132601 in the 36 pre-B ALL cases, as a control group we used age and sex matched children without leukemia. DNA was extracted from whole blood and purified by standard phenol/chloroform extraction protocol. The presence of polymorphism was analyzed using PCR with subsequent restriction enzyme *MboI* digestion and detected in polyacrylamide gel.
Results. SNP rs4132601 G allele frequency in patients was 0,458. Five out of 36 patients were homozygous for risk allele. There were statistically significant difference between ALL group and control group (p=0.042, OR=2,538, CI 95% 1,004-6,527).
Conclusions. In this study, we find statistically significant evidence about rs4132601 G allele as a risk allele. In order to detect SNP rs4132601 association with ALL have to be done larger study.

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J11.54

Enzymatic activity of PON1 and GST among patients with prostate cancer

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Prostate cancer is the most common type of cancer in men and the second leading cause of cancer death in men after lung cancer. Although prostate cancer is relatively common, there are genetic and environmental risk factors that increase the probability of developing prostate cancer. One of the factors involved in the development and aggressiveness of many cancers is the production of reactive oxygen species (ROS). Higher amounts of ROS play a role in the aging process as well as in a number of human disease states, including cancer, ischemia, and failures in immunity and endocrine functions. As a safeguard against the accumulation of ROS, several nonenzymatic and enzymatic antioxidant activities exist. Therefore, when oxidative stress arises as a consequence of a pathologic event, a defense system promotes the regulation and expression of these enzymes.
It is well known, that antioxidant processes are related to the development of prostate cancer. That is why, the study is focused in the determination of antioxidant activity by the analysis of paraoxonase 1 (PON 1) and glutation-S-transferase (GST) activity. Furthermore, genetic polymorphisms in these both enzymes will be determined in patients with prostate cancer. The enzymatic and genetic data obtained will be combined with clinical characteristics of the tumor and lifestyle of the patients, smoking habits, dietary style, exercise, etc; in order to adjust the dietary style of the patient improving their quality of life, as well as, the establishment of high-risk polymorphisms in these genes with prostate cancer aggressiveness.

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J11.55

Association of IGFBP5 and ERalpha with mir193b expression levels in breast cancer

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miRNA's are small, single stranded and non-coding RNAs that can be down or up regulated depending on the cell and cancer type. The role of miRNA's in cancer, became a new hope in cancer research.
IGF's plays an important role in tumor-genesis as well as cell growth, proliferation and apoptosis control. IGFBP5 inhibits cell growth in vivo and in vitro in human breast cancer, it is also associated with metastasis, bad prognosis and endocrine treatment.
miR-193b down-regulates in breast cancer patients and this results in tumor migration and invasion. miR-193b directly targets ERα at 3-UTR site and inhibits estrogen induced proliferation.
In this study, FFPE tissue sections of the patients with breast cancer analyzed by immunohistochemistry and confirmed with mRNA RT-PCR, to define IGFBP5 expressions. miR- 193b expression level is later analyzed with RT-PCR and the correlation between the expression levels and IGFBP5, plus ERα is analyzed.
It has been shown that Erα is regulated by mir-193b in some studies, but some studies show no correlation. In our study, we used tissue samples of 48 women with breast cancer, but we couldn't find any evidence that supports such regulation. No significant relation is found between IGFBP5 expression and mir-193b expression. The group of people that has been studied in our study showed no correlation between mir-193b expression and breast cancer sub types. There is also no difference between mir-193b expression in tumor tissue and in peripheral tissue. These results indicate that mir-193b is not a good bio-marker for breast cancer.

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J11.56

Association of polymorphic markers of xenobiotic biotransformation genes, tp53 and mdm2 genes with breast cancer in russian women of moscow region

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Abstract
The association of *+/-* polymorphic markers of *GSTM1* and *GSTT1* genes, *Ile105Val* of *GSTP1* gene, *Arg72Pro* of *TP53* gene and *T309G* of *MDM2* gene with risk of breast cancer in females of Moscow region has been studied. We have showed an association of null genotypes of *GSTT1* and *GSTM1* genes and *Ile/Val* and *Val/Val* genotypes of *GSTP1* gene with BC (*OR* = 1.89, 1.65, 1.47 and 1,33, respectively). We found an association of *Pro/Pro* genotype of polymorphic marker *Arg72Pro* with higher risk of BC development (*OR* = 1.41) and with ductal and lobular breast cancer development (*OR* = 5.32 and *OR* = 8.13 respectively). We have also found the highly significant association of the null genotype of *GSTT1* gene and *Ile/Val* and *Val/Val* genotypes of *GSTP1* gene with BC for patients with disease manifestation before 53 years (*OR* = 2.33, 2.07 and 2.31, respectively). We have found highly reliable association of combined susceptible genotypes: null genotypes of *GSTT1* and *GSTM1* genes (*OR* = 3.18) and null genotypes of *GSTT1* and *GSTM1* genes and *Val/Val* genotype of *GSTP1* gene (*OR* = 16.42) with BC. In case of combined susceptible genotype *Pro/Pro* + *TG* of *TP53* and *MDM2* genes we have found highly reliable association with BC and its most frequent subtype - ductal breast carcinoma (*OR* = 10.8 and *OR* = 12.8, respectively). The results of our study are evidence that studied genes play an important role in the development of breast cancer in Russian women living in Moscow region.

A.M. Burdennyy: None. **V. Loginov:** None.

J11.57

Vitis vinifera seed extract modulates temozolomide sensitivity in human glioblastoma cells via apoptosis related miRNA alterations

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One of the most attractive cancer therapy methods to date is the induction of tumor cell death by certain phytochemicals. We investigated the active component of *Vitis vinifera* (grape) seed extract (GSE) and its effect on cell death and regulation of associated miRNA expressions in Glioblastoma multiforme (GBM) which is the most lethal form of brain tumors.
The active component of GSE was identified by HPLC/DAD. The anti-proliferative activity of GSE was tested in T98G, U-138MG and U-87MG cells alone and in combination with TMZ, using WST-1 assays. The effect of GSE on cell death was analysed using FITC Annexin V and miRNA PCR array analyses in T98G cells. Potential mRNA targets were analyzed bioinformatically.
The components of GSE were Catechin derivatives, Quercetin, Gallic acid and Chlorogenic acid. One The results of Way Anova and Tukey analysis showed that, the combination of 500ug/ml GSE and 325uM TMZ significantly decreased cell proliferation ($p<0.001$) in all cell lines. According to findings of FITC Annexin V assay, 70% of cells were killed by apoptosis and necrosis. SABioscience PCR Array Data Analysis demonstrated that, miR-9, miR-10a, miR-10b and miR-455-3p were significantly inhibited ($p<0.001$). Also, miRWalk database support our findings, the target genes for these miRNAs have been defined to be related to apoptosis, the cell cycle and VEGF signaling pathways.
Even further studies and validations are required, using the combination of GSE and TMZ may provide a novel approach to treat GBM through miRNA regulation.

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J11.58

HER2 polymorphisms and colon cancer in Croatian population

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Colon cancer is the third most common cancer worldwide and the second leading cause of cancer death. HER2 gene, a member of the EGF receptor family, is located at chromosome 17q21 and encodes a transmembrane protein (p185) with tyrosine kinase activity. HER2 has been associated with colon cancer development and progression. A large number of HER2 polymorphisms have been described. Any functional polymorphism can potentially affect colon cancer risk as well as cancer outcome. The aim of our study was to analyze three SNP polymorphisms in the HER2 gene, -344 C/T, +1963 A/G (I655V) and +3508 C/G (P1170A) in the Croatian population and in patients with colon cancer. DNAs obtained from 300 colon cancer patients and 300 unrelated healthy volunteers were genotyped for the HER2 SNPs using real-time PCR TaqMan® SNP genotyping assays. Allele G of I655V and allele G of P1170A polymorphism were more common in the population with colon cancer in the comparison to healthy volunteers and were associated with an increased risk of colon cancer. The role of HER2 polymorphism with colon cancer progression and prognosis has to be investigated in the future studies.

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J11.59

What is the actual impact of fluorescence in situ hybridization (FISH) in the diagnosis of myelodysplastic syndromes (MDS)?

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Fluorescence in situ hybridization (FISH) is increasingly

being used in the cytogenetic diagnosis of myelodysplastic syndromes (MDS). Clinical MDS diagnosis is complicated. Despite the challenges related to cytogenetic analyses in MDS diagnosis, there are still some standard requirements and integrated algorithms that must be added. Due to this, in this study we would like to evaluate the FISH analysis and their outcomes of 98 MDS patients who were referred over a one year period. Conventional karyotype analysis was performed in all patients. The results showed that 13 patients (13.3%) from conventional karyotype analysis and

17 patients (17.3%) from FISH analysis presented with clonal chromosomal aberrations. Surprisingly, none of chromosomal aberrations detected via cytogenetic analysis and FISH analyses

were compatible due to the limited number of FISH analysis requests. From the 18 requests of our study group, isolated 5/5q deletion presented as the most common (43.9%). 5q deletion and trisomy 8, seen in equal rates, were the most frequent anomalies (63.2%) in the study. It was seen that with 5 FISH

analysis (5q Del, 7q Del, trisomy 8, 13q Del, TP 53 Del), we could cover all of the chromosomal anomalies that were detected in all patients in this study. It is important to determine the chromosomal rearrangements using both cytogenetic and FISH analysis in MDS patients. A more reliable approach would be to standardize

wider FISH panels in order to detect more anomalies, synchronously, in this patient group.

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J11.60

The *PALB2* germline genetic variants in pediatric patients with classic type of *medulloblastoma*

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Medulloblastoma (MB) is the most common malignant brain tumor in the children which the etiology remains unknown. Recently, multiple molecular dysfunctions were detected in patients with MB. The majority of them were somatic changes. Our previous studies indicate that germline mutations in DNA repair genes (e.g. *NBN*) seem to be particularly important in the development of MB. The next of candidate is the *PALB2* gene, a partner and localizer of *BRCA2*, therefore the essential component of DNA repair system.
The aim of our study was to identify germline genetic variants of the *PALB2* gene in patients with classical type of MB. In first stage of analysis direct sequencing of coding sequence and flanking regions of *PALB2* in 50 MB patients was performed. We identified three nonsynonymous changes: rs152451 (Q559R), rs45624036 (V932M) and rs45478192 (L939W). To evaluate their frequency a total of 100 patients and 100 control samples were screened. The control group consisted of DNA samples from healthy persons with negative cancer family history and age and sex matched to the group of patients. Our results indicate that only carriers of the rs45478192 may exhibit increased susceptibility to developing MB (OR 3.00, p-value=0.5) but account for only a small proportion of cases. All results did ***not*** have ***enough statistical power*** therefore they need to be validated on a larger group of patients, what will be implemented in the next stage of the project.
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J11.61

The RET protooncogene mutations in Russian patients with multiple endocrine neoplasia type 2

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Multiple endocrine neoplasia type 2 (MEN 2) is a very rare genetic disease characterized by medullary thyroid carcinoma (MTC) associated with other

endocrine neoplasia (MEN 2A and MEN 2B) or not (familial MTC) due to germline *RET* gene mutations. We started MEN 2 genetic study 15 years ago. DNA was isolated from peripheral blood DNA. Exons 10, 11, and 13-16 of the *RET* protooncogene were amplified by polymerase chain reaction (PCR) and examined by direct sequencing of PCR products and/or restriction enzyme analysis. So we analysed 36 unrelated families (36 probands and 29 at risk relatives). A total of 15 different missense *RET* mutations were identified. A single mutation at codon 918 was found in all MEN 2B families (n=11). All of the MEN 2A mutations affected codon 634. Mutations outside of codon 634 occurred only in familial MTC. p.M918T was the most common mutation in our cohort followed by mutations p.C634R (n=6), p.C634Y (n=3), p.V804M (n=3), p.C634F (n=2), p.C620R (n=2), p.C630R (n=1), p.C634W (n=1), p.C634G (n=1), p.S649L (n=1), p.L790F (n=1), p.Y791F (n=1), p.R833C (n=1), p. M918V (n=1), and novel p.Y791F/p.I852M double germline mutation (n=1). Codon 634 at exon 11 was the most frequently altered codon and consequently, exon 11 was the most frequently altered exon. High prevalence of p.M918T in Russia with respect to other European countries is possibly explained by the fact, that Russian doctors conduct clinical diagnostics best of all for patients with the more severe MEN 2B syndrome.

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J11.62

Early detection of SOX2 and hTERC gene amplification of oropharyngeal squamous cell carcinomas by FISH on brush biopsy samples.

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Oral and oropharyngeal squamous cell carcinomas (OSCC) are among the most common cancers. The poor survival rate among oral cancer patients can be atributed to several factors, one of them being lack of early detection. A key approach to this problem would be to detect potentialy malignant lesion at their early stage. The only established method for their diagnosis is biopsy, which is carried out only when the lesions become symptomatic. Exfoliative cytology is an easy, non-invasive procedure and hence could be carried out even on slightest suspicion regarding the nature of the lesion. Performing FISH technique on oral brush cytology slides can be an easy and rapid screening approach for the malignant cell detection.
Gain of 3q26 region is frequently observed in various squamose cell carcinomas of mucosal origin, where hTERC and SOX2 genes are located. TERC gene amplifications were described in developement of various SCC, the laryngs, cervics, esophagus and lungs. Our resultes, obtaind on cervical smears demonstrated that TERC gene amplifications correlates with high squamouse interepithelial lessions which lead to invasive cervical cancer. SOX2 gene amplifications were also described in esophageal and lung squamous cell carcinomas. Because in oral cancer 3q26 amplifications are also regulary observed, amplifications of SOX2 gene could be important diagnostic and prognostic marker.
The present study was designed to detect hTERC and SOX2 amplifications in OSSC exfoliative mucosal cells and evaluate whether those two gene amplifications might serve as a supportive biomarker in early detection and diagnosis of oral and oropharingial SCC.

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J11.63

P53 mutations in Turkish bladder cancer patients

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Bladder cancer is the 7th most common cancer in men and 17th in women in the world. Two distinct pathways have been described in bladder cancer carcinogenesis; superficial papillary carcinoma and non-papillary invasive carcinoma, which are represented by FGFR3 and p53 pathways, respectively. P53 gene is the preferred target for environmental carcinogens, therefore investigation of the p53 mutation profiles may provide clues about the etiology of the tumors. In this study we aimed to investigate the p53 mutations in Turkish bladder cancer patients. For this purpose, tumor and healthy tissues were collected from 79 bladder cancer patients with ages ranging from 54 to 81 years. Upon DNA isolation, all exons of the p53 gene were amplified by PCR and sequenced. In one patient, we have identified a C>T mutation in codon 190, which changes the amino acid Pro to Ser in the p53 protein. This mutation has been shown in colorectal cancer, but this is the first study which reports cd190 mutation in bladder cancer. In addition to that, 10 more mutations in cd 181 (C>T), cd213 (A>G), cd280 (G>C), cd 238 (G>A), cd249 (G>T), cd234 (A>G), cd245 (G>A) were identified. The mutated tissues were either T1G3 or T2G3. The patients were all men and smokers for at least 20 years, 1pack/day. Screening of p53 mutations in bladder cancer patients will help in exploring the p53 mutational profile, and accordingly, expected to help in the development of screening strategies for at-risk individuals.

N. Ersoy Tunali: None. **S. Sahoglu:** None.

J11.64

Optimization of RNA extraction from urine samples for use in non-invasive diagnostic procedures

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Conventional diagnostic procedures for prostate cancer are invasive and painful and in most of the cases unnecessary. Aversion of men towards this screening tool is generally the reason for late oncology diagnosis and unsuccesful treatment. Development of methods for low copy templates i.e. separation and characterisation of circulating tumor cells opened new oportunities for molecular detection of tumor markers in other body fluids i.e. urine. There are several national studies for validation of tumor specific molecular markers in urine, such as PSA/KLK3, PSGR / OR51E2, PCA3 and TMPRESS2/ERG, that proved a principle to be used as screening tool in order to make a targeted biopsy and avoid unnecessary painful procedures and acompanying costs. For these reasons, the isolation of mRNA and measuring the expression of these genes in urine as a noninvasive technique is aimed to assist patients in reducing stress, and doctors as a further indication for biopsy and cystoscope. Potential advantages of this method are availability and easy sampling and measuring the expression of more marker genes for this type of tumor. In this work we have optimized total RNA extraction from various individual urine samples and estimated the quality of RNA for gene expression analysis using RT PCR methods.

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J11.65

Impact of TPMT and MTHFR genotype on development thiopurine related toxicity in IBD and ALL patients

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Thiopurine drugs are mainly and effectively used in treatment of autoimmune diseases such as Crohn´s disease and Ulcerative colitis but also in treatment acute lymphoblastic leukemia. Individual genetic variations result in differences of activity specific enzymes involved in drug metabolic pathway. Polymorphisms in TPMT gene, which is key enzyme responsible for biotransformation of thiopurines, modify activity of this enzyme. Decrease activity is responsible for observed adverse thiopurine effects such as myelotoxicity and hepatotoxicity in some patients. The aim of our work was to monitor the prevalence of the most commonly appearing deficiency alleles of *TPMT* gene as well as other deficiency alleles possibly occurring in our population. For this purpose we used direct sequencing of whole gene but our results show that *TPMT**3A, *TPMT**3B and *TPMT**3C are most frequently occurring alleles preferably found in patients with undesirable toxic effects. We also genotyped polymorphisms in *MTHFR* gene (*MTHFR* 677C>T and *MTHFR* 1298 A>T) to study the association between MTHFR and/or TPMT polymorphisms and probability of toxicity development. We develop genotyping method for quick detection of these SNPs using snapshot method. Our results suggest that there is association between TPMT and MTHFR genes and thiopurine therapy side effects.

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J11.66

The clinical features of the type 2 diabetes in relation to genotype rs 7903146 polymorphism of TCF7L2 gene in Uzbek populations

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TCF7L2 gene (chr. 10q25.3) involved in the Wnt signaling pathway, encodes a T-cell transcription factor. This factor inhibits the synthesis of proglucagon in enteroendokrin cells and glucose-induced insulin secretion, and is involved in the control of proliferation intestinal epithelial cells, adipogenesis and regulates the maturation of pancreas b-cells. The disease association studies have shown that T allele of the rs7903146 SNP TCF7L2 gene is strongly associated with T2D in various populations.
We genotyped 108 patients with T2D for rs7903146 of TCF7L2 and carried out various clinical characteristics. All patients were not related, aged over 45 years, and had disease duration for 5,78 ± 0,75 years.
It was revealed significantly higher frequency of TT-genotype: the TT genotype was verified in 56 (51.8%) patients, the CT genotype in 42 (38,9%), CC genotype in 10 (9,6%). Age and duration of diabetes of the "TT" patients were higher than "CC" patients. At "TT" patients had determined higher incidence of hypertension (82,1 ± 7,2% (P> 0.05)). The duration of hypertension was also more prolonged in this group of patients.
In the study of the glycemic parameters revealed significantly high levels of fasting plasma glucose in "TT" patients compared with the CC genotype (P> 0.05). No significant difference of blood glucose 2 hours after a meal, between CC, CT and TT genotype groups was found.

Y.A. Malikova: None. **G.N. Rakhimova:** None. **Z.S. Akbarov:** None. **S.U. Turdikulova:** None.

J11.67

Cytogenetic studies in Acute Lymphocytic Leukemia (ALL): A study from Haryana (India)

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Cytogenetic studies on acute lymphocytic leukemia have led to the identification of recurrent abnormalities specific to ALL and have helped in understanding the mechanism of leukemia genesis. Cytogenetic abnormalities also provide a strong and independent prognostic factor for treatment outcome. As the cytogenetic abnormalities are poorly studied in population of Haryana (India), the present study was performed in patients of acute lymphocytic leukemia (ALL) from Department of Medicine and Department of Pediatrics, Pt. Sharma University of health sciences, Rohtak during 2008-12. Ninety four patients of acute lymphocytic leukemia were subjected to cytogenetic analysis by conventional method however a successful cytogenetic preparation could be obtained in 77(81.9%) patients due to non-availability of samples in remaining cases. There were 50 children (<15 years) and 27adults(>15 years). The most common cytogenetic abnormalities in patients of acute lymphocytic leukemia were high hyperdiploidy(>50 chromosomes), low hyperdiploidy(47-50 chromosomes), hypodiploidy(<46chromosomes) & t(9;22). Translocations t(4;11), t(1;19), t(8;14) were less common with a frequency of 3.8%,3.8%, & 2.6% respectively. The deletion of long arm of chromosome 6 was reported in only 2.6% patients. High hyperdiploidy & t(9;22) were more commonly observed in adults than children. Translocations t(4;11) & t(1;19) were found in children but not in adults. Similarly t(8;14) & deletion del6q were found only in adults. Remission duration and survival were longest in patients with normal karyotype and high hyperddiploidy, moderate with low hyperdiploidy, t(9;22),t(1;19), & deletion del 6q and shortest with hypodiploidy, t(4;11), & t(8;14).

A. Kumar: None.

J11.68

Frequency determination of MSI mononucleotide markers in sporadic colorectal cancer patients

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Background : Colorectal cancers (CRCs) tumors are diagnosed by microsatellite instability (MSI) due to accumulation of insertion/deletion mutations in tandem repeats of short DNA motifs (1 - 6bp) called microsatellites. Microsatellite instability (MSI) is not only a halkmark marker for screening of hereditary nonpolyposis colorectal cancer(HNPCC),but also a prognostic and predictive marker for sporadic colorectal cancer.Our objective was to determine and study the status of five mononucleotide microsatellite status among Iranian patients with sporadic colorectal cancer .
Material and Methods : In the current investigation 80 sporadic CRC patients were evaluated for MSI. The pentaplex panel consisting of 5 quasi mononucleotide microsatellite markers (NR-21,BAT-26,BAT-25,NR-27 and NR-24) was used .
Results: Our findings reveals that the NR-21 was the most frequent unnstable marker among the other markers (23%).69.6% of specimens had instability in sporadic colorectal cancer.Furthermore,the frequency of instability BAT-25 was determined in 20% sporadic CRC samples .Interestingly our results demonstrated that the frequency of instability NR-24 was 20% that was so close to istability of BAT-25 Marker. Moreover,percentage of NR-27 was 0% in sporadic CRC.Finally , we could find 6.6% instability for BAT-26 in sporadic cases.
Conclusion : It seems that among 5 mononucleotide markers NR-21 was the most useful marker for determining the status of sporadic colorectal cancer.Following NR-21,BAT-25 and NR-24 are the most effective markers. Therefore using a triple panel consisting 3 mentioned MSI markers shoud be more promising markers for identifying MSI status in patients with sporadic colorectal cancer.

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J11.69

ALL L2 with t(8;14)(q24;q32) associated with tetrasomy 1q and homozygous deletion of p16

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Acute lymphoblastic leukaemia(ALL) is the most common cancer of childhood. Recurrent chromosomal abnormalities revealed in approximately 80% of ALL and associated with distinct immunologic phenotypes of ALL and characteristic outcomes. Chromosome 1q duplications and trisomy 1q are recurrent changes mainly in B-lineage ALL and strongly associated with t(8;14)(q24;q32) which has been very occasionally reported in ALL L2. In about 15% of ALL cases have homozygous deletion of p16 gene which is associated with unfavorable clinical outcome. We present a case of ALL L2 with t(8;14)(q24;q32) associated with tetrasomy 1q and homozygous deletion of p16.
A 1-year-old female patient admitted to hospital because of weakness and skin rash. Complete blood count disclosed hemoglobin of 6.4 g/dL, white blood cell count of $21.7x10^3$/uL, and platelet count of $15x10^3$/uL. Bone marrow aspiration demonstrated ALL L2 and flow-cytometry analysis was consistent with CALLA+ Pre B cell ALL. Cytogenetic analysis of bone marrow cells revealed 47,XX,+i(1)(qter→q11::q11→qter),t(8;14)(q24;q32) which was confirmed with FISH analysis, in addition to homozygous deletion of p16. The patient treated with St Jude Total XIII ALL chemotherapy protocol. She is in good condition with remission of bone marrow and also t(8;14) found negative with FISH analysis at fourth mounth of therapy.
Altough 1q abnormalities may be found in the majority of hematological malignancies, 1q tetrasomy is very rare, with t(8;14) association, especially in ALL L2. We aimed to contribute to literature with report of this rare and interesting cases.

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J11.70

The study of the polymorphism Arg72Pro of TP53 suppressor oncogene in gastric cancer patients in Uzbekistan

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Gastric cancer is the second leading cause of cancer death in the world.
The main goal of our research is to investigate the correlations between polymorphic variant of TP53 gene (Arg72Pro - rs1042522) and susceptibility to gastric cancer in Uzbekistan population. For the study we performed genotyping group of 130 patients with gastric cancer and 234 healthy individuals by means of PCR and PCR-RLFP methods. We determined the frequency of the three genotypes of the p53 gene in the patients with stomach cancer and controls: genotypes *Pro/Pro* (391- bp), *Arg/Arg* (277- and 114- bp), *Pro/Arg* (391-, 277- and 114-bp).Comparative analysis of resulting genotypes sho-

wed a statistically significant association between gastric cancer and Pro/Pro genotype in the group of young men (12-33 year) (p=0.01 Pearson's $\chi 2$ and p=0.003 according to the Fisher exact test, OR ($CI_{95\%}$) - 6.10 (1.64 - 2.71)) , but there were no association between polymorphism rs1042522 and gastric cancer in the group of elder men and women(59 ± 12 year). This study suggests that the p53 codon 72 polymorphism may be associated with gastric cancer in population of Uzbekistan.

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J11.71

Karyotypic identification of abnormal clones preceding morphological changes of B cell lymphoma

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We report a case with clonal cytogenetic abnormalities precede bone marrow morphological changes without definite morphological features of B cell lymphoma.
A 50 years old male who presented with intermittent fever and general weakness admitted our hospital in Jan 2010. However, we could not detect abnormal findings of malignant on physical examination and imaging studies except hepato-splenomegaly.
For evaluation of primary or concomitant disease in bone marrow, we performed bone marrow(BM) examination. The peripheral blood count revealed that WBC, hemoglobin and platelet count were 6.8 x 103/uL, 11.4g/dL and 208 x 103/uL, respectively. BM aspiration and biopsy showed about 20-30% cellularity marrow without abnormal cell clusters and cytogenetic analysis revealed 46,XY,del(6)(q16),i(8)(q10)[8]/46,XY[12]. However, morphologic changes and cytogenetic feature were insufficient to make a diagnosis. After conservative cares, he discharged. We also could not find any specific changes from follow up imaging studies and BM analysis in Jun 2010. In Aug 2010,the third BM analysis and cytogenetic study were performed and focal aggregation of intermediate to large B-cells as a suspicious morphologic features of B cell lymphoma were noted with clonal cytogenetic abnormalities 46,XY,del(6)(q16),i(8)(q10)[7]/46,XY[5]. Therefore, it was investigated for primary lesion, which was not identified.
In this case, specific morphologic features were not detectable at initial BM analysis and we got only a clonal cytogenetic abnormalities. So, we believed continuous closed follow-up with BM analysis and cytogenetic studies will be helful in some cases that showed only clonal cytogenetic abnormalities without definite morphological abnormalities.

S. Cho: None.

J11.72

Characterization of BRCA1 mutations in Uzbekistan ovarian cancer patients

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Ovarian cancer affects approximately 1 out of 70 women during their lifetime and is regarded as the most lethal gynaecological malignancy. Women with a germline mutation in the tumor suppressor genes BRCA1 or BRCA2 have a high risk of developing both breast and ovarian cancer. To reduce increased risk of developing cancer or to increase the likelihood of early detection, carries of BRCA1 or BRCA2 mutations are offered surveillance programs or risk-reducing surgery. Estimates of occurrence of BRCA mutations in ovarian cancer cases vary from 3% to 35%. The presence of founder mutations in some countries orethnic communities simplifies the identification of those with inherited predisposition to ovarian cancer. This will strongly facilitate the clinical application of genetic testing for these populations.
This study aimed to inves[[Unsupported Character - Codename]]tigate the contribution of BRCA1 to ovarian cancer in Uzbekistan patients. A total of 44 patients with ovarian cancer were included in this study. By means of SYBR Green based real-time allele-specific PCR we have analyzed DNA samples from peripheral blood of ovarian cancer patients for the presence of two BRCA1 mutations: 5382insC and BRCA1 300T>G.2 samples (4,54 %) were found to be positive for the heterozygous 5382insC BRCA1 mutation. We failed to register BRCA1 300T>G mutation. Taking into account a high possibility of ovarian cancer development in carriers of BRCA1 mutation, our data suggest the reasonability of the inclusion of 5382insC mutations test in screening programs for ovarian cancer prevention in Uzbekistan.

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J11.73

APC gene large deletions in Familial Adenomatous Polyposis patients

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Background: Colorectal cancer is one of the most common cancers. Familial adenomatous polyposis (FAP) is one type of hereditary colon cancer with a large number of precancerous polyps .They initiate to grow in colon in age 7 to 35 years old in the patients. Large deletions are one of the important types of alteration in adenomatous polyposis coli (APC) gene which led to FAP. However, they cannot be identified entirely by traditional techniques. Among of all large deletions investigation methods, Multiplex ligation probe Amplification (MLPA) is the most effective technique. The aim of the current study was to standardize MLPA method in the screening of APC large deletions for the first time in Iranian patients with FAP.Materials and method: DNA was extracted from 34 FAP peripheral blood patients using saluting out method. All patients were screened for APC large deletions by MLPA and when the result was positive, the given region was investigated by PCR sequencing.Result: Two large deletions was detected in different sites of exon 15 in two patients. Sequence analysis did not show any point mutations in the probe binding sites.Conclusion: Rate of large fragment deletions in APC was 5.8% that is close to result of previous studies in other population like Polish, Czech, Taiwanese and Spanish. Nonetheless, it seems that the sites of alteration were different .Therefore, it would be more promising that consider of MLPA at the first step for screening the FAP patients in our population would be useful.

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J11.74

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Helicobacter pylori (H. pylori) is a gram-negative pathogenic bacterium that chronically infects the stomach of approximately 25-50% of the world's human population. It is associated with the development of chronic gastritis, peptic ulcer and even gastric cancer. There is increasing evidence that the genetic variability of *H. pylori* may have a clinical importance. Several genes have been identified that may play a role in the pathogenesis of *H. pylori*, such as *vacA* and *iceA*. In Uzbekistan there is not sufficient information regarding the pattern of *H. pylori* genotypes in patients.
This study aimed to inves[[Unsupported Character - Codename]]tigate the prevalence of the *vacA* and *iceA* genotypes of *H. pylori* from patients with upper gastrointestinal diseases and the relationship with clinical outcome in Uzbekistan. A total of 135 patients with gastric cancer, gastroduodenal ulcer, chronic gastritis and ulcerative colitis were enrolled in this study. They had undergone endoscopy and gastroduodenal biopsy specimens were obtained from each patient. DNA extraction and polymerase chain reaction were used to assess the polymorphisms of *vacA* and *iceA* genes.
The obtained data indicate that *VacA* s1, *VacA* m1, *IceA1* genotypes were more prevalent among patients with gastric cancer, *VacA* s1, *VacA* m1, *VacA* m2, *IceA1* genotypes were more prevalent in patients with gastritis and *VacA* s1, *VacA* m2, *IceA1* genotypes were most common among patients with gastroduodenal ulcer and ulcerative colitis. In addition, the prevalence of *VacA*s1/*VacA*m1 and *VacA*s1/*VacA*m2 genotype combinations were found to be significantly higher in patients with gastric cancer and gastroduodenal ulcer/ulcerative colitis respectively.

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J11.75

Very complex karyotype and poor prognosis in AML

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Balanced translocations or inversions and specific gene mutations of acute myeloid leukaemia (AML) were known as related with prognostic significance. And AML with complex karyotype is typical type of poor prognostic AML. We present clinical and cytogenetic data on 1 case of very complex karyotype. 61 year-old man visited out-patient clinic with mild fever. Complete blood count and peripheral blood smear were performed, and pancytopenia (white blood cell, 1.8 $x10^9$/L; hemoglobin, 7.4 g/dL; hematocrit, 21%; platelet, 45 x 10^9/L) and 37% blast cells and shift to left finding in neutrophil lineage were noted. Bone marrow study was requested to diagnose leukaemia. Leukaemic blasts were admixed with three lineage hematopoietic cells and were 36.6% of all nucleated cells (ANC) from bone marrow aspirate and biopsy. In immunophenotyping, 26.7% of CD14 expressing cells and 28.4% of CD34 positive cells with negative CD14 were reported. Multiplex reverse-transcriptase polymerase chain reaction, Hemavision-7 System (DNA Technology A/S, Aarhus, Denmark), for 28 genetic abnormalities screening panel of AML were shown all negative results. Karyotype was reported as 44~46,XY,del(5)(q22q35),add(6)(p21.3),-7,add(11)(p15),del(11)(q22),der(12;17)(q10;q10),ins(12;?)(q13;?),-14,-16,add(17)(p11.2),-19,+1~3mar[cp20]. Acute myelomonocytic leukaemia was diagnosed. Patient had treated 1 cycle chemotherapy with Idarubicin, Cytarabine, and Dexamethasone. Bone marrow study was performed again 11 days after chemotherapy, and poor response for chemotherapy was shown with 19.9% blasts of ANCs. Karyotype was 41~46,XY,-3,del(5)(q22q35),add(6)(p21.3),-7,add(7)(p13),+8,-9,add(10)(q11.2),add(11)(p15),del(11)(p22),der(12;17)(q10;q10),ins(12;?)(q13;?),-14,-16,add(17)(p11.2),del(18)(q21.1),-19,+2~3mar[cp16]/46,XY[4]. To evaluate influence of specific genetic abnormalities or complex karyotype itself about poor response to treatment and poor prognosis, plentiful data from many patients would be needed.

Y. Park: None. **H. Lim:** None. **H. Lee:** None.

J11.76

Important role of MMR genes in Lynch syndrome prognosis and medical follow-up

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Lynch syndrome is an autosomal dominant inheritance and the most hereditary form of colorectal cancer. The identification of the molecular genetic basis of Lynch syndrome enabled the implementation of predictive testing in families with a proven mutation. A prerequisite to detect patients with Lynch syndrome is the knowledge of the clinical and histopathological features of this disease. The molecular genetics underlying mechanism is a mutation in one of the mismatch-repair genes (most commonly MLH1, MSH2, and MSH6), that has added significantly important information to the recognition of this disease and the search for high-risk individuals as well as offering them a genetic counseling. We have performed an analysis in 71 patients with Lynch syndrome; some of them are cluster in a high number of family members. The main genetic analysis has been developed in MMR genes (MLH1, MSH2 and MSH6 genes) which allows us to discover some undescribed mutations, as well as, the importance of these mutations for categorize the aggressiveness of the cancer.
In this case, we focus in the importance of a previous undescribed mutation and it aggressiveness in a family with members affected and unaffected by this syndrome. After this genetic analysis unaffected members of the family are classified as high-risk and they will follow frequent clinical examination for CRC. In our patients, a total of ten members of the same family, some of them affected by Lynch syndrome but all have the presence of 117-1 G>K polymorphism.

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J11.77

The C515S (TGC/AGC) in a case of MEN2A

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Introduction: The multiple endocrine neoplasia MEN2A is a syndrome that presents a medullary thyroid carcinoma, with pheochromocytoma and hyperparathyroidism, is a part of MEN2, with MEN2B and MTC familial. The MEN2 are rare hereditary diseases, transmitted as an autosomal dominant form and associated with mutations of RET proto-oncogene. The genotypic diagnosis of MEN2is based on the identification of mutation in RET proto-oncogene.
Materials and Methods: Our study included 01 patient with MEN2A and two clinically heathy relatives, the son of 19 years and one brother of 45 years. Genomic DNA was extracted from peripheral blood leukocytes, and the 7 exons of the RET gene (8, 10, 11, 13, 14, 15, 16) were amplified by polymerase chain reaction (PCR) and examined by DNA sequence analysis to detect mutations. The pentagastrin test is realized for the patients and relatives and the dosage of calcitonine is effect by the IRMA-hCT of CIS-Bio-international.
Results: The genetic analysis of the 7 exons of RET gene has not permit to identify a germline mutation of RET in the index case, but has found a variation of sequence is C515S (TGC/AGC), located in exon 8, in a homozygous form. This variation was found in homozygote form in the brother and in heterozygote form in the son. The dosages of basal calcitonin and after pentagastrin test were normal.
Conclusion: After these results, we estimate that, this C515S variation of DNA sequence is a rare variant non pathogenic and cannot be considered as a mutation causing the MEN2.

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J11.78

Bone marraw mesenchymal stromal cells in myelodysplastic syndrome and acute myeloid leukemia patients contain genome instability

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Genetic association between hematopoietic cell (HC) and mesenchymal stromal cell (MSC) in hematopoietic malignancies is a matter of controversy. This study compared genetic aberrations in HCs and MSCs of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) patients to clarify this issue. A total of 80 patients (22 MDS, 58 AML) were enrolled. Chromosomal aberrations of HC were detected in 13 MDS (57%) patients and 34 AML (59%) patients. Cytogenetic abnormalities in at least one metaphase were detected in 15 samples among 72 (21%) cases with MDS and AML whereas MSCs from normal individuals revealed normal karyotype. *LINE1* global methylation level was measured by pyrosequencing. *LINE1* hypomethylation was observed in MSCs from hematologic malignancies compared with MSCs from normal control. Chromosomal microarray of HCs and MSCs was performed using CGX cytogenetic array 12x175K (Roche NimbleGen, WI, USA) in 9 patients. Four HCs and three MSCs revealed genomic abnormalities, which were comparable and/or not comparable to chromosomal aberrations. The copy number changes in MSCs were observed in chromosome 2q, 5, 13q and 17q. Our study demonstrated that MSCs from MDS and AML patients contained genetic instability, which was evidenced by cytogenetic abnormalities and copy number changes in submicroscopic level. The abnormalities were different from those in HCs. In addition, differences in global methylation level may constitute a particular pathogenesis of MDS and AML pathogenesis in epigenetic level.

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J11.79

Cytogenetic and moleculer cytogenetic analysis of a multiple myeloma case with complex karyotype

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Multiple Myeloma (MM) is characterized by accumulation of malignant plasma cells with bone marrow. Clinically patients with MM usually present bone pain related to lytic bone lesions, frequent anemia and, less often, renal impairment.
Several recurrent cytogenetic abnormalities have been reported in the literature. Informative karyotypes are usually highly complex, with two main subgroups: first subgroup characterized by hyperdiploidy and second subgroup characterized peudo, hypo- or tetraploidy with the accumulation of many structural changes. Among structural abnormalities observed in the karyotypes the most frequent one those involving the long arm of chromosome 1 and the 14q32 chromosomal region.
1q triplication is a distinct secondary chromosomal abnormality . Most repeated region of 1q is q21-q32. Recurrent translocations of 1q10 to the short arms of different acrocentric chromosomes(chromosome 15, 21, 22) and non acrocentric chromosomes (chromosome 12,16,19) have been identified. The gene involved in trp(1)(q) is unknown. However, it was suggested that the most common region of duplication, 1q23-24, harbours genes associated with tumor cell invasiveness.
Three main 14q32 translocations were observed in MM, each acounting for about 25% of the 14q32 rearrengaments: t(11;14)(q13;q32), t(4;14)(p16;q32) and t(14;16)(q23;q32). Cloning of some of 14q32 translocations showed that these translocations leaded to over expression of oncogenes. It's suggested that these recurrent chromosomal abnormalities are not randomly distributed, but present fight links.
We herein report a MM case of a 65 years old female with a complex karyotype as 45,XX,der(1)(:p35→q21:),t(1;2;?9)(q21;p23;?q34),-9,der(12)(12pter→12q24::1q21→1qter), t(14;20)(q32;q12),der(18)(18pter→18q23::1q21→1qter)[14]/46,XX[1], confirmed by FISH analysis.

A. Acar: None. **A. Ugur Bilgin:** None. **A.G. Zamani:** None. **T. Akın:** None. **E. Tuncez:** None. **M.S. Yildirim:** None.

J11.80

Screening of *BRCA*1 (5382insC, 185deAG) and *BRCA*2 (6174delT) mutations in Breast cancer patients in Iran by Multiplex Mutagenically Separated PCR

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Breast cancer is the most common cancer in women in the world. Mutation of *BRCA*1 and *BRCA*2 genes has been linked to hereditary breast and ovarian cancer. Some mutations such as 185 delAG and 5382 insC in *BRCA*1 and 6174 delT in *BRCA*2 are present in higher frequencies than other mutations. many methods have been reported for the study of *BRCA* mutations, but allele-specific PCR and PCR- mediated site-directed mutagenesis seems more efficient for screening of specific characterized mutations. **Patients and methods**: In this study 140 hereditary breast cancer women were identified. DNA of patient and control samples were extracted from peripheral blood. A particular Multiplex Mutagenically Separaded PCR method was used to screen for the 185 delAG and 5382 insC mutations in *BRCA*1 and 6174 delT mutation in *BRCA*2. For each mutation, three primers (one common, one specific for the mutant and one specific for the wild-type allele) were used. Amplification and Sanger sequencing were used to confirm the presence of mutations. **Results:** The BRCA1 5382 insC mutation was found in three individual patients, but the BRCA1 185 del AG mutation and BRCA2 6174 del T were not seen in any of the studied patients. **Conclusion:** The results show MS-PCR is a simple and reliable method to detect known recurrent *BRCA*1 and *BRCA*2 mutations. This study performed for the first one in Iran and these results could be used for detection of the frequency of *BRCA*1 and *BRCA*2 gene mutations in Iranian population.

S. Parchami Barjui: Other; Significant; Other. **N. Sadrizade:** A. Employment (full or part-time); Significant; Employment. **A. Salehi:** Other; Significant; Other. **N. Abdian:** A. Employment (full or part-time); Significant; Employment. **F. Heibati Goojani:** A. Employment (full or part-time); Significant; Employment. **M. Hashemzadeh Chaleshtori:** A. Employment (full or part-time); Significant; Employment. **M. Hajhashemi:** A. Employment (full or part-time); Significant; Employment.

J11.81

Molecular study of gastrointestinal stromal tumors (GIST)

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Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumors of the gastrointestinal tract. They are characterized by the presence of gain function heterozygous mutations of the two target genes, c-Kit and PDGFRA. Throughout this work we conducted both immunohistochemical and genetic analysis of 25 gastrointestinal stromal tumors. Our study aimed to analyze c-Kit and PDGFRA activating mutations in order to establish a genotype-phenotype correlation and to implement a target therapy based on Imatinib. Immunohistochemical study revealed a positivity of c-Kit staining in 20 among 25 tumors. Tumoral DNA extracted from formalin-fixed tissues was sequenced for hot spot mutations of both genes c-Kit and PDGFRA.We were faced to poor DNA quality because of formalin tissues fixation. Thus we only managed to analyze 7 tumoral DNAs among 25. 4 activating mutations were revealed in 6 tumors. Mutations were located in exons 9 and 11 of the c-Kit gene. One tumor did not show any c-kit mutation and is then thought to be c-Kit wild type.Although we did not analyze mutations in all 25 tumors, the 4 revealed mutations should enable in 6 tumors a therapeutic benefit.

M. Haddaji Mastouri: None. **D. H'mida-Ben Brahim:** None. **S. Trabelsi:** None. **M. Chourabi:** None. **N. Labaied:** None. **S. Sassi:** None. **M. Yacoubi:** None. **A. Saad:** None.

J11.82

Analysis of *APC* Promoter Hypermethylation Number of Patients with Sporadic Colorectal Cancer

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Colorectal cancer (CRC) is one of the most leading causes of cancer-related deaths in Iran. *Adenomatous Polyposis Coli* (*APC*) is a tumor suppressor gene and it's inactivation by hypermethylation of promoter region in CpG islands has been observed in the early development of Colorectal Cancer. Methylation of promoter CpG islands (CGIs) belonging to *APC* causes transcriptional silencing of this gene leading to carcinogenesis and other disorders. In current study, methylation of CGIs in the promoter of *APC* was analyzed using bisulfite direct sequencing method in 20 tumor tissue samples together with an equal number of normal tissues belonging to patients with confirmed diagnosis of colorectal carcinoma in south-west of Iran. Assessment of *APC* promoter methylation revealed that normal tissues were unmethylated, while 20 out of 30 (66.66%) tumor tissues were hypermethylated. Among the tissues in which methylation was detected, all of 20 were hypermethylated in one of the two alleles of *APC*. We are also studying 50 patients of CRC in north-west of Iran.

A. Abdollahi: None. **S. Khatami:** None. **H. Galedari:** None. **A. Forughmand:** None. **M. Ziadi:** None.

J11.83

Novel nonsense mutation of BRCA2 gene in a Moroccan man with familial breast cancer

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Abstract
Background: Breast cancer is the most common cancer among women worldwide accounting for about 30% of all cancers. The majority are sporadic, where as 5 to 10% of cases are due to an inherited predisposition in two major genes, *BRCA1* and *BRCA2*, transmitted as an autosomal dominant form with incomplete penetrance. Male breast cancer is rare and is mainly due to *BRCA2* than *BRCA1* germline mutations.
Objective: Molecular study of *BRCA2* gene in man with familial breast cancer.
Methods: PCR and direct sequencing of *BRCA2* gene.
Results: Identification of novel heterozygous germline mutation c.6428C>A ; p.Ser2143Stop of *BRCA2* gene.
Keywords: male, breast cancer, *BRCA2* gene, mutation, genetic counseling.

S. guaoua: None. **I. Ratbi:** None. **A. Sefiani:** None.

J11.84

TP53 Arg72Pro polymorphism and bladder cancer risk

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TP53 is a tumor suppressor gene which regulates cellular stress response. Polymorphisms in the TP53 gene have been reported among possible genetic risk factors for various cancers. It has been shown that cells with Arg/Arg genotype tend to undergo apoptosis faster than cells with Pro/Pro genotypes. In this work, we aimed to investigate the role of TP53 Arg72Pro polymorphism in susceptibility to bladder cancer. 102 healthy controls and 79 Turkish bladder cancer patients were included in the study. The genomic DNA samples of the individulas were subjected to PCR and direct sequencing of the polymorphic region. The genotype frequencies were not significantly different (p=0.7) between patients and healthy subjects, therefore we could not find a significant association between the TP53 Arg72Pro polymorphism and bladder cancer risk. The frequency of TP53 codon72 polymorphism varies among ethnic populations, therefore genotype distribution and associated risk for cancer should be studied in different ethnic groups.

N. Ersoy Tunali: None. **S. Sahoglu:** None.

J11.85

The genetic analysis in women with cancer and other pathology of female reproductive system

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Introduction. The clinical and genealogical history in women with finding changes in genes can help determine individual chance of cancer development. The possible outcomes of these tests are very important for women with family history of female reproductive system cancer. The aim of the study was to compare the results of genetic analyzes among women with cancer of female reproductive system (CFRS) including cancer family syndrome and women with gynecological pathology.
Material and metods. We investigated 30 women aged 26-72 years. The genetic analyzes were performed among 14 women CFRS treated (group I) and among 16 women with gynecological pathology such as polycystic of ovary, ovarial cysts, endometrios, fibromyoma of utery (group II). The genetic analyzes included PCR-RFLP for determining T-397C, A-351G polymorphisms of ESR1 gene and G1846A polymorphism of Cyp2D6 gene. We compared the results using Fisher test.
Results. There were found no differences between two investigated groups in the frequency T-397C polymorphic variants of ESR1 gene. The G allele (GG, AG genotypes) of ESR1 gene frequency was significantly higher (p<0,05) among group I (71,4%) comparing group II (25,7%). On other hand, among group II was significantly higher (p<0,05) the frequency of A allele (GA, AA genotype) of Cyp2D6 gene (17,1%) as compare with group I (0,05%).
Conclusion.The further investigation may help to evaluate the individual cancer risk for women with cancer family syndrome.

O. Paliychuk: None. **N. Gorovenko:** None. **Z. Rossokha:** None.

J11.86

Establishment And Characterization Of A Cytogenetically Complex Multiple Myeloma Case With Novel 14q Rearrangement

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The objective is this presentation to report the clinical and laboratory characterization of a case of multiple myeloma with complex karyotype with novel 14q rearrangement.
A 63-year-old gentleman was referred to our hospital with back pain and anemia. Laboratory values showed anemia with a hemoglobin level of 8,3 g dl^{-1}. The white blood count (WBC) was 4690 μl^{-1} and the platelet count was 238 k μl^{-1}. Serum protein electrophoresis revealed IgA lambda monoclonal gammopathy. Rest of the serum chemistries were within normal. Bone marrow aspiration revealed 72% infiltration with plasma cells. Bone marrow biopsy showed presence of CD138 antigen and cytoplasmatic lambda chains in 90% of the plasma cells. Skeletal survey showed a decrease in height of the L2 vertebral body and several lytic lesions in calvarium. He was diagnosed with Multiple Myeloma. The ISS stage was II and Salmon Durie stage was IIIA.
The cytogenetic study showed t(5;14)(q22;q32) in all mitoses examined. An abnormal cellular clone with complex karyotype was found 9 out of evaluated thirteen metaphases: 46,XY,t(5;14)(q22;q32)[4]/52,X,-Y,+5,t(5;14)(q22;q32),del(6)(q21),+del(6)(q11),+del(6)(q11),+7,-8,+9,-10,+11,der(13)add(13)(p11.2),-14,+15,+15,der(16)add(16)(q24),del(17)(p13),+18,+der(19)t(1;19)(q23;p13), der(19)t(1;19)(q23;p13),-20 [9]. Although 14q rearrangements are frequently present in MM, t(5;14)(q22;q32) was not reported before.
Recognition of aberrant features of plasma cells has a paramount role in prognostic evaluation of myeloma patients. Identification of novel translocation t(5;14)(q22;q32) in single multiple myeloma case suggesting that new rearrangements of IGH, leading to new fusion transcripts, may be involved in the malignant progression of myeloma cells by promoting their proliferation and survival. However, the prognostic significance of this rearrangement is not clear.

G.A. Giran: None. **K. Yararbas:** None. **K. Acar:** None. **N.A. Baysal:** None. **M. Pamukcuoglu:** None. **A. Tukun:** None. **Y. Laleli:** None.

J11.87

Single Nucleotide Polymorphisms in the Loss of Heterozygosity and Uniparental Disomy Regions in a Colorectal Cancer Patient in Saudi Arabia

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The incidence of colorectal cancer (CRC) in Saudi Arabia is on the increase. Genetic factors specifically linked to CRC have not been investigated widely in the Saudi population. Loss of Heterozygosity (LOH) and Uniparental Disomy (UPD) are two Genetic aberrations that are associated with CRC. In this study, we searched for the presence of CRC associated single nucleotide polymorphisms (SNPs) in the regions of LOH and UPD. Out of 27 SNPs we found 5 of them in LOH/UPD regions of chromosome eight in a patients diagnosed with CRC from King Fahd National Guard Hospital, Riyadh, Saudi Arabia.
Methods: DNA from tumor (case) and non-tumor (control) tissue from 15 CRC patients excised from each patient to represent samples and controls, respectively was analyzed using the AFFYMETRIX GeneChip Cytoscan HD array.
Results: Five SNPs (rs6983267, rs7014346, rs7837328, rs10505477, rs10808555) associated with CRC were found in a single patient in the UPD and LOH regions at chromosome 8.
Conclusion: The presence of SNPs in the regions of LOH/UPD may have a causal effect in the onset of CRC. Further studies are needed to establish this relationship.

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J11.88

Exploring signaling pathways to identify new molecular targets for cancer therapy

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It is widely accepted that cancer or other complex diseases are not caused by a single gene, rather an array of genes communicate with each other in a complex manner to determine the fate of a cell. In recent years, the research in this area has been focused on the understanding of biological pathways involved in a disease in order to develop effective drugs. In order to contribute to these efforts, we have established a work-flow in our laboratory, from initial high-throughput screening to mechanism of action level studies. It involves- *in vitro* anticancer screening focusing on cell death mechanisms, microarray gene expression analysis to identify differentially expressed genes, pathway mapping, and experimental validation of the identified pathway. The results of our study are presented here which not only led us to identify a new candidate therapeutic compound for estrogen positive breast cancer treatment but also facilitated the identification of new genes with potential role in cell death mechanisms, which may represent new molecular targets for developing anticancer therapies.

M. Kaur: None. **L. Esau:** None. **S. Sagar:** None. **V.B. Bajic:** None.

J11.89

The role of cox gene in hepatocellular cancer patients

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Cox-2 gene has an adverse effect on tumor suppressor p53,which is an apoptotic transcription factor normally found in the cytosol.Two of the metabolites of cox-2 prostaglandins A1and A2,when present in higher quantities bind to the cytosol and inhibits ability to cross into the nuclues. Aim of work,to proove the role of cox -2 gene in HCC patient.Subjects and methods,17 hepatocellular carcinoma tissue samples and twenty normal distant tumor samples,were taken from 22 patients underwent hepatectomy as a primary modality for HCC,Results:,All normal patients were cox-2 negative ,3 out of 4distant metastassis were cox -2 gene positive and 7 out of 13 were cox -2 positive,Cox-2 gene was 100% specific and 58% sensitive

R.M.T. Issa: None.

J11.90

Multitarget FISH assays in detecting abnormal cells in washing and bronchial biopsied lung cancer cells; a pilot study in Egyptian patients

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Background. Lung carcinomas are associated with molecular abnormalities. We performed a pilot study to determine the feasibility of an interphase FISH assay for the detection of lung carcinoma in bronchial washing specimens and tissue biopsy. **Methods.** Twenty bronchial washing specimens were analyzed. The LAVysion multicolor probe set contains locus-specific probes to 5p15, 7p12 (*EGFR*), 8q24 (*CMYC*), and a centromeric probe to chromosome 6 was used, hTERT gene (5p15) red and control region EGR1 gene (5q31) green probe was used also. **Results.** FISH positivity in the samples of this study was 60% (12/20) for LAVysion probes and for hTERT probe 33.3 % (6 / 20) only.
Conclusions. The current study indicated that Multitarget FISH assays have shown higher sensitivity in detecting abnormal cells in washing and bronchial biopsied cells from patients with lung cancer, also, hTERT gene amplification or high copy number could be a marker for poorer prognosis in early-stage NSCLC patients.

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J11.91

Investigation of genomic instability in cholesteatoma tissues

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Cholesteatoma is defined as 'skin in the wrong place' that means 'the presence of squamous epithelium in middle air'. They are locally invasive and destructive lesions around bone and have high recurrence rate after the surgery. Genetic basis of cholesteatoma has not been understood yet. In this study, we searched genetic basis of cholestetaoma by using LOH (Loss of Heterozygosity) analyse including MSI (Microsatellite Instability) which has an important role in genome-wide screening. For this, microsatellite markers are used. Microsatellites are polymorphic and basically similar although they contain small differences among individuals in the field of molecular genetic that makes them appropriate to use as genetic markers. Capillary electrophoresis fragment analysis was performed by examining seven loci. Markers used were selected according to the data obtained the study including epithelial cancers in literature. MSI was observed in 6 out of 39 cases in some regions resulting in increased size of one allele. One of these six patients is recurrent. The markers of observed MSI are D6S273, D6S473, D8S261, D9S157, D9S162. In addition, we found one LOH region (D15S153) in one patient. To our knowledge, this is the first study screening of LOH for cholesteatoma. This finding indicates different genetic basis of cholesteatoma. The further study is needed to carry out the molecular basis of cholestetaoma.

Z.B. Bulut: Other; Modest; Selcuk University Science Research Foundation. **H. Acar:** None. **K. Ozturk:** None.

J12.01

The ICS (Institut Clinique de la souris) genetic engineering and model validation department: Generation of customized and read-to-use genetically engineered mice

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The Institut Clinique de la Souris (ICS) is part of PHENOMIN the National research infrastructure that provides extensive services ranging from development of mouse models to comprehensive phenotyping.
The Genetic Engineering and Model validation Department at ICS is dedicated to the development and molecular validation of new mouse models. They can be valuable tools to better understand the molecular processes of monogenic diseases or to test drugs in vivo. We can generate duplications and deletions of defined genomic fragment (CNVs) as observed in human disease or small/large deletion or insertions, point mutations, humanizations and conditional mutations. We always work in strong interaction with the scientists and make sure to define their needs. Many publications have already arised from mice generated at ICS.
Model mice are generated either through introduction of DNA into zygotes or through injection of genetically manipulated embryonic stem cells (ESC) into blastocysts. We can either use ESC from consortium (http://www.knockoutmouse.org/) or introduce the desired mutation in our ESC. We are also able to derived ESC from your favorite mouse model if you wish to perform in cellulo experiments.
Please note that ICS phenotyping department is presented in another poster.
www.ics-mci.fr/ contact email : ics@igbmc.fr

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J13.01

Exome Sequencing in Primary Chronic Myeloid Leukemia Patients

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In chronic myeloid leukemia (CML) about 35% of patients either remain resistant or develop resistance to the most effective therapy by inhibitors of tyrosine kinase. Exome analysis is now-a-days the most powerful tool in the search for the new prognostic markers and molecular targets for individual therapy. We launched prospective study aiming to sequence exomes of leukemic cells in CML patients to find out possible exomic differences between responders and non-responders. This will help not only to find predictors to the therapeutic outcomes but also will allow to look in deep mechanisms of the disease progression which basically remain unclear. To the date there are 5 CML exomes sequenced with 20K variations found in average. About 6K of the found variants are non-synonymous. Preliminary analysis demonstrated that 123 of these nonsynonimous variants are not described in dbSNP and can potentially contribute to the pathogenesis of CML and/or resistance to the TKIs. These and new data with deep analysis of the findings will be discussed in the presentation.

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J13.02

The use of genetic testing for personalized optimization of warfarin therapy in patients of the West Siberian region of Russia.

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The aim of our study was to investigate the frequency of occurrence of CYP2C9 *2, *3 (R144S and I359L), C+1173T VKORC and G>A rs2108622 (V433M) CYP 4F2 and C>G rs 11676382 GGCX genes polymorphisms in patients of the West Siberian region of Russia, as well as to assess the contribution of these genetic markers to the change of therapeutic dosage of warfarin for this group of patients.
The study included 118 patients (mean age 51) from the West Siberian region taking warfarin more than 1 year. Effectiveness of warfarin was assessed by the International Normalized Ratio. Presence of alleles was determined

by polymerase chain reaction.
Significant differences of average daily dose of warfarin depending on the VKORC genotype were revealed: for patients with genotype CC average dose was 7,1+2,3mg, with genotype CT - 4,8+1,9mg, with genotype TT - 2,8+0,6mg (p(CC-CT)=10-6, p(CT-TT)=8*10-4, p(CC-TT)=10-9). There were not significant differences in selected doses of warfarin depending on the CYP2C9 *2 genotypes. Patients who had a „slow allele" CYP2C9*3 received low doses of warfarin (*1/*1 5.8±2.5mg and *1/*3 - 4.1±2.6mg, p=0.03). Presence of CYP2C9 *2 or *3 alleles resulted in tendency to decrease of average daily dose of warfarin based on VCORK1 genotype. Thus, genotype VKORC1 and CYP2C9*3 among other genetic markers contributes most to the titration of a therapeutic dosage of warfarin in the West Siberian population. The results of the pharmacogenetics testing on CYP2C9 and VKORC1 can predict the fluctuation range of the daily maintaining dose of warfarin.

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J13.03

The assessment of human plasminogen activator inhibitor - 1 (PAI-1) gene polymorphism association with deceased kidney graft initial function and survival

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Delayed kidney graft function (DGF) is a poor prognostic sign combined with reduced graft survival.We retrospectively investigated the relationship of DGF and the deceased donor kidney graft survival with inherited polymorphism of human plasminogen activator inhibitor (PAI-1) gene in transplant recipients (n=185) and corresponding donors (n=185). Genomic DNA was isolated from frozen and stored at -50C blood or internal organ tissue fragment. PCR-Taqman technique was used to type for PAI-1 gene mutation -675 5G → 4G (Synthol, Russia). The criterion for DGF was equivalent to at least one dialysis session during the first 7 days after surgery. Binary logit model and Cox's proportional hazards model were used to assess the reliability of the effect of mutation on DGF and graft survival at 1 year, resp.Only homozygous 4G/4G PAI-1 genotype of the donor showed significant correlation with the DGF (p = 0,008) in the logit model adjusted for the transplant order, cause of death of the donor, donor age, duration of cold ischemia time of the graft, the level of panel-reactive preformed cytotoxic antibodies, compatibility for HLA class I and HLA-DR, anti-CD25 therapy, and PAI -1 genotype of the recipient. In the Cox model with the same arguments both incompatibility for HLA class I (p = 0,0009) and the 4G/4G PAI-1 genotype of the donor (p = 0,037) demonstrated significant association with the fall graft survival.Conclusion. Homozygosity of the donor, but not the recipient of cadaveric kidneys for mutation -675 4G PAI-1 gene predisposes to DGF and reduced graft survival.

V. Morozova: None. **N. Kaluzina:** None. **J. Moisiuk:** None. **V. Novoseletsky:** None. **V. Abramov:** None.

J13.04

The influence of A313G polymorphism in GSTP1 gene on perinatal asphyxia development and necessity of reanimation supplementation in full-term neonates

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The GSTP1gene is involved in the deactivation of free radicals, in the inflammatory response and affect the lung development. The presence of 313G polymorphic variant is changing the enzymatic activity of enzyme-isomers, which may predispose to the development of pathological conditions. The aim was to examine the relationship between the gene polymorphism and the perinatal asphyxia risk and the necessity of support in the neonatal intensive care.
Methods. We examined 70 full-term neonates with different severity of perinatal asphyxia (PA) and 71 full-term healthy neonates. The genetic analyzes were performed using PCR-RFLP. There were evaluated the frequency of mechanical ventilation and hemodynamics medical support in the neonates with PA. Statistical analysis was implemented using SPSS 16.0.
Results. We have found no differences in the frequencies of GSTP1 genotypes between two groups. The investigated polymorphic variants had also no influence on the PA severity. The presence of GG genotype did not increase the relative risk of mechanical ventilation, but the neonates with genotype GG were required the necessity of hemodynamics medical support in 50% of cases comparing with 22% of cases for the neonates with genotype AA. The maximum dose of dopamine for hemodynamic support was for neonates with AA genotype - 6,03 mg / kg / min, with GG genotype - 9,0 mcg / kg / min. The neonates with GG genotype treated significantly higher dose of dopamine.
Conclusion. The further investigation may help to optimize the medical support and dopamine dose in the neonates with PA.

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J13.05

The Role of Genetic Factors in the Prediction of Myocardial Infarction Complications Within One Year Follow Up

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A sample of 165 patients with myocardial infarction (IM) with elevated ST segment has been studied to construct a prediction model for one-year period complications (recurrent nonfatal IM or cardiac death). 32 polymorphic genetic markers with confirmed role in cardiovascular disease pathogenesis were analyzed, the best model to stratify patients by risk of post-IM complications included variants rs4291 (A-240T) in the ACE gene, rs6025 (G1691A, Leiden mutation) in the F5, and rs5918 (Leu59Pro) in the IGTB3. C statistics for the genetic model was 0,75 (0,64; 0,86) p=0,001, which is comparable with characteristics of the GRACE scale for the same patients' population: 0,73 (0,61; 0,85). Thereby, analysis of a limited number of genetic markers was sufficient to create a risk prediction model for post-IM complications comparable by it characteristics to the models, which are currently in use in clinical practice. To confirm the clinical validity, the predictive model obtained in the study should be confirmed on independent samples of patients with MI.

O.A. Makeeva: None. **M.V. Zykov:** None. **E.V. Kulish:** None. **M.V. Golubenko:** None. **V.V. Kashtalap:** None. **I.A. Goncharova:** None. **O.L. Barbarash:** None. **V.P. Puzyrev:** None.

J13.06

Analysis of the associations between angiotensin-converting enzyme gene polymorphism and development of intraventricular hemorrhage in preterm infants

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Introduction. The objective of this study was to examine the associations between angiotensin-converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism and development of intraventricular hemorrhage in the preterm infants.
Material and methods. Prospective observational study included 50 preterm infants at less than 36 weeks gestation (median birthweight 1586 g, range 700-2499 g; gestation 30,8 weeks, range 27-36; 22 male) admitted to the neonatal intensive care units of children's hospitals of Poltava region. Control group consisted of healthy term infants from the same region (n=38). Both groups of patients were genotyped for the insertion/deletion polymorphism of the ACE gene. Intraventricular hemorrhage were compared in patients with II, ID, and DD genotypes of the ACE gene. Associations between different ACE genotypes and risk of intraventricular hemorrhage were analyzed.
Results. Distribution of infants in relation to the three variations of ACE gene I/D polymorphism was identical in the study (26,0 %, 54,0 % and 20,0 %) and control groups (39,5 %, 44,7 % and 15,8 %, p=0,404). Severe intraventricular hemorrhage was significantly associated with DD genotype (OR 6,938; p=0,050) while no associations with II and ID genotypes were found.
Conclusion. Results of the study confirm the potential role for genetic factors, particularly for gene variations of renin-angiotensin system, in the pa-

thogenesis of intraventricular hemorrhage.

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J13.07

Congenital Right Tibia Diaphysis Amputation and Bilateral Nephrolithiasis in a 6-Months-old child

M. A. SOYLEMEZ, *K. Delil, H. Simsek, A. Tafazzoli, P. Gultepe, M. Avsar, S. Ergunsu, N. Haklıgur, G. Girgin, H. Kazan, G. Koc, T. Avcılar, C. Erzik, M. jovaroq, A. I. Guney; MARMARA UNIVERSITY MEDICAL SCHOOL, ISTANBUL, Turkey.*

Objective: We report the case of a 6-Month-old boy with congenital right tibia diaphysis amputation which was nephrolithiasis of the bilateral kidney with hypercalcemia. Nephrolithiasis is a condition characterized by the formation of crystallized material in the urinary system . The occurrence of urolithiasis is most significantly influenced by metabolic disorders, hypercalcemia, urinary tract obstruction with urine stasis and urinary tract infection. Co-existence of congenital right diaphysis amputation and bilateral nephrolithiasis has not been reported previously in neonatal period.

Case Report:

A 6-month-old boy was sent to our medical genetics center with the diagnosis of Congenital right diaphysis amputation. The child was finally referred to our department for further diagnosis and treatment. There was no evidence of previous severe diseases of urinary tract. The Family history was positive for nephrolithiasis. His serum parathormon was in the normal range. However, his serum calcium and ionized calcium levels were elevated. Nevertheless, 25-OH vitamin level was normal for the patient. Renal ultrasonography showed some bilateral multiple renal micro-stones without hydronephrosis.

Conclusion: Congenital amputation of lower extremities and neonathal hypercalcemia with bilateral nephrolithiasis is not a common co-morbid disease. Early diagnosis and early treatment are essential for metabolic abnormalities of calcium. Correction and monitoring of metabolic abnormalities of calcium can be a life saving procedure.

M.A. Soylemez: None. **K. Delil:** None. **H. Simsek:** None. **A. Tafazzoli:** None. **P. Gultepe:** None. **M. Avsar:** None. **S. Ergunsu:** None. **N. Haklıgur:** None. **G. Girgin:** None. **H. Kazan:** None. **G. Koc:** None. **T. Avcılar:** None. **C. Erzik:** None. **M. jovaroq:** None. **A.I. Guney:** None.

J13.08

Incidental findings in large scale sequencing studies

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With the increasing adoption of sequencing technologies in large scale genetic studies, there is a strong possibility of discovering clinically relevant genetic information (incidental findings) about a research participant, outside the scope of the original research objective. These findings can relate to carrier status for a heritable condition or increased susceptibility to a medical condition.

In the context of the SardiNIA Medical Sequencing Discovery Project (dbGaP Study Accession: phs000313.v3.p2), we have sequenced the whole-genome of 2,120 Sardinian individuals enrolled either in the SardiNIA project or in a parallel project on autoimmune diseases, at an average depth of ~4X. We successfully identified ~17M single nucleotide polymorphisms with an error rate of 0.2%. The draft sequences allowed us to screen for clinically relevant variants, as those responsible for mendelian diseases (such as beta-thalassemia, cystic fibrosis, hemochromatosis, thrombophilia), and those conferring high-risk for complex diseases (such as breast cancer). Interestingly, ~6% of the 2,120 samples, on average, are carriers for 5 of the 9 mendelian conditions examined. This information could be clinically relevant for their life, as simple genetic counseling for couples or as warning for late-onset diseases. Interestingly, we also observed putative risk variants for multifactorial diseases to be instead common and in disagreement with the available clinical information for the same volunteers. These analyses emphasize the need of a close collaboration with clinicians and counselors in large scale sequencing studies, as well as the need for an improved annotation of disease alleles described in literature.

J13.09

A novel desmoplakin dominant mutation responsible for Carvajal/Naxos syndrome identified by exome sequencing

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Naxos (OMIM#601214) and Carvajal (OMIM#605676) syndromes are rare forms of recessive Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC). We report the case of an Italian 37-year-old woman with a form of ARVC with phenotypic features overlapping Naxos/Carvajal syndrome due to a novel desmoplakin (DSP) mutation identified by Whole Exome Sequencing (WES), but with a dominant model of inheritance.

The woman was admitted to the hospital after resuscitated cardiac arrest. Clinical and instrumental evaluations showed mild biventricular systolic dysfunction, with dilated ventricles. She also has palmoplantar keratoderma and family history of sudden cardiac death. Molecular analysis was performed by WES on the Illumina HiScan SQ.

Family's pedigree showed a dominant model of inheritance. 65993 variants were individuated by bioinformatics analysis; after data filtering and integration of filtered results with patient's clinical features 4 potential mutations were selected. Only two of those variants cosegregated with the disease in the family: c.878A>T, p.Glu293Val in the DSP gene and c.626A>C, p.Tyr209Ser in the cytochrome c oxidase assembly gene (SCO2). Since the cardiomyopathy related to SCO2 gene mutations is usually associated with encephalopathy and is fatal in children, we excluded the causative role of such gene in our family. Therefore, we considered the DSP- Glu293Val variant as disease-causing and we hypothesized a modifier role for the SCO2 variant.

We identified a novel DSP gene variant associated to a dominant form of Carvajal/Naxos syndrome by Whole Exome Sequencing. This report demonstrates the utility of Next Generation Sequencing in the clinical setting for syndromic cardiomyopathies.

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J14.01

A discovery that ensures uniform efficiency of assays between samples and across all genome regions

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For many analytical methods the efficiency of DNA analysis varies across the genome and between samples. This tends to correlate with high C+G content in a complex way that does not explain why the direction and magnitude of effects varies between samples. We provide evidence that sequence elements that have unusually structured C+G content do not melt even when aggressive melting conditions are applied. These elements remain duplexed, thereby preventing the strands of flanking melted sequences from diffusing away from each other.

Consequently, neighboring strands, whose length will depend on the quality of the DNA (via nicking, breaks, etc), can quickly re-anneal as non-denaturing conditions are re-established. This prevents the access of oligonucleotide primers (or probes), thereby reducing amplification (or hybridization) efficiency for broad domains that we have termed 'Thermodynamically Ultra-Fastened' (TUF) regions.

This mechanism explains why some genome regions are particularly difficult to amplify and assay in many procedures, such as PCR or WGA steps of NGS and GWAS. Importantly it also explains inter-sample variability of this behavior, DNA samples of varying quality will carry more or fewer nicks and breaks, hence their intact TUF regions will have different lengths and so be differentially affected by this amplification suppression - with 'higher' quality DNAs being the most vulnerable.

A major practical consequence of this discovery is that inter-region and

inter-sample variability can be largely overcome by employing routine fragmentation methods (e.g. sonication or enzyme digestion) prior to sample amplification.

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J14.02

Detection of broad range of DNA damage with Two-Dimensional Strandness-Dependent Electrophoresis (2D-SDE)

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Two-Dimensional Strandness-Dependent Electrophoresis (2D-SDE) in manual minigels or premade microgels is a novel technique for nucleic acid analysis. In the first dimension nucleic acid fragments are separated based on length and strandness i.e. double-stranded DNA, single-stranded DNA and RNA•DNA hybrids. The nucleic acids are heat denatured before the second dimension electrophoresis and in the second dimension all fragments are single-stranded and separate only based on length.

We tested if 2D-SDE could detect various types of DNA damage *in vitro* and *in vivo*. Each sample was run in duplicate both uncut and cut with *Mbo* I which cuts both single- and double-stranded DNA. Single-stranded breaks, either nicks or gaps, were detected as horizontal streaks on 2D-SDE extending from uncut DNA molecules too large to size separate in the gel. Double-stranded breaks generated an arc in the gel. In contrast, DNA with interstrand crosslinks and bulky adducts were bent and migrated in front of that arc. Single-stranded DNA molecules, too damaged for complementary strand binding, formed a diagonal line. 2D-SDE detected DNA damage at comparable level of sensitivity to the well known comet assay. However, 2D-SDE allowed direct detection of damaged molecules and subfractions could be isolated form the gel.

2D-SDE can be used to detect many common types of DNA damage. Applications include testing quality of biosamples and efficiency of various procedures. Applications also include genotoxicity testing, chemosensitivity testing and diagnosis of genome instablity and DNA repair disorders.

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J14.03

Array-DASH: Dynamic microarrays for highly-sensitive and robust DNA fingerprinting, scanning, genotyping and re-sequencing in various diagnostics settings

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To enable widespread DNA diagnostics in myriad settings, we have developed and optimised a new, low cost, simple and universal technology. The method is termed ‚array-based dynamic allele-specific hybridisation' (array-DASH), which exploits the proven diagnostic utility of melt-curve analysis, but transfers it to the content rich format of a micro-array.

Key features include; unprecedented sensitivity (directly detects all allelic variants at 1% representation), virtually error-free mutation detection, robustness to C+G extremes and secondary structures, and the use of completely standard run conditions - serving genotyping, scanning, fingerprinting, re-sequencing, and combinations thereof.

We are now exploring the real-world utility of this system for several important applications, including prenatal testing of fetal genomes in maternal plasma, detection of emerging clonal sequences in cancer biopsies, detecting rare drug resistance variants in HIV infections, and purity testing of harvested crops. In so doing, we also aim to create a commercially available array-DASH device with inbuilt software for automated data analysis.

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J14.04

A novel, generic, preimplantation genetic diagnosis (PGD) protocol applied to Cystic Fibrosis (CF) involving mutation detection through High Resolution Melting Analysis (HRMA) and simultaneous haplotype analysis through QF-PCR.

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CF is the most common genetic disease in Caucasians and a common indication for PGD. Due to CF's extremely heterogeneous mutation spectrum (>1800 mutations), most CF-PGD protocols reported to date, apply single-cell multiplex fluorescent PCR for segregation analysis (STR-linkage) along with p.Phe508del detection, when indicated. This approach is limiting for cases with other mutations when segregation analysis is unavailable. We report the development and successful clinical application, of a novel, generic PCR-PGD protocol to facilitate direct detection of any CFTR mutation by HRMA, and simultaneous confirmation of genotyping through STR-haplotype analysis. An optimized touch-down, multiplex PCR supports the co-amplifcation of: i). any CFTR exon-region combination using primers previously described (Montgomery et al. 2007) and ii. 6 STRs (4 intragenic and 2 extragenic). 1st PCR products were used: i. as a template for a nested PCR for HRMA and ii. to confirm the CFTR genotypes by STR haplotype analysis. Pre-clinical protocol validation involved testing 208 single isolated lymphocytes from whole blood samples of candidate PGD patients. Four clinical PGD cycles were performed, for 4 CF carrier-couples with genotype combinations as follows: p.Arg334Gln and c.489+3A>G, p.Phe508del and p.Phe508del, p.Phe508del and c.489+1G>T, p.Phe508del and p.Leu732X. 3 pregnancies were achieved and PGD genotypes were confirmed following conventional CF prenatal diagnosis after amniocentesis or trophoblast sampling.

The reported PGD method is a flexible and robust tool which facilitates direct CF genotype analysis, genotype confirmation and contamination detection in single cells, with minimal family work-up (generic) and rapid completion of PGD.

A. Destouni: None. **M. Poulou:** None. **G. Kakourou:** None. **C. Vrettou:** None. **J. Traeger-Synodinos:** None. **E. Kanavakis:** None. **M. Tzetis:** None.

J14.05

A new technology for Targeted Genome Enrichment, based upon High-Stringency Co-Operative Sequence Capture and dramatically improved amplification of synthetic oligonucleotide pools

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Targeted enrichment (TE) of genome sequences combined with Next Generation Sequencing (NGS) is a costly and imperfect method. It typically yields products with highly uneven representation of targeted regions regions and considerable off-target contamination.

By re-designing existing solution phase TE, we have developed a cost-effective and highly-automatable technique based on co-operative binding of oligonucleotide probes. This enables high-stringency target selection conditions, generating more specific and even sequence recovery.

A pilot application achieved a target specific enrichment factor of ~1000 fold when selecting a >200kb region, using 454 (Roche) sequencing. This was performed without repetitive element blocking. We have also designed and are now testing a novel repetitive-element blocking strategy that should

further improve performance.
A major practical and cost hurdle relevant to many TE methods is the requirement for uniform and efficient mass amplification of synthetic oligonucleotide pools. To specifically solve this problem, we designed a new, automatable and emulsion-free oligo-pool PCR procedure. This yields 2-3µg of 100% full-length, double-stranded product from ~200fg of synthesized oligo-pool sequences (e.g, comprising 99% truncated oligos and 1% full-length oligos). A simple purification step then yields >1µg of single-stranded oligo-pool material, which may be used directly in a TE reaction.
Furthermore, we applied lessons learned in optimizing oligo-pool PCRs, to improve linker-ligation methods for the production of long fragment genome libraries. This will further enhance our new TE method, with experiments now underway to determine the ideal range of fragment sizes that the technology will efficiently recover.

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J14.06
"Prenatal diagnosis (PND) for Cystic Fibrosis using High Resolution Melting (HRM) analysis and simultaneous haplotype analysis through QF-PCR."

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Cystic Fibrosis (CF) is caused by mutations of the *CFTR* gene and is the most common autosomal recessive disorder in Caucasians (carrier frequency 1 in 20) with >1800 disease causing variants. Prenatal diagnosis (PND) is offered to all CF carrier couples and includes detection of the causative mutations as well as haplotype analysis for exclusion of maternal contamination and non paternity.
High resolution melting analysis (HRM) is a validated, robust, low-cost, high throughput screening method applied in our laboratory for *CFTR* testing. Here, we report the development and retrospective evaluation of the diagnostic value of a novel multiplex HRM, genotyping and haplotyping method for CF prenatal diagnosis that overcomes the reported limitations of the HRM methodology.
Eighty study samples from 20 carrier couples referred for PND (whole blood in EDTA and CVS or amniotic fluid) were genotyped retrospectively. A multiplex 1st round PCR was optimized to co-amplify 9 *CFTR* exons along with two intragenic STRs. Haplotyping was achieved by running the 1st round PCR products on an automatic sequencer. Mutation detection was achieved by HRM analysis of a 2nd round nested PCR optimized to amplify the exon of interest.
All DNA samples (variable sources, extraction methods and unknown concentrations) were successfully amplified and genotyped by the 1st and 2nd round PCR. This generic protocol using HRM, facilitates the simultaneous analysis of DNA samples from various sources, is a robust and efficient method for CF PND and could easily be implemented for PND analysis of other genetic disorders.

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J14.07
Reprogramming of HDF cells to iPSc using polycistronic lentiviral vectors delivering the OSKM

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An induced pluripotent stem (iPS) cell is an adult cell that has been reprogrammed to form an artificially pluripotent stem cell. iPS cells have potential to differentiate into different cell types of body and can be generated through forced expression of specific genes in a non-pluripotent cell (typically human dermal fibroblast). The aim of the our study was the full reprogramming of human dermal fibroblast (HDF) cells into iPSCs using a single polycistronic lentiviral vectors delivering the Oct4, Sox2, Klf4, and c-Myc (OSKM) factors without extra less lentiviral integration into genome. In this study psPAX, pMD2G, and FUW plasmids were transfected into HEK-293T cell line for production lentiviral vectors containing OSKM genes. HDFs were isolated from foreskin samples and were transduced at passages 3. In this study we used only OSKM without control from tetracycline inducible lentiviral vector (rtTA). Our results show that polycistronic lentiviral vector contain OSKM genes are capable to induce full reprogrammed iPSc without transduction of extra lentiviral vector containing Tet.OP system for OSKM expression control. So we can generate iPSc with less insertional mutagenesis.

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J14.08
Quantitative real-time PCR technique for rapid diagnosis of TAR syndrome

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Thrombocytopenia absent radius (TAR) Syndrome is a rare genetic disorder inherited in and with autosomal recessive fashion. Chromosomes at 1q21.1 in most individuals harbor a 200-kb deletion. RBM8A gene is located in this region. High throughput real-time PCR technique was used to develop a reliable and specific technique for rapid diagnosis of microdeletion in patient with TAR syndrome. Blood samples were collected after informed and written consent from a consanguineous family clinically diagnosed as TAR syndrome. Quantitative real-time PCR applied to measure the RBM8A gene dosage of genomic DNA from lymphocytes of tar syndrome patient and normal people, Compared to conventional cytogenetic karyotype analysis. Real-time PCR results were calculated for measuring of the RBM8A/PMP22 ratio using the 2-ΔΔCt formula in TAR syndrome to normal subjects showing 0.45 and 1.0 (95% CI), respectively. Real-time PCR technique effectively differentiates the TAR syndrome from the normal subjects. Because the RBM8A/PMP22 ratio of TAR syndrome is significantly lower than that of normal, Quantitative real-time PCR could be the first choice of method for molecular diagnosis of microdeletion in TAR syndrome rather than cytogenetic study.

V.R. Yassaee: None. **F. Hashemi Gorji:** None.

J14.09
New platform for real-time PCR using microchip analyzer

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Nowadays a real-time polymerase chain reaction (PCR) method is a routine technique applied in various diagnostic fields. One of the most promising directions for the improvement of PCR analysis is an equipment facilities miniaturization. Indeed such miniaturization leads the laboratory diagnostics to be cost-effective and rapid.
We propose a new platform for real-time PCR with microchips containing lyophilized PCR mixtures which allow decreasing reagents consumption and reduce time of analysis. Microchips consist of silicon plate with 30 cells of 1.2 µl volume each. The inner surface of the cells is hydrophilic whereas the intercellular space is hydrophobic that helps to inject PCR mixtures inside the cells properly and reduce the risk of cross-contamination. Thermocycling and dual-channel fluorescence detection are carried out by microchip analyzer AriaDNA. To prevent sample evaporation a sealing liquid is used.
The new designed platform was used to identify some single nucleotide polymorphisms (SNPs) associated with genetic disorders and individual susceptibility to drugs. Particularly, F5 20210G/A, F2 1691G/A and MTHFR 677C/T polymorphisms in patients with vasculitis were tested. These SNPs are known to be associated with congenital thrombophilia and increase the

risk of deep vein thrombosis. With standard sample preparation protocol the analytical sensitivity was 1-10 ng/µl of genomic DNA. Single analysis (one microchip) has taken about 30 minutes. Thus encouraging assays characteristics have been obtained.
In summary, real-time PCR using microchips containing lyophilized PCR mixture provides reliable results and can be recommended for routine laboratory tests especially for screening programs.

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J14.10
Genetic diseases and congenital abnormalities of the children born in the families with reproductive losses (miscarriages)

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The administration of lymphocyte therapy within complex treatment of the families with miscarriages provided successful childbearing in 92% of cases. The 8-year observation of 141 children born in the families with reproductive losses showed that those children were facing a high risk of congenital abnormalities + Down Syndrome R=2,6319 (95% CI 1,073-6,455) and autosomal dominant diseases with a more prominent gene expression in comparison with that of their parents OR=3,64(95% CI 1,23-10,81). Besides, in this group of children such rare genetic diseases as Fanconi's anemia and tuberous sclerosis were registered. Minor disorders with the frequency of 2,41 per 1 child statistically validly with the level of significance p=0,0162 on test X2 were more often registered in the children born in the families with miscarriages in comparison with the children of the control group, where the frequency of minor disorders amounted to 2,14 per one child.
Therefore we may consequently state that findings have been obtained on the accumulation of genetic diseases, congenital abnormalities and minor disorders in the generation of the families with miscarriages, which shows the increase of conditions on the border-line of "adaptive" norm or crossing its borders. Despite the fact that the children of this sampling were born «in vivo», the same problems arise in the children born «in vitro», which denotes the presence of similar biological problems in the families with low fertility.

B. Ginzburg: None.

J15.01
In silico analysis of HMIP region (HBS1L-MYB intergenic region) and transcription factor identification in a region associated with fetal hemoglobin variation

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The aim of this study was to evaluate the HMIP region, especially the portions flanking the SNP rs9399137, since this is the most significantly associated with fetal hemoglobin (Hb F) through comparative analysis of sequences (phylogenetic footprinting) based on the identification of highly conserved regions in non-coding regions from different species, indicating the possible existence of functional regions, such as transcription factors (TF). For comparative analysis were used HMIP region of *Homo sapiens*, *Pan troglodytes*, *Pongo abelli* and *Canis familiaris*, deposited in GenBank. The computational scheme for alignment and conservation analysis was based on the local alignment program BLAT and then the sequences were processed and globally aligned using the program mLAGAN. To identify FT was used rVISTA that associates the database TRANSFAC with comparative analysis of sequences. We observed the occurrence of motifs associated with embryo viability compared to normal erythropoiesis, expansion of hematopoietic stem cells and progenitor cells, acceleration of differentiation of erythroid progenitors, erythroid differentiation and control of hematopoiesis in the fetal liver, and transcription activiation of α-globin and erythroid genes. It is noteworthy here the occurrence of recognition sequences FT group SOX, which belongs SOX6, which is related to the proliferation and maturation of erythroid cells during erythropoiesis and mainly cooperates with BCL11A in silencing of gene expression γ-globin gene, affecting the levels of Hb F. Therefore, this region may be associated with the control of factors responsible for the formation and phenotypic characterization of hematopoietic stem cells in the embryo and fetus, reflecting in adulthood.

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J15.02
GWAS Central: A Comprehensive Aggregate-level Genome Wide Association Database

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Genome Wide Association Studies (GWAS) produce high-dimensionality individual and aggregate level genetic data by assaying hundreds of thousands of common Single Nucleotide Polymorphisms (SNPs) in hundreds of thousands of individuals in the search for genetic variants that may have causative effects on a disease phenotype or trait.
GWAS Central (http://www.gwascentral.org/)is a comprehensive database resource developed to enable researchers to easily visualise and search aggregate-level GWAS data in the context of genes, genome regions, phenotypes or traits [Thorisson GA *et al,* 2009, Nucleic Acids Res. 37: 797]. The database currently provides >34 million p-values for over 1,000 studies collected from public databases, outreach gathering, and direct submissions.
Key features include:
•Interactive genome browser allowing visual comparison of selected GWAS in relation to other information
•Ability to upload (not submit or share) a researcher's own GWAS p-values to compare to other GWAS studies
•Manually curated phenotype ontology annotations, assigned using MeSH and HPO, searchable via a graphical tree displays, or auto-complete text
•Nano-publications that provide key results for each study, including individual markers, phenotypes and results (<10-5) in RDF. The resulting triple store can be queried using SPARQL: http://fuseki.gwascentral.org
•A virtual machine version of the GWAS Central software, for other projects to adopt and install (e.g., GWAS India http://gcindia.igib.res.in/)
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J15.03
Identification of alternative splicing variation in RNA-seq time series data

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Alternative splicing (AS) involves different multiple choices of splice sites to generate protein diversity. A single mRNA may code for different forms of a protein (isoform) as a result of alternative splicing, which increases the complexity of mammalian transcriptomes. Recent predictions based on deep sequencing suggest that more than 95% of human genes undergo alternative splicing, and that disruption of splicing can cause disease. Thus in order to increase our understanding of disease processes, it is essential to be able to detect alternative splicing. But no statistical methods of identifying this across time are available that would enable the new molecular techniques to assess the transcriptome. The ultimate objective of this study is to elucidate statistical methods of identifying alternative splicing in time series data. We propose fully bayesian approaches to characterize alternative splicing diversity in a variety of time course RNA-seq data and simulation sets. This methodology is based on dirichlet prior, shrinkage, and normalization method. We expect this statistical methodology to provide proof that our proposed methods will identify alternative splicing in temporal analysis. Ultimately, these methods can be used to better understand disease processes over time and to identify novel therapeutic targets by exploring variation in exon/splice expression in temporal analysis as a function of developmental stage and tissue type, so that abnormal splicing patterns can be used to help target human diseases.

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J15.04

Gene-scale functional impact prediction based on conservation and selection

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Whole-genome sequencing analysis indicates that conservation and natural selection show distinctive signals between neutral and deleterious genes, making them suitable features for functional impact prediction. Approaches such as SIFT, Polyphen-2 prioritize deleterious variants on conservation and other features. We extend it to gene level and score all genes from RefGene. Another feature we include here is natural selection, which is proved to have notable stronger negative signal within genes associated with hereditary diseases. Using logistic regression and ten-fold cross validation, we estimate the probability of each gene to be deleterious. Distributions of the scores within neutral genes and disease related genes from OMIM suggest that our method can classify functional impact on gene scale well. Besides annotation on known genes, the model is also developed for prediction on novel genes.

R. Chen: None. **W. Yang:** None. **J. Yang:** None.

J15.05

The effect of MicroRNA-377 on methylation pattern of tumor suppressor gene in pancreatic cancer cell line

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MicroRNAs (miRs) are small, noncoding RNAs that regulate expression of many genes. Recent studies suggest the role of miR in carcinogenesis. It is now well recognized that cancer is a consequence of genetic and epigenetic alterations while disruption of epigenetic mechanisms is the hallmark of cancer. Overexpressed DNA methyltransferase 1 (DNMT1) strongly contributes to silencing tumor suppressor gene in pancreatic cancer. However, the underlying mechanism of DNMT1 overexpression is still unclear. In this study, we investigated whether miR 377 is involved in the regulation of DNMT1 and aberrant patterns of methylation in two pancreatic cancer cell line (MiaPaca2). Quantitative RT-PCR was performed to check the effect of miR-377 on mRNA level of DNMT1. Upregulation of miR-377 resulted in a dramatic reduction of DNMT expression (53%, $p<0.05$). Then high resolution melt (HRM) was applied to check the effect of miR-377 on methylation pattern in promoters of tumor suppressor genes, such as BNIP3. The enforced expression of miR-377 in pancreatic cancer cell line restores normal patterns of DNA methylation (30% after 48h), induces re-expression of these methylation-silenced tumor suppressor genes (42% after 48h $p<0.05$). These findings support the role of mir-377 in epigenetic normalization of pancreatic cancer cell lines, which can be a motivation for the development of miRNA based strategies for the treatment of pancreatic cancer.

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J15.07

Distribution of 5-methylcytosine and 5-hydroxymethylcytosine in metaphase chromosomes from triploid human zygotes

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We analyzed distribution of DNA methylation marker - 5-methylcytosine (5-MeC) and active demethylation marker - 5-hydroxymethylcytosine (5-hMeC) in metaphase chromosomes from triploid human zygotes. IVF-produced triploid zygotes, discarded for uterus transfer, were enrolled in the study. All samples were donated for research with written informed consent of the patients. Zygotes were treated with 0,1% colchicines, 0.9% sodium citrate and fixed with freshly prepared 3:1 methanol:acetic acid. 5-MeC and 5-hMeC were detected by indirect immunofluorescence with anti-5-methylcytosine antibodies (Eurogentec) and anti-5-hydroxymethylcytosine antibodies (Active Motif) in QFH/AcD-banded chromosomes. Parental origin of chromosomes was determined in separately lying pronuclei on the basis of chromosome condensation type and presence of chromosome Y. Distribution of 5-MeC and 5-hMeC was analyzed in every chromosome of homologous triad.
First, distribution of 5-MeC and 5-hMeC in parental genomes demonstrated reverse patterns. In all homologues triads paternal chromosomes contained little 5-MeC, but were enriched in 5-hMeC, while maternal chromosomes were heavily methylated and contained little 5-hMeC. This shows that active demethylation involves both parental genomes, but is more intensive in paternal genome.
Second, the distribution of 5-MeC and 5-hMeC along chromosomes was not uniform and showed band-specificity. In both maternal and paternal chromosomes R- and especially T-bands were enriched in 5-MeC and 5-hMeC with 5-MeC showed less pronounced banding pattern than 5-hMeC. Constitutive heterochromatin (1q12,9q12,16q11.2,Yq12) contained neither 5-MeC nor 5-hMeC. This data suggests that active demethylation is band-specific, having the highest intensity in R- and T-bands.
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J15.08

ESR1 gene promoter methylation in adipose tissue

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AIM: Estrogen receptor alpha (ESR1) is a candidate gene for obesity. It was known that, obesity is formed in ESR1 knockout mice and ESR1 gene transcription decreases in obese individuals. Thus, in this study, we investigated the methylation status of ESR1 promoter in adipose tissue to sign the effect of methylation on decreased ESR1 gene transcription in obese individuals and to indicate the relationship with obesity.
METHOD: The study consisted of obese and normal 23 individuals totally. DNA and RNA are extracted from subcutaneous/omental adipose tissue. A, C, E2 and F promoter regions of ESR1 gene was analyzed by Methylation-Specific PCR.
RESULTS: As a result of the examination of ESR1 gene promoter region's methylation status in adipose tissue among obese individuals, methylation is detected as 33,3 % in promoter A and 100 % in promoter C despite no methylation in promoter F. 100 % methylation is defined in ESR1 gene E2 promoter region in adipose tissue in all groups. Although ESR1 mRNA level decreases in adipose tissue in obese individuals whose ESR1 promoter C is methylated, no significant association is detected.
CONCLUSION: In this study, methylation status of ESR1 gene promoter in adipose tissue is examined. Due to our findings, we assume that ESR1 mRNA level in adipose tissue decreases significantly depending on methylation status of ESR1 gene promoter C region in obesity. The study continues with increasing number of samples.

F. Guclu - Geyik: A. Employment (full or part-time); Significant; Istanbul University, Institute of Experimental Medicine, Department of genetics. **T. Erginel:** A. Employment (full or part-time); Significant; Istanbul Education and Research Hospital, Department of General Surgery. **N. Erginel-Unaltuna:** A. Employment (full or part-time); Significant; Istanbul University, Institute of Experimental Medicine, Department of Genetics.

J15.09

Methylation index as a prognostic marker in bladder cancer patients

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Bladder cancer (BC) is a common malignancy worldwide. At the time point of diagnosis 70% of BCs present as superficial BC (SBC), which do not penetrate the muscle layer of the bladder. The rest 30% of cases present muscle-invasive BC (MIBC). SBC has better prognosis, though recurrences happen in 30% of cases after the primary tumor removal. MIBC has much worse prognosis and survival. Therefore it is of a prior importance to reveal possible markers of recurrences and progression of SBC into MIBC. It is proposed that methylation pattern might reflect the ability of BC to recur and/or to progress. We examined 122 tumor samples from 108 SBC patients and 14 MIBC patients. Recurrence status after 1 year was known for 39 SBC patients: 31 developed relapses, 8 did not. Genomic DNA was extracted from fresh tissue. We investigated promoter methylation of *RASSF1A, RARb, P16, p14, CDH1* using methyl-sensitive PCR. Methylation index (MI) (or mean frequency of methylation) was defined as the ratio between the numbers of methylated genes to total number of examined genes in each sample. Statistical significance was evaluated using the Mann Whitney U-test.SBC had a significantly lower extent of methylation (median MI 0.1) than MIBC (medi-

an MI 0,25) (p 0,017). Recurrent within 1 year SBCs showed median MI 0,0 while non-recurrent tumors had median MI 0,2 (p 0,047). Our results show that MI might be used as a sensitive marker for the assessment of recurrence and progression potential of SBCs.

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J15.10

The interactive map of NF-kappaB-dependent inflammatory molecular interactions in human

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Inflammation disorders are considered nowadays to be associated with a broad range of pathologies, so making it a possible and promising target for diagnostics and therapy. Systemic understanding of molecular basis of inflammation has quantitatively and qualitatively advanced in recent years due to powerful molecular biology approaches. From this point, the novel challenge is adapting enormous amount of information for analytical and prospective purposes. Interactomic approach seems to be the most appropriate nowadays, as its idea relies on functional fusion of all cellular organization levels - from simple molecules and up to epigenome and its regulation. Considering this, we have developed (project № 14.A18.21.0199 funded by Russian Ministry of Science and Education) an interactive xml-based referenced and manually curated map of human NF-kappaB-inflammation system interactions - NFkBIM - allowing for systematization of the related most up-to-date experimental data. The map comprises more than 70 individual macromolecular factors subdivided hierarchically for logical analysis purposes, including protein, RNA, miRNA, SNP and epigenetic information fused with metabolomic effects. Much attention is paid to the description of the expression control for all the immediate participants of the system. The system of specified interactions is also integrated with two-step-neighboring higher cellular regulatory factors. NFkBIM allows for effective strategy planning and experimental design, selecting clinically significant SNPs associated with specific diseases for enhanced diagnostics purposes, and revealing feed-back and feed-forward systems for therapeutical concept development.

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J15.11

In silico prospection of transcription factors related to Fetal Hemoglobin regulation

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In adults, fetal hemoglobin (Hb F) expression comprises up to 1% of the total, suffering influence of transcription factors in its regulation. We aimed screening γ-globin genes regulatory elements by phylogenetic footprinting, in order to know the Hb F levels genetic determinants. We compared *Homo sapiens*, *Pan troglodytes*, *Macaca mulatta* and *Cebus apella* sequences, in the *National Center for Biotechnology Information*. Alignment and analysis were based on conservation programs BLAT and mLAGAN, visualized by VISTA Browser. For evaluation of the conservation was used as parameters 70% identity to at least 100 bp non-coding regions. To identify transcription factors was used rVISTA program. Among the 529 motifs of transcription factors indicated we highlight CDC5, c-MYB, CP2, BP1, KLF1, KLF11, GATA1 and GATA2. The transcription factors CP2, GATA1, GATA2 and KLF11 are direct positive regulators of Hb F, with binding sites in the promoters of the γ-globin gene. BP1, CDC5 and c-MYB act as indirect positive regulators of Hb F, from the negative regulation of β-globin gene and binding to specific sites, the -198 region of γ^G gene (chromosome 11) and *HMIP* region (chromosome 6), known to influence Hb F levels. KLF1 is a negative regulator of Hb F, act directly by interaction with the locus control region (LCR), and indirectly by the regulation of genes that silencing the γ-globin. Knowledge about the regulatory elements of Hb F can contribute in different therapeutic strategies discovery that may improve on clinical pathways when considering patients with hemoglobinopathies.

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J15.12

Restriction endonuclease selection for the reduction of RRBS libraries

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Reduced Representation Bisulfite Sequencing (RRBS) genomic library preparation by DNA hydrolysis with restriction endonucleases provides limitations on target sampling composition. Original RRBS protocol puts to use endonuclease MspI (methylation insensitive HpaII isoshizomer), assuming that CpG islands (CGIs) are HpaII tiny fragments. We suggest that other endonucleases might select CGIs more precisely allowing more sophisticated reduction of the RRBS libraries.

We have developed ReMark computer program (http://www.epigenetic.ru/projects/remark/) to calculate restriction endonuclease recognition site likelihood-ratio test in Markov Chain sequence models (output as "score"). The practical value of the scores calculated for selected enzymes has been estimated with the criteria of CGIs selection quality for 40-220 bp RRBS libraries.

ReMark scores and RRBS library attributes for a selected set of endonucleases.

Restriction endonuclease	MspI	HinP1I	TaqI	XmaI	AagI	NaeI	SfrI	BsePI	NotI	MauBI
Recognition site	CCGG	GCGC	CGCG	CCCGGG	CGGCCG	GCCGGC	CCGCGG	GCGCGC	GCGGCCGC	CGCGCGCG
CpG methylation sensitivity	-	+	+	-	?	+	?	+	+	+
Available RSA	+	+	+	-	-	+	-	-	+	-
library size, b.p.	70248053	40841839	15212336	3187500	1652457	1327517	1270563	977068	83604	21994
ReMark score	2.45	2.74	4.09	3.08	4.72	3.37	4.72	5.01	5.65	8.64
Library fragments overlapping CGIs, %	20	31	66	49	55	59	88	91	90	85
CGIs CpG genome coverage, %	49	42	36	7,9	5,2	4,3	6,2	5	0,4	0,1
CGIs CpGs library fraction, %	37	46	75	73	68	74	90	91	88	88
Promoter CGI library fraction, %	24	30	49	50	51	53	64	65	63	67
Exon CGI library fraction, %	14	17	27	22	29	30	32	36	38	26
Intron CGI library fraction, %	13	16	25	29	21	23	30	28	25	26
Non CGI CpGs in library, %	63	54	25	27	32	26	10	8	12	12

Higher scored endonucleases select CGIs more precisely with much smaller library sizes in spite of the fact that rare cutters omit many regions of interest. Some high-scored enzymes are methylation sensitive preventing their use for RRBS library construction. Taken that Roentgen stereophotogrammetric analysis (RSA) data is available for some, methylation insensitive mutants development may be expected.

In conclusion, ReMark software is useful for choosing endonucleases effectively selecting regions of interest for genome and epigenome research.

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J15.13

Prognostic significance in biochemical recurrence of prostate cancer in preoperative serum glutation S-transferase P1 gene in men following radical prostatectomy

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Hypermethylation of GSTP1 gene in the preoperative serum of men with localized prostate cancer(PCa) predicts early biochemical prostate specific antigen(PSA) failure , following surgical treatment. In our study we investigated the hypermethylation profile GSTP1 gene in the preoperative serum of men with clinically localized PCa who underwent radical prostatectomy, with PSA recurrence on a period of 40 months (3 years and 6 months), as a prognostic biomarker of recurrence in PCa men following radical prostatectomy.

Materials and methods:

We included a number of 77 men with clinically localized PCa, who underwent radical prostatectomy at the Urology Clinic from County Emergency Hospital Timisoara, and 38 men with negative prostate biopsy. All serum samples were collected before prostate biopsy or at least 4 months after prostate biopsy. PSA recurrence was defined as a single postoperative PSA level ≥0.2 ng/ml. To analyze the methylation status of gene GSTP1 before the surgery intervention we used the quantitative methylation-specific polymerase chain reaction(QMSP) method.

Results:

From the 77 men who underwent radical prostatectomy included in our stu-

dy, 16 (20.8 %) experienced PSA recurrence within the study period. From the 38 men with negative prostate biopsy only 4(10.6 %) presented positive GSTP1 hypermethylation
Conclusions:
Our study suggests that preoperative serum GSTP1 may be a useful prognostic biomarker for men with clinically localized PCa treated with radical prostatectomy.

R. Dumache: None. **S. Negru:** None. **R. Minciu:** None. **S. Putnoky:** None. **D. Ionescu:** None. **M. Puiu:** None.

J15.14
Computational research in a case with t(14;17)(q32;q11.2) causing 22q11 microdeletion syndrome-like phenotype

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Abstract:
A 22q11.2 deletion syndrome was suspected in a patient with language impairment, crowded teeth, immunodeficiency, long philtrum and dismorphic features. Conventional cytogenetic analysis showed the karyotype: 46, XY, t (14;17)(q32;q11.2). This translocation has not being reported previously in a patient with a constitutional disease. Despite the association of genes presents in critical region in deletion 22q11 syndrome with the phenotype it causes is not clearly understood, recent papers show that haploinsufficiency of TBX1 can recreate many aspects of this genetic condition.
We hypothesize that the presence of genes near the breakpoints code for proteins acting in the same process that those promoting phenotype in 22q11.2 or with a similar action.
Methods: A selection of genes were collated from literature trough GoPubmed (www.gopubmed.com) with the terms "22q11.2[tiab] AND critical region[tiab] AND Genes[mesh]". Genes retrieved where compared using GeneCards (www.genecards.org) and those with high-GIFtS peak (GeneCards Inferred Functionality Score) value were selected. Also two sites were consulted for an interaction of those genes with genes located in the breakpoints: Biocarta (http://www.biocarta.com/genes/index.asp) and Biogrid (http://thebiogrid.org).
Results: No interaction where was found between genes in 22q11.2 and those in 14q32 or 17q11.2. Despite this, there are genes in 14q32 related with coronary heart disease, parathyroid secretion, thyroid hormones and immunology.
Conclusions: Genes present in 14q32 could explain in part the phenotype but additional molecular genetic testing detection of affected genes is necessary once there could be affected genes outside the breakpoints and contributing to phenotype.
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M. Soriano: None. **N.E. De León:** None. **E. Morales:** None. **A. Castelví:** None. **L.A. Méndez:** None.

J15.15
Clinical implications of simultaneous promoter methylation of ERα and ERβ on sporadic breast cancers in North Indian women

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DNA methylation, an epigenetic marker, has critical roles in regulation of gene activity. Aberrant promoter hypermethylation leading to gene silencing underlie altered gene expression in cancers. Estrogens play a fundamental role in the development and progression of breast cancer, activating estrogen receptors (ERs), ERα and ERβ. ERα expression level prognosticates hormonal therapy responses of patients and is perhaps the most important biomarker for determining breast cancer progression. After the discovery of ERβ, it was quite evident that the role of estrogen is far more intricate.
We have investigated the promoter methylation status of ERα and ERβ and its possible correlation with protein expression in 110 sporadic breast cancer cases from North India. Methylation-Specific Polymerase chain reaction (MSP) method was used to analyze CpG methylation of promoter region of these genes. Immunohistochemical (IHC) staining for ERα and ERβ protein was performed to correlate promoter methylation with expression.
In general, we found that ERα was methylated in 64.54% (71/110) tumors, including 51 of 61 ERα negative tumors (83.6%, *P<0.0001*). ERβ showed methylation in 52.72% (58/110) tumors, including 38 of 58 ERβ negative tumors (65.5%, *P=0.007*). In addition, we observed a strong correlation between promoter methylation of ERα and ERβ with significance of *P=0.01*. An inverse correlation was found between promoter methylation and protein expressions.
Our results show a simultaneous involvement of epigenetic regulation for ERα and ERβ, indicating that methylation pattern of these genes can be exploited as biomarkers for the prediction of breast cancer outcome.

S. Chattopadhyay: None. **S. Deo:** None. **N. Shukla:** None. **S.A. Husain:** None.

J16.01
Family-based linkage, exome chips, and association to identify high impact coding variants for complex traits

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Whole exome sequencing has enabled geneticists to explore the influence of low frequency coding variants on biomedical traits. With simple association, exonic association has the limitations of GWAS: large numbers of subjects are required. Linkage methods in families are a powerful tool to identify biomedically relevant coding variants in relatively modest collections of families especially when combined with exome chip data and conventional association analysis. African American (AA) families (45 families; 500 subjects) from the IRAS Family Study were genotyped with the Illumina Exome Beadchip resulting in 92,150 genotyped SNPs. Two-point linkage and association analysis was performed for 38 cardiovascular and metabolic traits. The most striking result was the APOE-coding variant Arg158Cys linked with apolipoprotein B(APOB) levels with a LOD=4.91 and association with APOB (P=4.4XE-19). This variant is part of the ε2 haplotype and ε2 homozygotes (1.7% haplotype frequency) have APOB levels that are 53% of normal. . When "exome-wide" results of linkage and association were summarized, numerous regions of linkage and association were observed at less striking levels of significance. Further analysis may reveal similar high impact variants. In a similar analysis of the adiponectin gene in Hispanic Americans and AAs we have identified a class of coding variants which reduce circulating adiponectin levels to <20% of normal. Different variants are observed: G45R and R55C, respectively; both 1% frequency. These studies show the power of family-based methods to identify high-impact coding variants. Extension to whole exome sequencing of the families will test whether private mutations contribute to biomedical traits.

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J16.02
TRAF3IP2 Gene and Systemic Lupus Erythematosus: association with disease susceptibility and pericarditis development

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Systemic Lupus Erythematosus (SLE) is a chronic relapsing-remitting multifactorial autoimmune disease characterized by the production of a wide number of autoantibodies and the occurrence of diverse clinical phenotypes. Although genetic factors confer a predisposition to the development of the disease, only 15% of the genetic contribution is known. Recently, *TRAF3IP2* gene has been found associated with susceptibility to psoriatic arthritis and psoriasis. This gene is involved in the regulation of adaptive immunity acting as a negative regulator of humoral immunity and as a positive signalling adaptor in IL-17-mediated cellular immune responses. These evidences suggest that TRAF3IP2 gene variability could be implicated also in SLE susceptibility. We enrolled 239 consecutive SLE Italian patients and 278 healthy subjects as controls. Three *TRAF3IP2* polymorphisms were analyzed by allelic discrimination assay. A case-control association study and a genotype-phenotype correlation analysis were performed. All genotypes were in Hardy-Weinberg Equilibrium. The rs33980500 and rs13193677 resulted significantly associated with SLE susceptibility (P=0.021, OR=1.71, and P=0.046, OR=1.73, respectively). All three *TRAF3IP2* SNPs resulted associated with the development of pericarditis: in particular rs33980500 showed the strongest association (P=0.002, OR 2.59). The *TRAF3IP2* involvement was further highlighted by binary logistic regression analysis. In conclusion, our data show for the first time the contribution of *TRAF3IP2* genetic variability in SLE susceptibility, providing further suggestions that common variation in genes involved in the adaptive and innate immune

system are important in establishing SLE risk. Our study also shows that this gene may affect disease phenotype and, particularly, the occurrence of pericarditis.

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J16.03

Y-chromosome haplogroup analysis in the Besermyan ethnic group

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Besermyans are an ethnic group in the Volga-Ural region which speaks a language belonging to Finno-Ugric language family and which is close to Udmurt language but at the same time containing some Turkic traits. This group inhabiting North-West Udmurtia is very small and it has not been described in the terms of Y-chromosome haplogroups yet. We analyzed 53 individuals from Besermyan population using 12 Y-chromosome markers (M9, M89, YAP, M35, M130, 12f2, M170, M231, Tat, P43, M207, Page07) and found that Y-chromosome genetic pool of this ethnic group consists of haplogroups R1a1a-Page07, N1c-Tat, N1b-P43, I-M170, R1b1b-M269 and E1b1b1-M35. The majority of the Y-chromosome haplogroups in Besermyans is represented by N1c-Tat and R1a1a-Page07 lineages comprising 73,6% of all Y-chromosomes. The third leading haplogroup is N1b-P43 (13,2%) so the main share of the Y-chromosome lineages in Besermyan group is actually N-M231 (54,7%). In other words the main pattern of the Y-chromosome haplogroup distribution shows their relatedness to the Volga-Ural populations.

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J16.04

Application of model based approach and Bayesian networks for eye color prediction for forensic purposes

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Advances both in genotyping technology and human genetics research resulted in enormous progress in field of forensic genetics. Forensic molecular phenotyping allows to narrow subset of possible suspects. Here we would like to present model based approach to create panel of SNP markers for eye color and ancestry prediction.
Population sample contained 131 unrelated individuals: 100 (76%) Caucasians, 31 (24%) Asian (Kazakhs). Samples were binned according to eye color: 47 (36%) light eye color and 84 (64%) dark eye color. Genotyping was performed using TaqMan MGB assays. All samples were analyzed using panel of 22 SNP markers selected from literature.
Data were divided into two sets: training (100 Caucasian, 31 Asian) and testing (47 Caucasian, 81 Asian). Caucasian data for testing set were obtained by genotyping, Asian data for testing set were extracted from HapMap. Firstly, SNP data of the training set were filtered using ReliefF, which minimizes risk of SNP false positive association with studied phenotype. Consequently, multifactor dimensionality reduction was performed to uncover possible epistatic regulation between studied loci. For validation of selected markers Bayesian network was constructed. Network contained ancestry determination using rs1426654 and 16891982 and eye color determination using rs12913832, rs7495174 and rs916977. Sensitivity of the model reached 0.96, specificity was 0.99. Prediction of eye color using presented Bayesian network could be useful in case of unknown remnants identification and verification of eye witness testimony. The study was supported by the grant No. I/328 of the Ministry of Industry and Trade of the Czech Republic.

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J16.05

Checking the hypothesis of a Balkan origin of the Armenians

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The origin of Armenians is a controversial subject for anthropologists, archaeologists, historians and linguists. Among several hypotheses on this point, one prevails. The ancient Greek historian Herodotus described the Armenians as Phrygian colonists because of their speech and the garments they wore. Here, we tested the Balkan version of the origin of Armenians based on the Y-chromosomal markers. We used the results of high-resolution typing (applying 50-70 SNPs) in 1171 DNA samples representing 10 Armenian geographic groups covering the whole area of the Armenian plateau and the database of the Armenian DNA project at Family Tree DNA comprising a general Armenian population. As possible signals of Greek influence the presence of the E1b1b1a1-M78 haplogroup with its major sub-branches (E1b1b1a1b-V13, E1b1b1a1a-V12 and E1b1b1a1c-V22) were considered. The frequencies of the E1b1b1a1-M78 clade in Armenians are quite low in nine out of ten geographic groups and in the general dataset, ranging from 0 to 3.8%. The highest rate (8%) of the supposed Balkan lineages is observed in a sample representing the south-eastern part of the Armenian Highland (currently north-west Iran). The mean age of this haplogroup using 14 STR markers is 14.5 ky based on evolutionary mutation rates. This value is much higher than that shown for the Greek samples which indicates that the E1b1b1a1-M78 haplogroup among south-eastern Armenians is indigenous and clearly was not introduced by back-migration from Balkan region. Thus, the patrilineal genetic structure of modern Armenians groups does not support their Balkan origin.

L. Yepiskoposyan: None. **P. Hrechdakian:** None. **H. Simonian:** None.

J16.06

Molecular genetics of familial hypercholesterolemia in Petrozavodsk: known variants and new mutations

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Aims. Familial hypercholesterolemia (FH) is a common single gene disorder, which leads to premature atherosclerosis and coronary artery disease. FH is mainly caused by defects in the low-density lipoprotein receptor (LDLR) gene, which lead to dysfunction of the receptor. Spectra of mutations in FH are population-specific and currently more than 1000 mutations are characterized worldwide. We aimed to investigate the spectrum of mutations in Petrozavodsk and to compare our data to recent research in St.-Petersburg.
Methods. We studied a group of 94 patients from Petrozavodsk by means of automated single-strand conformation polymorphism followed by direct PCR amplified DNA sequencing.
Results. Thirteen different mutations of LDLR gene were characterized in Petrozavodsk: c.58 G>A, c.192del10/ins8, c.195-196insT, c.618T>G, c.925-931del7, c.1194 C>T, c.1277 T>C, c.1340 C>G, c.1532 T>C, c.1686del8/insT, c.1920 C>T, c.1936 C>A, c.2191delG. Only one mutation (c.925-931del7) was earlier described in St.-Petersburg population;however, it is specific for Finland where it is common in FH patients. Six of other twelve mutations were not characterized earlier in the world.
Conclusions. Our results show that the founder effect in Petrozavodsk population is absent and the spectrum of mutations in LDLR gene is unique for Petrozavodsk.
Research was supported in part by RFBR grant 10-04-00563a.

T.Y. Komarova: None.

J16.07

The birth defects incidence in Russian Federation in 2000-2009

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The main aim of our long-term study of birth defects (BD) is to obtain the incidence of BD in studied populations and to analyze variations in the incidences of BD.
Material and methods. The Russian united database consists of all cases of congenital anomalies notified by regional registries including live born cases and stillbirths. We analyzed data for10-year periods (2000-2009).
Results. The total incidence of BD is 21,33 per 1000 births. The incidence of isolated congenital anomaly present in table.
Conclusions. For most defects there are no changes in incidence during 10 years and observed frequency fluctuations are random. Decreasing trends among newborn were detected for neural tube defects (anencephalus, spina bifida, encephalocele) due to effective prenatal diagnosis.

Results of Russian BD Monitoring, 2000-2009

Anomaly	Number of cases	Incidence per 1000 births
Anencephalus and similar	1481	0,24
Encephalocele	432	0,07
Spina bifida	2861	0,46
Hydrocephaly	2755	0,44
Anophthalmos/micropthalmos	115	0,02
Anotia/microtia	369	0,06
Transposition of great vessels	962	0,16
Hypoplastic left heart	451	0,07
Cleft lip with or without palate	4109	0,66
Cleft palate	2612	0,42
Esophageal atresia with/ without tracheo-esophageal fistula	1179	0,19
Anorectal atresia and stenosis	993	0,16
Diaphragmatic hernia	1127	0,18
Gastroschisis	1357	0,22
Omphalocele	802	0,13
Bilateral renal agenesis	227	0,04
Bladder exstrophy and epispadia	218	0,03
Hypospadias	7677	1,24
Limb reduction	1789	0,29
Down syndrome	6294	1,02

N.S. Demikova: None. **A.S. Lapina:** None. **A.J. Asanov:** None.

J16.08

The FABP2 gene Ala54Thr polymorphism is associated with endurance athlete status

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The intestinal fatty acid-binding protein 2 (FABP2) belongs to the family of cytoplasmatic proteins involved in the intracellular transport and metabolism of long-chain fatty acids (Albala et al., 2006). These proteins thought to participate in the uptake, intracellular metabolism, and/or transport of long-chain fatty acids. The Thr54 variant of the FABP2 gene Ala54Thr polymorphism has been shown to be associated with a two-fold greater binding affinity for the long-chain fatty acids (Baier et al.,1995, Baier et al., 1996), increased fat absorption and lipid oxidation rates (Weiss et al., 2007). Several studies have demonstrated significantly improved prolonged exercise time when high-fat diets were compared with low-fat diets. It was suggested that increasing fat intake increases endurance, perhaps by improving fat oxidation and sparing glycogen during exercise. We therefore have hypothesized that the Thr54 allele of the FABP2 gene may give some advantage to endurance-oriented athletes. In order to test this hypothesis we genotyped 290 Russian athletes and 620 controls. Genotyping was performed by PCR-RFLP. The frequencies of the Thr54 allele in groups of power-oriented athletes (n=84, 29.7 vs. 32.2%, P=0.5877), athletes with mixed (endurance and power) activity (n=88, 26.1 vs. 32.2%, P=0.1183) and a whole cohort of athletes (n=290, 32.4 vs. 32.2%, P=0.9571) were not significantly different from the controls. However, the frequency of the Thr54 allele in endurance-oriented athletes (n=118, 39.0 vs. 32.2%, P=0.0498) was significantly higher than in controls. Although more evidence is needed, one might suggest that the FABP2 gene Ala54Thr polymorphism is associated with endurance athlete status.

E.S. Nasibulina: None. **I.I. Ahmetov:** None.

J16.09

Genetic structure and expansion patterns of Iranian populations based on complete mitochondrial DNA variation data

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Due to its pivotal geographical location and proximity to transcontinental migratory routes Iran has played a key role in subsequent migrations, both prehistoric and historic, between Africa, Asia and Europe. To shed some light on the genetic structure of the Iranian population as well as on the expansion patterns and population movements which affected this region, the complete mtDNA genomes of 352 Iranians were obtained. All Iranian populations exhibit similarly high diversity values comparable to the other groups from the Caucasus, Near East and Europe. The results of AMOVA and MDS analyses did not associate any regional and/or linguistic group of populations in the Near East/Caucasus and Iran region pointing to close genetic positions of Persians and Qashqai to each other and to Armenians, and Azerbaijanians from Iran to Georgians. By reconstructing the complete mtDNA phylogeny of haplogroups U7, R2, H13, and U3 we have found a previously unexplored, genetic connection between Iranian populations and the Arabian Peninsula, Near East and Europe, likely the result of both ancient and recent gene flow. Bayesian skyline plots (BSPs) of population size change through time show a population expansion around 40-42 kya, followed by a gradual decrease of population size up to ~24 kya. Then BSP for Persians show a continuous increase to the present, whereas the BSP for the Qashqai separates two steps (~10 kya and ~2.5 kya). This study was supported by Russian Foundation for Basic Research (11-04-00620) and by Far-East Branch of the Russian Academy of Sciences (12-III-A-06-101).

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J16.10

mtDNA analysis in the Volga-Ural populations.

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The population genetics study of the Volga-Ural region populations can be very fruitful since this region experienced different historical migrations and from the other hand there are ethnic groups that inhabit it for quite a long time.
In our study we used the method of HVS-I sequencing, RFLP analysis and mitochondrial DNA (mtDNA) complete sequencing. We have sequenced HVS-I in 1079 samples from nine ethnic groups: Tatars, Bashkirs, Chuvashes, Russians, Udmurts, Mordovians, Komis, Maris and Besermyans. For further research, we isolated samples with haplogroup H that were not identified with the RFLP analysis, and performed complete mtDNA sequencing. At this point we analyzed 18 complete sequences of haplogroup H.
We have discovered two previously not described mutations in the Udmurt population. Interestingly one of those mutations defines a new H subhaplogroup which is absent in any population except Udmurts. Another mutation defines a new subhaplogroup within haplogroup H55 and it was found in the following populations: Mari (5), Chuvash (2) and Bashkirs (2).
This study was supported by European Social Fund's Doctoral Studies and Internationalisation Programme DoRa, Program of fundamental research of the Presidium of RAS «Biodiversity and Dynamics gene pool" routine „The dynamics of gene pools», Russian Foundation for Basic Research (#11-04-00652) «Molecular phylogeography and ethnogenomics peoples of Eurasia".

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J16.11

PTPN22 polymorphism: associations with Type 1 Diabetes in several populations of the Russian Federation.

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After HLA and insulin gene, the next-strongest genetic association with Type 1Diabetes (T1D) is seen for the *PTPN22* gene. The *PTPN22* gene encodes a protein tyrosine phosphatase important in down-regulation of the immune response. The aim of this study is cross-ethnic group comparisons of frequencies and analysis of associations with T1D *PTPN22* single nucleotide polymorphisms (in coding part of the gene rs2476601 1858C>T R620W and in promoter region rs2488457 -1123G>C) in Bashkir, Buryat, Udmurt and Russian ethnic group of the Russian Federation. Case-control design was applied for assessment of 417 patients with T1D and 323 healthy sex and ethnos matched individuals. Allele identification was performed with RFLP or Real-Time PCR technique. Association of genetic markers with pathology was evaluated according to odds ratio index (*OR*), association was considered statistically significant when p-value<0,05). 1858T+ genotype is associated T1D in Russian(*OR*=2), Udmurt(*OR*=4) and Bashkir(*OR*=3,5) populations; -1123C+ genotype is associated with T1D in Buryat population (*OR*=2,4). Cross-ethnic comparison of frequencies of alleles showed statistically significant differences. Only in the Buryat population frequency of allele -1123G (71,3%) is much higher than the frequency of allele C. Russian population is different from other high frequency of allele 1858T (13% vs. 2,5-3,6%). It is concluded that in the Buryat ethnic group (with low incidence of T1D) diabetogenic marker is C+ genotype of rs2488457; in the Bashkir, Udmurt and Russian ethnic groups diabetogenic marker is T+ genotype of rs2476601.

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J16.12

Two decades of Pre-marital Screening for Beta-thalassaemia in central Iran

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Objectives: Pre-marital screening for beta-Thalassaemia, implemented for almost two decades, is one of the most important preventive screening programmes in the Iranian public health system,. We present the results of this programme between 1992-2010 in the Isfahan Province (central Iran).
Methods: Retrospective descriptive and cross-sectional study using data from the district health centers of the Isfahan Province analyzed using SPSS-19 and Excel softwares.
Results: A total of 703,082 couples were screened. Couples at risk were suspected by persisting abnormal indices (eventually after Iron therapy) and by HbA2 determination at a rate of 0.63% and 0.19% respectively and after molecular testing at 0.31% frequency.
In this way a total of 661 couples at risk have been receiving care in this period and genetic testing has been provided in 85% of the cases. The birth prevalence of severely affected children decreased from 43.7 cases per 100,000 live births in 1997 to 1.5 cases in 2010.
Conclusions: In spite of the programme's good results, shortcomings are still present in our programme that we would like to share with dedicated staff and policy makers of other countries offering premarital screening for these diseases.

M. Zeinalian: None. **A. Moafi:** None. **M. Hashemzadeh Chaleshtori:** None.

J16.13

Saudi Biobanks: importance, implications and opportunities.

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Saudi Biobank (SBB) is a nationwide biorepository project recruiting blood components) Buffy coats, red blood cells; serum, plasma), tissues, and urine from 200.000 local Saudis; half of them will be healthy donors. The DNA banking services are open for NGHA hospitals and other national healthcare providers. The SBB is functioning through many teams; Medical team, Laboratory team, IT team and Ethics team.
The initial operational processes approach a total of 4,432 participants while the DNA banking possesses 3,622 aliquots through a complete automated DNA extraction/storage system. In addition, 4,852 buffy coat aliquots as backup materials and 430 cord blood units with their DNA extracts are already deposited at the biobank. Plasma and serum fractionations have yielded 732 plasma and 690 serum aliquots. The biobank laboratory serves many projects at NGHA that include HLA registry, Saudi Genome Project (SGP), ICU patients···etc. Preoperational trials and QC measurements for these fractions were carried out using AST, ALT, ALB, Bili T, Na, and K. Random selections of serum and plasma banked samples were sent to the clinical biochemistry section; consistency of measurement was seen when compared to the diagnostic samples drawn at the same day.
Next trial phase will recruit an automated high throughput blood fractionation system that automatically separates blood components and urine to be stored in the BiOS automated storage system (-80°C) and LN2.

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J16.14

In Search of the Origin of Haplogroup J1-P58

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Y-chromosomal haplogroup J1, one of the most frequent male lineages in the Near East, is believed to have originated around 10-15 kya in Northern Mesopotamia. J1 along with R1b and J2is generally considered as a genetic marker for the Neolithic expansion, therefore the study of its origin and spread is essential for tracing back ancient human migrations and expansions from the Near-East. In this study, we report a new potential source population and geographic location for the origin of J1-P58, a major sub-clade of haplogroup J1.
Previous studies did not explore the region of Armenian Highland when investigating J1-P58 origin. For this study we have genotyped 453 Armenian samples representing eastern, central and western parts of the highland, 297 Azeri and102 Qashqai samples from Iran, as well as used already published results of different comparative data sets.
The highest J1-P58 variance was observed in the Armenian population from the central part of the highland (regions of Alashkert and Bayazet). The mean age of J1-P58 in this region based on 8 STR markers, was estimated to be the oldest among the studied populations, dating back to 19.4 ky when using the evolutionary mutation rates. It is worth mentioning that the obtained result is based on the analysis of one ethnically homogenous territorial group located in a geographically restricted region in the central part of the Armenian plateau. We believe that this approach leads to better time estimates and significantly narrows down the geographic area where J1-P58 could have originated.

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J16.15

HFE hemochromatosis: influence of dietary iron intakes on the phenotype of C282Y/C282Y patients

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Introduction: Many genetic and environmental factors are viewed to aggravate the iron overload phenotype of patients with *HFE* hemochromatosis but only few of them have been firmly confirmed. In this study, we aimed to determine the role of an iron-rich diet.
Methods: The study focused on 286 C282Y homozygous patients who were enrolled in a phlebotomy program (inclusion period: 2004-2010). Dietary intakes were assessed using an iron-rich foods frequency questionnaire. Iron parameters and the quantity of iron removed by phlebotomies (IR) were available for all patients. Potential confounding factors, such as BMI and alcohol consumption, were taking into account.
Results: Patients with iron-rich diet (n=74) and the other group (n=148) showed a similar proportion of men (58.1% vs 53.4%, p=0.50) and a similar mean age at diagnosis (48.3 vs. 50.3 y., p=0.28). The proportions of overweight patients (41.4% vs 43.6%, p=0.77) and of alcohol abusers (10.8% vs 11.0%, p=0.96) did not differ between groups. Although the median transferrin saturation coefficient was comparable between the two groups (82.0% vs 83.0%, p=0.48), patients consuming more iron had a higher degree of iron overload: higher serum ferritin concentration (749.5 vs 530.5 µg/L, p=0.06) and larger amount of iron removed (3.78 vs 3.10 g, p=0.02). These results were confirmed by multivariate linear regression analysis.
Conclusion: This study establishes a link between dietary iron intakes and the degree of iron overload in *HFE* hemochromatosis patients.

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J16.16

The rs3185480 SNP of the APCDD1 gene is associated with androgenic alopecia

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Adenomatosis polyposis coli down-regulated 1 (APCDD1) gene is an inhibitor of the Wnt signaling pathway therefore it may have a role in the development of the skin appendages, moreover a mutation of this gene has been shown to be associated with a rare hair condition, hereditary hypotrichosis simplex. In this study we aimed to investigate whether SNPs of APCDD1 gene contribute to the development of androgenic alopecia. 210 patients with androgenic alopecia and 98 controls have been enrolled to the study. The genotypes of 9 SNPs in the coding region of the gene have been determined with direct sequencing. We found a significant difference in the distribution of the genotypes of the c.1781C/T, p.L476L SNP (rs3185480) of the APCDD1 gene in exon 5, causing a 3.5 and a 2.8 times increased risk for the development of androgenic alopecia for the homozygote (CI 0.933 - 13.125; Nominal Regression p=0.063) and the heterozygote carriers (CI 1.086 - 7.217; Nominal Regression p=0.033) of the alleles respectively. Regarding the possible function of the rs3185480 SNP, an in silico investigation suggested that this polymorphism is located in an exonic splicing regulatory

element thus may alter mRNA splicing. Moreover, this SNP alters the codon usage of leucine from a preferred codon (CTC) to a rare codon (CTT), which might influence the efficacy of the translation and thus the APCDD1 protein level. Currently we are working on the functional characterization of this rs3185480 APCDD1 SNP.

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J16.17

Direct investigation of the genetic susceptibility to the most common multifactorial auto-immune disease, rheumatoid arthritis (RA)

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Introduction: RA's prevalence is 0.4 % in the European population, with a sex ratio of 1:3. The monozygotic concordance rate of 12% is 3 times higher than that of same-sex dizygotic twins and the sib recurrence rate is 2-10. RA incidence in first degree relatives (FRD) is 100/100.000/year (mean age of onset at 46 years). Out of 30 RA genetics factors, 2 have been associated with genes, *HLA-DRB1* and *PTPN22*. Only one environmental factor has been definitely identified, smoking. Those factors explain about half of the increased familial risk, leaving new factors to be identified.
Aim: To set up a large European cohort of RA un-affected FDR for direct investigation of RA genetic susceptibility.
Methods: Recruitment was performed in Switzerland and France. A yearly internet health questionnaire was used, checking RA diagnosis with the Rheumatologist in charge. Yearly blood and hair samples were obtained.
Results

Characteristics at inclusion (N = 1077 since 2011)	Switzerland (N = 663)	France (N=414)
Age [years] : mean (SD)	41 (15)	48 (15)
Sex [%] : Female	73	74
Ethnicity [%]: Caucasians	93	99
RA relatives : mean (SD)	1.2 (0.5)	1.1 (0.4)

RA onset occurred in 2 Swiss participants, a 74 years old female non-smoker and a 56 years old male smoker.
Conclusion: We have set up a cohort of RA FDR, suitable for direct investigation of RA susceptibility. As 10% of the population has an increased RA risk, having a RA first or second degree relative, the cohort is extended to other family members, to search for new factors and initial biological events that could shed light on the susceptibility mechanisms.

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J16.18

Drug transporter and metabolic enzyme gene variants and fluvastatin adverse drug reactions in renal transplant recipients

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Background. Statin use in transplant recipients had been hindered by concern about adverse drug reactions (ADRs). Polymorphisms in genes encoding metabolic enzymes and drug transporters could be valuable predictors of fluvastatin ADRs. Fluvastatin is substrate of CYP2C9 and drug transporters ABCB1, ABCG2 and to a lesser extent of OATP1B1 and MRP2. Methods. 52 renal transplant recipients (RTRs) that experienced fluvastatin induced ADRs and 52 control patients, were enrolled in the study. Blood samples of all participants were genotyped for *CYP2C9*2,*3; ABCG2* 421C>A; *ABCB1* 2677C>T/A, 3435C>T, 1236C>T; *SLCO1B1* 388 A>G, 521T>C; MRP2 -24C>T, 1249G>A by means of Real-Time PCR methods. Results. We found that variants of *ABCG2* 421C>A (p=0.005), *CYP2C9*3* (p=0.012) and *ABCB1* 1236C>T (p=0.05), were associated with fluvastatin ADRs. Genotypes *ABCG2* 421CA, *CYP2C9* *1/*3 and *ABCB1* 1236CC were statistically significantly more prevalent in the group of patients with adverse effects than in controls. Our results showed that polymorphism of *ABCG2* gene (OR=3.81; 95% CI=1.27-11.45) is of more importance than of *CYP2C9* (OR=2.44; 95% CI=1.05-5.71) and *ABCB1* 1236C>T (OR=2.38; 95% CI=1.04-5.47) in a subgroup of RTRs, being different from results of pharmacokinetic studies on healthy volunteers. Carriers of *CYP2C9* mutant alleles (*2, *3), who had inhibitor in their therapy, had more than six times greater odds of having adverse effects than those without inhibitor in their therapy (OR=6.59; 95% CI=1.24-35.08).
Conclusions. *ABCG2*, *CYP2C9* and *ABCB1* gene variants could be valuable predictors of fluvastatin ADRs. In real clinical settings pharmacogenetic predisposition could be of even more importance than in healthy volunteers.

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J16.19

Sex and ESR1 genotype may influence the response to treatment with donepezil and rivastigmine in patients with Alzheimer's disease

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Many factors could be responsible of the different response to treatment with cholinesterase inhibitors (ChEIs) donepezil and rivastigmine among late-onset sporadic Alzheimer's disease (AD) patients. Sex and the variants of the estrogen receptor α (ESR1) gene are reported to modulate the susceptibility to AD or the course of the disease.
Aim of the present study was to verify whether the two above factors could influence the response to ChEIs treatment, as there is evidence that estrogens affect cholinergic system functioning.
Two ESR1 intronic polymorphisms (PvuII, or rs2234693; XbaI, or rs9340799) were examined in 184 AD patients. 157 were treated with donepezil or rivastigmine and were compared with no treated subjects. The cognitive status was quantified using the the Mini Mental State Examination.
Females responded markedly to treatment with ChEIs, and the effects were statistically significant for donepezil and at borderline significance for rivastigmine. A significant effect of ESR1 genotypes was observed for treatment with donepezil, as treated patients carrying at least one copy of P and X alleles showed a significantly lower cognitive decline than non-carriers.
Present data seem to confirm the role of sex in AD, as women seem to be more sensitive to treatment and found to have benefits with regard to cognitive decline. ESR1 may be another gene contributing to to inter-individual variability in response to ChEIs.

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J16.20

Marked differences of haplotype tagging SNP distribution, linkage, and haplotype profile of APOA5 gene in Roma population samples

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Polymorphisms of the apolipoprotein A5 (APOA5) gene have been found to play an important role in the development of diseases like metabolic syndrome, stroke and cardiovascular disease. We examined four major haplotype tagging variants (rs2072560 [T1259C], rs2266788 [IVS3+G476A], rs3135506 [C56G/S19W], rs662799 [T-1131C]) of the APOA5 gene in pooled DNA of healthy Roma (Romani, Gipsy) and Hungarian population samples to determine the genetic variability, the haplotype profile and linkage of APOA5. We analyzed 366 healthy Roma and 404 average Hungarian DNA samples using PCR/RFLP assay. For T-1131C, T1259C, IVS3+G476A and C56G/S19W we found elevated plasma triglyceride levels in the risk allele carriers compared to the non-carriers in both populations We found that the minor allele frequencies in Roma population were significantly higher

compared to the Hungarians (-1131C: 13.5% vs. 5.32%, IVS3+476A: 6.32% vs. 3.59%, 56G: 9.34% vs.4.70%, p<0.05), except the T1259C variant (6.69% vs. 7.55%). Furthermore, the haplotype analysis revealed significant increase of the APOA5*2, APOA5*4 in Romas (5.4 vs. 3.2; 6.3 vs. 2.0, p<0.05), while the APOA5*5 in Hungarians (0.1 vs. 4.1, p<0.05), but we did not found difference in APOA5*1 and APOA5*3. We found different linkage disequilibrium between the IVS3+G476A and C56G/S19W variants in Roma population (97%) compared to the Hungarians (42%). The data presented here show profound differences in the APOA5 genetic profiles in the Roma population, which likely has also clinical implications in respect their possible role in the development of certain diseases, taking to consideration the differences of triglyceride level changes associated with the minor alleles.

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J16.21

APOE E4 allele - the negative predictor of longevity

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The apolipoprotein E (APOE) gene is one of the rare candidate genes consistently found to be associated with the longevity. Its product, ApoE enzyme, is essential for the normal catabolism of total and LDL-cholesterol, and therefore highly correlates with the risk for the development of atherosclerotic cardiovascular disease (CVD) and dementia. We tested the association of APOE gene epsilon polymorphisms (combinations of rs429358 and rs7412) with longevity, CVD risk factors and CVD endpoints in the Croatian 80+ population (80-101 yrs; mean=88.28 yrs; N=324; 74.4% of women; 65.1% hypertensives). None of our E44 genotype carriers was older than 95 years (p=0.034), which suggested E4 being a risk allele that does not contribute to longevity. This result was confirmed by the meta-analysis of E4 allele frequencies in general population compared with 80+ yrs population: E4 allele was more frequent in younger people in all 14 tested European populations (p<0.001). In addition to meta-analysis, we found correlations between genetic and geographic distances across Europe (p<0.001).
Considering the other tested CVD risk factors, all E4 carriers had waist/hip ratio ≥0.80 (p=0.034). At the same time E2 carriers less frequently had elevated LDL-cholesterol levels (p=0.047) and diastolic blood pressure (p=0.033), in comparison with non-carriers.
Our findings confirm previously reported deleterious effect of E4 allele to the longevity.

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J16.22

Founder Effect of Fanconi Anemia, Complementation Group G In East Asian Using Haplotype Analysis of Korean Population

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Fanconi anemia (FA) is a rare disorder characterized by physical abnormalities, bone marrow failure, increased risk of malignancies, and cellular hypersensitivity to DNA cross-linking agents. We previously demonstrated that founder mutations of *FANCA* and *FANCG* account for most of Korean FA like as described in Japanese FA. In this study, we performed haplotype analysis and investigated the founder effect in Korean FA-A and -G using microsatellite markers designed by Yagasaki H. The patients were 12 FA-A and 11 FA-G patients and their family members who have at least one founder mutation; c.2546delC or c.3720_3724delAAACA of *FANCA* and c.307+1G>C or c.1066C>T of *FANCG*. Haplotype analyses only revealed significantly shared haplotypes in both c.307+1G>C and c.1066C>T of *FANCG* as well as c.1589_1591delATA identified in unrelated Fanconi anemia, complementation group G (FA-G), indicating the presence of founder effects. But all FA-A patients did not share same haplotypes, suggesting that the mutation occurred independently on two different alleles and originated from different ancestors. Although *FANCA* may be a primary candidate for screening in FA patients, it is difficult to explain the founder effect in Korean and Japanese FA-A because of the diversity of the mutation profile, absence of a mutation-clustered region, and variously ethnic immigration. Haplotype analysis by means of 8 microsatellite markers spanning the *FANCG* locus indicates that c.307+1G>C (type I), c.1066C>T (type II), and c.1589_1591delATA (type III) are in complete association with distinct ancestry haplotypes.

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J16.23

Association of a G6PC2 gene variation with fasting plasma glucose level in elite athletes

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Glucose-6-phosphatase type 2 enzyme is likely to be involved in glucose-stimulated insulin secretion in pancreatic beta cells. Blood glucose levels were reduced in G6pc2 knockout mice (1). G6PC2 gene G/A polymorphism in intron 3 (rs560887) was shown to be associated with fasting plasma glucose levels, but not with type 2 diabetes risk (2). The aim of the study was to compare genotype and allele frequencies of the G6PC2 gene polymorphism in 220 elite Russian athletes and 837 non-athletic controls, and to investigate the association of the G6PC2 gene G/A polymorphism with fasting plasma glucose levels and its changes while performing physical exercise to failure in 60 elite athletes involved in cross-country skiing. Genotyping was performed by RFLP-PCR analysis. The concentration of glucose in blood serum was determined by enzymatic colorimetric method before and after step-increase load on a treadmill. Frequencies of the G6PC2*A allele (17.3 vs. 25.7%; P = 0.0003) and G6PC2*AA genotype (3.6 vs. 6.6%, P = 0.001) were significantly lower in a group of athletes compared with controls. Carriage of G6PC2*A allele was associated with significantly lower level of blood glucose in a group of cross-country skiers (P = 0.02), as well as with lower increase in blood glucose level after a step-increase load to failure (P = 0.05). These data suggest that G6PC2 gene G/A polymorphism due to its functional role might be associated with athletic performance and therefore with elite athlete status.
1. Pound et al. Diabetes. 2012. doi:10.2337/db12-1067
2. Bouatia-Naji et al. Science. 2008. 320(5879):1085-8

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J16.24

Association of glutathione S- transferase M1 and T1 polymorphism with male infertility and possible protective effect of P1 polymorphisms in Tunisian infertile men

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Introduction: Genetic causes are responsible of 60% of cases of idiopathic male infertility. Polymorphisms of genes that encode Glutathione S-transferases (GSTs), a group of phase II enzymes that detoxify electrophiles, can affect the biotransformation of toxic compounds to which the male productive system is exposed. Some reports attested the association of GSTs gene polymorphisms with male infertility. In order to investigate whether there is an impact of genetic variations of GSTs on semen quality and male fertility, we studied three genetic polymorphisms in GSTT1, GSTM1 and GSTP1 in infertile men and controls from Tunisia.
Methods: Participant's were 159 men with idiopathic infertility and 102 presumed fertile men. Basic semen analysis was performed including total sperm count and concentration, motility and morphology. Genotyping of GSTM1 and GSTT1 polymorphisms were performed using the multiplex PCR. The GSTP1 Ile 105 Val polymorphism was determined using PCR-RFLP.
Results: GSTM1 null genotype (GSTM1 0/0) was significantly associated with reduced sperm count in infertile men semen (oligozoospermia) (P=.001) and GSTT1 null genotype (GSTT1 0/0) was significantly associated with low sperm motility (P=.001). However, infertile men had a higher prevalence of the wide type of GSTP1 allele (GSTP1 Ile 105) than the fertile group (80.5% and 72.54%, respectively; P=.034) and the presence of the homozygote mutant genotype (GSTP1 Val/Val) was less common in infertile men than in fertile group.
Conclusion. Our results suggest that both GSTM1 and GSTT1 gene polymorphisms have a negative impact on semen quality and are associated with male infertility in Tunisia.

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J16.25

NOD2/CARD15 gene in Moroccan population

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Background: IBD (Crohn's disease and Ulcerative Colitis) are chronic and multifactorial diseases of the gastrointestinal tract. Till now, their pathogenesis remains unclear. They involve innate immunity, environmental component and genetic predisposition. Polymorphisms in NOD2/CARD15 have been implicated in Crohn's disease in several ethnic groups. The purpose of our study was to assess the frequency of the three major variants of this gene (Leu1007fsinsC, Arg702Trp, and Gly908Arg) in Moroccan IBD patients and to determine a possible effect of these variants on Disease's phenotype and clinical course.
Methods: A total of 96 Moroccan unrelated IBD patients and 114 healthy controls were genotyped (PCR-RFLP method) for the three main polymorphisms.
Results: In this study, no correlation was found between NOD2/CARD15 polymorphisms and ulcerative colitis or Crohn's disease in our population. Nevertheless, 32629insC variant was associated to a structuring behaviour on CD patients.
Conclusion: These findings suggest that NOD2/CARD15 influences disease behaviour but not susceptibility to crohn's disease in Moroccan IBD patients.

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J16.26

Molecular characterization of the genotype of sickle cell disease patients in the state of Rio de Janeiro, Brazil

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Sickle-cell disease (SCD) is a multisystem disease and is one of the most common severe monogenic disorders worldwide. SCD manifests itself as a chronic hemolytic disease with acute vaso-occlusive complications that require frequent hospitalizations and therefore represents a substantial burden to national healthcare systems. In Brazil, the geographical distribution of the disease is broad and in many Brazilian Blood Centers the diagnosis is made only by electrophoresis and chromatography tests. Thus, the objective of this study was to characterize the genotypes of sickle cell disease in the second state with highest frequency of SCD patients in Brazil. The study evaluated 1250 patients from Institute of Hematology - HEMORIO/ Rio de Janeiro. We performed electrophoresis in alkaline pH and acid pH, high performance liquid chromatography (HPLC) and molecular analysis using PCR-RFLP for identification hemoglobin (Hb) S and Hb D-Los Angeles and PCR-EA for identification of HbC and beta thalassemia mutations (IVS-I-110, IVS-I-6, IVS-I-1 e CD39). The genotypes found were 992 (79.4%) patients homozygous for HbS, 165 (13.2%) with HbSC disease, 73 (5.8%) with interaction HbS/beta thalassemia, 14 (1.1%) with HbSD-Los Angeles disease and 6 (0,5%) with the interaction of HbS with another unidentified Hb variant. For alleles beta thalassemia, we find 14 CD39 mutations, 14 with the IVS-I-6 mutation, 7 with IVS-110 mutation and 38 did not exhibit the four mutations assessed and require gene beta sequencing. These results show the importance of the molecular diagnosis of SCD, since the genotypic characterization assists in monitoring the disease and specific therapy for patients.

E. Belini-Junior: None. **L.S. Torres:** None. **J.V. Okumura:** None. **G.C.S. Carrocini:** None. **W.M. Barberino:** None. **R.G. Oliveira:** None. **V.U. Teixeira:** None. **D.G.H. Silva:** None. **C.L.C. Lobo:** None. **C.R. Bonini-Domingos:** None.

J16.27

Armenian Highland as a transition corridor for the spread of Neolithic agriculturists

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The routes of Neolithic migrations from the Near East are presently intensively debated among scholars of various disciplines. Recent studies suggest that haplogroup R1b1a2-M269, which is the most common lineage in the European populations, was spread with first farmers via Anatolia to Europe during the Neolithic transition. These studies, however, did not include indigenous populations from the Armenian plateau, though it has played a key role in the ancient human migrations since early Paleolithic.
We used a total of 358 Y-chromosomal data collected in three Armenian geographic groups from eastern and western parts of the Armenian plateau and comparative datasets of various European populations to assess the genetic contribution of the region to the spread of haplogroup R1b1a2-M269 north- and westward.
The frequency of this lineage in eastern Armenian populations is higher compared with eastern European populations (including Anatolia) and lower than in Western Europe. The rate of the variance and age of the R1b1a2-M269 is the highest in western Armenian population among all datasets considered. In addition, there is a strong correlation between the genetic and geographic distances of the populations studied thus reflecting the directions of pre-Neolithic and Neolithic migrations from the Near East.
In conclusion, the southwestern area of Armenian Highland deserves to be more thoroughly examined as one of the principal transition regions for the spread of first agriculturists from Levant to Europe.

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J16.28

Genome-wide CNV association study with osteoarthritis in Korean

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Osteoarthritis (OA) is a mechanical abnormality such as joints degradation. Some of causes (hereditary, metabolic, and mechanical) may lead to loss of cartilage. However, genetic studies have not identified any genes definitely conferring major risk for OA and only a small number of many genes that influence risk for OA collectively have been found to date. Copy number variations (CNVs) are quantitative structural variants including duplications and deletions. CNVs analysis is an alternative genome-wide molecular genetics approach to identify genetic aberrations underlying common complex. Given the need of more genetic information of OA and the growing implicated value of CNVs in complex disease, we performed genome-wide CNV association study in patients with OA using array comparative genomic hybridization (aCGH) method (371 cases and 467 controls).
The findings of our OA cases were compared with those in a large sample of controls. After statistical analysis, we found significant gene regions showing CNVs in patients with OA. To ensure the reliability of our CNV detection method, we experimentally validated the significant CNV using quantitative PCR (qPCR). Our data indicate that deletion of this locus is associated with OA and might be potentially important for OA.

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J16.29

A new approach to meta-analysis of population-specific sequencing association studies

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Since the joint effects of the loci in genome-wide association studies (GWAS) typically explain only a small proportion of the heritability, genetic association studies require a very large sample size to achieve the desired power and minimize the detection of false associations. Meta-analysis is an effective approach to achieve higher power in GWAS by combining summary results from multiple different studies. Although large sample sizes can help guard against false-negative and false-positive results, a major potential problem is that allelic heterogeneity across populations could lead to dramatic dilution of power to detect true positive signals. Population heterogeneity is also poised to be a major concern in sequencing studies investigating the association of low-frequency and rare variants with complex traits. The effects of population heterogeneity are likely specific to particular ancestral groups. Therefore, differences in nucleotide diversity across populations become important when looking at variants in aggregate. In this study, we propose a population-differentiated test of association with quantitative or dichotomous traits, which allows for heterogeneity of allelic effects among populations. Simulations are used to demonstrate the comparison of power between proposed population-differentiated meta-analysis and traditional population combined approach. Furthermore, a real data analysis with multiethnic Prostate cancer dataset from dbGaP are used to evaluate the performance of the proposed method. Our preliminary results indicate that the proposed method has the potential to investigate the population-specific

effect for complex disease using summary data from sequencing studies in meta-analysis.

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J16.30

HFE gene polymorphism in Sickle Cell Disease - a way to understand the phenotypic variability

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The sickle hemoglobin is a point mutation (GAG → GTG) in the *β g*lobin gene resulting in the substitution of glutamic acid by valine at position 6 of the *β* globin polypeptide chain. The multiple phenotypic expressions associated with complex genetic interactions and modifiers are not well understood. Hemochromatosis is a clinically heterogeneous condition and both genetic and environmental factors can modulate the evolution and the severity of the disease. The *HFE* gene is the more frequent gene screened in conditions that curse with iron overload, as hemoglobinopathies. Were evaluated 1256 blood samples of Sickle Cell Disease (SCD) patients from Brazil by molecular procedures. The frequency of Hb SS was 79.38%, Hb SC 13.14%, Hb S/beta thalassemia 5.89%, Hb SD 1.59% and Hb S/other variants 0.39%, that reflect the admixture of Brazilian population. The genotypic frequency of the *HFE* gene polymorphisms (C282Y, H63D and S65C) show a higher percentage of H63D heterozygotes in all Hb phenotype evaluated, mainly in Hb S/Beta Thalassemia (74.3%), Hb SS (60.6%) and Hb SC (46.7%) with significant difference (p=0.0008). The frequency of C282Y and S65C mutation were very low and without significant differences. The blood transfusion is the current treatment for SCD, and the iron overload is a consequence. The *HFE* gene profile in SCD patients improves the therapeutic strategy especially in the Brazilian multiethnic population. Identify risks of complications and understand the wide phenotypic variability of SCD will require markers of underlying processes.

C.R. Bonini-Domingos: None. **E. Belini Junior:** None. **A.M. Mach:** None. **C.C. Lobo:** None.

J16.31

Population based study of permanent teeth agenesis in Japanese

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Permanent tooth agenesis is one of the most common developmental anomalies in humans (OMIM: #106600, #604625) and may occur independently, or as one feature of a syndrome. The nonsyndromic forms may be sporadic or familial with prevalence varying by tooth type. Nonsyndromic tooth agenesis has sometimes been divided into two types: oligodontia, defined as agenesis of six or more permanent teeth, and hypodontia, defined as agenesis of less than six teeth. The number in both cases does not include absence of third molars (wisdom teeth). The purpose of this study, which was based on a large sample of 4088 Japanese school children, was to describe the occurrence of agenesis of permanent teeth. In addition, this study described and analyzed the sibling reoccurrence ratio in 71 Japanese families with tooth agenesis.
The prevalence of hypodontia and oligodontia were 6.84%(95% CI: 6.06%; 7.68%) and 0.13%(95% CI: 0.04%; 0.30%), respectively. Sibling recurrence ratio in absence of one, two, three to five, and six or more teeth were 0.245% (95% CI: 0.138%; 0.383%), 0.250% (95% CI: 0.073%; 0.524%), and 0.222% (95% CI: 0.064%; 0.476%), and 0.438% (95% CI: 0.264%; 0.623%), respectively. This suggests severe phenotype, oligodontia fitted with an autosomal dominant inheritance, whereas mild phenotype, hypodontia is most compatible with an autosomal dominant trait with incomplete penetrance and/or polygenic inheritance. In addition, we estimated a model of missing ratio of individual tooth.

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J16.32

Investigation of Type 2 Diabetes susceptibility loci in the Cypriot population

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Type 2 diabetes (T2D) mellitus is a chronic complex multifactorial disease with a substantial contribution of genetic factors that usually occurs over the age of 40 years old. It is characterized by high blood glucose levels caused by the combination of insulin resistance and relative insulin deficiency. T2D is a serious worldwide public health problem which has reached epidemic proportions and its prevalence is increasing. The disease leads to morbidity with the life expectancy being reduced, while additional implications include premature coronary heart disease, peripheral vascular disease, renal failure, stroke and amputation. Major steps have been made in the identification of T2D susceptibility loci, both through genome-wide association (GWA) scans and meta-analysis of these scans and several genetic loci have been reported with possible association. Our study is focused on the Cypriot population. We collected a representative number of diabetic and non-diabetic blood samples, extracted DNA and created the first Cypriot T2D DNA bank. Study of nineteen already established susceptibility loci (in genes *WFS1, PPARG, TCF2, KCNJ11, TCF7L2, CDKN2A/B, CDKAL1, SLC30A8, IGF2BP2, HHEX/IDE, FTO, KCNQ1, MTNR1B, NOTCH2, CDC123/CAMK1D, ADAMTS9, THADA, TSPAN8/LGR5, JAZF1*) is underway in order to examine if they are confirmed in our population. Samples were genotyped using the TaqMan technology. Data mining and interaction testing will follow in an attempt to extract additional information regarding T2D susceptibility in our population. Study results will be presented.
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J16.33

Complex analysis of association between inflammatory cytokines gene variants and essential hypertension

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Essential hypertension is a common disease with severe cardiovascular complications that are the leading cause of death in the majority of developed countries in the world. The term "essential" suggests that etiology of the disease, despite the extensive research in this field, remains unknown. It is hypothesized that inflammation and endothelial dysfunction are pathophysiological processes that are underlying the disease development.
The aim of our study was to investigate an association between candidate genes, whose products are involved in inflammation, endothelial function and blood pressure control, and essential hypertension. Nine loci in *IL1RN, LTA, TNF, IL1B, IL12B, IL10, IL6, NOS3* and *AGT* genes were genotyped in the group of 480 individuals (298 patients, 192 controls). Genotyping data were analyzed using APSampler algorithm (p-values<0.05 were considered significant). We found that carrier status of *IL1B* rs16944C allele, *IL10* rs1800872A allele and *IL6* rs1800796G allele was most significantly associated with essential hypertension (P=0.0009, OR=2.00, CI: 1.30-3.06). Carriers of *IL1B* rs16944C allele and *IL10* rs1800872A allele combination were also at the increased risk of essential hypertension (P=0.001, OR=1.97, CI: 1.29-3.01). It is worth noting that the associations observed for biallelic and triallelic combinations were more pronounced than those for the same loci when analyzed separately. Our results provide an evidence for association between cytokines genetic variants and essential hypertension. IL-1B and IL-6 are potent pro-inflammatory and pro-atherogenic cytokines, and, while IL-10 exerts anti-inflammatory action, the - rs1800872A allele of *IL10* gene is known to be associated with decreased IL-10 expression.

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J16.34

Structure of congenital malformations strictly accounting in the early neonatal period

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The birth defects were analyzed in order to study the frequency and structure of the „sentinel phenotypes" and their contribution to the early neonatal

mortality. Defects were detected in the first 6 days of life. Personalized card was filled for each patient. The diagnosis was being set in according to ICD 10. 772 diagnosed congenital defects in total, including defects „strict accountability" - 25%. The share of defects among newborns was 1.33% for the analyzed period.
Structure of congenital malformations „strict accountability": the malformations of the central nervous system (anencephaly, spinal hernia) - 9%, esophageal atresia - 3%, intestinal atresia - 2%, atresia of the anus - 3%, poly-syndactyly - 8%, reduction defects of extremities - 1%, omphalocele - 7%, gastroschisis - 2%, multiplex birth defects - 10%, Down's syndrome - 33%, other 2% chromosomal disease, cleft palate, cleft lip - 17%, achondroplasia - 3%.
Thus, study of the structure of congenital malformations will be more accurate to talk about the frequency of this disease, to monitor the dynamics of cargo of congenital disorders in the population and to take early preventive measures.

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J16.35
Consanguinity, endogamy and genetic disorders in Tunisia

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Background: Consanguineous marriages are common in many Arab countries including Tunisia. However, little information exists on their impact on genetic disorders in the Tunisian population. The objective of this study was to evaluate the current rate of consanguinity in Tunisia and to examine its impact on the occurrence of some specific genetic disorders.
Methods: Consanguinity profiles were retrospectively studied among 1121 Tunisian patients suffering from different monogenic autosomal recessive and dominant disorders and some complex conditions and compared to that of a healthy control sample.
Results: Consanguinity rates were 78.36% among autosomal recessive condition group, 38.04% among dominant group, 34.65% among multifactorial group and 29.80% among the control group with a first cousin union rate of 31.76%, 20.65%, 16.23% and 16.72%, respectively. The differences in consanguinity pattern were highly significant when comparing control group with recessive disorder group, but not significant compared to dominant or multifactorial groups. Consanguinity was associated with a nearly six-time increased risk to develop autosomal recessive disorders. This excess risk is proportional to the degree of consanguinity and the frequency of disease allele in the family. Furthermore, recessive disorders were associated with a very high level of regional endogamy (91.87%).
Conclusion: This study emphasizes the persistence of a high level of consanguinity in the Tunisian population (29.80%) and reveals a high impact of inbreeding and endogamy on the etiology of autosomal recessive diseases. In contrast, no significant association was found between consanguinity and complex disorders suggesting that these conditions are mainly influenced by environmental factors.

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J16.36
Atopic Eczema: genetic heterogeneity in European populations

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Atopic dermatitis (AD), or eczema (AE), the most common chronic inflammatory skin condition, is a complex multifactorial disease. The clinical manifestations of AE vary with age of onset (infancy/childhood, adolescence and adulthood), but common symptoms are: red blotches on the body, itchy rash that can quickly develop into painful papule. AE affects almost 1-3% of people in world-wide even if its causes are not clear. Currently the background of AE is very heterogeneous; in fact apart from the gene encoding filaggrin (*FLG*), the genes causing AE are unknown. In recent years studies on several populations have found different rare mutations (R501X, 2282del4, R2447X, S3247X, 3321delA, S2554X, S2889X and S3296X) in *FLG* gene. These mutations are prevalent in north Europe and Asia (Japan, Korea and Taiwan) and have been associated with the onset of AE. Recent case/control studies carried out on populations of south Europe (Italy, Croatia and Turkey) failed to reveal variations in *FLG* gene. These results suggest that *FLG* gene should not be considered as major risk factor to populations of south Europe. In order to confirm this hypothesis, we are resequencing the entire third exon of *FLG* gene in Egyptian and Greek populations. Preliminary genotyping of R501X and 2282del4 mutations, has failed to reveal evidence of mutations in patient samples.
These preliminary data suggest that *FLG* mutations are not common in these populations. Further experiments are needed to disclose different susceptibility genes in these populations.

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J16.37
Increased risk of hypertensive emergencies and polymorphism of the ACE gene

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Hypertensive crisis (HC) is characterized by a rapid, inappropriate, intense, and symptomatic blood pressure elevation. A hypertensive emergency is characterized by rapid deterioration of target-organs and poses an immediate threat to life, a situation not found in hypertensive urgency. Whereas the angiotensin-converting enzyme (ACE) plays an important role in regulating blood pressure through the renin - angiotensin - aldosterone system (RAAS), this study aim to analyze the insertion/deletion (I/D) polymorphism in the ACE gene, on HC. We collected blood samples, after consent, from 308 individuals, regardless of ethnicity and gender. 139 were normotensive individuals which compound the control group and the test group (169) with hypertensive crisis episodes, was divided into urgency (68) and emergency (101). The clinical data was obtained in the medical care. The DNA was extracted and polymorphism analysis performed by PCR. The genotypes polymorphism frequency evaluated in the control group was consistent with the Brazilian population. There no differences in gender frequencies. For the ACE genotypes there was a significant difference in the control and test group (p=0.00021). The II genotype differences were observed between control (21.58%), urgency (11.76%) and emergency (2.97%) groups (p= 0.0014). For the ID genotype the difference was in emergency (84.16%), urgency (77.94%) and control (61.15%) groups (p= 0.00021). There is a risk of emergency event associated with the D allele (Odds 5.41; p=0.02, CI:1.38-21.21). Based on these results and on the polymorphism frequency in Brazil, we consider essential to assess the gene, whereas hypertensive emergencies can be extremely harmful to individuals.

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J16.38
Mutation Leu40Arg of gene ITGB3 linked to mutation Leu33Pro among inhabitants of the Novosibirsk region

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The central role in realization of a primary hemostasis belongs to complex IIb/IIIa. The major mutation of gene ITGB3 - Leu33Pro, strengthening alarm functions of the given receptor and associated with development of arterial thromboses is àt the moment known. Among inhabitants of the Novosibirsk region we reveal replacement T on G in 1585 position ITGB3, leading to replacement Leu on Arg in 40 position amino acids sequences and linked to mutation Leu33Pro. In the Russian Federation Leu40Arg mutation has been given revealed earlier among inhabitants of St.-Petersburg.
At inspection of 246 healthy inhabitants of the Novosibirsk region the genital age, selected by epidemiological criteria, the Leu40Arg of ITGB3 have made frequencies of 0,0081. Thus the mutation always was in coupling with Leu33Pro and formed one of genotypes: Leu33Pro33/Leu40Arg40 or Pro33Pro33/Leu40Arg40 gene ITGB3.
Particular interest submit data received by us about authentic to higher frequency replacement T on G in 1585 position ITGB3 and genotypes Leu33-Pro33/Leu40Arg40 and Pro33Pro33/Leu40Arg40 gene ITGB3 among pati-

ents with thromboses, at children with infringement of intellectual function and in the families having reproductive problems.
The given fact confirms the importance testing mutations Leu40Arg and Leu33Pro ITGB3 at mediko-genetic consultation of families planning pregnancy.

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J16.39

Association of common KIBRA variants with cognitive performance in young adults

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KIBRA is a cytoplasmatic protein mainly expressed in the memory related structures of the brain where it has a role in synaptic plasticity and memory formation. Several studies have linked T<C substitution in the intron 9 of KIBRA gene to episodic memory performance and Alzheimer's disease risk but results still remain controversial.
The aim of our study was to investigate the association of T<C polymorphism, mentioned above, with general cognitive abilities, working memory and episodic memory of young adults. Our study was carried out on 538 students of Faculty of Medicine, University of Belgrade (mean age 20±0.8, 66.91% women). Subjects general cognitive abilities were assessed by the Raven Standard Progressive Matrices, and working memory with the Digit Ordering task maximal span scores. Episodic memory was tested with the Rey Auditory Verbal Learning Task (RAVLT) for semantically unrelated word stimuli and with Wechsler memory scale-revised form (WMS-R) Logical Memory subtest for semantically related material.
Statistical analysis showed that TT carriers had significantly better RAVLT immediate recall scores and better RAVLT delayed recall scores but without statistical significance. No association between KIBRA genotype and general cognitive abilities, working memory nor with the results of WMS-R Logical Memory subtest have been observed.
These findings suggest that KIBRA might modulate episodic memory performance specifically in case of semantically unrelated items.

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J16.40

Identify LD Blocks Using biplot function method

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In recent years methods of trait mapping based on theories of linkage disequilibrium analysis have been developing rapidly. In DNA sequences, domain hotspots exist at which recombinations have occurred briskly. Conversely, large domains with infrequent recombinations in which linkage disequilibrium is maintained also exist. Such a domain is called a haplotype block or LD block. Although the value of D' represents one of the disequilibrium parameters important for identifying LD blocks. We proposed that it's used biplot function and subspace method to identify LD block, based on technology developed by eigen value problem. We will show results and discussion on the day of our presentation.

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J16.41

First results of monitoring of health of the students - possibilities of modern genetics

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The aim of this study was to develop a method based on genetic testing for assessing health status of students of North-West Region of Russia within 4-6 years of training in higher education institution starting with the first course. For this purpose we conducted analysis of questionnaire data, laboratory tests and more than 25 genes of „hypertension" and „fibrinolysis" systems, and others like interleukins. Before participating in the research, all participants gave their informed consent.
The polymorphisms of three genes (ACE (I/D), NOS3 (4/5), TNFA (-238G/A; -308G/A) associated with some multifactorial diseases (cardiovascular diseases, diabetes) were studied by PCR-RFLP analysis in student population of North-West region of Russia (107 people). A population group consisted of unrelated individuals of age 25-45 (59 men and 58 women) - the group 2.
Distribution of relevant polymorphisms frequencies for studied genes was similar in student group compared to the group 2. Distribution of genotypes of NOS3 gene was significantly different between women students in comparison to women of group 2 (p=0.0032, df=2) for 4/4 genotype (6.2% and 1.6%, respectively). Higher frequency of D/D (ACE) genotype was found for men in student group compared to the group 2 (D/D - 40.0% and 27.6%, respectively).
The first results allow assuming that the group of students differs from population group on the frequency of genotypes associated with cardiovascular diseases. Further monitoring of health of students, analysis of data of their laboratory tests and questionnaires, and analysis of other genes will confirm or disprove this assumption.

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J16.42

Genetic variance in metabolically healthy obesity

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Common obesity is increasingly seen as a worldwide epidemic severely threatening global well being, as it is associated to a broad range of various pathologies. Some obese individuals seem, however, to be protected or more resistant to the development of metabolic abnormalities and pathologies associated with obesity. We know these individuals as "metabolically healthy but obese" (MHO). Mechanisms underlying this protective phenotype are yet unknown.
At the population level, common obesity can be seen as a complex disease which is based on limited number of gene alleles of which each allele slightly increases the risk of obesity to emerge. Even though the relative participation of environmental and genetic factors to the emergence of obesity is rather unclear, it is probable that the occurrence of certain polymorphisms makes some individuals more prone to weight gain compared to others. It is equally probable, that genetic factors affecting healthy obesity phenotype are complex by nature. While genetics of common obesity is extensively studied, studies about genetics of metabolically healthy obesity have not been done to date.
We have characterized metabolically healthy obese individuals in ten European population based cohorts and will test an interaction between genotype and metabolically healthy obesity phenotype.
Characterization of genetic and, in future also a metabolic profile of MHO individuals can lead to the better knowledge about the pathology of obesity overall. We believe that understanding genetic and metabolic differences between healthy- and sick obesity is likely to have a great impact on the obesity research on the whole.

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J16.43

Ancient mtDNA diversity in Bulgaria

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Background: To understand the biological evolution of Bulgarians, we need to clarify the changes of the Bulgarian gene pool over the times. This can be achieved by describing the genetic structure of the populations that inhabited Bulgarian lands in the past and comparing it with the results obtained from the study of contemporary Bulgarians.
Results: Using the most stringent criteria for ancient DNA analysis we analyzed 122 ancient samples from different regions of Bulgaria, dating from III

Millennium B.C. to VIII- X Century A.D. Preliminary results on 14 individuals (360 bp of HVS I sequence of Mitochondrial DNA) have shown that part of the ancient haplotypes are present among present-day Bulgarians, but we have also found haplotypes which are not observed in the contemporary population. Conclusion: Our results revealed discrepancies and overlaps between the ancient population and the modern Bulgarian mtDNA diversity.
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J16.44
Analysis of DFNB1-associated hearing loss in Tatar population (Russia)

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Connexins are the key components of the gap junction, which regulate various physiological and developmental processes. According to recent data, mutations in different connexin genes are associated with nonsyndromic sensorineural hearing loss (NSHL). Hearing loss is the most frequent in terms of incidence and prevalence. We conducted comprehensive medical and population genetic studies in seven regions of Tatarstan resulting in detection of 96 patients with congenital recessive deafness (Kukmorsky-18, Drozhzhanovsky-13, Buinsky-31, Atninsky - 6, Arsky - 8, Aktanyshsky - 11, Muslyumovsky - 9). Molecular genetic testing of patients DNA samples was carried out by sequencing the coding region of GJB2 gene. The frequency of the major mutation 35delG was 29,17%. The second frequent mutation was 313del14 (4,69%). The frequency of M34T mutation was 1,56%, the frequency of polymorphism V27I - 4,69%. The following mutations 299-300delAT, R165W, V153I, R127H, delV38, each was met with frequency of 0,52%, were revealed in the studied sample of patients with NSHL. A new deletion of three nucleotides (111-113delTGT), leading to a loss of one amino acid in the 38th position (delV38) was found. This mutation, 111-113delTGT, was detected in one patient with another mutant allele not yet identified. Two GJB2 mutations were detected in 8% patients, in 51% and 41% of patients one or none mutations, correspondently, were identified in GJB2 gene. Unexpectedly, screening of 35delG mutation in 710 healthy donors from three Tatar ethnographic groups showed a relatively high population frequency of 35delG mutation (1,19%). The further analysis of other connexins genes is planned.

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J16.45
Mutational screening for cathepsin C gene in Saudi patients with Papillon Lefevre-Syndrome

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Papillon Lefevre-Syndrome (PLS) is a rare genetic neuromuscular disease autosomal recessive disorder characterized by the development of palmar-plantar hyperkeratosis and periodontium associated with mutations in cathepsin C gene (CTSC). The disease manifestation is present during childhood characterized by severe dermal and dental manifestations.
A total of 6 families were genetically examine for the presence of CTSC gene mutation and carrier statues that included 14 PLS patients, along with their parents plus one sibling who showed no signs of PLS. Additionally, 20 healthy individuals were enrolled as study control.
The results showed that all PLS patients who participated in this study shared the same type of mutation on the CTSC gene; homozygous for c.815 G>C (p.R272P) mutation, while their parents who are first-degree cousins were all found heterozygous for the same mutation type. All control subjects in this investigation were homozygous normal.
This study is pointing into the consequence of consanguineous marriages as a major factor for increasing the risk of genetic diseases among Saudis. Additional community educational efforts should be adopted through Saudi health authorities to spread the awareness among the general population about these deformities as results of consanguinity relations.

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J16.46
The association of PPARD gene T294C polymorphism with endurance athlete status: a replication study

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Peroxisome proliferator-activated receptor delta (PPARD) regulates the genes involved in the oxidation of fatty acids, cholesterol metabolism, thermogenesis, embryogenesis, regeneration, inflammation and carcinogenesis (Furnsinn et al., 2007). The functional T294C single nucleotide polymorphism in exon 4 of the PPARD gene is located 87 nucleotides upstream of the start codon. The C allele rather than the T allele of this polymorphism has been associated with higher transcriptional activity of the PPAR-delta promoter by inducing a binding site for Sp-1 transcription factor (Skogsberg et al., 2003). It was hypothesized that the C allele is associated with increased fatty acid oxidation (endurance enhancing factor). Recently we have shown that the PPARD C allele is over-represented in Russian endurance athletes (Ahmetov et al., 2007). The aim of the study was to investigate the distribution of genotypes and the frequency of alleles of the PPARD gene in athletes (n=430) and controls (n=677) and thus replicate recent findings in independent cohorts. Genotyping was performed by PCR-RFLP. The frequencies of the C allele and CC genotypes in endurance-orientated athletes were significantly different from controls (n=106, 20.3 vs. 13.0%, P=0.0074; 6.6 vs. 3.1%, P=0.0262). Furthermore, the distribution of genotypes in athletes involved in sports games (n=114, P=0.0075) was significantly different from the controls. The distribution of genotypes and the frequencies of alleles in power-oriented athletes and a whole cohort of athletes were not different from control. Thus, we confirm that the PPARD gene T294C polymorphism is associated with endurance athlete status in Russians.

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J16.47
Role F2, F5, AGT, ENOS and MTHFR genes in the development of preeclampsia

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Preeclampsia is one of the most frequent and complications of pregnancy. The risk of repeated development of a preeclampsia can be raised in case of a carried of some polymorphisms in genes of F2, F5, AGT, eNOS and MTHFR.
The present study might suggest roles for genes F2, F5, AGT, eNOS and MTHFR in the development of preeclampsia.
Polymorphic variants were analyzed of 142 pregnant women with preeclampsia using PCR followed by RFLP analysis.
Analysis of F2, F5, AGT, eNOS and MTHFR genes allelic variants of preeclampsia. Following genotypes have been identified: GG (3%), GA (97%) for F2 gene 20210G>A; GG (42%), GA (58%) for F5 gene 1691G>A; CC (3%), CT (76%), TT (21%) for eNOS gene 786C>T; CC (64%), CT (33%), TT (3%) for AGT gene 521C>T; CC (39%), CT (55%), TT (6%) for MTHFR gene 677C>T; AA (39%), AC (49%), CC (12%) for MTHFR gene 1298A>C.
Although the exact genes involved in development of preeclampsia are still not discovered, an important role for genes F2, F5, AGT, eNOS and MTHFR in its pathogenesis is accepted.

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J16.48
Genetic variation of 15 autosomal STR loci in Bulgarians

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Recently the understanding of the genetic variation of Bulgarians has improved in different aspects. Still, despite its forensic application and importance for inferring population structure and affinity, the autosomal STR variation in Bulgarians has not been studied. In order to fill this void we have analyzed 128 blood DNA samples from healthy, unrelated Bulgarians from across the country. They were genotyped for 15 autosomal STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA). The amplification was performed using the AmpFlSTR Identifiler PCR Amplification Kit. DNA fragment analy-

sis was done on ABI 310 Genetic Analyzer and the results were processed with GeneMapperID software.
Among loci, the power of discrimination ranges from 0.835 for TPOX to 0.977 for D2S1338 and the polymorphism information content ranges from 0.65 for TPOX to 0.93 for D2S1338, which hints that D2S1338 is the most polymorphic and discriminating locus.
The combined power of exclusion for all the loci is estimated as 0,999999880570892 and the combined match probability as 5.73472982535149 x 10-18 (or 1 in 1.7 x 1017).
Our results indicate that the set of 15 STR loci we have analyzed is a useful and powerful tool not only for personal identification and parentage testing in Bulgarians but also for revealing different aspects of their genetic structure and relationships to other populations.

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J16.49
Common polymorphism in glutathione-S-transferase theta 1 gene and its association with dietary vitamin C in the Central-European population

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Background: Glutathione S-transferases (GSTs) represent an important group of isoenzymes that play a pivotal role in the detoxification of carcinogens. It is also known that GSTs largely contribute to the glutathione-ascorbic acid (vitamin C) antioxidant cycle. It has been reported previously that the polymorphisms in GSTs influence the dietary intake of vitamin C. The aim of the study was to determine whether GST genotypes modify the dietary intake of vitamin C and general eating behaviour with respect to food items containing vitamin C in the Central-European population.
Material and Methods: Nonsmoking men and women [n = 764, 309 non-obese ones (78M/231F) and 455 obese ones (120M/355F)] between 16 and 80 y of age were participants in the study. The common polymorphisms in GSTM1 and GSTT1 and an Ile105Val substitution in GSTP1 were genotyped. A FFQ as well as 7-day food records were used to estimate dietary vitamin C intake and basic anthropometric parameters (% of body fat, etc.) were also measured.
Results: In the multivariate regression modelling across the whole cohort, neither GSTM1 nor GSTP1 polymorphisms expressed any prediction role for the native dietary structure (% of macronutrients) or dietary intake of vitamin C. In GSTT1 polymorphism, however, the wt allele was associated with significantly lower dietary intake of vitamin C (88.6±57.6 for males, 94.0±54.0 for females) than the del allele (109.0±85 for males, 100.4±61 for females, p = 0.04).
Discussion: In our cohort, we observed significant association of the GSTT1 polymorphism with native dietary intake of vitamin C.

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J16.50
Frequency of MYBPC3 gene mutations in hypertrophic cardiomyopathy patients from North-West of Iran

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Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiovascular disease with a prevalence of 1 in 500 general populations. The disease is the major cause of sudden cardiac death in the young and morbidity in the elderly individuals. HCM is inherited as an autosomal dominant single gene disease, characterized by unexplained ventricular myocardial hypertrophy. Cmybc is a sarcomeric thick filament protein that interacts with titin, myosin and actin to regulate sarcomeric assembly. Mutations in MYBPC3 gene are one of the most frequent genetic causes of the HCM disease. The aim of the present study was to investigate the frequency and kind of MYBPC3 gene muations in the population of north-west of Iran. DNA was extracted from 42 HCM patients by salting out method after obtaining informed consent. All exons and exon-intron flanking regions of MYBPC3 gene were evaluated by PCR-SSCP assay. A cohort of 42 index patients with mean age onset of 38 including 25 familial, 10 sporadic and 8 inconclusive cases were examined. Twelve mutations (28%) were identified including three frameshift, 2-splice site, 1 nonsence and 6 missense mutations that four of them were novel. The onset mean age was 35 in patients carrying MYBPC3 mutations. In conclusion, screening of genetic variation in MYBPC3 is useful tool for management of HCM in North west of Iran population.

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J16.51
The association analysis of lipid metabolism gene polymorphisms with BMI, waist circumference and blood lipidogramm parameters in women

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Using the PCR-RFLP method we have studied polymorphism of 36 genes involved in lipid metabolism in 212 women, residents of St. Petersburg, aged 18 to 77. We found an association of polymorphisms in several candidate genes with body mass index (BMI), waist circumference (WC) and total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) levels. We proposed a logistic regression models for a primary assessment of these parameters in women based on corresponding genetic markers tests. We estimated the efficiency of these models on the basis of the adjusted determination coefficient (adjusted R2). The highest rates of adjusted R2 are received for the VLDL-C parameter (R2=0.101) with the smallest forecasting error (±0.21 mmol/litre) in comparison with an forecasting error of the BMI (±4.1 kg/m2), WC (±10.33 cm), TC (±0.95 mmol/litre) and the LDL-C levels (±0.83 mmol/litre). After the division of the group into premenopausal and postmenopausal women it emerged that adjusted R2 was higher in the group of postmenopausal women in comparison with the group of premenopausal women for all studied parameters: BMI (R2=0.44 and R2=0.043), WC (R2=0.38 and R2=0.098), TC (R2=0.32 and R2=0.143), LDL-C (R2=0.33 and R2=0.112), VLDL-C (R2=0.43 and R2=0.043), respectively. Our results apparently indicate higher genetic determinacy of the VLDL-C in comparison with other parameters, and also indicatethat after the menopause genes make more contribution to levels of studied parameters than environmental factors. Complimentarily, we performed Kendall's correlation analysis, which partially confirmed the association found with thelinear regression model.

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J16.52
Molecular characterization of deletional forms of beta-thalassemia in Antalya, Turkey

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Beta-thalassemia is the most common hemoglobinopathies with a high frequencies (13%) in Antalya. It is caused by the different types of mutations of beta-globin gene. In this study, we aimed to report the different types identified of deletional mutations and the methodological approaches to them in beta-thalassemia.
Beta-thalassemia carriers who were referred from Thalassemia Unit and AKHAV were screened by HPLC in 1171 postnatal cases in Molecular Genetic Laboratory, in this study. Following gDNA extraction, beta-globin gene was amplified by PCR, and mutations were screened by RDBH, DNA sequencing, MLPA and Gap-PCR.
Seven different types (Cod8 (-AA), Cod5 (-CT), Cod44 (-C), Cod 37/38/39 (-GACCCAG), FSC 22/23/24 (-AAGTTGG), FSC 74/75 (-C) and Turkish type inv/del) of deletional mutations were found in beta-globin gene in Antalya. Cod44 (-C) was the most common deletional mutation with frequency of

4.8%. One of the mutations is located at 5'-control region, three were on exon-1, remaining three were on the exon-2 of beta-globin gene. When the deletions of one, two and seven nucleotides were caused the HbA2 increasing, Turkish type inv/del that was detected by MLPA and Gap-PCR.increased the HbF level.
Our data showed seven different types of deletional mutations in beta-globin gene in Antalya population. No any deletional mutation on exon-3 may be an important data for the function of the gene. In conclusion, our results confirm that cases who could not be detected the mutations by classical molecular genetic tests should be screened by techniques such as MLPA and Gap-PCR for large deletions.

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J16.53

Association of MRAS and HNF1A gene polymorphisms and coronary artery diseases in the Tunisian population

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Cardiovascular diseases are the leading causes of death in Tunisia and in the world. Coronary artery diseases (CADs) are complex disorders where environmental factors interact with particular genetic susceptibility background to trigger their pathogenic mechanisms.
To unravel some of the genetic aspects of these diseases, we studied the effect of MRAS and HNF1A genes on the occurrence of CADs in Tunisia since they encode proteins involved, respectively, in the cell transformation, proliferation and adhesion representing key-steps of atherosclerosis, and a transcription factor (HNF1A) in the regulation of the transcription of several genes encoding CRP, fibrin, angiotensin,...
We investigated the association of single nucleotide polymorphisms (SNPs) rs98188870 (MRAS gene) and rs2259816 (HNF1A gene) in Tunisian patients presenting a coronary artery disease compared to normal controls. Genotyping of all participating individuals was performed using mutagenically separated polymerase chain reaction.
Considering the dominant pattern of inheritance for SNP rs9818870 (MRAS gene), carriers of T allele (TT+TC) have significantly lower risk of myocardial infarction (MI) (OR= 0,59 ; 95% CI [0,36-0,95]) when compared to homozygous CC.
However, the polymorphism rs2259816 (HNF1A gene) was significantly associated with CADs, (p=0,019) since individuals carrying the A-allele are at a higher risk for CADs (OR = 1,51 ; 95% CI=[1,07-2,14]). Interestingly, the A-allele was significantly associated with a higher risk of developing CADs within non smokers.
In conclusion, in the Tunisian population the T-allele (SNP rs9818870, MRAS gene) seems to have a protective role against MI. However, A-allele (SNP rs2259816, HNF1A gene) is a risk factor for CADs.

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J16.54

Population carrier screening for Cystic Fibrosis: Puzzles to be solved

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Cystic fibrosis (CF) is the second more frequent genetic disease in Greece following Thalaessemia. Carrier testing in general population is growing as an increasing number of obstetricians includes CF test in the antenatal test list.
Because of the great genetic heterogeneity the mutation detection rate ranges between 75% - 95% in different laboratories.
Carrier screening is offered to separate partners or couples planning a pregnancy, or during pregnancy. Some of them report family history, while the majority ask the test for prevention reasons only. DGGE and recently HRM analysis are used for the scanning of multiple exons and their corresponding intron boundaries of the CFTR gene. Eleven exons are scanned routinely covering about 85% of CFTR gene mutations identified in the Greek population. Carriers are offered genetic counselling and partner testing while couples at risk are offered prenatal diagnosis. The recorded results are from January 2005 to December 2012.
A total of 5500 individuals were screened and 207 carriers (3.8%) were identified. All carrier partners were screened, and subsequently 3 carrier couples were identified.
Twenty five mutations, seventeen polymorphisms and 5 novel alterations are recorded.
Intriquingly, scanning methods, in contrast to commercially available kits, may reveal novel non described gene alterations with unpredicted significance, rendering interpretation and genetic counseling rather complex. Additional questions emerge concerning the pathogenicity of alterations already referred to as mutations (F1052V, D1312G), due to their higher incidence in general population relatively to their corresponding frequency in CF chromosomes.

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J16.55

Population analysis of non-syndromic sensorineural deafness in Mexico: profiles of its genomic distribution.

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Mexico is a multiethnic and multicultural country. Deafness is the 2nd most common disability in some geographical regions and has predominated for more than a century. Mutations in GJB2 gene cause most cases of non-syndromic sensorineural hearing loss. In this study, 104 families were studied to determine the geographical distribution and type of causative mutations in four regions of the center-west of the country. Except for a northern region, the rest of regions were in Hardy-Weinberg equilibrium. Taking in account the Rh gene frequency and ABO, Fy and MN systems we found admixture of Amerindians (78% to 85%), Europeans (13% to 20%) and Africans (1% to 2%). Six mutations were found to be present (three transitions, two transversions and one deletion: c.250G> A, c.132G> A, c.79G> A, c.427C> T, c.91T> A and c.35DelG). The wild type frequency was 43%. Only one case showed alterations in GJB6 gene and other one showed no mutation.

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J16.56

GJB2 and GJB6 genes mutations associated with prelingual nonsyndromic hearing loss

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Introduction: The DFNB1 locus accounts for approximately 50% of congenital, severe-to-profound, autosomal recessive nonsyndromic hearing loss. Mutations in the GJB2 and GJB6 are associated with deafness at the DFNB1 locus. More than half of Caucasians with two identifiable GJB2 mutations are homozygous for the c.35delG point mutation. Approximately 2% of individuals with DFNB1 have one identifiable GJB2 mutation and one of two large deletions that include a portion of GJB6. In this respect, our aim was to use molecular testing of children at risk for confirmation of diagnosis and early intervention. Material and Methods: The study was carried out on 310 children with prelingual hearing loss. The 35delG GJB2 mutation was detected by ARMS-PCR using primers for normal and mutant alleles. We included beta-actin sequence detection as internal control. Analysis of del(GJB6-D13S1830) and del(GJB6-D13S1854) was performed by multiplex PCR. The GJB2 gene sequencing was carried out on 27 children. Results: 35delG mutation was detected in 36.12% of the 310 children tested. Homozygous status for 35delG mutation was found in 110 patients and 2 patients were heterozygous for this mutation. None of 310 children was found to have GJB6 mutations. GJB2 gene sequencing was performing for 27 children with negative results for the three mentioned mutations.The GJB2 gene sequencing revealed the recessive splice site IVS1+1G>A mutation in a homozygous status in two children.
Conclusions: Our results were in accordance with the main causes of congenital hearing loss allowing early detection and timely intervention at children with hearing impairment.

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J16.57

Analysis of GSTP1 and GSTA1 gene polymorphisms in Polish population using pyrosequencing.

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Glutathione S-transferases (GSTs) are a family of enzymes that play an important role in detoxification of many hydrophobic and electrophilic compounds including drugs, xenobiotics and carcinogens by catalyzing the conjugation with reduced glutathione. *GSTA1* gene encodes a glutathione S-transferase belonging to the alpha class (α-GST), which is dominant in the liver. Product of the *GSTP1* gene is a pi class glutathione S-transferase (π-GST) also present in the liver. These proteins level measurements are used in multiple studies as a hepatotoxicity indicator of different anaesthetics, including sevoflurane. Both of genes selected for analysis are known to be highly polymorphic. Genetic variations can influence for the GST level and an individual's susceptibility of the toxicity and efficacy of drugs detoxicated in the liver, including sevoflurane.
As a first step the aim of our study was to determine the frequency distribution of nucleotide changes -52C/T (rs3957356), -69G/A (rs3957357) and -567A/C (rs4715332) in *GSTA1* promoter region and 313A/G (rs1695), 341C/T (rs1138272) in *GSTP1*, known to decrease the enzymes activity, in the Polish patients under sevoflurane anaesthesia. We analyzed DNA samples from 135 patients anaesthesized with sevoflurane using nested-PCR and pyrosequencing as a genotyping method. Allele frequencies estimated in our study for *GSTA1* gene were: 44,6% (-52T), 20,5% (-69A), 1,5% (-567C). Allele 313G and 341T in the *GSTP1* gene were identified with frequency 36% and 13% respectively. The obtained results in our study are similar to the determined in others European populations based on the literature data.

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J16.58

Marked differences of haplotype tagging SNP distribution, linkage, and haplotype profile of IL23 receptor gene in Roma population samples

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Polymorphisms of the interleukin-23 receptor (IL23R) gene have been found to play an important role in the development of several autoimmune diseases (ankylosing spondylitis, inflammatory bowel diseases, psoriasis, Sjögren syndrome, systemic lupus erythematosus). We examined five susceptible (rs10889677, rs1004819, rs2201841, rs11805303, rs11209032), one protective (rs7517847) and two neutral variants (rs7530511, rs1884444) of the IL23R gene in pooled DNA of healthy Roma (Gipsy) and Hungarian population samples. Our aim was to determine the genetic variability of the major haplotype tagging polymorphisms, and the haplotype profile of IL23R between the two groups. A total of 273 Roma cases and 253 Hungarian controls were genotyped using PCR/RFLP assay. Comparing the five susceptible conferring alleles, there were significant increase ($p < 0.05$), while in the protective alleles, there were decrease in the allele frequencies in Roma population ($p < 0.05$). One of the neutral alleles showed increase, the another one did not differ between the two groups. The haplotype analysis of the SNPs revealed fundamentally different association types of SNPs in the two groups; moreover, the frequencies of the various haplotypes also exhibited strong differences, as of ht4 and ht5 haplotypes were significantly higher, whereas the frequencies of ht2 and ht3 haplotypes were significantly lower in the Roma population than in Hungarians ($p< 0.05$). The data presented here show profound differences in the IL23R genetic profiles in the Roma population, that likely has also clinical implications in respect their possible role in the development of certain immunological diseases.

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J16.59

Analysis of mitochondrial mutations related to hearing loss in Brazilian patients using mass spectrometry

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Sensorineural hearing loss is the most common cause of sensory loss affecting at least 1 every 1000 births and presenting a diverse etiology. Among the genetic causes, mitochondrial mutations are present in about 1% of prelingual hearing loss in children, and reaching 5% in post-lingual hearing loss in the Caucasian population, thus being the second most common cause, behind only the 35delG mutation in the GJB2 gene. So far 61 mutations in mtDNA have been reported as possible causes of deafness, and the analysis of all these changes by conventional techniques such as PCR or sequencing generates a high cost and demand a very long time, so it is necessary new techniques for analyzing large amounts of data. The use of platform mass spectrometer - Sequenom, Inc. (San Diego, CA) using the technique MALDI-TOF MS (Matrix Assisted Laser Desoption Ionization-Time of Flight Mass Spectrometry) has important advantages for high-throughput analysis as the Multiplex PCR. Therefore, the objective of this study was to trace the 52 main mutations in mtDNA related with hearing loss through mass spectrometry. Our sample was consisted of 50 Brazilian patients with non-syndromic sensorineural hearing loss and 50 controls. The results have presented 11 patients with the A827G mutation, five patients with the A1555G mutation and 1 patient with G15927A and G8363A mutations. The Mass Spectrometer platform proved highly efficient for the analysis of 5200 changes simultaneously with a cost and time significantly shorter than traditional techniques.

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J16.60

Study of F2, F5, MTHFR, MTR, MTRR genes in association with recurrent miscarriage

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In order to determine the frequency of polymorphic alleles of genes F2, F5, MTHFR, MTR, MTRR in the development of miscarriage were studied the 132 women with recurrent abortions. Single nucleotide polymorphisms - C677T and 1298 A> C gene MTHFR, G1691A gene F5, G20210A gene F2, A2756G gene MTR, as well as 66 A> G MTRR gene were typed by PCR.
The frequency of homozygotes for the MTHFR C677T favorable variant - 44.1% (C / C), the frequency of heterozygotes - 55.9% (C / T). The frequency of homozygotes for the favorable variant of the gene MTHFR (1298 A> C) is 44.2% (A / A), homozygous for mutant variants - 2.9% (C / C), the frequency of heterozygotes - 52.9% (A / C).
F5 G1691A: frequency of homozygotes for the favorable variant is 58, 3% G / G), the frequency of heterozygotes - 42, 2% (G / A). Homozygous for the favorable gene variant F2 G20210A - 25% G / G, the frequency of heterozygotes was - 75% GA. MTR (A2756G): the frequency of homozygotes for the favorable variant - 13.8%, the frequency of heterozygotes was 86.2%. MTRR (66 A> G): the frequency of homozygotes for the favorable option was 13.50%, 81.1% was heterozygous, homozygotes for mutant variants amounted to - 5.4%.
In conclusion, polymorphic alleles of the gene (MTHFR 1298S, MTRR 66G) have a greater impact on the development of disease in comparison with others (MTHFR 677T, MTR 2756G) and their combination increases the risk of recurrent miscarriage.

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J16.61

Phenotypic features in Beta thalassemia mutations Heterozygotes from Brazil

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Thalassemias occur in individuals of all ethnic backgrounds and are the most common genetic diseases worldwide. Heterozygotes for β-thalassemia are, in general, free of symptoms but have hypochromic microcytic red cells with low mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV). In this work, 328 blood samples of β-thalassemia Heterozygotes were analyzed, by molecular procedures, to identify correlations between

hematological data and theβ-globin gene mutation. The results show the predominance (39.79%) of the CD39 mutation in the southeastern region and 78.78% of the IVS1-6 mutation in the northeastern region from Brazil. These results reflect the ethnical differences in each Brazilian region which originate mainly due to the admixture of the population. The IVS1-6 (T>C) heterozygotes, a mutation expressing a β^+ phenotype had higher MCV and MCH. A higher Hb A_2 was observed for the IVS1-1 (G>A) mutation and a higher Hb F for the CD39 mutation, both expressing β^0 phenotypes ($p<0.0001$). The data observed were different to published results of molecular thalassemia analyses in the Brazil. Hence it is very important to consider the formation of the population, the method of analysis used, the type of thalassemia, the influence of and the environment in relation to the phenotype evaluation. To establish β-Thalassemia preventive programs using the most up-to-date techniques it is important to identify the real prevalence of the mutants in each Brazilian region.

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J16.62

Phenotypic variability in a Hungarian family with a novel TSC1 mutation

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Tuberous sclerosis is a multiorgan, autosomal dominant disorder, caused by mutations in the *TSC1* or *TSC2* genes. The encoded proteins-hamartin and tuberin- are involved in the control of cell migration, proliferation and differentiation. Mutation in either gene results in loss of control of cell growth and division, and therefore predispose to tumor formation. Clinical signs include non-malignant brain tumors, skin, eye, heart and kidney abnormalities. We investigated a Hungarian family, with epilepsy of various age onset, and MRI studies raising the possibility of tuberous sclerosis. While epilepsy in the grandmother started only at the age of 39 years, seizures in her younger daughter occurred at age 17; her eldest daughter is still asymptomatic at the age of 33 years. In the 5 year old boy the first seizure was seen at 15 months, his maternal uncle is treated with epilepsy since 8 month of age; the corresponding MRI alterations correspond with the severity of the symptoms seen in the male family members. Our department started molecular genetical analysis of *TSC1* and *TSC2* genes in 2012, the investigation of this family confirmed a previously non described, de novo heterozygous point mutation (c.2523 C>T) in exon 20 of *TSC1* gene leading to a premature stop codon. The mutation was detected in all examined family members even in the so far asymptomatic mother. Despite the increasing data on the pathomechanism of tuberous sclerosis, there is still little known about the genetic modifying factors influencing the broad intra-and interfamilial phenotypic variability.

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J16.63

Genetic polymorphisms at TNFα, TLR2, TLR4, TGFβ, CD14 and CCR2 genes in patients with lower limbs infections.

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Introduction: Chronic wounds are still growing challenge due to demographic changes and increased risk of artherosclerosis as well as diabetes. In innate response during bacterial infections toll like receptors, pro- and anti-inflammatory cytokines, chemokines as well as their receptors takes part. Few of them could have an essential role in the healing of pathological wounds like venous leg ulcer.
Aim of the study: The purpose of this study was to define genetic markers which could be responsible for predisposition to bacterial infections development in human lower limbs. Material and methods: The study population with serious infections (including sepsa) and with long-healing wounds consisted of 59 patients in each group and 25 patients with severe ulcers. A control group of 130 blood donors was ethnically matched to the study groups and randomly selected to comparing the study data.
The presence of polymorphic variants in six genes was investigated by PCR-RFLP method. Results: In group of serious infections we noted statistical importance of polymorphic genotypes in TNF-α
(-308G/A), TGF-β 29 codon (T/C),and TLR4 (1363C/T) genes. Group of long-healing wounds was characterized by additional importance of genotype TT in TLR2 gene (2029C/T). In the group with severe ulcers only polymorphisms of TGF-β and TLR4 genes, in described positions, were statistically significant. Conclusions: We cannot define single genetic marker responsible for occurrence of individual infection however it seems that TNF-α polymorphism is not crucial in severe ulcers and TLR2 polymorphic variant could predispose to long time of healing wound.

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J16.64

Effect of estrogen receptor α PvuII and XbaI polymorphism on growth and bone mineral density in thalassemia major patients

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Background: The etiology of bone disease in thalassemia is poorly understood. Numerous data indicate that polymorphism of estrogen receptor α (ERα) may predict lipid levels, lipid response to hormone replacement therapy (HRT), bone fracture risk, bone mineral density (BMD) and changes in BMD over time. In humans, estrogens influence many physiological processes, which include not only reproduction, cardiovascular health, bone integrity, but also cognition and behaviour.
Aim: In this study we aimed to evaluate distribution of ERα PvuII and XbaI genotypes and their effect on growth and bone mineral density in Thalassemia major patients of North India.
Material and Method: 150 β thalassemia major patients were assigned for the study. ERα PvuII and XbaI polymorphism was determined by PCR-restriction fragment length polymorphism (RFLP).
Result: PvuII genotype was distributed as follows: PP 24.5% , Pp 35.8% , pp 38.7% . Frequency of XbaI genotype was: XX 12.3% (n=13), Xx 34.9% (n=37), xx 51.9% (n=55). Four haplotypes with following frequencies were recognized: PX 17.3%, px 47.4%, Px 24.4% and pX 10.9%. We have observed significant association of PvuII digested estrogen polymorphism with low bone density at spine (P=0.010) and BMI (P=0.012) and in case of Xba I association was observed with height (P=0.001), weight (P=0.09) and BMI (P=0.007).
Conclusion: ERα gene polymorphism is probably one of the genetic factors contributing for low bone mass so it may be used as diagnostic or therapeutic marker.

R. Kumar: None. **K. Singh:** None. **S. Agarwal:** None.

J16.65

Carrier frequency of SLC26A5 (Prestin) gene mutation c.-53-2A>G among 16 ethnic populations of Eurasia

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Hearing impairment is one of the most common sensorineural disorders and the incidence of profound deafness is about one per 1000 at birth. The GJB2 gene mutations make the largest contribution to hereditary hearing impairment. A single nucleotide change IVS2-2A>G (NM_198999.1:c.-53-2A>G) in second intron of SLC26A5 gene has been reported in association with hearing loss. This study presents the data on the carrier frequencies of major SLC26A5 mutation c.-53-2A>G among 1310 healthy persons from 16 various populations of Eurasia: Bashkirs, Tatars, Chuvashes, Udmurts, Komi-Permyaks, and Mordvins, (Volga-Ural region of Russia), Russians, Belarusians, Ukrainians, Veps, and Karelians (East Europe), Abkhazians, Kazakhs, and Uzbeks (Central Asia), Yakuts, and Altaians (Siberia). Mutation c.-53-2A>G in heterozygous state was found in 24 subjects out of 1310 examined individuals: Belarusians (1), Ukrainians (2), Tatars (3), Chuvashes (1), Mordvins (4), Komi-Permyaks (6), Russians (3), Kazakhs (2), Abkhazians (1), Altaians (1) . Highest carrier frequency of c.-53-2A>G was revealed in Finno-Ugric populations of Mordvins (5.4%) and Komi-Permyaks (6.0%). Lower rates of this mutation were found in Slavic populations of Russians

(2.3%), Ukrainians (2.5%) and Belarusians (1.3%), and in Turkic populations of Tatars (2.5%), Chuvashes (2,6%), Kazakhs (2,5%), Altaians (1,3%) and this mutation was absent in Bashkirs, Yakuts, Uzbeks, Veps, and Karelians.
The data on the mutation c.-53-2A>G (SLC26A5) rates in studied ethnic groups can be used to investigate the prospective founder effect in origin and prevalence of this mutation in Eurasia and consequently in populations worldwide.This study was supported by RFBR grants (11-04-01221-a), (12-04-00342-a), (12-04-98520-p_vostok_a), (12-04-97004-p_povolje_a), (12-04-31230-mol_a).

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J16.66
Investigation of IL23R gene variants in Romanian ankylosing spondylitis patients

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Ankylosing spondylitis (AS) is a chronic rheumatic disease characterized by inflammation in the spine and sacroiliac joints. Interleukin 23 (IL23) is an important cytokine that acts through the signaling complex IL12Rb1-IL23R. Recent studies have reported the associations of IL23R gene single nucleotide polymorphisms (SNPs) with the risk of AS in Caucasian populations.
Objectives: The aim of this study was to investigate the association of IL23R gene SNPs with the risk of AS in Romanian population.
Methods: Nine SNPs mapping to IL23R gene (rs11805303, rs7530511, rs10489629, rs2201841, rs11465804, rs11209026=Arg381Gln, rs1343151, rs11209032, rs1495965) were genotyped in 168 consecutive AS patients and 161 healthy controls of Romanian ethnicity using the Sequenom MassARRAY platform (Sequenom, San Diego, CA) and/or TaqMan assays (Applied Biosystems, USA). Statistical analyses were performed with PLINK and Haploview softwares.
Results: We found no association between AS and individual SNPs in IL23R gene. The combined analysis of the studied SNPs revealed the association between AS and the 3-marker haplotype GCG rs11209026/rs1343151/rs11209032 (30% in patients versus 38% in controls, p=0.02, OR 0.69). The extended haplotype TTGCG rs2201841/rs11465804/rs11209026/rs1343151/rs11209032 was also significantly underrepresented in patients (24.5% versus 33.4% in controls, p=0.01, OR 0.65), thus conferring a lower risk for the disease. The prevented fraction associated with these haplotypes was 9.3% for GCG and 8.3% for TTGCG haplotype.
Conclusion: These findings confirm the contribution of IL23R gene variants to AS susceptibility in the Romanian population.
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J16.67
Distribution of HIV resistance/susceptibility associated genetic markers in a healthy brazilian urban population sample

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ABSTRACT: The Brazilian people have a peculiar history, considering the influence of Amerindian, European and African genetics. Population genetics is a powerfull instrument to better understand the formation of a people, even their susceptibility or resistance to diseases. The knowledge of human genetic variability and the analysis of the possible resistance to diseases, like the infection of human immunodeficiency virus (HIV), are as important as the study of the etiology or the biological cycles of the virus. OBJECTIVE: Evaluate genotypic distribution of a 32 base pairs deletion in the CCR5 gene, CCR5Δ32, and microsatellite markers TNFc, TNFd and TNFe, which are genetic markers associated with resistance/susceptibility to HIV, in a Brazilian healthy urban population sample. RESULTS: The frequency of the 32 base pairs deletion was 5.5%, the allele 2 of TNFc was found with a frequency of 9%, lower than previously reported in other populations in Brazil, Africa and Europe. TNFd and TNFe had the allele 3 as the most frequent and the haplotype formed by alleles TNFc *1, TNFd *3 and TNFe *3 was the most common. CONCLUSION: The frequency of the CCR5Δ32 has allowed the proposal of a gradient associated with the presence of European ancestry and corroborates the view that the population of Brasilia and also from this geographical region is a brief of the population. The description of the distribution of alleles for loci TNFc, TNFd and TNFe contributes to a better understanding of these markers, never studed before in Brasilia.

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J16.68
Relation of the CD36 gene polymorphisms to the risk of nephropathy

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Introduction:
CD36, also called thrombospondin receptor and platelet glycoprotein IV (GPIV), is a membrane glycoprotein acting as a receptor for matrix proteins including collagen and thrombospondin. A role for CD36 in the pathogenesis of atherosclerosis, inflammation and lipid metabolism has been well-documented. However, little is known about the role of CD36 in renal diseases. Based on these data, we are currently studying the relationship between three polymorphisms of the CD36 gene and susceptibility to renal diseases in the Moroccan population.
Methods:
A population of 400 subjects from morocco are analyzed according to clinical parameters.Out of them 200 controls and 200 nephropathic patients are genotyped for three SNPs : rs1761667 (G > A), rs1527479 (A>G) using polymerase chain reaction and restriction fragment length polymorphism (PCR–RFLP) and (A>C) -178 by PCR and sequencing analysis. Statistical analysis is followed.
Results and discussion:
In patients population :
For rs1527479 (A>G), the A/G genotype was prevalent in 48.95%, the A/A was 33.33% and the G/G was 17.70%.
For rs1761667(G>A), the G/A haplotype was predominant with 45.83%, the A/A was 35.41% and the G/G was present in 18.75%.
We notice the predominace of the heterozygous and the homozygous haplotypes of this two SNP in patients population.
The sequencing of the third SNP and all controls are being studied actually.
Conclusion:
Once the study is completed, the intent of this review is to develop the concept that CD36 is a prototypic inflammatory receptor that contributes to the pathogenesis of various nephropathies.

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J16.69
Inherited thrombophilia and pregnancy complications in Georgian population

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Background: Inherited thrombophilia (Factor V Leiden (FVL G1691A), Prothrombin (PTH G20210A) and Methylenetetrahydrofolate reductase (MTHFR C677T) gene mutations, as a risk factors for pregnancy complications have gained much attention in the scientific community. Published results suggest that inherited thrombophilia plays a significant role in the development of gestational vascular complications and represents a cause of maternal and fetal morbidity and mortality.
Aim: The aim of study was to evaluate how inherited thrombophilia is associated with Gestational vascular complications in women of Georgian population.
Methods: 140 patients with gestational vascular complications and 50 healthy controls were genotyped by PCR.
Results: The presence of MTHFR homo/heterozygous forms was significantly higher in women with pregnancy complications (84.69%) than in controls (32%).
In patients homozygosity for FVL/PTH/MTHFR was found to be 0%, 0%,16.32% respectively and heterozygosity was found to be 8.16%, 4.08%, 68.37% respectively, while frequency of heterozygosity in healthy individu-

als has been reported as 0%, 0%, 32%.
Our results showed that triple heterozygous forms for FVL/PTH/MTHFR (2 cases) and double heterozygosity for FVL/MTHFR mutations (1 case) were founded in patients, while no mutations were founded in controls except heterozygous form of MTHFR.
Conclusion: The preliminary analysis indicate that investigated mutations, especially MTHFR have the significant role in increasing of risk of development of pregnancy complications in Georgian population and the future investigations in this area would be especially important.
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J16.70
Association between IL28B and the response of treatment to HCV3 infection

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Hepatitis C virus infection affects about 3% of world population, and about 80% of them will develop chronic active hepatitis that may evolve toward cirrhosis and hepatocellular carcinoma. Reports from several independent GWAS studies and other sources showed that some polymorphisms close to IL28B gene were strongly associated to therapeutic response to interferon based treatment, and also with spontaneous clearance of HCV. These studies indicated a strong correlation between genotypes of polymorphisms RS12979860 and RS8099917 and the outcome of the treatment with interferon in patients infected with genotypes 1 and 4 of HCV. This association was not clear with patients infected with HCV genotypes 2 or 3 due to discrepant results from different studies, although study performed with an Italian sample infected with these genotypes showed an association between polymorphism RS12979860 and therapeutic response of patients that do not achieve a rapid virologic response (no RVR). In Brazil, the genotype 3 is the second most prevalent HCV genotype with about 25-30% of reported cases. In order to verify the influence of the IL28B polymorphisms in this highly admixed population, a meta-analysis of 5 independent cohorts was conducted aiming to associate treatment outcome and the polymorphisms RS12979860 e RS8099917 genotypes. The results clearly indicated that both SNPs are associated with response, but the RS8099917 (effect $\chi 21=14.6$; heterogeneity $\chi 24=1.32$) showed a stronger association, indicating that it is a more reliable marker for HCV3 IFN therapy. (Supported by CNpq)

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J16.71
Investigating the mode of KIV-2 CNV size changes at the LPA locus by SNP haplotyping of its flanking regions

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The lipoprotein(a) (LPA) locus (MIM 152200) harbours a transcribed, multi-allelic copy number variation (CNV) formed by a 5.5 kb KringleIV-2 repeat (KIV-2 CNV; 1-46 copies). The actual mode of KIV-2 CNV size evolution, i.e. whether it mainly changes by mutations of +/- one KIV-2 repeat or by larger, arbitrary size changes, is unknown and currently debated.
Previously, we had identified two SNP rich regions directly flanking the KIV-2 CNV. SNP haplotyping in these regions was experimentally conducted in 20 individuals each from six world populations by segregation analysis and/or allele separation by pulsed field gel electrophoresis (PFGE) followed by re-sequencing. KIV-2 CNV size was assessed by PFGE /Southern blotting. Linkage between the SNP haplotypes and KIV-2 CNV allele size was investigated.
We observed significant linkage between the haplotypes in the two flanking regions in all populations studied, with SNP haplotypes spanning over a wide range of KIV-2 CNV sizes. These results indicate a high mutation rate for the KIV-2 CNV. Certain SNP haplotypes were restricted to CNV alleles of neighbouring sizes, indicating that the CNV does indeed mainly change by mutations of +/- one KIV-2 repeat or in small steps. However, incomplete linkage between SNP haplotypes points to crossing over events also resulting in multi-step CNV size changes.

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J16.72
Incidence, trends and genetic assessment of neural tube defects

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Neural tube defects represent severe congenital anomalies that result from failure of neural tube to close during early development. Given their unknown incidence in our region and complexity of the related problems, we performed an epidemiological study in newborns from Timisoara, Romania, between 2007 and 2011 and compared data with those obtained through a retrospective study, between 1989-1994. The main goals were to highlight the incidence, etiological aspects and the trend in occurrence of spina bifida and anencephaly. We found an overall incidence of 0.034‰ in 2007-2011, compared to the incidence of 1.58‰, calculated for the interval 1989-1994. In the last years there were 9 cases with spina bifida and 1 with anencephaly, compared to 51 with spina bifida and 9 with anencephaly. Studying sex ratio, the following results were obtained: females prevailed among cases with spina bifida, but the only case with anencephaly from 2007 was a male and males also prevailed in our previous study, sex ratio 2/1. 60% of the cases from the 2007-2011 study reported previous spontaneous abortions, compared to 23.33%, discussible percentages not only from the statistical point of view, but also knowing the fear of speaking about abortions, even spontaneous, in 1989 and the early 90s. Two couples had two affected children, both with spina bifida. In some cases associated anomalies were found, such as hydrocephaly or limb defects. The incidence reduction is probably due to prenatal screening and diagnosis and genetic counseling, which were not performed in our country in the early 90s.

D. Stoicanescu: None. **M. Cevei:** None.

J16.73
Frequency of Polymorphism Plasminogen Activator Inhibitor-1 (PAI-1) in Tuzla region population (Bosnia and Herzegovina): preliminary results

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The -675 4G/5G polymorphism discovered in the promoter region of the PAI-1 gene causes the increased interest of PAI-1 gene polymorphism as genetic risk factor for development of venous thromboembolism. The 4G/5G insertion/deletion polymorphism is related to increased concentration of PAI -1 in plasma which is common finding in patients with deep vein thrombosis (DVT).
The aim of this study was to analyze the role of polymorphism -675 4G/5G of PAI-1 gene and increased risk of venous thrombosis in population of Tuzla region (B&H).
Genotyping of 4G/5G polymorphisms was done with polymerase chain reaction followed by simultaneous restriction of digestion with Bsl I. Compared were the frequencies of genotype of this polymorphism between 100 individuals with personal history of DVT and a control group of 100 healthy subjects without known risk factors for DVT.
The frequency of the 4G allele was 46, 99% in the group of patients with DVT and 38, 71% in the control healthy subjects, OR confidence interval [95%CI]) =1,403 (0,918-21, 45); P=0,117. Leading to the P=0,117 it did not seem to significantly influence the risk of DVT.
This study did not confirm the association the -675 4G/5G polymorphism of PAI-1 gene with DVT in Tuzla region population but it may be risk factor in combination with common genetics polymorphism associated with VTE.

R. Terzic: None. **M. Pođanin:** None. **A. Karic:** None. **Z. Jerkic:** None.

J16.74

Distribution of the most common genetic variants associated with variable drug response in the population of the Republic of Macedonia

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Information regarding geographic structure and multiethnic distribution of clinically relevant variants in genes coding for Phase I, Phase II drug metabolizing enzymes (DMEs) and drug targets that control DME activity levels is becoming increasingly useful for improving drug therapy and explaining inter-individual and inter-ethnic differences in drug response. We evaluated the frequency distribution of most common allelic variants in three broad gene categories: CYP450 (CYP2C9, CYP2C19, CYP3A5, CYP2D6), phase II DME (GST, SULT; UGT) and drug target (TYMS and MTHFR) in the R. Macedonia using a combination of PCR/RFLP, capillary electrophoresis and Real-time PCR allelic discrimination method. The allelic frequencies are presented in Table 1. All results were in accordance with the expected genotype distributions, calculated by the Hardy-Weinberg equilibrium (P>0.05).

Table . Population frequencies of analyzed genetic variants.

Gene Group	Gene/ genetic variant	Allele frequency	
Phase I (CYP450)	CYP2C9		
	*1	0.79	
	*2[rs1799853]	0.14	
	*3[rs1057910]	0.07	
	CYP2C19		
	*1	0.65	
	*2[rs4244285]	0.14	
	*17[rs12248560]	0.2	
	CYP3A5		
	*1	0.76	
	*3[rs28365095]	0.14	
	*10[rs15524]	0.1	
	CYP2D6*		
	*2[rs16947]	0.39	
	*2XN[rs1135840]	0.43	
	*3[rs35742686]	0.008	
	*4[3892097]	0.19	
	*6[rs5030655]	0.005	
	*9[hCV32407229]	0.02	
	*10[rs1065852]	0.22	
	*33[rs28371717]	0.02	
	*35[rs769258]	0.11	
	*41[rs28371725]	0.1	
Phase II	SULT1A1	G	C
	213G>C	0.63	0.37
	UGT1A1	(TA)6	(TA)7
	*28	0.69	0.61
	GSTT1	null	no null
	null/no null [chr22:24343276]	0.13	0.87
Drug target	MTHFR	C	T
	677C>T[rs1801133]	0.59	0.41
	TYMS-TSER		
	2R	0.44	
	3R	0.56	
	3C	0.32	
	3G	0.24	

Our investigation adds to the evidence regarding the distribution of clinically important variant alleles in DME and drug target genes in population with European ancestry.

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J16.75

Genetic differentiations of populations of Russia on genes of hereditary disorders

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The medical genetics consulting should take into account patterns of genetic diversity of populations on genes of hereditary disorders (HDs). The genetic territorial diversity and differentiation of the genes of monogenic hereditary diseases (HDS) in 13 populations of Russia (7 ethnic groups: Russians from 7 regions, Maries, Udmurts, Chuvashes, Bashkirs, Tatars, Adigeans) was identified. Genetic diversity of HDs in the investigated populations was revealed more that 500 HDs. Each population was characterized by specific spectrum and prevalence of HDs. Differences was found not only in the prevalence of diseases, but the frequencies of mutations. More that 3480 healthy donors from six ethnic groups - Maries, Udmurths, Chuvashs, Tatars, Bashkirs and Russians from 3 regions of Russia were analyzed for major mutation in three genes - CFTR (Cystic fibrosis), connexine 26 gene (Deafness) and LIPH (Hypotrichosis). At least 800 chromosomes from each ethnic group with the ethnographic division were analyzed: Maries (1010 chromosomes), Udmurts (892), Chuvashes (868), Tatars (1420), Bashkirs (808) and Russians (1970). Twelve CFTR mutations (CFTRdele2,3(21kb), F508del, Idel507, 1677delTA, 2184insA, 2143delT, 2183AA>G, 2184delA, 394delTT, 3821delT, L138ins; E92K), 35delG mutation in Cx26, ΔEx 4 mutation in LIPH gene were studied. Significant differentiation in mutations frequencies between different ethnic groups was discovered between Russians and Maries, Udmurts, Chuvashs, Bashkirs. The greatest similarity of the analyzed ethnic groups on CFTR mutations and 35delG mutation in Cx26 was found between Russians and Tatars. Volga-Ural ethnic groups and Russians were found to be distinct in the frequencies of ΔEx 4 mutation in LIPH gene.

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J16.76

PPARα and Apo proteins target genes polymorphism, lipid and glucose metabolism parameters in children, having risk factors of the development of coronary heart disease (CHD).

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Study population: The main group consisted of 95 children and adolescents, having risk factors of cardio-vascular pathology, such as: atherogenic dyslipidemia, obesity, hypertension. The control group consisted of 55 children and adolescents of comparable age without such risk factors.

Methods: In all children cholesterol, triglycerides, HDL, LDL, glucose, insulin and C-peptide were analyzed. Molecular-genetic analysis of polymorphisms of PPARα genes and L162V PPAR α target genes (G75-A Apo A1 and C+83T Apo A1, C1131T ApoA5 and S19W Apo A5, Sst I Apo C3) was performed.

Results: In the main group 10 children and adolescents, having risk factors of CHD, had 162V allele in the heterozygous state and 1 had 162V allele in the homozygous state. In the control group only 3 children had 162V allele in the heterozygous state.

In the main group the correlation analysis of lipid and glucose metabolism parameters depending on presence or absence of 162V allele of PPARα gene was performed. In the group of children, having 162V allele of PPAR α gene, correlations between triglycerides and C peptide (0.49) and between HDL and C-peptide levels (-0.48) were found. No differences in the distribution of alleles and genotypes of studied genes were found in the main group. Comparative analysis of distribution alleles and genotypes of the PPAR α target genes (ApoA1, ApoA5, ApoC3) in the carriers of 162V allele PPARα gene and LL genotype carriers was not performed due to the small sample.

A. Vasina: None. **A. Nikitina:** None. **A. Voitovich:** None. **M. Didur:** None. **V. Larionova:** None.

J16.77

Chromosome studies in 639 Tunisian couples with repeated spontaneous abortions

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Recurrent pregnancy loss (RPL) is a devastating reproductive problem. The cause is not apparent and often requires intensive and expensive laboratory investigations, despite which there is still a limited understanding of recurrent miscarriage (RM).

A retrospective study was done in couples with 2 or more fetal loss from Mai 2005 to Mai 2011. Karyotypes were performed from peripheral blood lymphocyte culture, and cytogenetic analysis was performed by using R-banding.

Among the 639 couples studied, 27 anomalies were detected (4.22%). There was no apparent relation with number of abortions. However, women were more frequently affected then men with a prevalence of 65% and 35% respectively (p<0.05). The abnormalities were 19 translocations, 7 cases of mosaicism and an inversion of chromosome 12 in one case. In 15 cases were reciprocal and in 4 were robertsonian. Most mosaicism involved the maternal X chromosome aneuploidy. When a history of normal living children, repetitive abortions, malformative syndrome or mental retardation was found in the family of one of two parents, the risk of finding chromosomal

aberrations was significantly higher.
In conclusion, parental chromosome abnormalities represent an important etiology of (RM). But, face to unknown proportion of parents who appeared chromosomally normal, we hypothesize that cryptic subtelomere rearrangements can be implicated.

A.C. Hajlaoui: None. **A.I.M.I. S Dimassi:** None. **A.D.M.S. A saad:** None.

J16.78

The gene pool of Argyn in the context of generic structure of Kazakhs according to data on SNP-Y-Chromosome markers.

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Study of Y-chromosome polymorphism with consideration of the genealogical structure provides information about the history and the fine structure of gene pool, and it can help identify major patterns of variation in gene pool, determination of migration routes, and genetic boundary between the relatively homogeneous gene pools.
The study was performed on the Kazakh tribe of Argyn, which are included in the three "Juz" (ethno-territorial entities of Kazakh tribes) in the „Shezhire" system of Kazakhs. The Shezhire system characterizes the tribal structure of the Kazakh people, which was the ethno-social basis of social organization. It is therefore important to examine the association of Y-chromosome variability with the genealogical structure, and clarify the relationship between biological and social kinship.
We analyzed 40 SNP and 17 STR Y-chromosomal markers. In the study, besides the descendants of Argyn (sample size N = 287), 14 tribes of Kazakhs and 12 tribes Mongolians (in all N = 2186) were analyzed. In the tribe of Argyn, twenty haplogroups were identified, among which there were one major haplogroups - G1a - P20 (71%) and three minor haplogroups R1a* - M198(xM458) (6%), C3c - M48 (5%) and C3* - M217(xM48) (3%). Other haplogroups represent less than three percent.
We calculated the genetic distances and plot multidimensional scaling, comparing the gene pools of the studied genus with other tribes of the Eurasian steppe from the frequencies of haplogroups. Tribe Argyn took on graph an isolated position, demonstrating the absence of genetic links with other Kazakh tribes.

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J16.79

Molecular spectrum of β-globin mutations and their linkage to β-globin gene haplotypes in North-Western Iran

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In β-thalassmia, a variety of mutations (>400) have been identified influencing gene transcription, translation or mRNA processing. Number of studies demonstrated a nonrandom linkage of particular RFLP haplotypes with specific β-thalassemia mutations. β-globin cluster haplotypes are a valuable tool for indirect mutation detection.
In this study, we characterised the β-thalassmia mutation spectrum using ARMS-PCR and DNA sequencing for common and non frequent mutations in Iranian Azeri subjects respectively. In addition β-globin cluster haplotypes were determined by PCR-RFLP for 35 βthal and 101 βA chromosomes.
We detected 20 different mutations in the 105 chromosomes. IVSII-1(G>A) (30.47%), was the most frequent mutation followed by IVSI-110 (G>A) (16.19%), and CD8 (-AA), CD8/9(G) both (7.6%).
We determined the haplotypes belonging to 35 βthal and 101 βA chromosomes. Haplotype I was found to be the most common haplotype in both normal (25.74%) and mutant (20%) chromosomes, as is the case in the Mediterranean region. The subsequent common haplotype associated with mutant chromosomes was haplotype IV in 5 (17.14%) and IX, V in 11(10.89%) normal chromosomes. It is found that the highest observed heterozygosity was related to HindIII Gγ, Hind II 5'ψß of all polymorphic sites with a value of 48% whereas the highest expected heterozygosity was for HindIII Gγ, HindII ε with a value of 50%. These two markers (HindIII Gγ, HindII ε) have the highest PIC, 0.37. In this study for 20 out of 26 couples at-risk of having a child with major β-thalassmia, prenatal detection of β-thalassemia mutations accomplished successfully by ß-cluster haplotyping.

S. Mansoori Derakhshan: A. Employment (full or part-time); Significant; Tabriz University of Medical Sciences. **A. khorrami:** A. Employment (full or part-time); Modest; Ebne Sina Medical Genetics Lab. **A. Hosseinpour Feizi:** A. Employment (full or part-time); Significant; Tabriz University of medical Sciences. **P. Derogar:** A. Employment (full or part-time); Modest; Ebne Sina Medical Genetics Lab. **M. Niusha:** None. **M. Shekari Khaniani:** A. Employment (full or part-time); Significant; Tabriz University of Medical Sciences.

J16.80

The association of AGTR2 gene polymorphism with athlete status and body composition

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The angiotensin II type 2 receptor (encoded by AGTR2 gene in the X chromosome) by mediating the effect of the angiotensin II play an important role in the development of muscle fibers (Johnston et al. 2011). The CC genotype of the C3123A (rs11091046) polymorphism of the AGTR2 gene located within the 3-untranslated region of exon 3 have been reported to be associated with increased proportion of slow-twitch (fatigue resistant) muscle fibers (Ahmetov et al. 2006). The aim of present study was to investigate the association between the C3123A polymorphism of the AGTR2 gene, athlete status and body composition in Russians. Two hundred and five Russian athletes (51 females and 154 males) from different sporting disciplines were involved in the study. AGTR2 genotype and allele frequencies were compared to 159 controls (85 females and 74 males). Body composition parameters were assessed by bioelectrical impedance analyzer Tanita MC 980 (Japan) in 79 athletes (16 females and 63 males). We found that the frequency of the AGTR2 C allele was significantly higher in endurance-oriented male athletes compared to controls (62.3 vs 43.2%; P=0.0001). Similarly, the frequency of the AGTR2 C allele was significantly higher in female cross-country skiers compared to female controls (83.3 vs 50.6%; P = 0.036). Furthermore, CC genotype was associated with higher relative muscle mass in female athletes (P=0.0268). In conclusion, we have shown that the C3123A polymorphism of the AGTR2 gene is associated with endurance athlete status and body composition in Russians.

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J16.81

Polygenic profile of Russian power athletes

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Power performance is a complex phenotype, subject to the influence of both environmental and genetic factors. The aim of the present study was to investigate the associations of multiple common gene polymorphisms (involved in anaerobic glycolysis, glucose, insulin and lipid metabolism, and regulation of muscle fiber type composition) with power athlete status. The study involved 1161 Russian athletes (272 endurance athletes, 462 athletes with mixed endurance/power activity, 427 power athletes) and 934 controls. ACE I/D, ACTN3 R577X, HIF1A P582S, PPARA intron 7 G/C and PPARG P12A gene polymorphisms were determined by PCR-RLFP. To assess the combined impact of all 5 gene polymorphisms, all athletes were classified according to the number of 'power' alleles they possessed. We also determined a „total genotype score" (TGS, from the accumulated combination of the 5 polymorphisms, with a maximum value of „100" for the theoretically optimal polygenic score) in athletes and controls. The proportion of subjects with a high (4-8) number of 'power' alleles was greater in elite power athletes compared to controls (50.0% vs. 34.1%, P=0.0049). Furthermore, the mean TGS was significantly higher in elite power athletes (35.9 (15.8) vs. 30.2 (12.8); P=0.0003) compared with controls, whereas it did not differ between endurance athletes and controls (P=0.906), nor between athletes

with mixed activity and controls (P=0.995). These data suggest that the likelihood of becoming an elite power athlete depends on the carriage of a high number of power-related alleles.

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J16.82

The monitoring of the congenital malformations and inheritable disorders in population of the Kazakhstan

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In order to monitor the frequency and the spectra of congenital malformations and inheritance disorders the computerized database was developed which consist of several blocs: the private data, the anamnesis, the objective status, and the results of examination. Diagnosis was detected in according to ICD (International Classification of Diseases).
The database contains information about 2005 cases of patients and the fetuses with congenital malformations and inheritance disorders with predominance of the congenital malformations according EUROCAT. The frequency of congenital malformations makes 49% of all cases. The most frequent cases represent the neural tube defects in 157 (18%), the multiple malformations in 132 (15%) cases, congenital malformations of urinary and genital in 122 (14%) cases, the congenital heart defects in 112 (14%) cases, the malformations of digestive system in 103 (12%) cases.
The chromosomal abnormalities were detected in 428 (26%) cases. The diagnosis of chromosomal abnormalities was confirmed by cytogenetic and molecular cytogenetic methods. The genetic registrar contains information about 250 fetuses with chromosomal abnormalities revealed by prenatal invasive diagnostics. The aneuploidy and the structural chromosomal abnormalities were diagnosed by cytogenetic research.
The monogenic pathology was discovered in 452 (26%) cases. The molecular genetic tests (RFLP, MLPA, mass spectrometry and fluorimetry) were applied for diagnostics. The autosomal dominant diseases were detected in 35% cases, the recessive diseases in 50% cases, X liked diseases in 15% cases.

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J16.83

Peculiarities of *HLA-G* polymorphism in St. Petersburg population (North-West of Russia)

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HLA-G is a key gene involved in the maintaining of the maternal-fetal tolerance during pregnancy. Less-polymorphic than the majority of *HLA* genes, *HLA-G* being expressed by extracellular trophoblast is involved in silencing of the maternal immune response, thus some *HLA-G* alleles could negatively effect on the fetal development. In our study 116 healthy individuals were genotyped for *14bp ins/del* in exon 8, *-725G>C* and for 6 other most frequent alleles G**0101-G*0106*. Frequencies of 14b.p. *del* and *ins* alleles were 59% and 41% respectively. Hardy-Weinberg distribution was not affected (χ^2=0,22; p>0,05). Genotype *-725G/G* was not found, rates of *-725C* and *-725G* alleles were 87% and 13% respectively, thus observed frequencies deviate significantly from those expected according to the Hardy-Weinberg ratio (χ^2=15,82; p=0,0001). Absence of this genotype could be explained by evolutionary pressure due to allele *-725G is* associated with increasing miscarriage rates. Frequency of Alleles *G*0101, G*0102, G*0103, G0104 and G*0105* were 89%, 1%, 2%, 6.5% and 2 % respectively. *G*0106* allele was not detected. Our data are significantly different from studies in other populations (p= 0.005, p= 0.001), it might be treated as a specific trait of St. Petersburg population.

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FGB and FV genes polymorphism and their association with longevity

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Changes of hemostasis parameters are traced with age. Genetic background, which determinant protection of organism in conditions of hypercoagulability, may be predisposed to increase of lifespan and promote human longevity. Our objective was to estimate alleles and genotypes frequencies dynamic of FGB (rs1800790) and FV (rs2269648) genes with age. Gene polymorphism was analyzed by PCR-RFLP. There was composed group of 1520 unrelated individuals, from 1 to 109 years, ethnic Tatars (Russia). For comparison of age groups was used Fisher's two-tailed exact test (P<0.05 was considered significant). In aged and senile groups, in compare with middle-age I group, FGB*A/*G genotype frequency was higher and FGB*G/*G genotype frequency was lower. Frequencies of FGB*G/*G genotype and FGB*G allele differed between senile individuals, in one hand, and middle-age II persons, on the other. Among senile and long-lived male FGB*A/*G genotype frequency was higher, than in middle-age I group; FGB*G/*G genotype frequency was lower in group of long-livers than in middle-age I individuals. Using logistic regression analysis (SPSS18.0) there was established that FV*C/*C genotype frequency was an increase in age from 45 until 81 years (p=0.037, OR=1.015) in total group and also in age from 55 until 82 years (p=0.045, OR=1.027) among female. As well, within male in age from 25 years FGB*A/*G genotype frequency was rise (p=0.008, OR=1.011), FGB*G/*G genotype frequency was reduce (p=0.009, OR=0.990). Thus, received results suggests that rs2269648 polymorphism of FV gene, probably, is associated with achievement of senile age and rs1800790 polymorphism of FGB gene- with attainment of longevity status.

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J16.85

Molecular heterogeneity of familial hypercholesterolemia in Russia

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Background: Familial hypercholesterolemia is a common monogenic disorder usually caused by mutations in the low density lipoprotein (LDL) receptor gene and leading to premature atherosclerosis development.
Aim of the study: To evaluate variability of the LDL receptor gene mutation spectra in different regions of Russia.
Results:

Region	Number of FH-causing mutations/Mutations common with St.Petersburg	Number of mutations specific for Russian subregion/Mutations common with other countries of the world
St.Petersburg	33/33	18/15
Moscow	17/2	14/3
Novosibirsk	21/5	10/11
Petrozavodsk	13/2	7/6

Conclusion: High variability of mutation spectra in different regions of Russia argues against strong founder effect in Russian population. Mutation carriers have high level of blood cholesterol and increased risk of coronary heart disease.

M.Y. Mandelshtam: None. **A.B. Maslennikov:** None.

J16.86

Association of single nucleotide polymorphism in *ERCC6* gene with indivuduals including dental materials

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The aim of this study was to investigate the role of excision repair cross-complementing group 6 (*ERCC6)* gene polymorphisms, which has very rare been reported to be associated with indivuduals including dental materials. A variety of materials are used for filling and the bonding of restoration materials to the tooth cavity. Particularly, amalgam is known for its toxicity potential as it releases mercury and shown to be genotoxic as well. The defects in the DNA repair gene *ERCC6* is thought to play a role in the development of malignancy in humans. The association of *ERCC6* codon 399 polymorphism were compared between the individuals having at least one or more amal-

gam fillings together with composite fillings or crowns with control group; individuals who does not contain any dental material in the oral cavity with RFLP-PCR method. We found that frequency of the *ERCC6* codon 399 genotypes (G/A, A/A) is rates 20% in control groups and 45% in indivuduals including dental materials. Our results showed that the heterozygous and homozygous A allele of the *ERCC6* codon 399 may be associated with dental materials and a useful marker for primary prevention in oral cancer.

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Genetic risk factors of bronchial asthma in the Buryat population, Russia

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Asthma is one of the most frequent chronic disease due to the interactions between many genetic and environmental factors. Several candidate genes have been implicated in the development of asthma. The prevalence of polymorphisms in these genes can vary considerably by ethnicity, so the importance of specific polymorphisms may be very different in different ethnic groups. We assessed whether polymorphisms in *FCER2*, *ADRB2*, *NOS2*, *NOS3*, *GSTM1*, *TNFA* and *GSDMB* genes are associated with asthma in Agin Buryat schoolchildren. Agin Buryat is an ethnogeographic group of Buryats inhabiting the Agin Buryat Autonomous Region of Zabaykalsky Kray, Russia. 68 schollchildren with asthma and 100 healthy control individuals were included in the survey. The significant association of rs7216389 polymorphism in *GSDMB* gene and VNTR polymorphism in exon 4 of *NOS3* gene with development of bronchial asthma is confirmed in children group of the Buryat population. The frequency of allele T of rs7216389 *GSDMB* polymorphism was significantly higher in patients than in control group (0.788 vs 0.580; p=0.00011). Odd rates were 2.66 [CI95% 1.59- 4.39] for rs7216389 T allele. The homozygous rs7216389 T/T genotype may be a risk factor for BA (OR=6.55 [CI95% 3.30-12.99]; $p=3\times10^{-8}$). The frequency of allele 4 in VNTR polymorphism of *NOS3* gene in Buryat patients with BA was 0,103, in healthy control - 0.045 (p=0.04), OR=2.44 [CI95% 1.02-5.80]. The risk of BA was significantly higher in persons carried allele 4 of *NOS3* gene (genotypes 4/4 and 4/5) than in homozygotes for allele 5 (OR=2.72 [CI95% 1.06-6.97]; p=0.030).

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J16.88

Correlation between cardiovascular gene polymorphisms and functional capacities of the students

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Associations of genes polymorphisms with different parameters of cardiovascular system are well-known. The goal of the study was to disclose feasible correlation between genetic factors participating in regulation of metabolic processes with relevant indicators of cardiovascular system state in students.
The polymorphisms of *NOS3* (5/4), *AGT* (Met235Thr), *ACE* (I/D), *AGTR1* (1166A>C), *AGTR2* (3123C>A), *BDKRB2* (-58T>C), *REN* (-83G>A), *F5* (1691G>A), *PAI1* (5G>4G), *ITGB3* (1565 T>C), *F2* (20210G>A), *F1* (455G>A), *MTHFR* (677C>T), *ADRB2* (48A>G,
81C>G), *PPARA* (2528G>C), *PPARD* (294T>C), *PPARG (*Pro12Ala), *UCP2* (Ala55Val), *UCP3* (-55C>T), *PPP3R1* (5I/5D) were studied in students (N=170). Gene's polymorphisms were determined by PCR-RFLP and PCR-biochip methods. Assessment of the cardiovascular system state included: the concentration of fibrinogen and homocysteine in serum, blood pressure, volume of blood, stroke volume of blood and others. Correlation between genotypes and parameters was determined by the Pearson correlation.
Strong positive relationship was proved between stroke volumes and minute blood volume and T/T genotype of *AGT* (r<0,63; p<0,01) or C/C of *ITGB3* (r<0,8; p<0,01). Correlation between body surface area index and T/T genotype of *AGT* (r=0,66; p<0,005), diastolic and average arterial pressures and G/G of *ADRB2* (r<0,51; p<0,05), average arterial pressure and 4G/4G of *PAI1* (r=0,75; p<0,05), total peripheral resistance and M/M of *AGT* and T/T of *GP3A* (r=0,69; p<0,005 and r=0,77; p<0,01) was assessed.
Our data confirm clear cut genotype contribution in functional capacity of cardiovascular system. Combined analysis of more representative cohorts of the samples is now in progress.
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J16.89

Genetic aspects of essential hypertension

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Cardiovascular diseases are the leading cause of premature death in the worldwide adults. Overall prevalence of hypertension in Morocco is there about 33.6% being a real public health issue.
Causes of elevated peripheral resistance are certainly multiple and involve several types of factors: vascular endothelial factors involving sympathetic stimulation and the renin angiotensin aldosterone system (RAAS).
Several candidate genes that encode for proteins involved in the pathophysiology of hypertension have been studied, to highlight a link between polymorphisms of these genes and the level of blood pressure.
Our study focused on 132 patients enrolled in the department of Cardiology of the University Hospital of Casablanca Ibn Rushd. Our population was stratified according to cardiovascular risk (CVR) and in which we studied the genetic variability of two genes namely the MTHFR gene (C677T) involved in vascular factors and ACE gene (I / D) which is part of renin angiotensin aldosterone system.
According to the RRS, we found 35% of our population (sex combined) respectively having a medium risk and high risk, while 29.2% were low risk, by ethnicity, we found that whites have low risk with a percentage of 19.40%.
Concerning the genetic study, we found an allelic frequency (D) of the gene ACE about 67.74% and allele frequency (T) around 29.5% of the gene MTHFR and are related with a high cardiovascular risk.

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J16.90

The study of monogenic hereditary dermatosis in the Rostov region (Russia)

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In the literature, more than 200 different genodermatosis or hereditary dermatosis (HD) were described. Genodermatosis can be divided into isolated (violation of only bodies of the skin) and syndromal (a combination of disorders of the skin to other organs and systems). At HD affected different systems of skin covers: skin, hair, nails, glands. The main group of HD up monogenic autosomal dominant disease (AD), less common autosomal recessive (AR) dermatosis, and a small part are of X-linked, dermatitis. Information on the prevalence of HD in different countries is few and hard to compare with each other because of differences in the approach to data acquisition. Hereditary dermatosis in the population 12 districts of the Rostov region was studies (surveyed 497460 people). A basic group was made by AD diseases: ichthyosis vulgaris (prevalence 1:6140 people), neurofibromatosis, type 1 (1:12133), keratosis palmoplantaris (1:19900), epidermolytic palmoplantar keratoderma (1: 124365), monilethrix (1:22611), multiple lipomatosis (1:33164), tuberous sclerosis (1:62182), epidermolysis bullosa simplex, Koebner type (1:124365), LEOPARD syndrome (1:62182), and met rarely alopecia, Von Hippel-Lindau syndrome, ectrodactyly with ectodermal dysplasia, and cleft lip/palate syndrome. The AR diseases were presented: congenital ichthyosiform erythroderma, epidermolysis bullosa dystrophica, Hallopeau-Siemens type, keratosis palmoplantaris with periodontopathia, albinism oculocutaneous, acrodermatitis enteropathica. The X-linked diseases were presented: X-linked ichthyosis and Christ-Siemens-Touraine syndrome. Total prevalence of HD was 5.97±0.35/10000 people (5.07-AD,

0.38-AR and 0.64-X-linked). We examined the patient with monilethrix by means of molecular genetic tests. We detect previously known mutation in 7 exon of the hHb6 gene.

E. Degtereva: None. **N. Vetrova:** None. **S. Amelina:** None. **R. Zinchenko:** None.

J16.91

Search of the prevalence of recurrent mutation R138Q NPHS2 gene in steroid-resistant nephrotic syndrome among Moroccan patients.

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Familial idiopathic steroid-resistant nephrotic syndrome is characterized by a nephrotic syndrome with often early onset. Prevalence in the general population is unknown. The nephrotic syndrome is defined by severe proteinuria with low serum albumin and possible edemas.This disease is rare but severe as it usually progresses to end-stage renal failure. Steroid-resistant idiopathic nephrotic syndrome does not respond to cortico therapy or immunosuppressors and progresses to terminal renal insufficiency. Recurrence after transplantation is exceptional. Several causative mutations have recently been identified. Among these, mutations in the *NPHS2* gene (chromosome 1q25-q31 and encoding podocine) have been found to be involved in autosomal recessive forms of the disease. Mutations in the podocine gene have also been detected in later-onset forms and in apparently sporadic forms (10 to 30% of the cases depending on the series). The R138Q mutation has been reported as recurrent mutation in patients with steroid-resistant nephrotic syndrome, especiallly familial cases.

Objective: Search by targeted sequencing the recurrent mutation R138Q in the NPHS2 gene in Moroccan patients with steroid-resistant nephrotic syndrome.
Determine the prevalence of this mutation in Moroccan patients with steroid-resistant nephrotic syndrome with the aim to propose as a first-line diagnostic strategy in this disease.
Provide genetic counseling to families and allow early treatment of children, to improve the prognosis of this condition.

Materials and methods:

- Recruitment of patients with familial steroid-resistant nephrotic syndrome or sporadic cases with consanguinity,
- Search of the mutation R138Q NPHS2 gene by molecular biology techniques.

L. Boualla: None. **S. Chafai Elalaoui:** None. **N. Oulhiane:** None. **H. Ait Ouamar:** None. **A. Sefiani:** None.

J16.92

Analysis of eight polymorphic nuclear genome DNA loci in Tatar population

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Population genetic survey of the indigenious populations of the Tatarstan Republic (Russian Federarion) belonged to the three Tatar ethnographic groups: Kazan (Arskiy, Atninskiy districts) and Mishary (Buinskiy, Drojjanovskiy districts) and Teptyary (Muslimovskiy, Aktanishskiy districts), was carried out. DNA samples of 675 individuals were examined at eight polymorphic DNA loci of nuclear genome, diallelic: *CCR5 (del32)*, *ACE (del/ins)*, *DATI (del/ins)*, *NOS3 (VNTR)*, and polyallelic: *THOI (STR)*, *FABP2 (STR)*, *CFTR (IVS6aGATT)*, *PAH (VNTR)*. Allele and genotype frequency distributions, indexes of heterozygosity (H_s) and intrapopulation differences ($F_{ST)}$ were obtained for each subpopulation (district) as well as for the ethnic group overall. Analysis of allele's frequency of autosomal DNA markers in Tatar subpopulations shows considerable genetic differentiation between them. The highest level of genetic diversity in diallelic system was established at locus *ACE (del/ins)*, H_{obs}=0,4359, in multiallelic system - at locus *THOI (STR)*, H_{obs}=0,7950. The index of mean heterozygosity is 0,4665. The index of mean intrapopulation differentiation (F_{ST}) was 0,0260. The highest level of intrapopulation differentiation was revealed at locus *FABP2* (F_{ST}=0,1129), the lowest one - at locus *ACE* (F_{ST}=0,0039). The analysis of dendrograms, based on correlations between the matrix of genetic distances, and multidimentional scaling analysis prompted us to conclude that subpopulations of Kazan and Mishary Tatars are genetically closer to each other than subpopulation of Teptyary Tatars. Analysis of genetic distances between populations of the Volga-Ural region shows that the population of Tatars joins the cluster of Udmurts and Bashkirs, where as Mari and Chuvash populations form distinct cluster.

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J16.93

A genome -wide survey of copy number variations in population of Lithuania

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Background: Copy-number variation (CNV) is the most prevalent type of structural variation in the human genome, and contributes significantly to genetic heterogeneity. The extent of CNV and its frequency in different ethnic populations is still largely unknown. Population studies and reference databases for control and disease-associated samples are required to provide an information resource about CNVs frequencies and their relative contribution to phenotypic outcomes.**Objective**: The accessment of CNVs frequencies and distribution in population of Lithuania. **Methods:** A healthy cohort from population of Lithuanian (n=150) were genotyped using the *Agilent* 400K microarray.**Results:** We detected 1073 CNVRs encompassing 166 Mb and accounting for 5.1% of the human genome. The median size of CNVRs was 65 kb (range 20 - 5200 kb). Copy number losses were 1.4 times more frequent than copy number gains. 87% (969 out of 1073) of these CNVRs were common. In this study we found four common CNVRs that were not reported CNV in database of genomic variants (DGV). Interestingly, in Lithuania population 53% (72 out of 137) of rare CNVRs are well known from previously studies. 47% (65 out of 137) of rare CNVRs were novel and not present in the Database of Genomic Variants (DGV).**Conclusion:** In this study the spectrum and frequencies of identified CNVRs supplemented the knowledge of genome peculiarity of Lithuanian population and will be valuable resources for studying human genome diversity and its association with disease.

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J16.94

Genetic dissection of ascending aorta diameter: microsatellites and SNPs linkage analyses in an isolated population of Cilento.

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The ascending aorta is the most vulnerable segment of the aorta. In fact, this segment faces the entire cardiac output volume coming from the left ventricle, since it receives little support from surrounding tissue and no arteries branch from it. The weakening of the aortic wall and loss of elasticity could produce a dilatation and degenerate into a dissection and rupture, becoming a potentially life-threatening disease.
So far, the genetic contribution underlying the ascending aorta diameter (AAD) variability remains unknown. Through echocardiography, we evaluated the AAD in an isolated population from Cilento area in South Italy (N=1,435). The estimation of heritability was of 0.43. Sex, age, BMI and blood pressure were used as covariates. To identify the loci influencing the AAD variability we performed a genome-wide linkage analysis with a regression-based approach using 1122 microsatellites. A significant maximum LOD score of 5.96 was detected on chromosome 4q25. To confirm this results, a linkage analysis using a SNP map was performed. Applying the MASEL method, a subset of SNPs highly informative and with a minimum linkage disequilibrium was selected on chromosome 4. A maximum LOD score of 3.2 was detected in the same region identified with the microsatellites confirming the involvement of the 4q25 region in the ADD variability. Moreover, the SNP analysis allowed us to refine the linkage region from 7.9 to a 6.7 Mb. In this work, for the first time, we found a strong linkage signal on chromosome 4q25 linked to AAD in an isolated population of Cilento.

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J17.01

Assessment of detection of proviral DNA and RT (MET 184 VAL) gene resistance mutation in HIV-1 identified by polymerase chain reaction and restriction fragment digestion assay

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ABSTRACT

Proviral DNA and ART (Antiretroviral Therapy) resistance forms a corner stone of a short chemotherapy course for post-exposure prophylaxis. The aim of this study was to rapidly identify the proviral DNA and screen M184V

ART (Antiretroviral) resistance mutation in HIV-1 reverse transcriptase by using PCR-RFLP (Polymerase Chain Reaction- Restriction Fragment Length Polymorphism).The male patient's outnumbered female. Among 13 male subject 2(15.4%) were proviral DNA positive and 11(84.6%) were proviral negative. Among 2 female subject 1(50.0%) were proviral DNA positive and 1(50.0%) were proviral negative. RFDA (Restriction Fragment Digestion Assay) were performed using restriction enzymes CViAII allowing determination, on Agarose gel electrophoresis, of 184M wild-type (ATG) or 184V mutant (GTG) strains. All isolates were only digested by CViAII restriction enzyme and confirmed as wild type. No mutation was detected in the analyzed sample. In summary. Potent antiretroviral therapies suppress cell-free plasma viral RNA levels below the limit of current assay detection necessitate other complementary approaches for assessing viral burden, such as quantification of cell associated proviral DNA. Therefore, it is recommended to use this technique for the diagnosis of Drug Resistance Mutation in HIV-1 patients and the response of ART for their Better Health Care.

Keywords: HIV/AIDS, HAART, Drug resistance, mutation, env gene, RT gene, PCR, RFDA.

R. Shrestha: None.

J18.01

Screening for Fragile X syndrome in females: A molecular approach in a Brazilian Public Genetic Service routine

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ABSTRACT: Fragile X syndrome (FXS) is caused by the expansion of trinucleotide repeat citosin-guanin-guanin $(CGG)_n$ in the 5´untraslated region (UTR) in exon 1 on *FMR1* gene on X chromosome. OBJECTIVE: Implement a molecular screening methodology for SXF on females in a Brazilian Public Genetic Service in the aim to reduce the necessity to use Southern Bloting. METHODS: Sixty-three females tested by PCR-ssp, when resulted inconclusive were tested on PCR-*fam* by capillary electrophoresis. RESULT: It was possible to determinate a final result in 31.75% of females tested by PCR-ssp, 68.25% resulted inconclusive. On PCR-*fam*, 74.42% resulted in heterozygosis, 4.65% presenting gray-zones and 20,93% of all was homozygosis. CONCLUSION: The techniques show efficiently identification of homozygosis and heterozygosis females, reducing of 85,71% of the necessity to apply SB to give a final diagnostic on FXS in females.

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J18.02

The relationship between BRCA2 mutation risk and pedigree size

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Introduction. Genetic counselling for HBOC in Iceland uses a population-based electronic genealogy database and cancer registry, ClinicalPedigree and Boadicea risk assessment programs. All mutations in Iceland are rare except c.771_775del5c in the BRCA2 gene (prevalence 0.6%). This unique situation makes it possible to study the relationship between pedigree-size and accuracy of risk assessment.

Materials and methods. 340 individuals (136 probands) received cancer genetic counselling during 2009-2011 at Landspitali. Two electronic pedigrees were obtained for each counselee, one for each family side. After exclusions, calculations for the relevant side were carried out for 102 individuals. Included were 12 belonging to previously known BRCA2 families. From this group, 82 probands had BRCA2 mutation testing.

Results and Discussion. On average, the pedigrees sides included 260 individuals (range 21 to 1288). Of 82 tested, 20 had BRCA2 and 1 BRCA1 mutation. Nine were sent for full mutation screening with no new mutation found. Truncation of pedigrees to 3° relatives included 43.4 (8 to 139), 2° relatives 22.8 (5 to 63) and 1° relatives 8.7 (3 to 17). The average BRCA2 mutation likelihood score for BRCA2 positive individuals in Boadicea with 3° truncations was 0.2280 (range 0.0063 to 0.7426). Average score for non-tested individuals was 0.0165 (0.0010 to 0.0897). Average score for further analyses was 0.1759 (0.0131 to 0.3871). By using ≥10% as a cut off for increased risk, the sensitivity for BRCA2 mutation increased with pedigree size, i.e. for 1° truncation the sensitivity was 45%, for 2° truncation 55% and for 3° truncations 70%.

V. Stefansdottir: None. **O.T. Johannsson:** None. **C. Chapman:** None. **L. Tryggvadottir:** None. **H. Skirton:** None. **H. Tulinius:** None. **J.J. Jonsson:** None.

J18.03

Microarray Comparative Genomic Hybridization analysis (array-CGH) as a diagnostic tool for the investigation of patients with autism or autistic spectrum disorders

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Aim: Autism or autistic spectrum disorders (ASD) are a lifelong neurodevelopmental disorder and α few cases of chromosomal abnormalities are found by conventional cytogenetic techniques, while the microarray Comparative Genomic Hybridization analysis (array-CGH) allows the identification of submicroscopic genomic rearrangements. This study describes the application of array-CGH, as a diagnostic tool for the investigation of patients with autism or ASD.

Materials- Methods: During the last 4 years, 88/480 patients were studied with autism or ASD of unknown aetiology but with normal previous conventional karyotype and other genetic tests. High resolution 4X180K and 1x244K Agilent arrays were used in the study (>170.000 and > 236.000 probes respectively, average resolution of 8.9 Kb).

Results: Submicroscopic genomic rearrangements (CNVs), ranging in size from 0.08 to19.01 Mb were detected in 51/88 (58%), some of which also presented: seizures (8/51), hypotonia (1/51), obesity (2/51), mental retardation (9/51), hearing loss (1/51) and dysmorphic features (2 / 51). CNVs detected were in loci at high risk for autism: 2q37.2, 2p16.3, 5p15.32p14.3, 7p22.3, 10q26.3, 12q24.22q24.3, 15q11.2q11.3, 16p11.2, 18q22.3q23, 21q22.3, Xq21.31. Genes associated with autism or ASD which were identified included: NRXN1, SHANK3, DOCK8, ZNF92, ASMT, HSFX1, KCNH7, CHRFAM7A, CHRNA7, KCND2, CNTNAP3, MAOA, MAOB, STS, VCX.

Conclusions: In patients with autism or ASD and non-syndromic phenotype, array CGH analysis is mandatory to detect possible submicroscopic chromosomal abnormalities. For the family, positive results of array-CGH, provides comprehensive genetic counseling, which includes determining the patient's prognosis and planning antenatal diagnosis in subsequent pregnancies.

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J18.04

Genetic counseling for patients with intellectual disability in IRAN

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Intellectual disability (ID) is an unresolved health care problem worldwide and an enormous socio-economic burden. Our goal for genetic counseling was providing reliable data for the best decision in families which suffering ID.

Over last10 years total of 985 Iranian families with ID have been referred to Genetics Research Center of USWR. After obtaining informed consent form, completing clinical profile was performed. As a primary investigation, karyotyping, fragile X testing and metabolic screening have been performed.

From 985 families, a total of 859(87.2%) had autosomal recessive pattern of inheritance, 32(3.2%) with autosomal dominant, 94(9.5%) with X-linked recessive pattern. Two hundred forty (24.3%) out of 985 families were associated with microcephaly of whom 56 families MCPH genes have been detected. A total of 46(4.6%) families identified with fragile X syndrome. In 18(1.8%) cases different types of chromosomal abnormalities have been detected and 36(3.65%) families were positive for metabolic disorders. In 27(2.7%) families different known ID syndromes has been detected and 68(6.9%) families had mutations in recently identified genes responsible for hereditary ID.

Genetic counseling is helpful for families by providing information about recurrence risk, carrier detection and offering prenatal diagnosis for future

pregnancies. Therefore genetic counseling is a suitable method for reducing the prevalence of ID in each population.

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J18.05

Unusual splicing mutations in patients with neurofibromatosis type 1

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Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder caused by inactivating mutations within the NF1 gene. Protein product of NF1, neurobibromin, is a negative regulator of Ras proto onkogen. In Slovak population we identified 85 NF1 gene mutations. 14 of them (16,5%) represent splicing defects, that were easily uncovered thanks to the sequencing of cDNA prepared from a total RNA extracted from the patients' cultivated lymphocytes. Consequently, by sequencing of affected exon on gDNA level individual nucleotide changes causing such incorrect splicing were described. In 9 cases (64,3%) these mutations involved consensus splicing regulatory sequences, in remaining 5 patients (35,7%) non-typical splicing pathogenic variants were uncovered. In one of them skipping of exon 29 or 29 and 30 was caused by short genomic deletion including part of intron 28 and exon 29 that eliminated acceptor splice site. In another case a single nucleotide substitution in intron 9 created new splice site, leading into insertion of last 17 nucleotides of intron 9 into transcript. Another 3 mutations were single nucleotide substitutions affecting exons 7, 10b and 24 that would normally be described as missense mutations, however, cDNA sequencing uncovered their splicing defects. Prediction program HSF (Human Splicing Finder) indicates in all of them activation of cryptic splice sites. Three of the unusual splicing mutations are novel, whereas two missense mutations causing skipping of exon 24 and partial deletion of exon 10b have already been described. Our work confirms the advantage of using RNA based method for NF1 gene mutation detection.

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J18.06

Molecular genetic analysis in Russian families with neurofibromatosis type 2

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¬¬Neurofibromatosis type 2 (NF2) is an autosomal dominant disease (prevalence around 1 in 60,000), that is caused by inactivating mutations of the NF2 gene localized on chromosome 22q12.2. NF2 is characterized by the development of schwannomas (the most typical feature is bilateral vestibular schwannomas), meningiomas, spinal tumors, peripheral nerve tumors, ocular abnormalities. Previous studies have stated that half of NF2 patients have no family history and have de novo mutations in the NF2 gene, 25-33% of these new cases are mosaic with the mutation only detected in tumour and not in lymphocyte DNA. We describe NF2 gene molecular diagnosis in 15 individuals from 13 families from Russia with (7) or without (8) family history of NF2, mostly patients of Burdenko Neurosurgery Institute (Moscow) after surgery vestibular shwannoma removing. We have screened lymphocytes and tumor DNA samples for germ line and somatic mutations in NF2 gene. Mutation analysis was performed by direct sequencing using 17 primers pairs covering the coding sequences and their intron-exon junctions and MLPA (MRC Holland). Overall, we found 16 mutations in 15 patients (as in lymphocytes and in tumors): one large deletion (including exon 4), two duplications (one includs the regeon promotor-exon 4 and the other one is duplication of 11-17 exons) and 13 point mutations (4 of which have been described by other authors: R196X, R198X, R57X, Y153X). The remaining 9 point mutations are not announced in HGMD: S87X, Q121X, V219M, c.241-3C>T, Y266D, K278X, K284X, R335W, E378X. This is the first survey of NF2 in Russia.

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J18.07

The first Iranian patient affected with Oguchi disease

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Oguchi disease is characterized by congenital static night blindness and diffuse yellow or gray coloration of the fundus. After 2 or 3 hours in total darkness, the normal color of the fundus returns.
Based on mutations in two genes, namely, the arrestin (also called S-antigen, *SAG*) and rhodopsin kinase (also called G-protein-dependent receptor kinase 1, *GRK1*), the disease is classified as type 1 or type 2, respectively.
A 38-year-old boy with bilateral eye pain and headache on reading and non-progressive blurring of distance and near vision, especially in the dark, was referred by a neurologist.
To identify the genetic cause of the disease in the patient, blood samples were collected by venipuncture in EDTA vacutainer tube from the patient and the available family members. The samples were stored at 4 °C till DNA isolation was performed. Oguchi disease candidate gene analysis was performed in the family by sequencing *both SAG and GRK1 gene.*
The PCR products were sequenced with the 3710 Applied Biosystems, ABI. Carrier screening of the identified mutation in the family was also performed with sequence analysis of exon 4 in all family members. Sequencing analysis showed a homozygote deletion C, in codon 96 (in exon 4). Parents of patients were heterozygote for mentioned deletion as well.
In conclusion, we have identified the first deletion mutation in the *SAG* gene in a patient with Oguchi type 1 disease in IRAN.

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J18.08

Ambiguous genitalia in newborns - clinical variability due to rare genetic causes

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Disorders of sexual development (DSD) are among the most fascinating conditions encountered by clinicians. The ability to diagnose these disorders soon after birth is very important clinical issue with its different aspects relating to diagnosis, treatment and sex of rearing. The causes to DSD are frequently related to effects of sex hormones- congenital adrenal hyperplasia (21-hydroxylase deficiency) and androgen insensitivity syndrome are most widely recognised. Rare causes of DSD include other forms of congenital adrenal hyperplasia (CAH), chromosomal abnormalities including mosaic karyotypes and genetic syndromes where ambiguous genitalia is one of the presenting symptoms.
The aim of this study is to describe the rare genetic causes of DSD in the patients with ambiguous genitalia presenting in newborns.
Materials and methods. Patients referred to the genetic consultation due to ambiguous genitalia in the region of Northern Estonia are described.
Results. During 2007-2012 four babies were consulted as newborns due to ambiguous genitalia in neonatal period. Mosaic karyotypes were diagnosed in 2 patients: Down-Ullrich-Turner (DUT) syndrome (fibroblast karyotype 45,X (66%), 47,XY,+21 (34%)) and mosaic Turner syndrome (fibroblast karyotype 45,X[48]/46,XY[53]) were diagnosed. 17-α-hydroxylase deficiency, a rare form of CAH was diagnosed in a girl with 46,XY karyotype and Smith-Lemli-Opitz syndrome was diagnosed in a dysmorphic child with the karyotype 46,XY.
Conclusion. Wide range investigations including hormonal, metabolic and genetic studies are necessary to establish the cause of ambiguous genitalia. Identifying the underlying cause of is necessary for the individual prognosis and genetic counselling of the family.

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J18.09

Patient with chronic pancreatitis and polycystic kidney disease

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Introduction: The major ethiological factor for recurrent acute pancreatitis/chronic pancreatitis (RAP/CP) in adults is the excessive alcohol consumption. Among children anatomical anomalies of pancreas and biliary

tract and genetic factors seem to be crucial.
Autosomal dominant polycystic kidney disease (ADPKD) is the most frequent hereditary renal disease, leading to terminal renal failure of around the fifth decade. *PKD1* and *PKD2* genes are responsible for its clinical manifestation. ADPKD is usually asymptomatic during childhood.
Case report: A 10-years-old girl with acute exacerbation of chronic pancreatitis (2nd attack) was admitted to the hospital. Ethiologic causes including autoimmune, metabolic, infectious, drug and systemic were excluded. The MRCP was performed and structural anomalies were ruled out, but multiple cysts in both kidneys were the accidental finding.
Family history was negative for CP, but positive for ADPKD. Hence unexplained CP in the girl, molecular-genetic analysis of 4 genes associated with CP (*PRSS1, SPINK1, CFTR, CTRC*) was performed. The homozygous c.180C>T mutation of *CTRC* gene was confirmed. The linkage analysis of ADPKD genes validated the presence of the risk haplotype and carriage of germ line mutation in *PKD1* gene.
Conclusion: We present a girl with two genetically determined disorders. The CP is in this case caused by the homozygous c.180C>T mutation of *CTRC* gene. It increases the risk of CP about 10-fold and is associated with early-onset RAP/CP.
The risk genotype of ADPKD with *PKD1* gene involvement expects more severe course of the disease, consequently requires early management to prevent complications.

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J18.10
One month exercise training effect on body composition and muscular performance in young cystic fibrosis patients

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Aim: To evaluate the effectiveness of one month intervention programme on body composition and muscular performance in young cystic fibrosis patients.
Material and Methods: We performed a one month prospective study, on 19 cystic fibrosis patients, aged between 6 and 12 years old. According to their availability to spend a period of 4 weeks at the Romanian National Cystic Fibrosis Center, patients were divided in 2 groups. Intervention group (10 patients-accepted to remain in the hospital for the entire period of intervention) performed intensive exercise training and received specific diet and nutritional counselling; and Control group (9 patients) who returned home after receiving nutritional and exercise training advices. Patients were evaluated, at baseline and at the end of the intervention period in regard to body composition (using a four-paired electrode bioimpedance device - InBody 720) and muscular performance of the lower limbs (using Myotest system).
Results: At the end of the study we observed a significant increase of skeletal muscle mass (from 19.90±7.27 to 21.82±7.45, p= 0.0003), explosive power of the lower limbs (from 19.23±2.28 to 21.89±1.87, p< 0.0001) and force of the lower limbs (from 19.77±2.72 to 20.81±2.95, p< 0.0001) in the intervention group. One month intervention did not influence significantly the patients in the Control group.
Conclusions: A one month in-patient intervention programme based on intensive exercise training associated with proper nutrition significantly improves the body composition and muscular performance in cystic fibrosis patients.
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J18.11
Should we offer preimplantation genetic screening to women with advanced maternal age?

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Chromosome abnormalities increase with advanced maternal age (AMA). This leads to an enhanced risk to have an offspring with aneuploidy and miscarriages.
Interventions for prenatal diagnostic purposes and spontaneous miscarriages due to aneuploidy following IVF can be often disappointing for the women in advanced age. Preimplantation genetic screening (PGS) a variant of preimplantation genetic diagnosis, screens for frequent numerical chromosomal abnormalities that are observed in miscarriages and widely recommended in AMA cases to select euploid embryos.
In this study, we analysed aneuploidy rates of 45 spontaneous miscarriage materials from women with AMA. 15 of these cases were IVF pregnancies. 20 of 45 cases revealed chromosomal abnormalities (44.4 %) and all of them were aneuploidy including trisomy 4, 13, 15, 16, 18, 21 and 22. 12 of 30 AMA cases (40 %) and 8 of 15 (%53.3) IVF+AMA pregnancies revealed aneuploidy.
These results supported the hypothesis offering PGS to women in advanced age for selection of euploid embryos would increase reproductive success.
PGS is routinely offered in many IVF centers by the way the low quantity and quality of oocytes after ovarian stimulation, which is closely related to the ovarian reserve, is still a problem. For this reason, we suggest if the response to the ovarian stimulation is enough we can offer PGS to the women in advanced age.

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J18.12
Renal Disease's Genetic Counseling- a Must for an Affected Family

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Introduction: genetic counseling represents a challenge in families with socio-economic problems, even in countries with a properly preventive medicine. We present a local family case report aiming to analyze how a precocious correct diagnose and a proper family counseling may improve the quality of life and requires a low grade of society's resources giving hope for severely affected family.
Methods: family tree, antecedents' analysis, the onset, diagnoses or suspicions of disease, genetic counseling.
Results: studying the family, it revealed 5 persons with inherited renal disease: 5 years old boy, 16 years old girl, 18 years old young woman, their mother and their grandmother. All children have no other brothers or sisters; their mother has 2 healthy sisters. All the members of the family that have the disease presented a precocious onset, with a progressive glomerulonephritis, the adult ones being on dialysis. They had a restricted access on medical information and they weren't investigated of an inherited renal disease till now. They have renal biopsy for a proper diagnosis in these days and we start a suitable genetic counseling for their family potential offspring. Our counseling started with genetic consult and psychological sessions, individual and with all family members.
Conclusions: After 10 years of dialysis (grandmother) and 5 - mother, this family receives now a good medical care but with a difficult management of their genetic disease, without a global viewing of their pathology.The correct diagnose followed by a proper counseling is their chance to improve next generation future and life.

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J18.13
A Novel mutation in POLH gene in Tunisian families with a severe phenotype of XP-V

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Xeroderma pigmentosum (XP) is a genetically heterogeneous autosomal recessive disorder with seven complementation groups and an additional variant form, XP-V, that is characterized by late onset of skin symptoms and absence of neurological abnormalities.
In the present study, we report on the molecular investigation of three patients belonging to two Tunisian unrelated families and manifesting severe phenotype. Homozygosity by descent analysis was carried out using two microsatellite markers: D6S1582 and D6S271. Haplotype analysis showed homozygous haplotype for all patients that share a common allele for D6S271 marker. Mutations were screened by direct sequencing of exons 5, 9, 10 and 11 of *POLH* gene. Results showed a new splice site mutation c.660+1G>A at the donor site of intron 5. Bioinformatic tools revealed abolition of the donor splice site. Assessment of mRNA by RT-PCR analysis showed total ab-

sence of *POLH* mRNA in all patients. Absence of mRNA could be explained by the involvement of the Nonsense Mediated Decay mechanism, responsible for the aberrant mRNA degradation and thus leading to the severe phenotype of XP-V patients.
In conclusion, molecular investigation allows identification of the genetic basis of the severe form of XP-V in Tunisian population. This will enable families to beneficiate from genetic counseling.

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J18.14
Parent's attitude towards prenatal diagnosis and termination of the pregnancy could be influenced by other factors rather than by the severity of the condition

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Objective: The aim of this study is to inevstiagete whether the severity of a particular condition alone influences parents attidues toward prenatal diaganosis (PND) and termination of pregnancy (TOP) or are there other factors involoved? Additionally, we aim to find similarity and different views toward PND and TOP for 29 conditions (variable severeities) compared to thalasseimia (severe condition).
Methods: A questionnaire which mainly focuses on parent's attitude toward PND and TOP for 30 different hypothetical scenarios for a series of genetic, non-genetic and non-medical conditions were completed by 400 Saudi parents. Results were compared and scored and parents comments were noted. Additionally, cross tabulation of thalassemia, considered the most severe and had most favorable PND and TOP, against the 29 other conditions were carried out to find similarities and different views toward TOP and PND.
Results: We found that parents' attitudes towards PND and TOP for thalassemia are significantly associated with their attitudes in relation to all of the other conditions (Fisher's exact P<0.01 in all cases). Some parent's attitudes toward TOP were clearly influenced by their religious-beliefs, others by cultural-values and some by the impact on their quality of life regardless of the severity of the condition.
Conclusion: Saudi Parents attitudes toward TOP and PND are not always influenced by the severity of the condition, but religious-beliefs, cultural-values and impact on parent's quality of life also play a role.

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J18.15
Genetic counselling and epigenetics

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When providing genetic counselling to a patient or a couple it is important to choose the most relevant and objective information and to assure its understanding. Only then the patient's future decisions are well-grounded.
The aim of this study was to discover the opinion of students regarding the influence of epigenetics on genetic counselling.
For this purpose 154 students of the Medical and Pharmacy University „Carol Davila" in Bucharest were asked to write their opinions in a two page essay. The first year medical students had learned in seminars and lectures about genetic counselling and epigenetics. In the essay entitled „Genetic counselling and epigenetics", it was specified that they had to address whether or not the new discoveries impede genetic counselling. The essay was part of their final evaluation in Medical Genetics.
Students' views at the end of the first academic term are reviewed, discussed and the most frequent opinions are summarized. They viewed as irreplaceable the followings: determining the risk of occurrence or recurrence of a disease, preimplantation and prenatal diagnosis, the use of predictive genetic testing, as well as the ongoing genomic research, which increases our understanding of how diseases are caused and progress.
As a conclusion, all students highlighted the importance of genetic counselling in present day medicine and the need to integrate all new data in a genetic consultation. So epigenetics can only be an essential tool in the management of inherited disorders shaping accordingly the genetic counselling.

R. Dragotoiu: None.

J18.16
Dynamic prenatal screening in the I and the II trimesters of pregnancy

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Combined screening in the 1st and 2nd trimesters of pregnancy, comparative analysis.
309 women 9-13 and 16-22 weeks of pregnancy are surveyed using noninvasive and invasive screening methods. The research methodology included undertaking ultrasonic screening; identification of the serum markers of-HCGβ and PAPP-A in the I trimester; human chorionic gonadotropin (hCG), Alpha Fetoprotein and Estriol - in the II trimester; computer analysis with application of the Life Cycle programs in the I trimester and PRISCA-in the II trimester; invasive methods of prenatal diagnostics.
At dynamic control in the I trimester of pregnancy from 47 (15,2%) women in the group of risk 9 (19,1%) fetuses were diagnosed with the Down syndrome (5), Edwards (3) and Patau (1). In the II trimester in the group of risk of 64 (20,7%) women no fetus were found with the chromosomal pathology i.e. all changes of a karyotype were established in the I trimester.
All chromosomal pathology cases were diagnosed in the I trimester of pregnancy. Therefore, based on the results of the analysis, the advantages of inspection in early times of pregnancy were identified.
The screening programs should be predominantly applied in the I trimester to identify pregnant women of risk group. When low risk is identified, inspection in dynamics is inexpedient. Noninvasive screening in the II trimester should be carried out only in the absence of risks in early periods of pregnancy. At high risks it is necessary to decide carrying out invasive methods of prenatal diagnostics in the I trimester of pregnancy.

D.T. Tursunova: None. **A.K. Yuldasheva:** None. **Z.G. Khvan:** None. **F.K. Odinaeva:** None.

J18.17
Childhood obesity and the MC4R gene: Necessity for early genetic testing

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According to a survey conducted be the WHO, globally around 170 million children (< 18 years) are estimated to be overweight.
Mutations in the MC4R (melanocortin-4 receptor gene) are the most common genetic cause of obesity. The *MC4R* gene codes for a protein that is primarily expressed in the brain and encoded by a single exon on 18q22. Mutations in this gene are associated with obesity in an autosomal-dominant fashion. In a study, the MC4R mutation accounted for 5.8% of the 500 morbidly obese children who participated. Symptoms accompanying MC4R-related early onset obesity are also seen with other types of obesity and may become apparent only over extended periods of time. Therefore, MC4R-related early onset obesity cannot be diagnosed via symptomatic clinical diagnosis. Genetic testing allows a diagnosis of MC4R-related early onset obesity at any age**. Genetic testing can also identify carriers of obesity-associated mutations.** Studies in cell culture have indicated that the functionality of a normal MC4R protein is not affected by presence of a mutated receptor protein in the same cell .Therefore, increased stimulation of the remaining healthy MC4R protein with specific drugs may be able to compensate for the loss of function in the mutated receptor. Early detection of this mutation shall help start treatment immediately. Also preventive measures such as dietary control can be immediately incorporated, thus reducing levels of obesity and bringing down the risk of associated disorders such as Diabetes and cardiovascular disorders.

R. Subramaniam: None.

J18.18
Comprehensive Phenotyping of Mouse Models

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The Institut Clinique de la Souris (ICS) is a technology platform that provides a comprehensive set of highly specialized mouse services to scientists from academia and industry. The ICS combines the capacity of generating mutant mice on a large scale with a high-throughput and comprehensive phenotypic analysis of mice. The ICS phenotyping platforms are adapted for the study of genetically engineered mouse models, as well as for pharmacological and toxicological studies, allowing better understanding of human diseases and their underlying physiological and pathological basis. The ICS

has successfully assembled a comprehensive phenotyping platform on 5 following core units: 1) Clinical chemistry laboratory covers biochemistry, hematology, coagulation, immunology, endocrinology exploration. 2) Metabolic exploration service is set up to explore energy balance including body composition, glucose homeostasis, and energy expenditure, as well as the skeletomuscular and the uro-genital systems, and the gastro-intestinal tract. 3) Cardiovascular and respiratory exploration including tests to analyze the cardiac function and anatomy, as well as the respiratory system such as in asthma models. 4) Behavior and nervous system. This core has developed a comprehensive tests battery to evaluate: general CNS function, affective behaviors (anxiety, depression), cognitive function, sensory thresholds and analgesia, as well as the sensory systems (visual and auditory functions). 5) Histology and pathology. This service provides a comprehensive histological and histopathological analysis of mutant and control mice, as well as embryology studies. The assays performed by each core unit will be presented, as well as validated flow schemes for applications in therapeutic areas.

M. Champy: None.

J18.19

Molecular and genetic aspects of the regulation of lipid metabolism in individuals with different levels of fitness

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In recent years, increased interest in the study of lipid metabolism in the adaptation to the effects of physical activity and its nature in athletes of different specialization and different levels of fitness. Long-term adaptation of athletes to physical activity of varying intensity accompanied by specific changes in the structure of the lipid metabolism.
Important role in the regulation of metabolism of triglyceride-rich lipoproteins and HDL genes belong, which is part of a gene cluster A1/C3/A4/A5 apolipoproteins, of which we have studied polymorphic variants of two genes - apolipoprotein A-I APOA-I (rs 1799837, A-75G) and gene apolipopotein C-III APOC-III (rs 76353203, C3238G), located on chromosome 11 (11q23 and 11q23.3, respectively).
The material for the study is based on 456 DNA samples of healthy individuals. Determining the level of the major lipid profile (total cholesterol, triglycerides, low density lipoprotein, high density lipoprotein) was performed by standard enzymatic methods. Analysis of polymorphic DNA loci APOA-I, APOC-III was performed by PCR-RFLP.
Univariate analysis of variance revealed a significant effect of genotype APOA-I A/A (F=4,967; p=0,028) at a lower concentration of triglycerides, and genotype APOA-I A/G (F=7,862; p=0,006) to increase the concentration of triglycerides.
A comparative analysis showed a group of individuals practicing significant increase genotype APOA-I A/A (p=0,0151; χ2=5,9795) and a decrease in genotype APOA-I A/G (p=0,0112; χ2=6,5579).
During the haplotype analysis in a group of individuals practicing found a significant increase in the haplotype G/C (p=0,0006; χ2=17,5365) and a decrease in the haplotype A/G (p=0,0348; χ2=4,4642; OR=1,6186).

L. Kayumova: None. **E. Vorobieva:** None. **V. Gorbunova:** None.

J18.20

Monosomy 1p36 case with left ventricular „noncompaction“ and right ventricular rhabdomyoma

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Monosomy 1p36 syndrome is characterized by many different dysmorphic features including large and late-closing anterior fontanelle, microcephaly, brachycephaly, deep-set eyes, depressed nasal bridge and a pointed nose and jaw structure. LVNC syndrome has been reported as associated with 1p36 deletion in pediatric population. On 31/05/2012, the patient, genetically unrelated three-year married couple's first baby, was born by caesarean section at 38 weeks. They applied to our clinic with complaints as fetal rhabdomyoma, left ventricular „noncompaction“, VSD, PDA. Large forehead, open and large anterior fontanelle with front starting point, flat and broad bridge of the nose, straight eyebrows, short palpebral fissures, narrow and slanted, both ear curved inward and helix, small corners of the mouth, bevel-down, long philtrum, high narrow palate, pointed chin, telangiectasia/hemangioma inside the thumb of the right hand, inner palmar of the left hand, hypopigmented macules on the right axillary region, the lower-right quadrant of the abdomen and lumbar region, open sinus on sacrococcygeal region, second fingers of both feet were top placement. 46, XX chromosome structure was determined according to the chromosome analysis of patient's peripheral blood sample with G-banding technique. Heterozygous 1p36.32-36.33 microdeletion was detected by the mental retardation panel of MLPA analysis method. 60 cells were examined by the method of FISH for the verification and 1p36 deletion was detected in all cells This case has importance because this is the first case of LVNC-monosomy 1p36 syndrome shown in Turkish population and different from recent studies with the presence of hypopigmented macules.

H. Gurkan: None. **S. Gokalp:** None. **H. Tozkir:** None. **G. Sari:** None. **U. Vatansever Ozbek:** None.

J18.21

Perceptions of physicians on pharmacogenetics in Abant Izzet Baysal University, Medical Faculty, Turkey

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Abant Izzet Baysal University (AIBU) Medical Faculty pharmacogenetics testing laboratory is recently established. To inform the availability and recent pharmacogenetics knowledge, two seminars are given to the total of 106 hospital attendees. After the availability of pharmacogenetic testing, we evaluated physicians' perceptions on pharmacogenetic testing and its implementation.
The survey was performed among 35 academic medical staff in the faculty and 35 recently graduated medical faculty students. The results were calculated with the EpiInfo statistics software using the χ2-test.
According to this survey performed at AIBU Medical Faculty, 2.9% of the responding academic medical staff (AMS) was trained for pharmacogenetics at undergraduate level, while 20% of recently graduated medical faculty students (GMS) declared the same. Belief in the influence of genotypes on drug response was as 91.4% and 100% of AMS and GMS, respectively. Relatively fewer AMS (31.4%), than GMS (25.7%) reported that they felt adequately informed about the availability of genetic testing and its application. Test results of the two groups were statistically significant (p<0.001).
In this survey we can conclude that there is a remarkable difference in the knowledge, approach and proceeding on pharmacogenetics between academic medical staff and recently graduated students. The cause of the difference can be speculated as experience or curriculum differences. Additionally, we can follow up, that this particular issue is now taught in faculties. To complete, one can postulate, that with the availability of pharmacogenetic clinic implementation knowledge and more education, future adopters will rise.

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J18.22

Rapid detection of fetal aneuploidies by Quantitative Fluorence PCR (QF-PCR) in prenatal dioagnosis

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Purpose to investigate which STR markers are better for prenatal diagnosis and the frequency of euplodies and aneuploidies in prenatal diagnosis of pregnant subjects in Cukurova, Adana region. QF-PCR, a novel technique, which is fast and reliable, is employed to detect aneuploidies (13,18, 21, X, Y) without the need of time consuming culturing process. QF-PCR can detect 5 different chromosome aneuploidies with 98.6% accuracy. Methods, 1804 amniotic fluid samples of pregnant subjects, who were referred to molecular biology section of our department, were analyzed with QF-PCR technique employing 35 STR markers for detecting chromosomes 13, 18, 21, X and Y aneuploidies. Results we detected 152 subjects (8,42%) out of 1804 with aneuploidies or euploidies. The average age of pregnant subjects was 32 (varies between 14 and 49). Abnormal karyotypes detected were as follows: 47,XX,+21 (34.87%, 53/151); 47,XY,+21 (40.13%, 61/151); 47,XX,+13 (0.66%, 1/151); 47,XY,+13 (1.32%, 2/151); 47,XX+18 (7.89%, 12/151); 47,XY+18 (5.93%, 9/151), 47,XXX (1.32%, 2/151); 69,XXX (1.32%, 2/151); 45,X (6.58%, 10/151). Conclusions QF-PCR can be regarded as an alternative method of conventional cytogenetic analysis since it is rapid and reliable, however in most cases it is required to be supported or validated with conventional cytogenetic karyotyping.

T. Tufan: None. **A. Pazarbasi:** None. **M.A. Erkoc:** None. **G. Comertpay:** None. **S.K. Sel:** None. **F.T. Ozgunen:** None. **M.B. Yilmaz:** None. **S. Buyukkurt:** None. **N.S. Ilgaz:** None. **L. Ozpak:** None. **I.N. Uslu:** None. **P. Pazarci:** None. **D. Alptekin:** None. **C. Evruke:** None. **H.U. Luleyap:** None.

J18.23

Coexistence of Townes-Brocks syndrome and Albinism in a case

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Townes-Brocks syndrome (TBS) is a rare genetic disorder. The most common features of this syndrome are anal atresia, abnormally shaped ears, and hand malformations that most often affect the thumb. TBS syndrome is caused by mutations in a SALL1 gene. This gene is located chromosome 16q12.1.2 Albinism is one of the archetypal inborn errors of metabolism described by Archibald Garrod, with a frequency of around 1:20.000. It is usually defined as a congenital hypopigmentation of the skin, hair, or eyes. There are a large number of genes responsible for the occurrence of albinism .One of this gene is The melanocortin 1 receptor gene (16q24.3).
Our patient is male child who his age is 9 months. He has an obstruction of the anal opening (imperforate anus), low and simple ears, ear tag, simian line, short palpebral fissure. The patient's mother and father were not consanguineous. We performed a cytogenetic analysis and the other laboratuary analysis. We have found that patient`s chromosome 16 has anormal structure. The patient was evaluated as TBS after we performed a cytogenetic analysis and the other laboratuary analysis. However, he has a congenital hypopigmentation of the skin, hair and eye. This new situation was evaluated as Albinism because his mother has albinism. Therefore, we thought that our patient is both Townes-Brocks syndrome and albinism. In addition, further research will be beneficial because the localization of these two genes which are responsible for the disease is closely.

K. Nadir: None. **S. Çelik:** None. **S. Çitli:** None. **T. Çora:** None.

J18.24

PhD, MD

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Background: peculiarities of clinical manifestations and cytogenetic features are analyzed in Turner syndrome, which is a gonosomal abnormality characterized by a loss of X chromosome (total or partial) with or without mosaics.
Materials and methods: A group of 68 children with Turner syndrome was investigated during medical genetic counseling in the study.
Results: The most characteristic features of patients with Turner syndrome are: short stature - 97%, incomplete sexual maturation 95%, cubitus valgus - 48%, pterygium colli - 44%, short neck - 42%, palatine arch - 37%, multiple nevus - 23%, lymphedema - 24%, nail dysplasia - 13%, scoliosis - 12%. The frequency of cytogenetic variants of Turner Syndrome: 1. homogeneous form or X monosomy: 45 X - 53% of cases; 2. mosaic form: 45, X/46 XX, 45, X/46, XY - 11.8% cases; 3. structural abnormalities of X chromosome: 46, xix, 46, X delX, 46, X dicX, 46, XRX - 17.6% cases; 4. mosaicism with structural abnormalities of X chromosome: 46, XX/46XiX/45, X - 2.9% of cases and other chromosome: 46, XY - 14.7% cases.
Conclusions: Peculiarities of phenotype manifestations in girls with Turner syndrome induce the diagnosis in the early neonatal period, however up to 60% of patients are not diagnosed during early childhood because of lack of complaints of family and the late visits to geneticist. Karyotype in Turner syndrome is highly variable and different, in 53% of cases being the homogeneous form or X monosomy - 45, X.

M.L. Sprincean: None. **E. Halabudenco:** None. **N. Barbova:** None. **A. Mişina:** None. **T. Samoilenco:** None.

J18.25

A Case Report: 46, XX, ins (3, 1) (q22; q25qter) karyotype in dizygotic twin sisters

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İnsertion is a chromosome abnormality that is due to a nonreciprocal type of translocation in which a segment is removed from one chromosome and then inserted into a broken region of a nonhomologous chromosome. This can happen due to unequal crossover during meiosis. Although, chromosomal insertions are considered as balanced chromosomal abnormalities, they can be hazardous if the affected region is in an exon. We present the case reports of 13 years old female s. Both patients were presented with short stature, pubertas tardas, dysmorphic features and mild mental retardation. Classic cytogenetic G banded karyotype analysis performed on peripheral blood samples demonstrated a 46,xx, ins (3, 1)(q22;q25qter) in both Twin A and Twin B. Abnormal karyotype ins (3, 1)(q22;q25qter) is rarely reported in the literature. The phenotypic effects of this karyotype is not fully described. The affected loci after insertion may contain genes associated with growth, cognitive ability and sexual development. We suggest that performing advanced molecular techniques will assist in determining the affected genes and their roles in phenotype-genotype relationship.

R.U. Akkoyunlu: None. **K.E. Gümüslü:** None. **S. Eren:** None. **B. DoĞruoĞlu:** None. **D. Sünnetçİ:** None. **N. Çİne:** None. **H. Savli:** None. **E. Özsu:** None. **F. ÇİzmecİoĞlu:** None.

J18.26

Phenotype variability in pericentric inversion of chromosome 9 - case series

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Background: Inherited pericentric inversion of chromosome 9 - inv(9)(p11-q13) is relatively commonly found in humans (1-4%), generally without apparent phenotypic alterations. However, the inv(9)(p11-q13) was also found to be associated with congenital malformations and certain cancers.
Aim: We aim to examine the relation between the inv(9)(p11-q13) and the various phenotype it is associated with in a series of 5 cases.
Methods: We present 4 boys, aged 7 months, 2 years, 4 years and 13 years, respectively and a five years old girl. The patients were examined and also had blood work alongside cytogenetic studies.
Results: We found various phenotypes ranging from facial dysmorphism (2 cases), delayed milestones (2 cases), micropenis and cryptorchidism (2 cases), mild growth retardation (1 case), atacsia (1 case). Mothers of two of the boys also presented the inversion. Interestingly, we also found the duplication 16q12.2q2 in one boy presenting facial dysmorphism.
Conclusion: The phenotype associated with pericentric inversion of chromosome 9 is extremely variable; consequently, further studies analyzing each breakpoint region of inv (9), using mo¬lecular cytogenetics are needed to understand the disease association.

A. Chirita-Emandi: None. **S. Dumitriu:** None. **C. Popa:** None. **S. Arghirescu:** None. **M. Puiu:** None.

J18.27

Karyotype and SRY gene analysis in patients with phenotype and genotype incompatibility

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The major factor in creation of the gender is the SRY gene on chromosome Y. SRY gene neighbors the pseodoautosomal region 1 *(PAR1)* crossing over region on the short arm of the chromosome Y. Rarely the SRY gene on the Y chromosome may pass to the X chromosome via erroneous crossing over. In this case, if the gamete carrying the X chromosome that received the SRY gene is inseminated, only male phenotype individual is created in XX genotype. In case the gamete carrying the Y chromosome that has lost the SRY gene is inseminated, only female phenotype individual is created with XY genotype. SRY genes of only females with 46,XY genotype and 45,X/46,XY mosaic individuals with genotype-phenotype incompatibility, that is detected as a result of the chromosome analysis carried out in our cytogenetic laboratory, are studied in the present study. Standard chromosomal analysis was performed by using giemsa-trypsin (GTG) banding. The SRY gene was amplified by polymerase chain reaction (PCR).
SRY is positive only in 46,XY genotype female phenotype patients. However, it gives rise to the thought that it may be caused by other factors such as noncreation of male phenotype, inactivity of the SRY gene, androgynous receptor dysfunction or 5 α-reductase deficiencies. Although SRY gene is available in 45,X/46,XY mosaic cases, it is insufficient in creation of male phenotype since the band level is thin in ratio proportional to the level of mosaic. Turner phenotype is emerged due to the excess number of Turner genotype cell ratio.

E. Akbas: None. **Z. Mert altintas:** None. **H. Senli:** None.

J18.28

Chromosome 15 alterations implicated in Silver-Russell Syndrome

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Background and aims: Silver-Russell Syndrome (SRS) is a clinically and genetically heterogeneous disorder. Most frequently the 7 and 11chromosomes are involved, other chromosomes considered as candidate loci for SRS are 1, 8, 15, 17, 18 and X.
We aim to present a case with classic Silver Russell syndrome phenotype with chromosome 15 alterations.
Methods: The infant was comprehensively examined trough clinical, lab work, imagistic and genetic studies.
Results: An 8 month old female infant with intrauterine growth retardation, severe hydramnios and single umbilical artery, born at 33 weeks of gestation, weighing 1200 grams (below 2 standard deviations), presented slow weight gain during the first 6 months of life (average 362 grames /month). She displayed a classical Silver Russell syndrome phenotype. The infant had delayed bone age (corresponding to 1-2 months of age). The patient's karyotype was 46,XX,dup(15)(pter-q22::q22-qter), the mother had normal karyotype, while the father's karyotype was 46,XY,1qh+ without phenotypical alterations. She has now started growth hormone treatment.
Conclusions: The patient shows a typical phenotype although the association between chromosome 15 alterations and Silver Russell Syndrome is rarely mentioned in specialized literature. This confirms that this phenotypic syndrome is genetically heterogeneous.
The case needs molecular cytogenetic investigations leading to a more detailed description of chromosomal rearrangements for both parents and patient.

A. Dobrescu: None. **S. Dumitriu:** None. **A. Chirita-Emandi:** None. **C. Borza:** None. **M. Puiu:** None.

J18.29

Genetics counseling in a case of Kabuki syndrome

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Kabuki syndrome (KS) is a rare disorder and has an estimated frequency of 1 in 32,000.
This paper reports the case of an 5-year-old male with KS who has the various features typical of the disease: hypotonia and postnatal growth deficiency, characteristics face (long palpebral fissures, ectropium of the lateral third of the lower eyelids, arching eyebrows with sparse lateral halves, depressed nasal tip, large and prominent ears, micrognathia, mid-facial hypoplasia, dental abnormalities), moderate mental retardation, distinctive behavioural features and learning difficulties, urogenital abnormalities (congenital inguinal hernia, ectopic testis), short stature and skeletal abnormality.
The etiology of the syndrome is unknown and is thought to be possibly due to sporadic mutation with no familial history. There is no prenatal screening, genetic test or consensual diagnostic criteria to confirm this condition. The patients are diagnosed according to the recognizable facial features. The identification of the basis of genetic diseases is essential to provide an accurate genetic counseling and to ensure proper disease prevention. In this case is difficult to estimate the risk of parent's patient having another child with KS.
The prognosis of survival to adulthood is relatively good as KS is not typically associated with severe medical complications.

D. Capatina: None. **G. Cozaru:** None.

J18.30

Distribution of trisomies in 96 cases of trisomic spontaneous abortions

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In thirteen years period, from 1999 to 2011, i<<<<<<<n Genetic Centre in Novi Sad, Serbia were analyzed 759 spontaneous abortions and we detected 96 trisomic karyotypes. The most frequent trisomies were: 18.75% Edwards syndrome (N= 18/96), 16.67% trisomy of
chromosome 15 (N=16/96), 13.54% Daun syndrome (N=13/96); 12.5% Patau syndrome (N=12/96); 9.37% trisomy of chromosome 22 (N=9/96); 14.58% trisomy of chromosome 16 (N=14/96); 3.12% trisomy of chromosome 9 (N=3/96); 3.12% trisomy of chromosome 14 (N=3/96); 8.33% others (N=8/96). Trisomies were predominantly detected in first trimester from 9th to 12th week of gestation (83%).
Cytogenetic abnormalities are one of most important cause of spontaneous abortions. Genetic counseling provide information that may be helpful for subsequent pregnancies.

J. Jovanovic Privrodski: None. **I. Kavecan:** None. **M. Kolarski:** None. **D. Radovanov:** None. **M. Obrenovic:** None.

J18.31

Mosaic trisomy 9 in a girl with mental retardation and dysmorphic syndrome

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Trisomy 9 is an uncommon chromosome abnormality that may be seen in a mosaic or non-mosaic state. As complete trisomy 9 is often lethal to the fetus, individuals affected by this disorder commonly display mosaicism, harboring a mixture of cells in which some cells contain the normal two copies of chromosome 9, while other cells contain a third copy of chromosome 9. Associated symptoms and findings may vary greatly in range and severity and include intrauterine growth retardation; mental retardation; congenital heart defects; distinctive craniofacial abnormalities; musculoskeletal, genital, kidney , and/or additional physical abnormalities.
We present the case of a 10 yrs old female patient born from the first pregnancy of a healthy couple with normal birth weight and length. The girl had found psychomotor retardation, abnormal behavior, truncal obesity, joint hypermobility and dysmorphic features (broad-based nose with bulbous tip , high arched palate, short palpebral fissures, microretrognatia, tapering fingers). The performed brain imaging did not find any structural brain anomalies. The routine cytogenetic test detected mosaic trysomy 9 (47,XX,+9[3]/46,XX[21]).
Mental retardation and non specific dysmorphism are leading clinical features in our patient. The wide phenotypic range in trisomy 9 mosaic syndrome is likely to explain by the percentage of trisomic cells in different tissues. Routine cytogenetic investigation remains important test in individuals with mental retardation and/or dysmorphic features. A large number of cells is needed in order to obtain a correct karyotype diagnosis in mosaic patients. Correct diagnosis is essential to define the prognosis and provide accurate genetic counseling.

I. Bradinova: None. **R. Vazharova:** None. **V. Bojinova:** None. **A. Savov:** None.

J18.32

A Genetic approach to understand the rare Liddle syndrome in India

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Genetic analysis of the rare syndrome, Liddle syndrome is carried out for understanding its etiology. It is reported in fewer than 30 families (or isolated cases) worldwide. It is known to be autosomal dominantly inherited and presented with hypertension and low plasma renin activity, metabolic alkalosis due to hypokalemia caused by elevated levels of sodium ions and loss of potassium from the renal tubule. Till now this syndrome has been identified by the biochemical parameters only and to the best of our knowledge no cytogenetic analysis has been carried out. In this study the proband was confirmed by the biochemical parameters. In addition, all the family members (n=7) were subjected to genetic analysis. The cytogenetic analysis included the chromosome aberration assay (CA) and cytokinesis block micronuclei assay (CBMN Cyt assay). The molecular analysis included the mutation study for the genes SCNN1B and SCNN1G exon 13. The frequency of CAs was significantly higher in the proband (0.18±0.43) than the other family members and controls (0.005±0.0085). In CBMN Cyt assay, the frequencies of micronuclei, nucleoplasmic bridges and nuclear buds were significantly higher than in the family members and controls. To the best of our knowledge this is the first study involving Genetic analysis of Liddle Syndrome and family members. This will help in understanding the etiology of this disease and affecteds in the family. This kind of study will be useful in genetic counseling for an early intervention.

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J19.01

Bend it like Beckham! The ethics of genetically testing children for athletic potential.

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In this paper I analyse the use of direct-to-consumer (DTC) genetic tests[1], sometimes coupled with more traditional methods of talent scouting such as intensive summer camps[2], to assess a child's predisposition to athletic performance. The recent boom of DTC-genetic tests, aimed at measuring children's athletic potential, is the latest wave in the "pre-professionalization" of children that has characterized, especially but not exclusively, the US in the last twenty years.[3] In this paper I discuss the scientific evidence at the basis of the DTC-genetic tests and challenge their predictive ability. I then discuss how the parental use of DTC-genetic tests impact on the children's right to an open future, and on their developing sense of autonomy and of self-determination. I argue that the use of DTC-genetic tests to allegedly 'measure' children's athletic potential is ethically problematic as it trumps the right-in-trust of children to an open future favouring a too broad interpretation of parental reproductive and child-rearing freedom. I conclude tht DTC-genetic tests to scout children's potential should be seen as a 'wake up' call for other problematic parental attitudes aimed at scouting and developing children's precocious talents, be they in sports or elsewhere.

Footnotes

[1] Among these, Atlas Sports Genetics (www.atlasgene.com/), Geneffect (http://www.geneffect.com/), Sports X Factor (www.sportsxfactor.com), Athleticode (http://athleticode.com/)

[2] Chang E (2009) In China, DNA tests on kids ID genetic gifts, careers, CNN NEWS http://edition.cnn.com/2009/WORLD/asiapcf/08/03/china.dna.children.ability/

C. Silvia: None.

J19.02

Reduced telomere length in placentas during pregnancies complicated by intrauterine growth restriction

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Objectives: Recent studies have shown that telomere length was significantly reduced in placentas collected at delivery from pregnancies complicated by intrauterine growth restriction (IUGR) secondary to placental insufficiency. Placental telomere length measurement during ongoing pregnancies complicated by IUGR has never been reported. *Methods:* In our center, late chorionic villus samplings were performed between 18 and 37 weeks of amenorrhea in 24 subjects with severe IUGR (cases) and in 28 subjects with other indications for prenatal diagnosis (controls). Placental insufficiency was assessed by histo-pathological examination. Relative measurement of telomere length was carried out prospectively by quantitative Fluorescent *In Situ* Hybridization (FISH) using Peptide Nucleic Acid probes on interphase nuclei obtained from long-term cultured villi and with an automated epifluorescent microscope. A quantitative Polymerase Chain Reaction (Q-PCR) technique was performed to confirm the quantitative FISH results. The number of copies of gene loci encoding the RNA template (*hTERC*) and the catalytic subunit (*hTERT*) of the enzyme complex telomerase were also estimated in these placentas by FISH. *Results:* Mean fluorescence intensity of telomere probes estimated by quantitative FISH was significantly less for cases compared to controls ($p<0.001$). This result indicated that mean telomere length was significantly reduced in placentas during pregnancies complicated by IUGR. Reduced telomere length was confirmed by the Q-PCR technique. No copy number variation of the *hTERC* and *hTERT* loci was noticed for cases, or for controls. *Conclusion:* This study clearly demonstrates a reduction of placental telomere length in ongoing pregnancies complicated by severe IUGR secondary to placental insufficiency.

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J19.03

Preimplantation Genetic Diagnosis (PGD) for beta-thalassaemia and sideroblastic anaemia, combined with HLA-typing, employing three different technologies for single cell genotyping: fragment analysis, nested real-time PCR and nested high-resolution melting (HRM) analysis.

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PGD with HLA-matching to select histo-compatible siblings to facilitate curative haematopoeitic stem-cell transplant (HSCT) is widely applied. We describe a rare case, where HLA-PGD was performed to exclude two familial single-gene disorders (SGDs). The couple were heterozygous for different beta-thalassaemia mutations as well as an identical sideroblastic-anaemia mutation.Their daughter, affected with sideroblastic-anaemia, was programmed to have HSCT. We optimized a one-step-multiplex-fluorescent-touchdown-PCR protocol for the simultaneous amplification of 19 genetic regions: the *HBB*-gene mutated regions (c.118C>T and c.25-26delAA), four linked-short tandem-repeats (STRs) in chr11p15.4 (4-138kb upstream and downstream of *HBB*), the *SLC25A38* gene mutation (c.726C>T), two linked-STRs in chr3p22.1 (208-245kb upstream-downstream of *SLC25A38*), plus eleven STRs for HLA-haplotyping (chr6p22.1-21.3). This was followed by nested real-time PCR and high-resolution melting (HRM) for direct mutation detection and analysis of all STRs on a genetic analyzer for indirect genotyping and HLA-haplotyping. The protocol was validated following PGD guidelines and successfully performed in a clinical cycle. A single blastomere was biopsied from each of 19 day-3 embryos and sent for genetic analysis. One blastomere failed to amplify at all loci. From the remaining 18 embryos, diagnosis was as follows:for beta-thalassaemia, 15/18 were unaffected, 2/18 affected and 1/18 showed only normal maternal alleles.For sideroblastic-anaemia: 9/18 were unaffected, 7/18 affected, 2/18 inconclusive.Two embryos were HLA-compatible, 14/18 not compatible, 2/18 inconclusive. One HLA-compatible embryo was also unaffected for both SGDs and transferred, however a pregnancy was not achieved.The couple plans a second cycle. The novel methodological PGD-approach described here is fast, accurate, clinically-validated and has a low cost.

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J19.04

Prenatal molecular cytogenetic analysis should be performed on mesenchymal core rather than on cytotrophoblast cells

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Objectives: To examine the reliability of interphase FISH analysis of the main aneuploidies performed on mesenchymal core when prenatal diagnosis was performed on pregnant women with first-trimester fetal abnormalities on ultrasound. *Methods:* 386 first trimester prenatal examinations were investigated from chorionic villus samplings for increased nuchal translucencies or other fetal ultrasound abnormalities. Interphase Fluorescence *In Situ* Hybridization (FISH) for the main aneuploidies (trisomies 13, 18, 21 and gonosomal aneuploidies) was performed on the mesenchymal core of villi. Molecular cytogenetic results were always complemented by conventional cytogenetic results on long-term cultured villi (LTC-villi). Short term cultured villi (STC-villi) preparations were retrospectively performed only when a chromosomal abnormality was observed with interphase FISH and/or LTC-villi. *Results:* 88 chromosomal abnormalities (88/386 = 22.8% of first-trimester diagnoses) which could discuss subsequent abortions were observed after LTC-villi preparations. All cases possibly detectable by interphase FISH were detected. Thus, 85 aneuploidies (85/386 = 22.0% of first-trimester diagnoses; 85/88 = 96.6% of chromosomal abnormalities) were detected by interphase FISH, allowing early abortion by curettage before week 14 amenorrhea. No discrepancy occurred between interphase FISH and LTC-villi results for the aneuploidies studied. Three false-negative results (3/386 = 0.77% of first-trimester diagnoses; 3/88 = 3.41% of chromosomal abnormalities) were observed with STC-villi. *Conclusion:* We observed a high rate of false-negative results on cytotrophoblast cells. Conversely, interphase FISH of the main aneuploidies on the mesenchymal core provided rapid and reliable results. We therefore postulate that prenatal molecular cytogenetic analysis should be performed on mesenchymal core rather than on cytotrophoblast cells.

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J19.05

The Interaction of 3.7kb alpha-globin gene triplication with beta-globin gene mutations in Iranian population

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The 3.7kb triplicated α-globin gene has been found in most populations. It results from unequal crossover between misalignmented homologous segments in the α-globin gene cluster during meiosis. The pathophysiology and clinical severity of ß-thalassemia (ß-thal) are associated with the degree of α-chain imbalance. The excess of α-globin chains plays an important role in the pathophysiology of ß-thal. When heterozygous/homozygous ß-thal coexists with alpha gene numerical alteration, the clinical and hematological phenotype is affected, showing a decrease (-α/) or increase (ααα/) in thalassemia carriers' anemia.
The coexistence of α-globin gene triplication (ααα/) consider as an important modulator of the severity of ß-thal, exacerbating the phenotypic severity of ß-thal by causing more globin chain imbalance.This type of phenotype modification has rarely been observed and reported in Iranian population. Here, we report co-inheritance of triple α-globin gene arrangement and heterozygous/homozygous ß-thal in 21 cases presenting thalassemia intermedia/major phenotype. Some of these patients consider as having mild intermedia phenotype, they need no transfusion, some receive blood occasionally in their time life (for example on delivery time) but some of them are dependent to regular blood transfusion (once every 20 to 40 days). Our study focus on the importance of detecting 3.7kb triplicated α-globin gene in genotype/phenotype prediction in Iranian thal patients.

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J19.06

Chromosome polymorphic variants in infertility and their effect on ongoing pregnancy rates

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INTRODUCTION: Polymorphic variants on chromosomes were considered normal without clinical significance, however previous studies have reported that incidence of these variants is higher in infertile patients. The aim of this study was investigate the correlation of chromosome polymorphic variants with infertile phenotype and its effect in IVF cycles outcome.
MATERIAL AND METHODS: We considered a total of 1551 cytogenetic studies involving infertile patients (n=866) and oocyte/sperm donors for the control group (n=685). Rate of aneuploid spermatozoa were evaluated using FISH in 145 infertile men. Outcome of IVF cycles were analyzed in 259 patients. RPL was defined as two or more miscarriages.
RESULTS: A statistically significant increase in the frequency of chromosome variants was observed in infertile patients (16.9% study group, 24.1% in RPL patients vs 12.8% controls; p<0.05). Significant differences were observed in rate of aneuploid spermatozoa between infertile men with and without polymorphisms (37,7% vs 16,3%; p<0.05). In the IVF cycles analyzed, biochemical and clinical miscarriage rates were higher in the group of patients without RPL carrying of polymorphisms compared with patients carrying of normal karyotype (35.6% vs 22.7% for biochemical miscarriage and 20.7% vs 9.8% for clinical miscarriage).
DISCUSSION: Higher frequencies of chromosomal variations are present in infertile patients compared with fertile population. Moreover, the presence of these variants is higher among patients with repeated pregnancy loss (RPL). Men carrying polymorphisms have a higher incidence of sperm aneuploidies. Moreover, the patients without RPL undergoing an infertility treatment have a higher incidence of miscarriage when are carriers of polymorphisms.

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J19.07

Correlation of 5-hydroxymethylcytosine-positive spermatozoa frequency in ejaculate with DNA integrity and head morphology

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Male gametes quality is essential for fertilization capacity and subsequent pre- and postimplantation embryogenesis. DNA integrity and correct chromatin epigenetic status are necessary for appropriate gamete function. Recently a new epigenetic marker was discovered - 5-hydroxymethylcytosine, which is a product of 5-methylcytosine oxidation by TET proteins.
We assessed correlation between rate of spermatozoa with fragmented DNA and rate of spermatozoa with hydroxymethylated DNA. Study group consisted of 3 sperm donors and 17 patients of IVF clinic. Semen analysis was performed according to WHO criteria. Sperm morphology was assessed using strict Kruger's criteria. Preparations were made from sperm samples fixed with ethanol:acetic acid (3:1). Spermatozoa with fragmented DNA were detected by TUNEL (CellDeath Detection kit, Roche). The rate of spermatozoa with fragmented DNA varied from 0,10% to 20,78% (n=2000 for each patient). The rate of spermatozoa with 5-hydroxymethylcytosine was evaluated after immunostaining of preparations with anti-5-hydroxymethylcytosine polyclonal antibodies (ActiveMotif). The rate of 5-hydroxymethylcytosine-positive spermatozoa in ejaculate of different patients ranged from 0,06% to 13,89% (n=5000 for each patient). The direct linear correlation between sperm DNA fragmentation and hydroxymethylation was found (r=0,40; P=0,048). Moreover the negative correlation was found between sperm DNA hydroxymethylation and the frequency of spermatozoa with morphologically normal head (r=-0,53; P=0,021).
Thus,the frequency of 5-hydroxymethylcytosine-positive spermatozoa is associated with markers of semen quality-DNA integrity and morphology of spermatozoa. This suggests that the rate of hydroxymethylated spermatozoa could be used as a new marker of semen quality.
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J19.08

Assessment of genetic variations of DPY19L2 and DYDC1 in men with total globozoospermia

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Background: Globozoospermia is a rare and severe type of teratozoospermia, characterized by round-headed spermatozoa lacking an acrosome. The pathogenesis of this anomaly is still unclear but genetic factors are likely to be involved. Recently, it has been shown that DPY19L2 deletion can be found in a vast majority of, but not all, globozoospermic patients, suggesting that probably other genes associated with the disorder. DYDC1 plays a crucial role during acrosome biogenesis and it is of particular interest in this context because of phenotypes in the null mutant mice that are very similar to human globozoospermia. The objective of this study was Assessment of genetic variations of DPY19L2 and DYDC1 in men with total globozoospermia.
Methods: 15 total globozoospermic men and 30 men with normal spermogram referred to Royan fertility and infertility Centre were included in the study. DPY19L2 deletion was assessed in men with total globozoospermia and noromozoospermic controls by performing both PCR of two exons and a long-range PCR across the deletion and then, sequenced the entire coding region of DYDC1 in the patients without DPY19l2 deletion.
Results: Our results showed that 73.3% of the patients (n=11) have DPY19L2 homozygous deletion. We did not find mutation in the coding regains of DYDC1 in other patients (n=4).
Conclusion: This study confirms that DPY19L2 is the major gene responsible for globozoospermia in Iranian men and also seems that there is no relationship between DYDC1 and globozoospermia in human.

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J19.09

Genetic analysis of gonadal disorders of sex development (46, XY DSD) by cytogenetic and molecular methods

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Among disorders of sex development (DSD) which result from abnormalities during gonadal determination and differentiation, there are especially rare forms such as 46, XY gonadal dysgenesis with mutations in SRY, NR5A1, DHH, DAX1 and WNT4 genes. Mutations of known genes are responsible for only a few percentages of these disorders and there are probably some other potential genes or loci that play a role in sexual disorders that are waiting for further analysis.
We recruited patients that were clinically suspicious for 46, XY gonadal dysgenesis. Cytogenetic analysis as well as the direct sequencing of the SRY, NR5A1and DHH genes was performed for all cases. MLPA technique was used to detect deletions and duplications in DAX1 and WNT4 genes and subsequent imbalances were confirmed by real time PCR. Additionally, other potential loci were investigated by whole genome Array CGH method.
One new chromosomal rearrangement and SRY deletion was found in one and five patients, respectively. Two heterozygous partial deletion and duplication were present in NR5A1 and WNT4 genes. Array CGH results confirmed the chromosomal rearrangement data and one partial deletion were detected in the SOX2OT gene.
Autosomal chromosome abnormalities could play a role in DSD. SRY gene deletion has a significant role in DSD and has a similar incidence in our patients compared with other reports. Del/dup mutations found to be more common than point mutations in our patients and might be preferred to check Del/dup mutations prior to point mutations. SOX2OT might have a potential role in gonadal dysgenesis.

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J19.10

Oligonucleotide-based array CGH analysis of blighted ovums

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Approximately half of first trimester miscarriages have abnormal karyotype according to conventional karyotyping. However, some additional cases of pregnancy loss may be caused by non-visible submicroscopic chromosomal rearrangements. The role of microdeletions/microduplications is even more expected for blighted ovum (empty fetal sac) - the most severe form of miscarriage. We were aimed to search for unbalanced chromosomal aberrations and genes essential for proper embryo development in 10 blighted ovums specimens using SurePrint G3 Human CGH+SNP 4×180K Microarray Kit (Agilent Technologies, USA). All specimens were shown to have normal karyotype by conventional karyotyping. Three to eighteen unbalanced aberrations were detected in each of 10 miscarriages (altogether 95 CNVs). The size of aberrations varied from 726 bp to 26 Mbp. Using Gene Ontology enrichment analysis and visualization toolkit (GOrilla) for complex CNVs analysis we found that genes of gamma-aminobutyric acid signaling pathway were significantly represented in the CNVs affected genomic regions in blighted ovums ($p<0.00001$). It is unexpected because this pathway is essential for the central nervous system development and functioning. But taking into consideration that the number of genes normally expressed in the brain is the greatest in comparison with other tissues and that it is the brain which controls a variety of processes within the organism our funding is of particular interest. After benign CNVs were excluded using Database of Genomic Variants, the analysis of the remaining dataset showed the prevalence of DNA-dependent transcription factors group ($p<0.001$). This study was supported by grants of Federal Program N 8276 and 8720.

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J19.11

Importance of Preimplantation Genetic Screening (PGS) in the reduction of multiple pregnancies in *in vitro* fertilization (IVF) cycles

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In general, the live birth rate of assisted reproduction techniques (ART) ranges about 20% worldwide, while the rate of clinical pregnancies varies between 25-45%. One of the most recent achievements of the continuously improving techniques of assisted reproduction is preimplantation genetic testing, which serves to genetically analyze the embryos prior to their transfer into the uterus. Several randomized controlled trials analysed the outcome of embryo transfer upon the application of the newly introduced preimplantation genetics methods and verified already a significant increase in clinical pregnancy rates (60-79 %) and a major decrease in miscarriage rates (3.4-6.9 %). Furthermore, frequent genetic disorders (such as Down syndrome, Patau syndrome etc.) can be selected before embryo implantation, thus their prevalence could be decreased.
One of the major scopes of preimplantation genetic screening is however the reduction of multiple pregnancy rates in *in vitro* fertilization (IVF) cycles. The proportion of multiple pregnancies in IVF is 20-60% based on the data of several international studies; in Hungary the average rate of multiple pregnancies is 24.9% (10-54%).
The preimplantation genetic screening with microarray comparative genomic hybridization (aCGH) technique was first applied in Hungary at the Versys Clinics, Human Reproduction Institute. We have analyzed more than 430 embryos and had more than 100 embryos transferred since 2011. PGS provides the possibility of selecting and transferring only euploid embryos, therefore can we perform mostly single embryo transfers (SET). Here we present about the strategy of decreasing multiple pregnancy rate with SET following preimplantation genetic screening.

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J19.12

Early diagnosis of a boy with adrenoleukodystrophy due to the ABCD1 gene mutation and prenatal genetic diagnosis of his sibling for bone marrow transplantation.

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Introduction: Adrenoleukodystrophy is an X-linked disorder (X-ALD) which is due to the mutations in the *ABCD1* gene. These mutations result in the defect of peroxisomal beta oxidation and leading to the accumulation of saturated very long chain fatty acids (VLCFA) in body tissues. The clinical menifestations of the disorder occur primarily in the adrenal cortex, the myelin of the central nervous system, and the Leydig cells of the testes. In this report, we analyzed the *ABCD1* gene of a boy whose uncle died because of the same disease.
Material- Method: The patient was diagnosed as X- ALD due to high serum amounts of VLCFA together with pristanic and phytanic acids. PCR- sequencing methodology was used in order to confirm the disease and identify *ABCD1* gene mutations. Amplification of the whole coding regions including exonic- intronic boundaries were assessed by using spesific primers and sequenced directly (ABI Prism 3100 Genetic Analyzer).
Results: Sequencing process revealed that the examined boy carries c.G1202A (p.R401A) mutation in the *ABCD1* gene and his hemizygous mother has the same mutation as heterozygous and therefore, she is carrier.
Discussion: In addition to biochemical analysis, genetic testing is required in determining the correct diagnosis to genetic diseases. *ABCD1* genetic analysis revealed that the boy is X- ALD and needs a bone marrow transplantation. Prenatal genetic diagnosis was assessed for HLA identical baby and his mother is pregnant for his sibling.

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J19.13

Investigating autosomal recessive gene defects in severe oligospermic and azoospermic infertile men

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Infertility is a serious public health issue and studies show out of every 100 couples, 15 are infertile. Furthermore; 50% of these infertile couples suffer from male factor infertility. Although many factors on sperm structure and functions have been investigated including environmental factors, ethiologies of many infertility cases are yet to be enlightened. From the genetic point of view, chromosome abnormalities and genetic mutations are important causes of male infertility. The aim of this study is to detect autosomal

recessive genetic mutations in oligospermic and azoospermic infertile men. Patients included in the study were required to fill special survey forms not only to exclude environmental factors, but also to choose patients with consanguineous marriages or patients with at least 2 infertile people in their family. Blood samples of 20 Turkish men; 6 of whom were azospermic, and 14 severe oligospermic, were evaluated using array technology. Several genes in the "Spermatogenesis-associated protein" SPATA family, "Sperm-associated antigen" (SPAG1, SPAG4, SPAG5, SPAG8, SPAG9, SPAG11A / SPAG11B, SPAG16) genes, "Sperm acrosome-associated protein" (SPACA3, SPACA4) genes, as well as a few other genes known to play roles in spermatogenesis were found to be defective. These results will guide physicians in genetic consultation and in choosing the best assisted reproductive therapy.

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J19.14

About 46,XY DSD: A rare mutation of the ligand-binding domain of the AR protein in three Tunisian sisters with complete androgen insensitivity syndrome

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The androgen insensitivity syndrome (AIS) is an X-linked recessive disorder in which affected males have female external genitalia, female breast development, blind vagina, absent uterus and female adnexa, and abdominal or inguinal testes, despite a normal male 46,XY karyotype. Mutations in the androgen receptor (AR) gene cause the AIS by impairing androgen-dependent male sexual differentiation to varying degrees. Thus they are associated with a variety of phenotypes, ranging from CAIS to PAIS or men with infertility. Through AR gene mutations Database (http://androgendb.mcgill.ca), AR gene mutations reported until 2012 overtake 1000.
Here we report a rare AR mutation in three Tunisian sisters presenting with all of the characteristics of CAIS. This Tunisian consanguineous family from Sfax town is composed of three daughters suffering from primary amenorrhea and one healthy son. DNA analysis was carried out by direct sequencing of PCR-amplified exonic fragments of the AR gene. A c.3474C>T substitution in exon 6 of the AR gene leading to a missense mutation p.(Arg787*) in the ligand-binding domain (LBD) of the AR protein, was confirmed in the three sisters as well as in their mother who was heterozygous for the mutation.
This mutation resulted in a truncated form of the receptor including a major part of the C-terminal LBD which is not only involved in binding to androgen, but also involved in binding of coactivator proteins and dimerization.
This mutation is a rare variant associated with CAIS and was described only three times. Furthermore, it was described in association with prostate cancer twice.

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The outcome of prenatal diagnostics in pregnancies with increased risk for chromosomal abnormalities

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Chromosomal abnormalities (HA) still remain a remarkable challenge, with prenatal detection and pregnancy termination, as the most effective negative eugenic method. The aim of this study was to evaluate the outcome of 10 years invasive prenatal diagnostics (2002-2012yr). A total of 3227 pregnant woman with increased risk to fetal HA were referred to pre-testing genetic counseling. Invasive prenatal diagnostics was provided for all (2747 amniocenteses and 181 chordocenteses), except 229 (9%) women, who refused the indicated diagnostics. Fetal karyotype was analyzed using GTG banding method. The fetal loss after invasive prenatal diagnostic was 0.27%, all after amniocentesis.
Advanced maternal age was the most frequently recognized risk factor for fetal HA (58%), followed by abnormal ultrasound findings, abnormal maternal serum screening and parental balanced translocations. Abnormal karyotypes were ascertained in 76 (2.6%) of all performed cytogenetic analyses. Aneuploidies were the most frequent HA (83%), predominantly trisomy 21 (49%), and followed by trisomy 18 (21%), gonosomal trisomias/monosomy X (11%, 8% respectively), trisomy 13 (6%), and others (5%). Unbalanced structural aberrations were found in 13 (17%) fetuses, as "de novo" event or resulted from parents' reciprocal/Robertsonian translocation. Balanced structural rearrangements and polymorphisms were found in additional 31 (1%) of all fetal karyotypes, and pregnancies were continued. After comprehensive and detailed genetic counseling, an option for pregnancy termination was offered in all cases of fetal abnormal karyotipe (except XYY status), and was accepted in 96%. Gonosomal aneuplodies presented a particular challenge for genetic counselor, requesting more delicate counseling, including covering some ethical issues.

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Prenatally diagnosed case of mosaic trisomy 13 associated with Currarino triad

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Currarino triad, also known as Currarino syndrome, is a complex of congenital caudal anomalies characterized by anorectal malformation, sacral bone abnormality and presacral mass. Currarino syndrome was first described in 1981, however its genetic basis still remains unclear. Although, mutations in the *HLXB9* gene were found in almost all cases of familial Currarino syndrome, they are present in less than 30% of patients with sporadic Currarino syndrome. To date, only few reports on genetic causes in cases of sporadic Currarino syndrome have been published.
Herein, we present prenatally detected case of Currarino triad associated with mosaic trisomy 13. A 32-year-old woman, G1P0, was referred to our hospital because of suspected fetal urogenital anomalies. Ultrasonic examination at 20 weeks gestation showed sacral bone defect, low presacral unechogenic mass of irregular shape, bilateral hydronephrosis, megaureters and urinary bladder with hypertrophied bladder wall and "keyhole sign". Amniocentesis was performed, and cytogenetic analysis revealed a female karyotype with 89% of trisomic cells. Parents elected to terminate the pregnancy, and pathological examination showed imperforate anus, kidneys with markedly dilated renal pelvis, distended urinary bladder, and cystic mass histologically identified as immature teratoma. The sacral bone was undeveloped and coccygeal was missing. Trisomy 13 mosaicism was confirmed in cultured skin fibroblasts and fetal cord blood lymphocytes.
Since our case is the fifth report on association of trisomy 13 and Currarino triad, it supports the idea that trisomy 13 in mosaic form could be involved in etiopathogenesis of sporadic cases of Currarino triad complex.

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Prenatal diagnosis of Fanconi Anemia in Tunisia: Cytogenetic and Molecular testing

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Fanconi anemia (FA) is a rare genetic disorder characterized by variable congenital anomalies, progressive bone marrow failure, and high predisposition to acute leukemia.
This study describes successful molecular diagnosis and subsequent prenatal diagnosis of FA in a Tunisian consanguineous family.
The index case, was an 8-year-old girl with typical clinical features of FA and born to first-cousins healthy parents. Physical examination of the younger sister has shown skeletal anomalies. Thus, the diagnosis of FA was suspected. According to sensitivity to MMC, the index case and the younger sister were diagnosed as FA. The parents requested prenatal diagnosis during the actual pregnancy.
Genotyping analysis using four markers flanking the FANCA gene was carried out using DNA from parents, the unaffected older sister, the affected younger sister and foetal DNA obtained from amniotic fluid at a gestational age of 16 weeks. Multiplex ligation-dependent probe amplification (MLPA) was performed in order to confirm cytogenetic and genotyping results. Haplotype analysis showed that the affected members present the founder haplotype at a homozygous state whereas the fetus shows a different haplotype likely segregating with the normal alleles. These results were confirmed by MLPA analysis that revealed a total deletion of exon 15 for the affected

child while this mutation was absent in the fetus showing that he is unaffected and is not at risk of developing FA.
The present study is the first report of molecular testing that was successfully applied for prenatal diagnosis of FA in Tunisia and in North Africa.

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J19.18

Revealing genetic reasons for fetal death by combining new and old methodology: three different cases

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Fetal death can occur for different reasons in any gestational age. The reason behind fetal death is important to resolve for information of the parents and estimation of recurrence risk. We report three cases where different methods were combined to unravel the genetic reason behind fetal death or abnormality.
Case 1: a monochorial twin pregnancy where both fetuses died in utero h23+5. Prenatal BoBs (PNBoBs) from placental sample showed a duplication of the chromosomal region 15q11. Genomic microarray analysis (GMA) showed trisomy 15. Karyotype of cultured cells was 47,XX,+15[1]/46,XX[8]. Thus, a mosaic trisomy 15 was detected in the placenta.
Case 2: a monochorial twin pregnancy where fetus B died in utero h36+5. A skin sample was studied by PNBoBs that showed a duplication of the region 22q11. Karyotype was unavailable due to absent cell growth. GMA confirmed a 2,8 Mb duplication of 22q11.21. Also, dup(22)(q11.21) was shown in peripheral blood of twin sister by PNBoBs.
Case 3: an abnormal karyotype 46,XX,?i(18q) in amniotic fluid sample (NT:4,39; T18-risk: 1:68). The pregnancy was terminated. GMA from fetal skin sample showed duplication of 18q12.1-18qtel and deletion of 18p11.32-18ptel while cell culture failed. Mother had an abnormal karyotype 46,XX,inv(18)(p11.32q12.1). Thus the fetus had inherited der(18) rising from inversion chromosome 18 from the mother. She has increased risk for unbalanced chromosome rearrangement also in future pregnancies.
To conclude, PNBoBs and GMA are valuable methods in revealing genetic reasons for fetal death or abnormality especially when cell culture is not successful, and in detecting submicroscopic aberrations.

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J19.19

First trimester screening - beyond trisomy 21, 18 and 13.

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First trimester screening is primarily used to establish the risk for trisomy 21, 18 and 13 in a fetus. If the risk exceeds an agreed threshold an invasive procedure is recommended and the karyotype of the fetus is ascertained. During the screening procedure and subsequent karyotyping other abnormalities apart from trisomy 21, 13 and 18 can be diagnosed. We reviewed all cases of first trimester screening performed in our department in 2010 and 2011; in total 5690 women were tested. Abnormal findings were encountered in 65 fetuses (1 in 87) - trisomy 21 and 18 (no trisomy 13 was found) in twenty three fetuses (35.4%) and other abnormalities (other chromosomal abnormalities, congenital abnormalities without abnormal karyotype, or NT above 3.5 mm without abnormal karyotype or other congenital abnormality) in forty two fetuses (64.6%). Trisomy 21 and 18 represented just over a third of abnormal findings encountered during the first trimester screening.

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J19.20

Men with LOC203413 minor T allele have an increased risk for impaired spermatogenesis

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Association studies have shown that a number of gene polymorphisms modulate the efficiency of spermatogenesis. Previously, we have investigated the association of male infertility with nine single nucleotide polymorphisms (SNPs) in eight different genes: FASLG, JMJDIA, LOC203413, TEX15, BRDT, OR2W3, INSR and TAS2R38 among two ethnic groups from the R. Macedonia. This study showed an association of rs5911500 in LOC203413, rs3088232 in BRDT and rs11204546 in OR2W3 gene with azoospermia and/or oligozoospermia in Macedonian and/or Albanian males. Here, we present an association study of the nine SNPs in 242 infertile men (82 azoospermic and 160 oligozoospermic) and 116 fertile men from Slovenia. The methodology included multiplex PCR/SNaPshot analysis, followed by capillary electrophoresis on ABI3130 Genetic Analyzer. Only two SNPs were found in association with male infertility among Slovenian men with borderline statistical significance; rs34605051 in JMJDIA gene was associated under the dominanat model, while rs2059807 in INSR gene showed an association under the recessive model. Although the difference was not statistically significant, the LOC203413 minor T allele was found with higher frequency among infertile Slovenian men (22.7%) when compared to the fertile controls (17.2%). When all three populations (Macedonian, Albanian and Slovenian) were analyzed together, statistically significant association was observed for the rs5911500 in LOC203413 (OR 1.73, 95% CI 1.14-2.62; p=0.0090) with male infertility. In conclusion, the association study of a total of 644 infertile men with azoospermia and oligozoospermia from three different ethnic populations shows that men with LOC203413 minor T allele have an increased risk for impaired spermatogenesis.

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Does inbreeding lead to decreased Tunisian human fertility?

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Although a high proportion of marriages in Tunisia are consanguineous there are very few reports about the association between consanguinity and fertility.
In the present study, one hundred and four infertile male patients and 100 fertile male attending the department of Cytogenetics of the Pasteur Institute of Tunis were asked if they are born from consanguineous parents and if they are married to close biological relatives.
Infertile patients were more likely than controls to report first-degree (parental) and second-degree (grandparental) consanguinity. Men with azoospermia and severe oligospermia showed high rates of both consanguinity and family clustering.
This study demonstrated a significant association between consanguinity and family clustering of male factor infertility cases, suggesting a strong genetic component.

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J19.22

Mutational analysis of MED12 exon 2 in uterine leiomyoma in patients from North-West of Russia

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Uterine leiomyomas arise from smooth muscle tissue in majority of women at the age of 45. MED12, encodes the 12th subunit of the Mediator complex, implicated in transcriptional regulation. The mutations in exon 2 of MED12 gene were identified in 70% of sporadic uterine leiomyomas. The mutation hot spot affected an evolutionary conserved region of the MED12 protein. DNA samples of 25 tumours from 15 patients with uterine leiomyoma from North-West of Russia have been analyzed by sequencing of exon 2 in MED12 gene. MED12 mutations were identified in 62% cases (16/25) with almost half of them (27%) were mutations in codon 44 of MED12 gene. Seven leio-

myomas (27%) displayed an exonic insertion-deletion mutation, one (4%) was represented by missens mutation in codon 36 and one (4%)- by somatic intronic T to A mutation eight base pairs upstream of the splice exon 2 acceptor site. No exon 2 mutations of MED12 were found in DNA samples isolated from the peripheral blood of the same patients thus proving somatic origin of MED12 mutations. The finding of two different mutations in the same leiomyoma tissues registered in two cases favors the origin of these mutations after separation of myoma cell clones. Our data are in line with other studies, proving substantial impact of exon 2 MED12 gene mutations in the origin and development of leiomyomas. Regular origin of these mutations in specific locus of particular gene and its actual contribution into molecular pathogenesis of leiomyoma remains obscure and requires further studies.

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J19.23

Noninvasive screening method for fetal gender determination in pregnancies at risk for Duchenne muscular dystrophy

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Introduction: The fetal sex determination at an early gestational age is of most importance for pregnant women carrying an X-linked recessive chromosomal abnormality. The identification of a male fetus indicates hemizygosity for the X chromosome and thus potential diseases such us Duchenne muscular dystrophy. Our aim was to demonstrate the applicability of the noninvasive fetal (NIPD) gender screening procedure in clinical practice of pregnancies at risk for Duchenne muscular dystrophy.
Materials and Methods: We applied a method based on PCR and high-resolution capillary electrophoresis for fetal gender determination from maternal plasma aiming two Y specific sequences - SRY and DYS14. Two pregnant women with extended deletion in the DMD gene were tested noninvasively. For prenatal DMD gene analysis we used the SALSA MLPA kit P034-A2/P035-A2 DMD/BECKER (MRC Holland).
Results: This NIPD protocol for fetal gender determination was applied in two cases with long histories regarding the DMD gene heritability. In the first case, the pregnant woman was a heterozygous carrier for the exons 51-55 deletion and in the second case for the exons 30-43. The NIPD gender screening showed a female fetus in the first case and we eliminate de invasive sampling procedure. For the second case we detected a male fetus, the invasive DMD gene test was performed and the result was negative for the 30-43 deletion.
Conclusion: The pregnancy management in the case of DMD-carrier mothers can be improved by this NIPD screening approach for fetal gender determination.

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J19.24

Preimplantation genetic diagnosis (PGD) for sixteen translocation carriers by FISH and clinical outcomes

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The incidence of balanced chromosome abnormalities including mostly reciprocal or Robertsonian translocations is approximately 4-5% in couples with recurrent miscarriage. Genetically unbalanced gametes can be produced through meiotic segregation in these couples. Pre-implantation genetic diagnosis (PGD) provides the detection of chromosomal abnormalities and their exclusion before embryo transfer in assisted reproductive techniques. In this study, we reviewed 24 cycles of PGD in 16 couples with balanced translocations (5 Robertsonian, 11 Reciprocal) and analyzed pregnancy outcomes and the meiotic segregation mode of gametes of the translocation carriers using fluorescent in situ hybridization (FISH). PGD couples had either one or two blastomeres biopsied from all embryos with ≥7 blastomeres on day 3 post oocyte collection. These blastomeres were assessed for the specific chromosome rearrangement using FISH. Clinical outcomes were assessed retrospectively. We found that 34 of 117 embryos (29.05%) were normal or balanced. Five couples had no normal or balanced embryos and transfer process were cancelled. Twelve clinical pregnancies (50%), including four spontaneous abortions (33.3%) and one ectopic pregnancy were established. Four pregnancies resulted in healthy babies with normal or balanced karyotypes, others are being followed. Our results suggested that specific probes of part of translocation chromosomes used for PGD beneficial for translocation carrier couples by reducing the risk of miscarriage and avoiding a pregnancy with an unbalanced form of the translocation. Besides these advantages, this method is technically limited for the detection of other potential chromosome anomalies at the same time.

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J19.25

Prenatal genetic diagnosis of a case whose sibling is a CRLF1 related Crisponi syndrome

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Introduction: Crisponi sydrome (CS; 601378), is caused by homozygous or compound heterozygous mutation in the cytokine receptor- like 1 (CRLF1) gene (604237) that is located on chromosome 19p12. CS is inherited in an autosomal recessive manner and characterized with busted sucking and swallowing resulting in poor feeding that drives individuals to medical intervention during neonatal life. In this study, we report a girl with CS, whose mother is pregnant to a prenatally genetic diagnosed embryo.
Material- Methods: *CRLF1* mutation analyzes were assessed in the effected girl and in her parents who have consanguinity. Peripheral blood samples were used as DNA sources, QIAamp DNA Blood Mini Kit was used for extraction. 9 of the exons, including intronic boundaries, were amplified and sequenced directly (ABI Prism 3100 Genetic Analyzer).
Results: We detected a homozygous duplication, c.713dupC, leading a asparagine to alanine substitution (p.P239Afsx92) in exon 5 of the girl. Her parents had the same duplication as heterozygous.
Discussion: *CRLF1* gene is accepted as an causative factor for CS. Scoliosis was the additional menifestation of the disease in our patient. To date, there are not adequate number of studies reporting c.713dupC homozygous mutation in CS patients. Her mother is pregnant to her sibling, who was prenetally screened and now mother has a healhy pregnancy. As a conclusion, we suggest to analyse *CRLF1* gene for genetic counselling or for preimplantation genetic diagnosis for releated diseases.

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J19.26

Evidence for association of the rs605059 polymorphism of the HSD17B1 gene with recurrent spontaneous abortions

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Objective: Estrogen production and levels of circulating estrogen are markedly increased during pregnancy. The HSD17B1 gene product, enzyme 17β-hydroxysteroid dehydrogenase type 1, mainly regulates the biological activity of specific steroid hormones by catalyzing their conversion. We investigated whether the rs605059 polymorphism of the HSD17B1 gene is related to an increased risk of recurrent spontaneous abortions (RSA).
Material and Methods: The study group consisted of 138 women with three or more unexplained spontaneous abortions, before the 20th week of gestation, while 140 women with at least two live births served as controls. All individuals were Greeks. For genotyping the subjects we used the PCR- RFLP method.
Results: The observed frequencies for AA, GA and GG genotypes were 0.18, 0.52, 0.30, respectively, for the patient group and 0.32, 0.49, 0.19, respectively, for the control group. The A allele frequencies were 0.45 and 0.57 for the patient and control group, respectively, while the G allele frequencies were 0.55 and 0.43 for the patient and control group, respectively. Statistical analysis of these results revealed significant differences in genotype and allele frequencies between the patient and control group.
Conclusions: The rs605059 polymorphism of the HSD17B1 gene has

been found to be strongly associated with RSA. Thus the G allele of this polymorphism could be considered as a risk factor for RSA in our population.

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J19.27

Combined QF-PCR and subtelomere MLPA molecular analysis for the detection of chromosomal abnormalities in early spontaneous miscarriages

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A miscarriage affects 10-15% of all clinically recognized pregnancies. Chromosomal abnormalities are the underlying cause in 40-50% of the miscarriages. The vast majority of the chromosomal abnormalities are numerical aberrations, of which trisomies are the most common, followed by polyploidies and monosomies. Conventional karyotyping is hampered by the high rate of culture failures and maternal cell contamination. Several studies have shown that molecular approach is an efficient and cost-effective diagnostic testing strategy for miscarriage products. The aim of this study was to evaluate the usefulness of a molecular strategy, involving quantitative fluorescent polymerase chain reaction (QF-PCR) and subtelomere multiplex ligation probe amplification (MLPA) for the detection of chromosomal abnormalities in early spontaneous miscarriages. The multiplex QF-PCR, including 17 STR markers on the chromosomes 13, 18, 21, X and Y was performed by in-house method, while MLPA was performed with commercial kits from MRC Holland (P036 and P070). A total of 86 spontaneous miscarriage product samples were studied. QF-PCR results showed that ten miscarriage samples (11.62%) were contaminated with maternal tissue. Chromosomal abnormality was detected in 38 samples (50%). The most frequent abnormality was trisomy 16 (37.84%), followed by Turner syndrome, trisomy 22 and trisomy 14 (10.81% each), trisomy 21 (8.11%), trisomy 18 (5.41%), trisomy 4, trisomy 7, trisomy 8, trisomy 10, trisomy 15, monosomy 21 and triploidy (2.70% each). No chromosomal abnormality was detected among 33 control artificial abortion samples. We conclude that QF-PCR and subtelomere MLPA is a suitable strategy for routine chromosome analysis of spontaneous miscarriages.

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J19.28

A Unique Case: Prenatal Diagnosis Of Paternal UPD (18)

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Uniparental disomy (UPD) is a genetic condition which describes inheritance of two homologue chromosomes/chromosome pairs from a single parent. Aberrant dosage of genes regulated by genomic imprinting and homozygosity of a recessive mutation are most important issues as a consequence of UPD. This atypical pattern of inheritance could be detected by newly developed technologies such as STR analysis. We report a patient which was referred to our department for prenatal diagnosis because of abnormal biochemical screening test results. QF-PCR test were performed from amniotic fluid samples for rapid aneuploidy screening. There was no numerical abnormality for 13, 21 and sex chromosomes. Although, all screening markers for chromosome 18 were uninformative. We used FISH test to exclude monosomy 18 and test results were compatible with disomy 18. Parental QF-PCR analysis was showed paternal UPD (18). Chromosome analysis from amniotic fluid was revealed normal karyotype. Results were discussed with family in several genetic counseling sections and they decided to continue pregnancy. The child, who has been followed up until the first year, is healthy and normal. According to our knowledge, this is the first prenatal case with paternal UPD (18). Detection of findings with unknown significance in prenatal diagnosis complicates the management of pregnancy. We believed that our case and diagnostic algorithm would be important and guiding for further management of such complex cases.

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J19.29

AZFc partial deletions are commonly associated with Y-chromosome N-haplogroup and R1a1-haplotypes among infertile men from Ukraine

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The relationship between Y-chromosome haplotypes with diversity and historic-ethnographical aspects as well association with the some conditions has been extensively studied. Heterogeneous phenotypes are associated with partial AZFc deletion and their role as a risk factor for impaired sperm production is unsettled because of controversial data for different populations. The purpose of the study to analyze Y chromosome haplotypes pattern in cohorts of fertile and infertile men of Ukrainian origin with and without AZFc partial deletions

Among 350 males with idiopathic spermatogenesis disturbance 28 (8.0%) were detected to have different types of AZFc partial deletions but in no one among control group (0/100) ($p<0.01$). 19 different Y chromosome haplotypes were identified among studied groups of Ukrainian men. The most frequent are R1a1 (18%), R1a1a (18%) and I2a (19%). Over 50% of males from both studied groups belong to haplogroup R. The Y-chromosome haplotypes were differentiated depending on the presence and the type of Y-chromosome AZFc partial deletions. The accessory haplogroup N was established only in patients with b2/b3 AZFc partial deletions. In contrast, 8 out of 10 men (80%) with gr/gr partial deletions belonged to haplotype R1a1, which was not identified among fertile males of control group. These results point to the following association: haplogroup N increases the risk of b2/b3 partial deletions (OR=11.05, 95% CI: 1.21-101.2, p=0.019), and the presence of R1a1 haplotype increases the risk of gr/gr partial deletion of AZFc region (OR = 20.09, 95% CI: 2.44 - 165.57, p=0.0004).

H. Makukh: None. **M. Tyrkus:** None. **V. Cherepanin:** None. **S. Gavrulushun:** None. **O. Hnateyko:** None.

J19.30

Complex chromosomal rearrangements in a patient with oligozoospermia and Charcot-Marie-Tooth disease

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BackgroundComplex chromosomal rearrangements (CCR) occurring in phenotypically normal persons are rare, about 255 cases have been reported. Most familial cases have a normal phenotype with apparently balanced rearrangements while de novo cases usually are unbalanced or apparently balanced but with associated multiple anomalies as well as mental retardation. **Method**A couple with fertility problems was investigated. He had oligozoospermia and Charcot-Marie-Tooth disease.

ResultsChromosomal analysis and fluorescence in situ hybridization with whole chromosome paint revealed that he had apparently balanced translocation between chromosome 2, 7 and 14. Array comparative genomic hybridization was normal.

ConclusionTo our knowledge, this is a new case of CCR identified in the human population and the first time a CCR is identified in a patient with CMT.

G.J. Braathen: None. **V. Haugan:** None. **A.G.F. Helland:** None. **K.O. Clausen:** None.

J19.31

"High Risk" maternal serum screen and adverse pregnancy outcome

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Aim: To compare adverse obstetric (PIH and oligohydramnios) and neonatal outcomes (prematurity, low birth weight, NICU admission) in women screened "high risk" or "low risk" for chromosomal aneuploidy by maternal serum screening and to find association between individual serum marker (PAPP-A, beta hCG (measured in the 1st trimester), AFP, UE3 and beta hCG (measured in the 2nd trimester) and adverse obstetric and/or neonatal outcome.

Methods: Records of delivered women who underwent maternal serum screening (1st trimester or second trimester) for chromosomal aneuploidy were studied retrospectively. Women undergoing invasive procedure wit-

hout fetal chromosome anomaly were grouped as "High risk" (n=111) while others were grouped as "low risk" (n=157).
Results: "High risk" women were older compared to the controls (31.5+ 4.7 & 29.1+ 3.9 yrs respectively, p<0.005) but did not differ significantly for parity. They experienced high risk for PIH [OR=2.15 (CI: 1.05-4.41)], oligohydramnios [OR=4.07 (CI: 1.70-9.75)] and prematurity [OR= 2.05 (CI: 1.10-3.83)] as well as LBW [OR= 2.12 (CI: 1.12- 4.01)] as compared with 'low risk' women, after adjusting for age. Birth weight was positively correlated with PAPP-A (r=0.274, p<0.05) and inversely with free beta hCG measured in the 1st trimester (r= -0.243, p<0.05) while the second trimester beta hCG was inversely associated with oligohydramnios (r=-0.30, p<0.05) and birth weight (r=-.0.24, p<0.05) after controlling for age.
Conclusion: It might be worth following all women with "high risk" screen for early detection and timely intervention to avoid obstetric and neonatal complications.

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J19.32
Preimplantation genetic testing of balanced translocation by haplotyping analysis in Gennet.

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In 2012, we first performed pre-case haplotyping (PGH) and subsequent preimplantation genetic diagnosis (PGD) of embryos in family with balanced structural aberration of chromosomes 13 and 21. We present atypical case in which affected embryos may not be unambiguously determined by cytogenetic analysis using standard FISH probes due to hybridisation problems.
In presented case, male partner was carrier of *de-novo* balanced structural aberration t(13;21)(q14.1;q22.1). Pre-case haplotyping (PGH) was concluded, based on analyses of STR markers located on the long arms of chromosome 13 and 21, in both partners and mother of the male partner.
During the IVF cycle haplotyping technique by multiplex PCR was used on products of multiple displacement amplification (MDA) from one blastomere biopsied from the cleavage-stage embryo on the day 3. In rare cases of ambiguous result from the blastomere, analysis may be repeated from trophectoderm and embryo is still managed to be transferred on the day 5.
During the PGD analysis we excluded from transfer embryos, carrying either trisomy, or monosomy of tested parts of chromosome 13 or 21. IVF cycle with PGD was performed in January 2013: 7 COCs were retrieved, 4 blastomeres were biopsied, 2 embryos were diagnosed as at low risk and 1 embryo was transferred on the day 5.
Haplotyping analysis using STR markers is technique suitable for indirect PGD testing of monogenic diseases, but can be used also for PGD of structural aberrations, when FISH approach cannot be applied. Confirmed pregnancy is currently on-going.

I. Soldatova: None. **L. Krautová:** None. **M. Hejtmánková:** None. **M. Brandejská:** None. **J. Míka:** None. **D. Stejskal:** None. **M. Putzová:** None.

J19.33
Complex fetoplacental rearrangement : discrepancies between array-CGH and FISH results

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We report the case of a 22-year-old woman, G1P0, who underwent prenatal diagnosis in our fetal medicine center for severe intrauterine growth restriction with decreased fetal movements and tinny lower limbs muscles.
Late chorionic villi sample was realized at 34 weeks of amenorrhea (WA). Conventional karyotyping on cultured placental villi revealed no abnormality. Array-CGH identified a complex 1p36 rearrangement, consisting of a terminal deletion of 14.6Mb, composed by a contiguous mosaic (~70%) 1p36.33p36.23 deletion (6.7Mb), a mosaic (~45%) 1p36.23p36.21 deletion (7.8Mb) and a non-mosaic 1p36.21 deletion (405kb). To confirm these results, FISH techniques were applied using two different probes targeting 1p36.33 locus. Unexpectedly, they showed 2 copies of this locus. Since 1p36.33 deletion was not confirmed, amniotic fluid was sampled at 36 WA. Array-CGH on amniocytes revealed the same non-mosaic 1p36.21 deletion as in the villi and identified a non-mosaic duplication, reciprocal to the 7.8Mb deleted region in the villi. Otherwise, no terminal 1p36.33 deletion was found in amniocytes by array-CGH.
Specific FISH analyses confirmed the presence of the non-mosaic 1p36.21 deletion in both amniocytes and villi. They also confirmed the presence of the 1p36.23p36.21 duplication in amniocytes, and surprisingly showed this duplication in the villi instead of the expected mosaic deletion. The terminal 1p36.33 deletion remained not confirmed.
Pregnancy termination was proposed, based on the ultrasound anomalies and array-CGH results. Indeed, despite discrepancies between FISH and array-CGH results, a complex rearrangement of the 1p36 region was highly suspected. The exact nature of this rearrangement is still to be determined.

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J19.34
IL-6 gene promoter polymorphisms: Genetic susceptibility to recurrent pregnancy loss

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Recurrent pregnancy loss (RPL) is defined as three or more pregnancy losses before 20 weeks. RPL is a multifactorial condition with several etiologic factors including genetic abnormalities of the parents, anatomical, endocrinological, hematologic and immunologic abnormalities, infections, nutritional and environmental factors. The causes of pregnancy loss in about half of the women with RPL even after extensive investigations remain unknown. We analyzed IL-6 -174 G/C, -572 G/C, -597 G/A, -1363 G/T, -2954 G/C promoter region polymorphisms in 113 RPL patients and 113 healthy subjects by using polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) assay. The -174G/C genotypic and -174C allelic frequency and the -2954G/C genotypic and -2954C allelic frequency of IL-6 was higher in RPL patients than healthy controls and significant association was found between RPL and -174G/C, -2954G/C polymorphisms (P: <0.0001, OR: 0.28, 95% CI: 0.15-0.51, P: <0.034, OR: 0.16, 95% CI: 0.01-1.12 respectively). We found remarkably similar frequencies in RPL patients compared with controls for IL-6 -572G/C,-597G/A and -1363G/T genotypes/alleles and no association was observed between RPL and these polymorphisms. Our study supported that IL-6 -174G/C and -2954G/C polymorphisms are associated with increased risk of RPL in Turkish patients.

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J19.35
Search for NLRP7 mutations in first trimester missed abortions with multiple methylation changes in imprinted genes

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NLRP7 gene has been identified as the gene involved in biparental hydatidiform moles (BiCHM) and associated with multiple loss of imprinting (LOI) of the maternal allele. Previously, we report about multiple epimutations affected from 4 to 12 imprinted genes in placental tissues of first trimester missed abortions (Sazhenova et. al., 2012). It is possible that BiCHM and miscarriage have common pathogenetic mechanisms of the epigenetic regulation. To investigate whether the *NLRP7* mutations might cause a miscarriage with LOI we sequenced this gene in the placental tissues of 11 first trimester missed abortions with previously detected hypo- or hypermethylation of multiple imprinted loci. We found nine *NLRP7* genetic variants. Seven of them had already been described in the Ensembl or INFEVERS databases in women with BiCHM and normal reproductive outcomes. In this study, we report the two new homozygous genetic variants c.1405delC and c.1444delC in the embryo with multiple LOI. This embryo had hypomethylation in imprinted genes *PEG10*, *KCNQ1*, *WT1*, *ZNF215* and hypermethylation in *INS*, *PWCR1*, *GABRA5*. These imprinted genes participate in apoptosis and cell differentiation (*PEG10*, *WT1*, *INS*), cell proliferation and growth (*INS, WT1*), reproduction (*WT1*), transport (*GABRA5*, *INS*) and cell cycle (*INS*). It is notable, that epimutations of all these genes, except *PEG10*, were confined by chorion cytotrophoblast or extraembryonic mesoderm indicating its postzygotic but not germinative origin. These findings provide new insights

into the etiology of miscarriages and associated abnormalities of genomic imprinting. Further studies are needed to evaluate the role of *NLRP7* mutations in early pregnancy losses.

M.V. Lepshin: None. **E.A. Sazhenova:** None. **I.N. Lebedev:** None.

J19.36

SRY- negative 46, XX male with complete virilization and infertility as the main anomaly

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Introduction : the 46, xx male syndrome is a rare cause of male infertility and a rare form of sex reversal with complex mechanisms leading to a large clinical features. We report a case of man with a normal male phenotype who presented for exploration of infertility and was found to be 46, xx male without SRY gene in the x chromosome.
Case report : A 34 year old man was referred to our institution because of history of primary infertility in whom repeated seminal analysis showed complete azoospermia. Physical examination showed normal phenotype exept bilateral gynaecomastia. Penis size and testes volume were normal. Serum LH, FSH and prolactin were normal but testosterone serum concentration was under the limit of normal range (1.98ng/ml). The testicular biopsy revealed germinal cell aplasia and fewer leydig cells without sertoli cells anomalies. karyotype analysis showed 46, xx chromosome complement without evidence of mosaicism in peripheral blood cells. Polymerase chain reaction analysis and fluorescence in situ hubridization were performed in lynphocyte culture and also confirm the absence of Y chromosome sequences including any detectable SRY gene.
Conclusion : This case presented here is a rare SRY negative, xx male with testicular differenciation, complete virilization and to our knowledge, it is the third case reported of diagnosed during investigations of infertility. This finding suggests that other genes downstream from SRY not yet identified, play an important role in sex determination such as the presence of mutations of an autosomal or x-chromosomal gene.

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J19.37

Identification of IVS-I (-1) (G>C) or Hb Monroe as a first report on the beta- globin gene with a beta thalassemia minor phenotype in South of Iran

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β-Thalassemia (β-thal) syndrome, is one of the most frequent hereditary diseases in the Mediterranean region, comprising about 280 mutations. We described the first report of IVS-I (-1), codon 30 (G>C)or Hb Monroe in five individuals from four unrealated families in Khuzestan province. PCR followed by sequencing of the beta-globin gene confirmed the presence of Hb Monroe in the heterozygous form which causes β0-thalassemia due to missplicing in the course of mRNA processing. This mutation has described in individuals originated from Arabic and Behbahani origins, Ahvaz city, Southern of Iran. The knowledge of the β-globin variants present in the Iranian population is essential for the molecular diagnosis and prevention of hemoglobinopathies.

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J19.38

Altered intra-nuclear organization of heterochromatin of chromosome 1 in interphase nuclei of patients with endometriosis by 3D-FISH

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Chromatin structure and spatial arrangement of genomic regions, including heterochromatin in interphase nuclei are important in the gene expression and genome function. Variations in heterochromatic segments are usually considered as normal ones without any direct harmful phenotypic effect. However, it is already known that changes in heterochromatin structure and organization in interphase nuclei are associated with two disorders, Roberts and ICF syndrome.
We report for the first time: 1) two females with endometriosis, who have decrease in length of heterochromatin on the long arm of chromosome 1 (1qh-) revealed on peripheral lymphocyte cultures by GTG and CBG banding methods, and 2) the influence of 1qh- on the intra-nuclear organization revealed by 3D-fluorescence in situ hybridization (3D-FISH) on interphase nuclei of these two patients and two healthy control women. Endometriosis is a common, gynaecological disease characterized by the presence of uterine endometrial tissue outside of the uterus. Its causal mechanisms are not fully understood. Our results of 3D-FISH showed statistically significant changes in sizes of volumes of hybridization D1Z1 signals (DNA probe for 1q12) and their relocation to the periphery in interphase nuclei of patients compared with controls. Thus, our results give new insights into the chromosome alterations in metaphase cells and spatial intra-nuclear organization of heterochromatin of chromosome 1 in interphase nuclei of endometriosis patients. We suggest that observed changes in the structure and location of heterochromatin of chromosome 1 in interphase nuclei have major role in the pathogenesis of endometriosis due to altered intra-nuclear architecture and genome function.

R. Mikelsaar: None. **H. Paves:** None.

J19.39

Chorionic Villi Sampling: Cytogenetic, Molecular and Clinical Findings in the Western part of Romania

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First-trimester chorionic villus sampling (CVS) was performed on a number of 80 pregnancies in the last 2 years. The test used in all cases was chromosomal analysis from mesenchymal cells belonging to long-term cultures combined with the Quantitative Fluorescent Polymerase Chain Reaction (QF-PCR) for the most common chromosomal aneuploidies. The main reasons for sampling were the presence of fetal malformations at ultrasound examination (42,10%), the biochemical risk (33,33%) and the maternal age (26,25%). Abnormal laboratory findings resulted in 10 terminations of pregnancy (12,5%). We have detected 4 fetuses with trisomy 21, 2 with trisomy 18, one with trisomy 13 in robertsonian translocation, one with mosaic X monosomy, one with double monosomy (-18,-19) and one with maternal duplication dup(1q12-14). In addition four unexpected balanced chromosome rearrangements were detected: two fetuses with maternal 9 inversion and two with paternal balanced translocation t(2;18)(q36;q21) and "de novo" t(1q;Xq),t(1p;Xp). There were no false negative findings. False-positive cytogenetic findings occurred in 2 cases, comprising with mosaicism confined to the trophoblast: 1 case with trisomy 16 in the CVS karyotype, presenting normal karyotype when amniocentesis was performed and a tripleX case that resulted after a QF-PCR test, turning out to be a 45,X/46,XX karyotype when amniocentesis was performed. Another discrepancy that arose was by fault of maternal contamination: a false XX/XY mosaicism which amniocentesis proved to be a normal 46,XY karyotype. QF-PCR has proven to be rapid (24-48h), relatively inexpensive, efficient in detecting major numerical abnormalities, reliable complementary diagnostic method alongside fetal karyotyping resulted from long-term cultures.

C. Gug: None. **C. Muresan:** None. **B. Muresan:** None. **F. Dorneanu:** None. **T. Cioata:** None. **V. Dumitrascu:** None. **D. Vlad:** None. **R. Mihaescu:** None.

J19.40

Identification of a novel mutation in the AR gene in a Tunisian Family with Complete Androgen Insensivity Syndrome in the daughter and with Primative Ovarian Failure in the mother

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The Androgen Insensitivity Syndrome (AIS) is a frequent etiology of 46, XY Disorders of Sexual Development and is caused by mutations in the Androgen Receptor (AR) gene, mapped to chromosome Xq11-12, the AR encodes the AR protein, which binds both testosterone (T) and dihydrotestosterone and acts as a transcriptional factor involved in the expression of genes responsible for the development of male sexual characteristics. The Complete AIS (CAIS) is characterized by external female genitalia, absence of Mullerian structures, a blind-ending vagina and a developed breast in patients with 46, XY karyotype formula.

We studied a female Tunisian patient diagnosed with CAIS at the puberty age. A novel mutation c.3162C >T leading to p.P683S was identified in the patient and in a heterozygous state in patient' mother. Using multiple alignments, we noticed that the Proline residue is conserved and the 3D modeling showed that the mutated AR conformation may be alerted. As previous reports of AR knockout mice model and Indian women with AR gene mutations showed POF (Premature Ovarian Failure) phenotype, the novel mutation p.P683S could be among the genetic causes of POF observed in the patient'mother.

As a conclusion, the novel mutation p.P683S is the first mutation to be described in a mother with POF and the daughter with CAIS; it substantiated the implication of the AR gene in the POF among 46, XX females subjects and in the Androgen Insensibility Syndrome in 46, XY patients.

B. Ben Rhouma: None. **N. Belguith:** None. **F. Abdelhedi:** None. **M.M. Feki:** None. **M. Kesentini:** None. **K. Chabeene:** None. **H. Kamoun:** None. **F. Fakhfakh:** None.

J19.41

Complex chromosomal rearrangement involving chromosome 2,5 and 10 in a fertile male: meiotic segregation and interchromosomal effects

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Complex chromosomal rearrangements (CCRs) are defined as rare structural abnormalities. They involve between more than two chromosome breaks and exchanges of chromosomal segments.CCRs are usually associated with male infertility as a result of the disruption of spermatogenesis due to complex meiotic configurations and the production of chromosomally abnormal sperm.

Here, we describe a rare case of an apparently balanced karyotype of 46,XY, t(2;5;10)(q21;p15.3;q22.1) in a fertile male. The couple admitted to our clinic because of recurrent spontaneous abortions. They had four abortions and a healthy 6-year-old son whose karyotype was normal. Parental karyotypes of our patient were normal,too. Thus it was assumed to be a de novo case. In general, most familial cases have a normal phenotype with apparently balanced rearrangements. In contrast, half of de novo CCRs are unbalanced, and remaining half are apparently balanced but associated with multiple structural anomalies as well as mental retardation.

Sperm fluorescence in situ hybridization (FISH) analysis was performed on each of the involved chromosomes to determine the patterns of segregation. FISH was also performed on chromosome 13, 18, 21, X and Y to determine any interchromosomal effect. An array-CGH analysis was planned. Findings of the patient are discussed and compared with literature.

A.G. Zamani: None. **E. Tuncez:** None. **A. Acar:** None. **M.S. Yildirim:** None.

J19.42

A patient with a Diamond-Blackfan Anemia-1due toa mutation in ribosomal protein S19 gene and prenatal genetic diagnosis for bone marrow transplantation.

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Introduction: Diamond-Blackfan anemia-1 (DBA1, MIM 105650) is a rare, mostly autozomal dominantly inherited condition characterized by congenital erythroid aplasia that usually presents in infancy. One of the candidate gene responsible for the condition is ribosomal protein S19 gene (RPS19, MIM 603474). In this case study, we report a girl with DBA1 and anaylzes of RPS19 gene in her family.

Material- Method: DNAs from the individuals enrolled in the study was extracted by using QIAamp DNA Blood Mini Kit from 8 ml perpheral blood. All coding regions and exonic- intronic regions were amplified and sequenced directly for mutation detection.

Results: The patient had a heterozygous mutation in its *RPS19*, c. 185G>A transition in exon 4, which leads a arginine to glutamine substitution, p.R185Q. We detected no mutation in her healty sibling and parents fort he same gene analysis. **Discussion:** *DBA1* has severe effects during the neonatal life and needs bone marrow transplantation for the effective treatment. In this case, we present a mutation in *RPS19* in agirl, and screened candidate embryonic cells for the detected mutation and nearby markers, and HLA, as well in the terms of appropriate embryo transfer. Mother is not pregnant now but the process is still going on for the treatment of the girl.

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J19.43

Non-immune hydrops fetalis: a prospective study of cases

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Non-immune hydrops fetalis (NIHF) is caused by a heterogeneous group of conditions and the investigation of cases constitutes a diagnostic challenge. This study aimed to evaluate prospective and systematically a series of NIHF cases from an expanded investigation protocol and review the frequency of inborn errors of metabolism in the published series of NIHF. During the period (2010-2011), 53 individuals with NIHF were evaluated. A clinical-etiological or pathogenic diagnosis was reached in 46 (86.8%) cases. The main diagnostic groups were chromosomal abnormalities (17 - 32.1%), syndromic (8 - 15%), isolated cardiovascular anomaly and congenital infection (4 - 7.5% each one). Metabolic disease was identified in three (5.7%) cases, being all due to lysosomal storage disorder (LSD). Seven (13.2%) cases were classified as idiopathic - unknown cause after wide investigation (three cases) and incomplete evaluation (four cases). The hydrops was identified in prenatal period in most cases (48 - 90.5%), and despite the poor prognosis commonly associated to NIHF, three (5.7%) cases had spontaneous and complete resolution of the hydrops during pregnancy. The overall mortality was 75.5%. The IEM frequency in the present study (5.7%) is higher than that usually reported in the literature, justifying the inclusion of a systematic approach of these conditions in NIHF. IEM investigation, however, should be performed after exclusion of the more common causes, i.e., chromosomal abnormalities, isolated cardiovascular anomaly, congenital infections. Furthermore, considering that placental examination and skeletal evaluation may be suggestive of LSD, these exams should not be neglected in the NIHF investigation.

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J19.44

Investigation of Interleukin 4 Gene -590 C>T Polymorphism and Distribution of Genotypes in Preeclampsia and Normal Pregnancies

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Preeclampsia is a complex disease of pregnancy with both feto-placental and maternal factors contributing to its pathogenesis. Although the cause of this disease is uncertain, imbalance between pro-and antiinflammatory cytokines has been implicated in the pathogenesis of preeclampsia. Functional single nucleotide polymorphisms (SNPs) of interleukin (IL)-4 -590 (C>T), was examined by polymerase chain reaction-restriction fragment length polymorphism to identify their merit as genetic markers for preeclampsia. The study cohort was a group of 128 woman with preeclampsia and 85 healthy pregnant woman subjects from the general turkish population. Among controls, the IL-4 Ava II genotypes of C/C, C/T, and T/T were observed in 74.1%, 24.7%, and 1.2% , respectively, whereas the C/C, C/T, and T/T genotypes were observed in 69.5%, 26.6%, and 3.9% of case patients, respectively. There was not significant difference in terms of genotype (p=0.456) frequencies of IL-4 gene -590 polymorphism. Polymorphisms of IL-4 gene in the immune system pathway was not associated significantly with preeclampsia risk.

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J19.45

Child outcome following mid - trimester amniocentesis: respiratory health and sleep habits

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OBJECTIVE. The aim of the study was to determine the incidence of respiratory problems and sleep disturbances (sleep habit, sleep scheduling and sleep maintenance) in babies born after second trimester amniocentesis. It is first Lithuanian study analysing child outcome following mid - trimester amniocentesis for sleep and respiratory health.
METHODS. Analysis was performed with 3 years babies of mothers who had undergone second trimester amniocentesis (n = 50) for fetal karyotyping because of advanced maternal age or family history of chromosomal abnormality. Data were compared with children whose mothers (n=47) declined the test (control group).
RESULTS. Amniocentesis was associated with a significantly higher incidence of child sleep disturbances: 30 child (60%), comparing with control group 11 child (23.4%) (P = 0.001). Although there was no significant difference in the frequency of acute respiratory infections rate, stridor, apnea, we observed that 7 children (14%) had asthma in postamniocentesis group, while in control group asthma was diagnosed only for 1 child (2,1%) (P = 0.06).
CONCLUSIONS. Second trimester amniocentesis does not appear to compromise child lung function development however amniocentesis may impair postnatal sleep habits.

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J19.46

Prenatal screening for Aneuploidies in Iranian families using QF-PCR

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Quantitative fluorescence polymerase chain reaction (QF-PCR) has been introduced in a number of genetic laboratories as an inexpensive, rapid and reliable method for prenatal recognition of aneuploidy in chromosomes 13, 18, 21, X and Y. We have investigated the efficacy of QF-PCR for the prenatal recognition of common aneuploidies and compared our findings with cytogenetic results in Iran.
Total of 1747 prenatal samples (1720 amniotic fluid and 27 chorionic villous samples) were analyzed by QF-PCR using several selected STR markers together with amelogenin.
Results were compared with those obtained by conventional cytogenetic analysis. We could detect 55 numerical abnormalities in our subject by QF-PCR. Concordant QF-PCR and karyotype results were obtained in 1716 (98.22%) of the samples. An abnormal karyotype associated with adverse clinical outcome undetected by QF-PCR was found in 0.5% of samples.
Using QF-PCR alone we were able to detect abnormalities in 98.56% of all referred families, however, the karyotyping results improved the detection rate to 99.60% in the referred cases.
In countries where large scale conventional cytogenetic is hampered by its high cost and lack of technical expertise, QF-PCR may be used as the first line of screening for detection of chromosomal abnormalities. In addition, karyotyping will also recommended for all prenatal cases to increase the power of detection. We also recommend for all the families that are seeking prenatal diagnosis of single gene disorders, additional aneuploidies screening to be added to their work up.

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J19.47

A complex fetal chromosomal aneuploidy due to maternal translocation t(1;9)

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Introduction: Chromosomal abnormalities- aneuploidy and structural anomalies are frequently associated with fetal malformations and postnatally mental retardation.
Aim was to present a case history of a complex fetal trisomy of 9q21.33-pter and 1q42-qter diagnosed antenatally. According to cytogenetical to molecular-genetical investigations this abnormality had maternal original.
Materials and methods: The family was consulted by clinical geneticist and antenatal invasive investigations were performed due to fetal abnormalities detected by ultrasound (US): brain anomaly and soft fetal US markers of possible chromosomal disease which was indication for fetal chromosomal analysis. There had been one early miscarriage in this family previously. Fetal G-banding was performed using the trypsin-Giemsa staining technique. At least 20 metaphases were analysed. Fetal karyotype was abnormal : 47,XX,+add(9)(q22). Parental cytogenetical investigation showed, that father had a normal man`s chromosomal finding, mother had a karyotype 46,XX,t(1;9)(q42;q21.33).To clarify the possible etiology of this change submicroscopical chromosomal analysis by HumanCytoSNP-12 array was performed. A pathological fetal result was achieved: arr[hg19] 1q42.12-q44(226,350,383-249,138,233)x3,2q13(110,859,672-110,982,530)x1,9p24.3-q21.33(1-88,226,038)x3.The final fetal karyotype was 47,XX,+der(9)t(1;9)(q42;q21.33)mat.Genetic counselling to the family was performed and the family decided to terminate the pregnancy. Pathological investigation of the fetus is presented.
Conclusion: Using different cytogenetical methods allowed us to verify fetal kayotype. A complex aneuploidy was associated with fetal anomalies and may play a role in the etiology of the miscarriage in this family. Clinical, laboratory and pathological data are presented and genetic counselling problems of this kind of complicated families are discussed.

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J19.48

A case of confined placental mosaicism for trisomy 21 and fetus with abnormal ultrasound findings

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Confined placental mosaicism (CPM) is found in 2% of the diagnostic chorion villus sampling (CVS). Clinically CPM may be associated with a spectrum of fetal manifestations ranging from normal pregnancy outcome to intrauterine death of a chromosomally normal fetus or intrauterine growth restriction (IUGR). Chromosome specific mosaicism could be responsible for suboptimal placental function and associated pregnancy complications.
Here we report a case of CPM for trisomy 21 detected by QF-PCR on 13 gw CVS. A 27-years old pregnant woman was referred for prenatal diagnosis with an increased nuchal translucency (5.3mm), hypoplastic nasal bone and calculated high risk for trisomy 21 (1:5) from the maternal serum screening; free bCG =5.8 MoM.
QF-PCR analysis was performed with Aneufast (Molgentix SL, Barcelona, Spain) and analyzed on ABI 3130xl. Two of the markers located on chromosome 21 showed ratios consistent with a trisomic condition, the other three markers were normal. The family opted for termination of the pregnancy due to poor prognosis. Chorionic villi and fetal tissue from the terminated pregnancy were analyzed by QF-PCR. The results from the villi were concordant with those from the CVS. The QF-PCR profile of the fetus was normal. Long term culture of the chorion showed normal male karyotype.
Based on these results, a low level placental mosaicism type I - confined to the trophoblast could be considered. In addition, an array CGH analysis is planned to be performed.

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J19.49

CYP21A2 gene analysis in Congenital Adrenal Hyperplasia patients from Bashkortostan Republic of Russia

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Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is the most common inherited defect of adrenal steroid biosynthesis.
The molecular genetic analysis of the CYP21A2 gene was performed in 126 CAH patients from Bashkortostan Republic of Russia. Our study showed that the gene deletions/large gene conversions were present in 27.7% of unrelated alleles. The most frequent point mutations I2splice and R356W

occurred in 13.5% and 11.1%, respectively. Other mutations, I172N, Q318X, V281L and P30L were rare (4.8%, 4%, 2.4% and 1.2%, respectively). Mutations R426C and delIle384 were identified in only 0.4% of alleles.
Furthermore, the clusters of mutations on one chromosome Q318X+R356W (5%), I172N+Q318X (1%), delA2orLGC+V281L (1%), I2splice+P453S (0.4%) were found in CYP21A2 gene in 8 CAH patients.
In one patient with simple virilizing form we identified the insertion of one nucleotide in exon7 of CYP21A2 gene. The frameshift mutation F307+1 nt was found in a heterozygous state with I172N mutation.
Moreover, the analysis of CYP21A2 gene detected several polymorphic variants. The frequency of alleles T of rs6462 (c.289+9C>T), rs6449 (c.289+67C>T) and S374S was40.5%, 22.5% and 11.3%, respectively. The allele A of rs6474 (R307K) was found in 40.5% of unrelated alleles.
Thus, studying of molecular-genetic nature of CAH represents the doubtless scientific and practical importance in respect of use of the received data for differential diagnostics of its various forms, medical and genetic consultation and prenatal diagnostics.

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J19.50
Small supernumerary marker chromosomes (sSMC) detected prenatally

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The restriction of the GTG-analysis at identification of small supernumerary marker chromosomes (sSMCs) represent a big problem in prenatal diagnosis. FISH-method with different DNA-probes allows to identify chromosomal origin of sSMCs and to ascertain if they include the euchromatic segments in order to estimate of fetal genetic risk. The FISH-analysis was carried out on a AxioImagerM1 (Zeiss) with Isis software (MetaSystems) and DNA probes: CEP15/LSI D15S11, wcp 13, 14, 15, 21, 22, LSI TUPLE I/ARSA (Abbott, Vysis). Also the multicolor FISH techniques were used. Four cases of autosomal sSMCs were found at GTG-analysis on fetal lymphocytes. In cases 1and 2 bisatellite sSMCs were derived from chromosome 15 (inv dup 15) of parental and maternal origin respectively containing only heterochromatic segments (15pter-q11.1). In case 3 sSMC was detected as inv dup 22(q11.1) de novo with wcp 22 and TUPLE/ARSA DNA-probes (Abbott, Vysis). Ultrasound examination of these fetuses was normal and pregnancies ended with normal childbirth. In case 4 two sSMCs were found in fetus with severe intrauterine growth retardation. Multicolor FISH (cen-mFISH, mFISH, mBAND) was used and the origin of these sSMCs was established: both sSMCs appeared as ring chromosomes 5 and 20 respectively. The fetal karyotype was defined as 48,XY, +r(5) (p13.2q11.2), +r(20) (p11.1q12) dn, there is a partial trisomy on (5) (p13.2→q11.2) and a partial trisomy on (20) (p11.1→q12). Interpretation of sSMCs origin and euchromatin involvement are a big clinical problem since it is difficult to establish connection between of these chromosomes presence and fetal anomalies.

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J19.51
Two cases of insertional translocations in patients with reproductive disorders

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Insertional translocations (ITs) are rare chromosomal rearrangements. Correct definition of IT is critical for preimplantation genetic diagnosis (PGD) and prenatal diagnosis.
In our practice there were 2 cases with ITs in 6500 karyotypes.
In the first case a couple was infertile. Analysis of GTG-banded male's chromosomes demonstrated a reciprocal translocation 46,XY,t(1;4)(p32;q21). During genetic counseling the couple refused PGD. In vitro fertilization (IVF) cycle was performed. Prenatal diagnosis detected a derivative chromosome 1 in a fetus karyotype. Unbalanced IT was established by whole chromosome painting (WCP) for chromosome 4, and the fetus was aborted. Reanalysis of male's karyotype using WCP demonstrated the balanced IT - 46,XY,ins(1;4)(p32;q22q28?). IT regions were detected through high-resolution banding. Locus specific probe LSI 4q22 validated IT. Individual FISH (fluorescent in situ hybridization) probes were composed for PGD. A pregnancy was not obtained in the second treatment cycle IVF/PGD.
The second case was a couple with two healthy daughters and four cases of miscarriages. Analysis of male's karyotype detected a reciprocal translocation - 46,XY,t(2;3)(p23;p21),1qh+. For PGD set-up we used centromeric and telomeric FISH probes, but the reciprocal translocation was not confirmed. WCP for chromosome 2 identified balanced IT - (2;3)(p23;p22p24?). Genetic counseling was carried out. The couple is suggesting the PGD cycle.
According to these cases we consider that identification of ITs by GTG-banding is complicated, and needs additional cytogenetic techniques.

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J19.52
The role of IL-6, IL-8, and IL-10 gene polymorphisms in the pathogenesis of recurrent pregnancy loss

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The maternal immune system is crucial for pregnancy establishment and maintenance. IL-6, IL-8 and IL-10 are key regulators of immune response in gestational tissues. Fluctuations of abovementioned cytokine levels are controlled by regulatory elements in promoter or intron regions of respective genes and are critical for pregnancy tolerance.
The study aimed to evaluate the role of *IL6* gene -174G/C, *IL8* gene -781C/T, *IL10* gene -592 C/A and -1082G/A variants in recurrent pregnancy loss (RPL) pathogenesis. Study material - genomic DNA extracted from peripheral blood of unrelated individuals from Ukrainian population. Case group (60 women with history of RPL) and control group (106 healthy women, who have given birth to at least one child conceived in natural way) were genotyped by a PCR based RFLP assay. Statistical analysis was performed using OpenEpi statistical package.
There was no significant difference in *IL6* -174G/C genotype and allele frequencies between case and control groups. Frequency of *IL8* -781T allele carriers was significantly higher (p<0,05; OR=1,866; CI95%: 1,073-3,244) in RPL group (0,716) comparing to control (0,576). Frequencies of *IL10* -592A and -1082A allele carriers were higher (p<0,05) in group with RPL (0,484 and 0,825 respectively) comparing to control (0,34 and 0,764). Odds ratio calculation showed association of both these variants with RPL (OR=1,8; CI95%: 1,062-3,116 and OR=2,16; CI95%: 1,07-4,362 respectively).
Obtained results suggest that *IL8* gene -781T, *IL10* gene -592A and -1082A alleles are associated with RPL risk in Ukrainian population.

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J19.53
A new case of pregnancy with X homogenous monosomy in a patient with Turner syndrome

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Monosomy X affects 1/2,500 girls and it generates the Turner syndrome (TS), characterized by: short stature, primary amenorrhea, deficit of sexualisation and sterility. The studies of TS indicated that 90% of patients have lack of pubertal development and primary amenorrhea. The normal puberty is more frequent in mosaic X monosomy, 10-15% of these having menarche, but usually they present a secondary amenorrhea. The pregnancy in TS patients is a rare situation, associated with a high risk for chromosomal abnormalities. Before introduction of IVF techniques was cited <150 pregnancies in TS. 10% of pregnancies in mosaic X monosomy patients presented an X monosomy. We present a case of 45,X/46,XX women, 22 years old, that have a pregnancy finished by spontaneous abortion in 10 weeks of gestation. The patient was diagnosed with TS during childhood, but not received therapy with GH or estro-progesterone. She developed a spontaneous puberty and has regulated menstrual cycles. The women have 143 cm and 40 kg, and she was directed to our service by obstetrician in 5 weeks of pregnancy, confirmed by level of β-hCG. Unfortunately, a second echographic examination of embryo (10 weeks) attested a cystic hygroma and the stop of vital activities. The obstetrician decided to produce an abortion, and he took a sample of chorionic tissue. We analysed the embryonic tissue by FISH and we also harvested the cells. Both analysis confirmed the presence of 45,X monosomy.

Our case indicates the importance of prenatal genetic analysis in all pregnancies at patients with TS.

E. Gorduza: None. **V. Martiniuc:** None. **M. Gramescu:** None. **C. Bujoran:** None. **M. Diaconu:** None. **L. Caba:** None. **E. Braha:** None. **R. Popescu:** None. **L. Butnariu:** None. **D. Nemescu:** None.

J19.54

Hb Ahvaz [α-83 (F4), Leu>Arg], a new hemoglobin variant of the α2-globin gene

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In general, α- globin variants result from point mutations in α1 or α2 globin genes leading to abnormal α-chain hemoglobins . Alpha thalassemia is also common in Iran and the most frequent lesion reported so far is the -α3.7 mutation. We report a novel mutation in the α2-globin gene, codon 83 (T>G), detected in two members of two unrelated families from Khuzestan province, South of Iran, which we named Hb Ahvaz. This mutation detected by cellulose acetate electrophoresis and characterized by molecular studies. Hb Ahvaz seems to be no responsible for hematological abnormalities in the carriers but with Alpha zero thalassaemia defects it might induce severe clinical symptoms.

M. Hamid: None. **G. Shariati:** None. **A. Saberi:** None. **H. Galehdari:** None. **B. Kaikhaei:** None. **M. Mohammadi -Anaei:** None.

J19.55

Comparison of multiplex ligation-dependent probe amplification (MLPA) technique and routine karyotype method in diagnosis of common chromosomal aneuploidies

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Introduction: The major aneuploidies diagnosed prenatally involve the autosomes 13, 18, 21, and sex chromosomes X and Y. Improvements in non-invasive screening methods for detection of common aneuploidies have radically changed the indications for prenatal diagnosis over the last decade. Using Multiplex ligation-dependent probe amplification (MLPA) technique instead of traditional culture method, allows for quick, easily automated multiplex testing of these aneuploidies in one polymerase chain reaction.
Material and Methods: Chromosome copy numbers were determined by analyzing size and peak area for each MLPA probe. Cytogenetic culture was attempted too. Results were confirmed by quantitative fluorescent-PCR (QF-PCR).
Results: In this blind study, MLPA with P095-A2 aneuploidy probe mixes was performed on 55 amniotic fluid (AF) and 10 control samples. The mean age of pregnant women was about 33 years old. Using this technique, one case with 45, X monosomy diagnosed. There were no false-positive results. In all conclusive tests, the MLPA results were concordant with cytogenetic and qf-PCR findings.
Discussion and Conclusion: The experiment demonstrates that MLPA can provide a rapid and accurate clinical method for prenatal identification of chromosome aneuploidies with 100% sensitivity and 100% specificity.

M. Omrani: None. **S. Saleh Gargari:** None. **F. Azizi:** None. **N. Safavi Naini:** None.

J19.56

Cytogenetic survey of 270 Iranian females with premature ovarian failure.

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Menopause normally occurs in women during their late 40s or early 50s. However, in almost 1% of cases, it occurs before age 40 called "premature ovarian failure". It known as an enigmatic and heterogeneous disorder, with poorly understood etiology. The importance of chromosomal abnormalities in the aetiology of premature ovarian failure (POF) is well-known but in many cases POF still remains idiopathic. To date, several studies have attempted to clarify association between type of chromosomal abnormality and incidence of disease.
The aim of this study was to investigate the frequency and type of chromosomal abnormalities in Iranian POF patients to assess the efficacy of cytogenetic screening for them. We investigated the frequency and type of chromosomal aberrations in Iranian females, diagnosed with idiopathic POF. Standard cytogenetic analysis was carried out in a total of 270 patients. Karyotype analysis of these patients revealed that 240 (88.88%) patients had normal female karyotype and 30 (11.11%) patients had abnormal karyotypes. The abnormal karyotypes included sex reverse SRY negative (7 Cases), X chromosome mosaicism (10 cases), abnormal X chromosomes (7 cases), abnormal autosomes (4 cases), and X-autosome translocation (4 cases).
It is clear that, if the diagnosis of POF made after complete follicular depletion, the infertility of patients with POF would not be restored, so, early diagnosis by genetic investigation may instead lead to the advice of early conception or oocyte harvesting and preservation. Chromosomal studies thus provide valuable clinical information for reproductive management and genetic counseling

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J19.57

The presence of one trisomic diallelic marker of a fetus for D21S1411 with normal karyotype

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Introduction: The purpose of this study was to evaluate the results of a Chorionic Villus Sample (CVS) with QF-PCR and conventional cytogenetic methods.
Methods: In this study, a CVS was obtained from pregnant women with positive maternal serum screening, attending to the Cukurova University, Department of Obstetrics and Gynecology outpatient clinic. QF-PCR was used for trisomy detection. Karyotypes of the fetus and the family were performed with standard GTG banding procedure.
Results: Except D21S1411, all markers of fetus were informative and normal. D21S1411 was at trisomic diallelic form. QF-PCR was used for identification of the parental origin of the trisomic diallelic marker of the fetus for D21S1411. The father had the one allelic form for D21S1411 marker. Chromosome analysis confirmed that all cells of CVS had 46,XX karyotype. Karyotypes of the parents also were normal.
Conclusion: Trisomic diallelic form of D21S141 was found as polymorphism in some populations. It could be a polymorphism but chromosome 21 of the fetus should be investigated with a powerful molecular technique. Although assessment of a normal or trisomic copy number is concluded when at least two informative markers were detected for the chromosome of interest we want to investigate the genes of this region of the fetus with high throughput sequencing technique. The pathological QF-PCR results require additional cytogenetic analysis that is still regarded as gold standards in order to clarify the underlying chromosomal rearrangement.

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J19.58

The microdeletions of Y chromosome and chromosomal abnormalities at idiopathic infertility

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Cytogenetic and molecular genetic studies were performed in order to identify the genetic causes of idiopathic infertility in men of reproductive age.
The material of the study were peripheral blood 203 men with a history of reproductive disorders (59% astenoteratozoospermia, oligoastenoteratozoospermia in 29% and azoospermia in 12% in cases).
Cytogenetic and molecular genetic studies were carried out. Cytogenetics differentially stained chromosomes, derived from 72-hour culture of peripheral blood lymphocytes was performed to identify numerical and structural chromosomal abnormalities.
Genomic DNA was extracted by the standard method. Multiplex polymerase chain reaction was performed using Taq-polymerase. The nine of STS-mar-

kers specifying of AZF-locus were used. The results of amplification reaction were evaluated by electrophoresis in 7% PAAG.
Normal karyotype - 46, XY was diagnosed in 176 cases. The 27 cases (10%) showed chromosomal abnormalities. The Klinefelter's syndrome (47, XXY) was determined in 74%, the disomy of Y chromosome (47, XYY) - 11% and the structural abnormality - 45,XY,der(14,15)(q10;q10); 46,XY,inv(9)(p11q13); 46,XY,t(X;21)(q21;q12); 46,X,t(Y;1)(q12;q21.1) in 15%.
The 91 men with normal karyotype were carried out molecular genetics investigations. The 11 patients had deletions of AZF locus such as AZFa in the 10%, AZFb in the 10%, AZFa+b in the 10%, AZFc in the 70%.
The obtaining results showed the genetics causes of idiopathic infertility and the tactics of IVF / ICSI program.

B. Kamaliyeva: None. **D. Zhumazhanova:** None. **A. Pshenbaev:** None. **G. Abildinova:** None.

J19.59

Maternal biochemical screening as an approach for genetic prevention - the experience of the Laboratory in Medical Genetics, University hospital "St.Marina " - Varna, Bulgaria

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Objective: Performance of eight year experience on prenatal screening for Down syndrome.
Methods: The Laboratory in medical genetics was the 2nd laboratory which started the prenatal biochemical screening for risk estimation of Down syndrome and neural tube defects in 2005.
Pregnant women underwent screening in second trimester (ST2) - 15-19+3 gestational week using serum AFP and free beta-hCG biochemical markers. At the end of 2009 first trimester test was implemented (11-13+6 gestational weeks). The combined screening test (CST1) was based on US measurements of NT (nuchal translucency) and NB (nasal bones) supplemented by biochemical markers of serum free beta-hCG, PAPP-A.
Results: The test was performed on 17348 pregnant women: 12968 by ST2 test (10903 at the age < 36 and 2065 at the age > 36) and 4380 - in the first trimester (3561 at the age < 36 and 719 at the age > 36). High risk for a chromosome disorder was found by ST2 test in 1095*:* 649 <36 and 448 >36. The more sensitive CST1 detected 33 and 60 women respectively to the age groups. For the eight year period prenatal diagnostic tests by DNA and cytogenetic analyses were performed. Among pregnancies tested for chromosomal diseases variety of abnormalities were found. Additional ultrasonographic scan confirmed the biochemical risk for a serious Neural Tube / Abdomainal Wall defects.
Conclusions: Based on screening and diagnostic results we comment on the sensitivity, limitations and the stepwise sequential testing way of achieving a high performance of screening.

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J19.60

Chromosomal rearrangement in Xq and CNVs in Xp in a Turkish patient with premature ovarian failure

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Premature ovarian failure (POF) is defined as amenorrhoea for more than 6 months, occurring before the age of 40, with a FSH level higher than 40mIU/ml and hypoestrogenism accounts for about 10% of all female infertility. Furthermore abnormalities of the X chromosome are associated with POF. Here we describe 30 year old woman who referred to our IVF clinic because of infertility and secondary amenorrhoea. She reported normal pubertal timing and progression, with menarche at age 13 years. At age 16 years, she experienced oligomenorrhea therefore she was placed on oral contraceptive pills for cycle regulation. At age 20 years although the oral contraseptive pills usage, she experienced amenorrhea. Baseline laboratory evaluation included day 3 FSH 113 mIU/ml, estradiol <20 pg/ml. She was diagnosed as POF. Analysing of her and available family members karyotype revealed that she had a karyotype of 46,XX, t(3,X) (p21.1,q22.3) and the translocation was de nova. Involvement of the gene disruption in the breakpoint of the X chromosome rearrangement can be cause of the POF clinic, therefore aCGH was performed. Data analysis revealed no major chromosomal alterations. The comparison with the literature data showed 2 CNV's significantly associated with the POF phenotype that overlapped with CNV's found in our case in Xp22.31 and Xp22.33. However in our patient these overlapping CNV's were smaller and include gene in one CNV. Although the particular overlapping was found, further studies are required to asses whether there really is an association between these CNV's and the POF phenotype.

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J19.61

Prenatal diagnosis and DNA analysis case of thrombocytopenia-absent-radius (TAR) syndrome from Russia

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Thrombocytopenia, aplasia of the radial (TAR) syndrome - a rare form of multiple birth defects with bilateral aplasia of the radius and the development of thrombocytopenia, an inherited autosomal recessive (MIM 274000). Most of the TAR-syndrome caused by a rare deletion in the 200-kb region 1q21.1, RBM8A related gene on one chromosome, and low-frequency noncoding single nucleotide polymorphisms (SNP) in RBM8A to another.
We report a case of prenatal diagnosis of TAR syndrome is not consanguine family at young parents. Sonography of the fetus at 32 weeks of pregnancy established bilateral aplasia of the radius while maintaining thumb, radial deviation of the hands. Upper limbs dramatically shortened by shortening and forearm strain, small-fetal gestation. The presence of thumb on both sides allowed excluding Holt-Oram syndrome and Fanconi anemia.
Birth 37 weeks of gestation by cesarean section, the weight 2460, is long 48 cm, half female. On examination, the child says: radial deviation of the hands, (thumbs saved), hemorrhagic rash on the skin of the trunk and extremities, gastric bleeding, thrombocytopenia (platelet counts are the 8000 to 45 000). X-ray confirmed aplasia radius and ulna shortening.
The child has been found by MLPA-analysis of gene RBM8A SNP rs139428292 G/A in the homo/ hemizygous state. DNA analysis allows early prenatal diagnosis in the family.

T.V. Fedotova: None. **A. Stepanova:** None. **V.P. Fedotov:** None. **A.V. Polyakov:** None.

J19.62

Prenatal diagnosis and molecular genetic analysis of isochoromosome 18 at amniocentesis

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A quantitative fluorescence polymerase chain reaction (QF-PCR) technique based on the determination of triple-dose chromosome-specific short tandem repeats (STR) has been recently developed for the prenatal diagnosis of numerical abnormalities of chromosomes 21, 18, 13 and X and Y. Specific 5 STR markers for chromosome 13,18 ,21 and sex chromosomes are used. If the patients have at least two trisomic markers for a spesific chromosome, result is detected as trisomy. A 32-year-old woman was referred to Ege University Medical Genetics Department due to abnormal prenatal USG finding (NT=6.8 mm). Amniocentesis was performed at the 16 week of gestation. The trisomy of 3 STR markers (D18S386 , D18S390 and D18S535) localized on the long arm of the chromosome 18 was detected whereas D18S391 marker located on the short arm of the chromosome 18 was found to be normal in the fetus. The pick areas rates for the markers mentioned above were 1:1.63,1:1.66, 1:2.1 and 1:1, respectively. QF-PCR analysis of parents was normal. Cytogenetic analysis of cultured amniocytes revealed a karyotype of 46,XX,i(18)(q10) in fetus. Pregnancy was terminated by the approval of the ethics committee. This report emphasizes the efficiency of QF-PCR in structural chromosomal abnormalities besides numerical abnormalities.

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J19.63

Associations between SNPs IL-10 and IFN-γ, level this cytokines in women and idiopathic recurrent pregnancy loss

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Successful pregnancy is dependent on maintaining a fine balance between Th1 and Th2 immunity. Taking into consideration the discussion of IL-10 and IFN-γ SNP influence on the level of their expression the aim of our research is to investigate the genetic polymorphisms of IL-10 and IFN-γ associated with cytokine levels in women with RPL in comparison with the blood levels of IL-10 and IFN-γ in these women. Methods: DNA extraction from peripheral blood cells, PCR, agarose gel electrophoresis, ELISA. Results: 225 women with RPL and 290 reproductively healthy women have been observed. The analysis of distribution of IL-10 SNP-1082G/A, -592C/A, -819C/T genotypes has shown the significantly higher 1082GG-genotype (P<0.01) and 592CC, 819 CC-genotypes (P<0.05) frequency in the group of women with RPL in comparison with the control group. The increasing of risk of RPL up to 4 times with 1082GG-genotype (OR=3.43;CI: 1.72-6.84) and 592CC, 819 CC-genotypes, (OR=3.87;CI: 1.23-12.20) has been established. Significantly lower IFN-γ +875AA genotype frequency (P<0.05) and significantly higher T allele frequency (P=0.01) has been shown in the group of women with RPL in comparison with the control group. The presence of +875TA or +875TT genotypes in women are associated with 3-fold increased risk of RPL (OR=3.15;CI: 1.18-8.36). Determining the level of IL-10 and IFN-γ in the serum showed the significantly higher of IFN-γ level in women with RPL (P<0.05) in comparison with the control group. Conclusions: IL-10 and IFN-γ influence on fetus development and their genetic regulation is a key to the development of a successful pregnancy.

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J19.64

Correlation between sperm DNA fragmentation and sperm head morphology

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Conventional semen analysis evaluating sperm concentration, motility and morphology are widely used to assess man's reproductive potential giving no information about sperm DNA characteristics. Among different sperm genome abnormalities DNA fragmentation is one of the most frequent especially in infertile patients. Sperm DNA damage is closely associated with embryo development, implantation, pregnancy outcome and the health of offspring conceived both naturally and by assisted reproductive technologies.

The aim of this study was to establish the correlation between sperm DNA fragmentation and some morphological forms.

Sperm DNA fragmentation and head morphology were assessed in semen samples from 70 patients with reduced fertility and 12 sperm donors. The following morphological forms were highlighted: normal, with small pathology, bulb, amorphous and vacuolated. 300 sperm heads per sample were analyzed. The method used to assess sperm DNA fragmentation was the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay. 2000 spermatozoa per sample were evaluated.

The direct linear correlation between the frequency of the spermatozoa with fragmented DNA and vacuolated sperm heads was found (r=0,55; P<0,05). DNA fragmentation rate was not associated with the prevalence of bulb or amorphous sperm heads.

Sperm head vacuoles are related to increased sperm DNA fragmentation rate. Therefore, vacuolated sperm heads may be a predictive mark of sperm DNA fragmentation. Such correlations may be useful tool for improving the choosing of the most competence gamete for fertilization.

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J19.65

Birth of healthy twin from hemophilia A carrier after transferring single zygote, a PGD case report

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An obligate hemophilia carrier mother had come to our center requesting PGD to have a healthy boy. We identified several STRs linked to the F8 gene and subsequently designed primers. We then used these STRs and five other X-chromosome specific STRs as well as amelogenin region for diagnostic purpose. The mother went through routine IVF procedure and mature oocytes well collected and each was microinjected with a single sperm. In day 3 a single blastomere was collected from each embryo and multiples STRs were performed. From eight embryos a single unaffected male and two unaffected female embryos were obtained. The mother insisted in only transferring a single male embryo. Pregnancy was obtained and later solography revealed twin pregnancy. Fetuses were tested at 11th week gestation and the twin was diagnosed unaffected identical male twin. The pregnancy ended up to term in June 2011 and both boys are healthy. This is the first report of PGD for hemophilia in Iran.

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J19.66

Clinical and cytogenetic correlation in primary amenorrhea: retrospective study on 493 patients

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Primary amenorrhea (PA, absence of menarche) have many causes including hypothalamic and pituitary disorders, gonadal dysgenesis and uterovaginal malformations.

We performed a retrospective study, with the purpose of establishing the frequency and the type of chromosomal abnormalities, in 493 patients with PA who were clinically and cytogenetically evaluated (1985-2009) in Iaşi Medical Genetics Center.

The X chromatin test, used as a screening test, was abnormal in 201 cases (40.8%) and normal in 292 cases (59.2%). The karyotype was normal in 224 cases (45.43%) and abnormal in 269 (54.56%) patients; the most frequent abnormality detected was X chromosome monosomy, homogeneous (137 cases - 27.78%) or mosaic (80 cases - 16.22%). Other 22 cases (4.46%) had X chromosome structural unbalanced abnormalities (homogeneous or in mosaic). One particular group, represented by 23 patients with PA, had a Y chromosome cell line and the final diagnosis was: pure gonadal dysgenesis (8 cases), CAIS (6 cases), mixed gonadal dysgenesis (4 cases) and true hermaphroditism (5 cases). Other 7 patients presented X trisomy (4 cases) and structural chromosomal abnormalities (3 cases).

Our results were similar with other reported studies and attest the importance of cytogenetic investigations in the etiologic diagnosis of amenorrhea.

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J19.67

Azoospermia and Skeletal dysplasia in a patient with a Y chromosome microdeletion: A case report

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Infertility is appreciated as a problem across all societies and affects about 10%-15% of couples. Half of these cases are due to male factors. Male infertility is a heterogeneous disorder, with various genetic and environmental factors. We here report a 30-year-old Iranian male with both primary infertility and skeletal abnormalities. His parents are non consanguineous with no family history of male factor infertility. The clinical signs were azoospermia with sertoli cell only syndrome, short stature, short hands, and brachydactyly. Routine chromosome analysis using standard GTG banding technique revealed 46,X,-Y,+mar. FISH technique confirmed that the marker chromosome was originated from Yp; accordingly, this deletion included the whole AZF region along with growth-control gene (GCY). By the way, MLPA analysis using subtelomeric kits showed a microdeletion of Short stature

homeobox (SHOX) gene, a pseudoautosomal gene in PAR1 of the Y chromosome. This is the first report concurrently indicating the presence of male infertility and short stature in a patient, who suffers from Yq deletion, including AZF region and the GCY gene and also SHOX haploinsufficiency. We hope this case report will facilitate genetic counseling when male infertility is associated with skeletal abnormalities.

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J19.68

Peculiarities of prenatal diagnosis of cystic fibrosis in Republic of Moldova.

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Cystic fibrosis (CF) remains important medical and social problem in Moldova, due to difficulties with diagnosis, treatment and social support. As a result of investigation in framefork of international scientific project, was revealed fact what mutations which are important in development of CF in Moldova, are: F508del (58,84%), 2789+5G>A (1,7%), G542X (1,36%). N1303 (1,36%.), 2184insA (1,36%), and 30% mutations of *CFTR* gene remains unidentified.
Aims - analysis of effectiveness of prenatal DNA diagnosis in families with high level of CF.
We observed 58 families with high risk of CF. Since 2001 to 2012 years within 20 families children were born: in 10 of families were used prenatal diagnosis. In Moldova we can made molecular diagnosis of five mutations in *CFTR* gene. In four cases the diagnosis was done jointly with our colleagues from France, Ukraine and Russia. Were studied 11 samples of DNA derived from amniocytes or chorion villi.
During the period of study by results of prenatal diagnosis of CF 8 pregnancies from 11 in 10 families were saved (in 2 fetuses mutations of *CFTR* gene were not detected, and in 6 fetuses were detected mutations, one in each case). In these cases in 100% of cases were prevented CF in newborns. In 10 families where parents refused from prenatal diagnosis were 10 children, 6 of them (60%) suffered from CF.
Conclusion. A prenatal diagnosis within the framework of international cooperation is an effective method of preventing of CF in families with high risk from Republic of Moldova.

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J19.69

FMR1 Premutation and Premature Ovarian Failure

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Background: Premature ovarian failure (POF) is a common cause of infertility. It is a hypergonadotropic ovarian deficiency with primary or secondary amenorrhoea affecting 1% of women before the age of 40. The Known causes of POF include iatrogenic agents, autoimmune disorder, exposure to environmental toxicants and genetic causes. Among the latter, POF can be associated with abnormalities affecting X chromosome such as premutation in *FMR1* gene (50- 200 CGG repeats).
Objective: We investigated of the *FMR1* premutation prevalence in women with POF.
Material &Method: This study included 90 women with POF reffered to Royan Institute. 11 women were excluded after finding X chromosomal mosaics and abnormal karyotype. 30 women with proved fertility and normal hormonal profile enrolled as the control group.
DNA was extracted from the blood samples by salting out method under the informed consent of patients. The repeated trinucleotide sequence was amplified by Hot Start PCR and was run on 4% agarose gel over night. Different DNA bands were purified from the gel and the number of trinucleotide repeats were determined by sequencing.
Result: 13 (16 %) of 79 women with POF had *FMR1* premutation, meanwhile none of the women in control group had *FMR1* premutation.
Conclusion: According to our results, determining the number of CGG repeats in *FMR1* gene can be used as a diagnostic method in the susceptible individuals. Thus, earlier screening of the *FMR1* premutation may help them to choose between oocyte freezing or earlier pregnancy.

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J19.70

Coexistence of SMN1 deletion and duplication in unrelated cases

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Spinal muscular atrophy (SMA) is the second most common autosomal recessive genetic disorder. About 95% of cases are characterized by homozygous deletion of exon 7 in SMN1 gene. Here we report an unusual presentation of SMA in cases that was referred to Dr. Zeinali Medical Genetic Lab.
SMA molecular diagnosis for cases with clinical impression of SMA was performed using specific MLPA kit P021B1, MRC-Holland. DNA was extracted and qualified. MLPA was performed according to the standard protocol including hybridization, ligation and amplification. PCR product was separated using ABI-3130 genetic analyzer. Raw data were analyzed by Gene Marker V1.95.
Through analysis of those referred to our Lab, we found 3 unrelated families with a deletion in one parent and normal MLPA pattern in another parent (i.e. two copy of SMN1 gene). Paternity and/or maternity were tested and confirmed. Pedigree analysis showed several consanguinity marriages in previous generations. Further investigation of the family and pedigree analysis showed that normal parents have received both deletion and duplication from their parents. So they had 2 copies of SMN1 gene in one chromosome (duplication) and no copy in another one. This feature couldn't be identified from normal cases by MLPA technique.
In spite of high prevalence of deletion in SMN gene, co-existence of a duplication in a normal people with family history of an affected child should be considered. Further investigation of other family members is recommended.

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J19.71

A case of a fetus with non-acrocentric sSMC

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We report a case of a fetus in thirty week of gestation having small supernumerary non-acrocentric marker chromosome (sSMC) wich was found after amniocenetesis and then confirmed by cordocentesis. Prenatal diagnostic was performed because mother was thirtyseven years old, but on ultrasound examination there were no abnormalities of fetus. Fetal karyotype was 47, XX+sSMC [63]/46, XX [37]. We also done an aneuploidy screening QF-PCR from fetal blood, showing one trialelic trisomic marker for chromosome 21 (D2S1411, for region 21q22.3). We performed chromosome analysis from peripheral blood of parents and normal karyotypies appeared. We did QF - PCR analysis for chromosome 21 from parents' DNA and for both of them, normal markers were found. Literature data are showing us that 70% of non-acrocentric sSMC do not have phenotype exspression, while 30% might have different clinical appearances. After genetic counseling parents decided to keep the pregnancy. In our case, phenotype of the newborn was normal, although it is believed that this marker (D2S1411, for region 21q22.3) is one of the most informative markers for the phenotype of Down syndrome.

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J19.72

Diagnosis and implication of structural chromosomal anomalies prenatally detected - our experience

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Introduction Since 70s when it was first used, till now new techniques for

screening and diagnosis of fetal chromosomal aberrations were introduced. Using the new screening tests that can select the pregnancies at risk, many syndromes can be identified before birth by performing prenatal diagnosis.
Material and method. We present here our experience in regard to the genetic diagnosis and counseling offered for pregnancies in which structural chromosomal aberrations were found. The study lot is formed by 528 prenatal samples of amniotic fluid and chorionic villi, received by our laboratory from 2006 through October 2012 for cytogenetic diagnosis.
Results. By using conventional citogenetic investigation accompain in some cases by FISH technique a total number of 21 structural chromosomal anomalies and polymorphic variants were identified in the study lot. The chromsomal aberrations identified included several deletions and microdeletions, situations with abnormal long p arm of acrocentric chromosomes, duplications, reciprocal translocations, inversions, additions, Robertsonian translocation associating trisomy 13, one 9q heteromorphism and one complex chromosome rearrangement.
Conclusion. An accurate characterization of the fetal chromosomal defects has implications in the couple decision regarding the continuing of the pregnancy or elective abortion and brings important information for the future reproductive options. Currently the prenatal genetic diagnosis can benefit from the advances of the molecular techniques that are very useful for the accurate characterization of the chromosomal anomalies. The genetic counseling, corroborating the cytogenetic information with the ultrasound abnormal findings, where present, and the results of the molecular investigations are essential for the pregnancy management.

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J19.73
X chromosome inactivation in Klinefelter syndrome and 46,XX testicular DSD

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We analyzed X-chromosome inactivation (XCI) in 50 Klinefelter syndrome (KS) and 15 46,XX testicular DSD patients. Chromosome analysis was performed on peripheral lymphocytes using GTG-technique. The Y-chromosome was analyzed by mPCR for SRY, AMELX/Y, ZFX/Y loci and 21 Y-specific STS markers. XCI pattern was determined by the identification of methylated (inactive) CAGn allele in exon 1 of the androgen receptor (AR) gene using methylation-sensitive restriction enzyme *Hpa*II. KS patients presented following karyotypes: 47,XXY, n=42; 48,XXXY, n=1; 48,XXYY, n=1; 47,XXY,t(3;8)(q23;p21), n=1; 47,XY,+der(X), n=1; mos46,XY/47,XXY, n=3, mos46,XX/47,XXY, n=1. No 'classic' AZF deletion was found; partial AZFc, deletion (b2/b3, n=3; gr/gr, n==2) were detected in 10% KS patients. Two of 15 (13.3%) 46,XX males were SRY-negative individuals with complete (n=1) or incomplete (n=1) masculinization. SRY+XX-males presented complete masculinization and a 46,XX karyotype (n=12), one SRY-positive patient had XX-sex reversal with Robertsonian translocation, 45,XX,der(13;14)(q10;q10). Breakpoints in the Yp11.2 locus were localized in all SRY-positive 46,XX patients. No significant difference in CAGn length between the groups was found. Also, no preference of X-inactivation toward the short and/or long AR-allele was revealed. XCI failed to evaluate in 21 KS patients and one SRY-XX-male that was homozygous for AR-CAGn allele. Skewed XCI (≥80%) was detected in 20% XX-males and 6.9% KS patients presented heterozygosity for AR-CAGn allele. Extremely skewed X-inactivation (ratio ≥90%/10%) was found only in 2 46,XX males (one SRY+ patient with complete masculinization and one SRY- patient with incomplete masculinization). Obtained data indicate that skewed XCI occurs in 46,XX-males more often that in KS patients.

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J19.74
Cytogenetic and molecular analysis of complex Y chromosome mosaicsm

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We report on newborn patient with sexual differentiation abnormality and complex Y chromosome mosaicism. The patient's mother was referred to invasive prenatal diagnosis due to cardio-vascular abnormalities detected by ultrasonography and elevated hHG. Non-mosaic 45,X karyotype was revealed by conventional chromosome analysis. FISH with DXZ1 and DYZ3 probes allowed to detect a mosaicism 45,X(72%)/46,XY(9%)/47,XYY(19%) in amniotic cells.
At the birth, following features were revealed: intrauterine dwarfism, micrognatia, high nasal bridge, ventricular septal defect. Genitalia ambiguous with penoscrotal hypospadias and bilateral cryptorchidism were prominent. The gonads were not found in the scrotum and inguinal canals. Ultrasonography demonstrated the presence of vagina, but not uterus.
Postnatal chromosome analysis was performed on cultured blood lymphocytes using GTG-staining. FISH with DXZ1, DYZ1, DYZ3 and LSI SRY probes was done on metaphase chromosomes and interphase nuclei of peripheral lymphocytes and interphase nuclei of buccal smears. Molecular analysis was performed on DNA extracted from peripheral lymphocytes with QF-PCR and mPCR for SRY, AMELX/Y, ZFX/Y, TAFL, 4SH loci and 20 Y-specific STS markers.
Postnatal chromosome analysis shown 45,X karyotype, but FISH results confirmed the presence of complex Y chromosome mosaicism: X(73.8%)/X,idic(Y)(p11.3)(13.8%)/XY(6.6%)/XY,idic(Y)(p11.3)(4%)X,idic(Y)(p11.3),idic(Y)(p11.3)(1.8%) and X(56%)/X,idic(Y)(p11.3)(26%)/XY(16%)/X,idic(Y)(p11.3),idic(Y)(p11.3)(2%) in peripheral lymphocytes and buccal smears, respectively.
QF-PCR analysis demonstrated the ratio X:Y signals approximately as 70%:30%. PCR amplification was positive for Y-chromosome loci, excluding sY1192. Detected 'b2/b3' deletion, partial AZFc region microdeletion of Y chromosome, presented in all Y-bearing cell lines. Yp breakpoint was localized distally to SRY locus in Pseudoautosomal region 1 that is characterized for idicYq chromosomes.

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J19.75
Clinical expression of de novo 18(q-) syndrome in a child with multiple congenital malformations

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Congenital malformations in children are generally manifestations of numerical or structural alterations at chromosomal level. Numerical alterations are mostly the result of meiotic non-disjunction and structural aberrations are inherited from parents in balance or unbalance form. De novo structural abnormalities are less common; however, cause severe congenital malformations and multiple systemic defects in children. We report one 18 month old boy born to non-consanguineous healthy parents as the second child at one month pre-term with 1.2 kg birth weight. Prominent malformations, including cleft lip and palate, hypertelorism, protruding tongue, micropenis, hypotonia, atopic dermatitis and bilateral overlapped and depressed mid toe. Pediatric echo cardiography detected congenital heart disease with malaligned VSD, absent pulmonary valve and moderate pulmonary stenosis. The child could follow light when it was close to face and could not respond to sound. Conventional karyotyping detected constitutive abnormality with 46,XY,del(18q21). Parental karyotyping confirmed de novo origin of the deletion. At this age, the child had severely delayed milestones and mental retardation. This child could not hold head and didn't have social smile. Distal deletion in 18(q) is a rare disorder with an epidemiological index of 1 in every 40,000 live birth; however, it is the second most common syndrome involving chromosome 18. The mother had continuous vomiting and swelling of hands and legs till delivery and that could have been considered significant for counseling and prenatal karyotyping for prevention of this rare de novo chromosomal aberration. Prenatal karyotyping is an established cost-effective measure of taking care of chromosomal aberrations.

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J19.76
Sperm DNA fragmentation rate among patients with reduced fertility

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Conventional semen parameters are widely used to assess man's reproductive potential. But they give us an incomplete picture of men's reproductive health. Information about sperm DNA integrity impacting on early embryo-

nic development also is very important. Sperm DNA integrity assessment is not a routine diagnostic, and specific mark seen performing standard spermogram are required to assign patients for DNA fragmentation assay.
The aim of this study was to determine sperm DNA fragmentation rate in men with reduced fertility.
Semen samples were collected from 58 men with reduced reproductive potential and from 12 sperm donors as a control. Semen analysis was performed according to WHO criteria. All patients were divided into four groups: normozoospermic, teratozoospermic, patients with reduced motility alone or in combination with reduced concentration. The method used to assess sperm DNA fragmentation was the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay. 2000 spermatozoa per sample were evaluated.
The DNA fragmentation rate in normozoospermic group (0,54±0,69) was equal to DNA fragmentation rate in control group (0,25±0,14) (P=0,14). Increased sperm DNA fragmentation rate was observed in teratozoospermic men (0,88±0,41). There was a statistically significant difference between DNA fragmentation rate in teratozoospermic group compare to control (P<0,05). No difference was found between mean DNA fragmentation rate in group with reduced motility alone or in combination with reduced concentration, so sperm DNA fragmentation has no effect on sperm motility.
On the foregoing in case of teratozoospermia sperm DNA fragmentation assessment should be recommended.
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J19.77

Genetic service as a part of Assisted Reproductive Technology - experiences of Center of Reproductive Medicine (Belarus)

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Genetic factors cause infertility, spontaneous abortions, repeated IVF failures, fetal abnormalities. At present, genetic service (GS) is a necessary part of Assisted Reproductive Technologies (ART). We present a model that includes combination of ART and GS in Center of Reproductive Medicine (CRM). Results. ART programs include insemination, IVF, IVF+ICSI, testicular biopsy, cryopreservation of sperm, oocytes, embryos, blastocysts. Effectiveness is 49.2%. Genetic investigations, included in practice of CRM since 2005, are expanding every year. GS provides: 1) Counseling (~850 per year) of infertile couples for outcome prognoses, persons with malformations, monogenic, chromosomal pathology; 2) Cytogenetic studies (2005-2012 years): 1.4% rearrangements were detected among 7116 karyotypes (numerical, structural aberrations, markers); 3) Molecular testing (2010-2012 years, 2152 patients) for: deletions of AZF region Y (22/423=5.4%), mutation 1691G>A Leiden (33/1209=2.7%), mutations dF508, CFTR2,3del, 2184insA of CFTR gene (10/448=2.2%), polymorphism G20210A of F2-protrombine gene (18/1209=1.5%), polymorphism 677T/T of MTHFR gene (114/1209=9.4%); 4) Prenatal diagnostics (2005-2012years) provides: combined screening in 1-2 trimesters (3344 pregnant women); counseling of pregnant with advanced age (25.6%), calculated risk for combined screening ≤1:360 (12,6% pregnant), parental balanced rearrangements, fetus abnormalities in need of fetus examination (karyotype, DNA). Reduction of affected fetuses in multiple pregnancies (18). Pre-implantation diagnostics of aneuploidies, monogenic diseases using polar body, FISH, DNA markers and sex-linked diseases detection using free fetal DNA in maternal plasma was started. Conclusion. Combination of above-mentioned methods in reduces loss of time for necessary examinations, increases efficiency of ART, which is confirmed by the birth of 5200 healthy children.

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J19.78

Chromosomal Abnormalities Identified at Prenatal Diagnosis

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Purpose: Amniocentesis is a very crucial diagnostic procedure for preventing the birth of genetically defective fetuses in order to decrease the prevalence of genetic diseases in populations.
Methods: The karyotyping of 5861 fetuses was carried out in our department during the years of 2000-2013 from the samples of amniotic fluids which were sent from departments of Gynecology and Obstetrics of Balcali Hospital and other regional hospitals.
Results: Among 5861 fetuses that were karyotyped; 2989 fetuses were males and 2872 fetuses were females and 351 (5.98%) fetuses had various chromosomal abnormalities. The mean age of pregnant women having fetuses with chromosomal abnormalities was found to be 33 years of age which suggest that fetal chromosomal abnormalities were associated with maternal age. Numerical chromosomal abnormalities predominated the structural chromosomal abnormalities (65% and 33%). Both numerical and structural chromosomal aberrations were observed in 9 (2%) of all abnormal cases. The numerical chromosomal abnormalities with an incidence of 28.5% trisomy 21, 11.4% trisomy 18, 7% monosomy X, 4% trisomy 13, 3.4% triploidy, 3% Klinefelter Syndrome, 1.1% trisomy X, 0.9% XYY Syndrome, 5.7% mosaics and the others represented the remaining. The frequent structural abnormalities were 7.7% 46,XX/XY, inv(9)(p11;q13) and 6% 46,XX/XY, inv(9)(p11;q12) in all abnormalities.
Conclusions: Corollary to literature and our findings revealed that the advanced maternal age and certain environmental factors can increase the risk of fetal chromosomal abnormalities. Fetal chromosomal abnormalities representing 5.98% in our study group is crucial and underlines the importance of prenatal diagnosis for healthier pregnancies.

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J19.79

Cell-free fetal DNA in maternal blood using for noninvasive prenatal diagnostics of RHD gene

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Noninvasive determination of RHD gene using cell-free fetal DNA was detected in 98 rhesus negative women in the 1nd and 2nd trimester of pregnancy.
To identify fetal DNA in maternal blood approach based on polymerase chain reaction (PCR) with fluorescence detection of PCR RT was used. Regions of exon 7 of RHD gene were subjected to amplification.
Material for molecular genetics study was total DNA extracted from the plasma of pregnant women with Rh negative blood factor. Pregnant women were of different nationalities and parity women. Determination of the Rh factor in the fetus was done in consisted steps.
Among 98 RhD-negative women the results from 43 samples diagnosed Rh negative factor and 55 cases - Rh-positive factor.
The analysis took into account the position of the curves of fluorescence amplification curve relative to the curve of positive control (PC). Indirect indicator of the quality of procedures for the allocation and taken for analysis is the value of the threshold cycle between the curves of RNase-P and PC.
The difference should be no more than 2-3 of the cycle. The big difference (5 cycles or more) means serious loss of DNA during isolation leading to a decrease in the number of fetal DNA in the reaction mixture less than the detection limit of the test system and can lead to the possibility of false-negative results.
Thus the minimum allocation to guarantee correct operation of the diagnostic kit and reliable results is approximately one milliliter of plasma, recommended - 2 milliliters.

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J19.80

Prenatal genetic diagnosis in cystic fibrosis

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Background and aim: Our goal was to detect CFTR mutations in fetal genomic DNA isolated from amniotic fluid collected by classic amniocentesis and early amniocentesis. **Methods**: Ten couples where selected for performing prenatal diagnosis. Molecular diagnostic was performed on Genomic DNA isolated from venous blood samples collected on EDTA from both parents and on amniotic fluid samples collected by classic amniocentesis (16th week

of pregnancy) and by early amniocentesis (13th week of pregnancy) . For detection of CFTR mutations we used the Elucigene CF29 kit. **Results** showed: 6 couples with negative genetic test, 3 with heterozygote for one of next mutation (DF508, G542X, 621+1 G-T), and one of them affected DF508/621+1 G-T). **Conclusion.** Prenatal diagnosis can be performed on samples of amniotic fluid collected by normal amniocentesis or by early amniocentesis however, due to the greater risks for pregnancy and fetus of this early procedure and because the volume of collected amniotic fluid is reduced . Also, for an accurate renatal diagnosis in mucoviscidosis it is required to have a good sampling technique for the amniotic fluid. Mutation detection by ARMS-PCR with Elucigene CF29 is applicable only to those couples in which at least one of the parents is a carrier of the ΔF508 mutation, or when both parents carry different CFTR mutations, other than ΔF508, because the kit can differentiate between the condition of heterozygote (carrier of a mutation) and homozygote (diseased) only in the case of ΔF508 mutation.

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J19.81

Pseudomosaicism in several cases of amniocentesis

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Introduction: Our study is based on a work of three years of amniocentesis.
It is known that sometimes the cultured cells develop subtle changes from original cells.
The presence of chromosomally abnormal cells in a single culture of amniocytes with only normal cells in all other cultures is considered to represent pseudomosaicism since the fetus is usually found to have a normal karyotype.
We have three cases of structural abnormality of chromosomes and ten cases of numerical chromosomal modifications.
For each case, three primary cultures were set up using Falcon flasks, Amniomax medium and classic GTG bands.
The first case was represented by a pregnant woman of 36 years old reffered for amniocentesis because a positive triple test; fetal karyotype revealed a normal 46,XX in two flasks, while in one flask was found a deletion on the long arm of chromosome X.
The second case was a 39 years old pregnant woman of 16 weeks pregnancy. The fetal karyotype was normal 46,XX but in one flask was found an inversion on 11 chromosome and one cell with a deletion on long arm on 10 chromosome.
The third case was a pregnant woman of 19 weeks of pregnancy with fetal karyotype 46,XY in two flasks and in one flask few metaphases revealed t(4;22) (p14;q11.2).
Conclusion : Later on, for a correct diagnosis, amniocentesis was repeated and in all cases fetal karyotypes were found to be normal.

L. Neagu: None. **S. Grigore:** None. **D. Albu:** None.

J19.82

Genetic basis of recurrent miscarriage in Kazakh population

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The recurrent miscarriage (RM) of pregnancy has a multifactor background as a results from interactions between environmental factors and functionally weak alleles of many susceptibility loci across the genome. The aim of this research was to determine the contribution of some genotypes of genes of RM predisposition in Kazakh population.
The polymorphisms of genes of coagulation system FV и FII, ITGB3, FGB, PLANH1; folate metabolism MTHFR, MTRR, MTR; II phase of detoxification system - GSTM1, GSTT1, GSTP1; lipid metabolism ApoC, ApoE; endothelial dysfunction eNOS3 and ACE genes were detected.
Molecular genetic and clinical laboratory assays were carried out in a group of 200 women with 2 and more spontaneous early abortions in anamnesis and 150 women with normal reproductive function. All were Kazakhs.
The most important contribution in RM was determined for unfavorable genotypes of FII и FV coagulation factors, carriers of which increase the risk of RM (OR) in 4-18 times, antiphospholipid syndrome (APS) in 5 times. The carriage of 677TT and 1298CC genotypes (MTHFR), 66GG (MTRR) and 2756GG (MTR) increase risk of RM in 2.5-8.3 times. The carriage of 3 unfavorable GST genotypes decrease the activity of antioxidant enzymes and increases the risk of RM (OR) in 2.3 to 12.2 times.
We observed the significant genetic input of functionally weak genotypes in RM, APS in Kazakh population. The algorithm of prognosis, preconceive prophylaxis of RM were worked out, which reduced to 2.5 times the frequency of RM for Kazakhs.

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J19.83

Still Births and Foetal Genetic Defects causing Recurrent Miscarriages in couples

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Recurrent pregnancy loss (RPL), defined as two or more miscarriages of fewer than 20 weeks of gestation, affects up to 5% of couples trying to establish a family, whereas still birth is the intrauterine foetal death (IUFD) at 20 weeks of gestation or greater. These abnormalities are caused by errors in the number or structure of chromosomes. Some chromosomal abnormalities result in miscarriage or stillbirth. However, clinicians and patients find it particularly frustrating that no convincing aetiology is identified in at least 50% of cases. This report describes known and suspected causes of recurrent miscarriages and stillbirth including genetic abnormalities, maternal and paternal age, infectious diseases, environmental toxins, obesity, infertility, and a variety of medical conditions in the mother, etc. The aim of this study is to detect the cause of foetal wastage and assess the frequency and nature of chromosomal abnormalities that contribute to the occurrence of foetal wastage in couples. Karyotype analysis of the abortus tissue was performed by our cytogenetic laboratory using standard G-banding techniques. Metaphase chromosome preparations from the amniotic fluid samples were also made according to standard cytogenetic protocols and studied. Chromosomal abnormalities were reported according to the current international standard nomenclature (ISCN). In almost 50% of the cases, the miscarriages were due to chromosomal abnormalities in the embryo. A common type of chromosomal abnormality is trisomy 21, affecting approximately 1 in 800 babies. This report helps to describe the cytological abnormalities and correlate the recurrent abnormalities in a given population.

N.J. Jain: None. **K. Gomes:** None. **N. Patil:** None. **S. Salvi:** None. **B. Ganguly:** None.

J19.84

Screening of chromosomal aberrations in infertility work-up

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Infertility is a serious societal concern for women for not being able to give birth to a child. Always priority is given to perform a series of investigations for the woman since she carries the pregnancy. Gynecologists advise karyotyping for the couple; however, generally the family decides to consider the woman for the test first, and the male partner agrees when wife's karyotype comes normal. Karyotypic configurations of the partners have been found as one of the major issues leading to reproductive failure. We report chromosomal status of 223 couples having history of primary infertility or recurrent first trimester miscarriage. Peripheral blood samples were collected from 446 partners and cultured in serum and phytohemagglutinin supplemented RPMI 1640 medium for 72 hours at 37_0C. Mitotic cells were collected following standard colcemid-hypotonic-fixative protocol. A total of 11150 G-banded metaphases were analysed with the help of IKAROS imaging software (MetaSystems, Germany). Five cells were classified in karyotypic form as per ISCN nomenclature. Chromosomal abnormalities, including small Y (30%), long Y (2.24%) and inv(Y) (1.34%) were recorded exclusively in Y chromosome in 34% male partners. Other constitutive aberrations, including t(2;7), t(6;20), t(3;6), t(1;7), t(17;20), t(3;4), inv(9) and del(9q), inv(7), XX/XXX mosaicism and XY female were present in 2.69% with an equal ratio in males and females. Small Y could be the result of microdeletion in AZF factor and warrants seriously for considering a molecular test for every infertile male partner. Thus, in general, karyotyping of the couples should invariably be included in infertility management.

B.B. Ganguly: None. **G. Khastgir:** None.

J19.85

The possibilities of prenatal DNA diagnostics of complex chromosome pathology using fluorescent quantitative PCR analysis

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The most frequent cause of congenital human pathology are numerical ab-

normalities of chromosomes (aneuploidies). For diagnostics of numerical abnormalities of human chromosomes there was developed a quantitative multiplex PCR analysis based on the simultaneous testing of 15 microsatellite markers of chromosomes 13, 18, 21, X, Y.
We analyzed 200 DNA samples from fetuses having the high risk of chromosomal pathology. DNA was extracted from the amniotic fluid cells or cell culture of chorionic villus. Numerical abnormalities of human chromosomes were detected in 19 cases (9.5%). Diagnosis determined by DNA analysis was confirmed by karyotyping in all the cases. Revealed pathology included trisomy of chromosome 21 (8 fetuses - 4%), trisomy of chromosome 18 (5 fetuses - 2.5%), trisomy of chromosome 13 (1 fetus - 0.5%), triploidy (3 fetuses - 1.5%), mosaic form of chromosome X monosomy (1 fetus - 0.5%, karyotype 45,X[50]/46,XY[5]). Molecular-genetic study has also identified an unusual pathology (1 fetus - 0.5%) with three chromosomes 13 and 21 and two chromosomes 18 and X. Cytogenetic study determined karyotype 67,XX,-18[14]/47,XX,+18[1] with mosaicism both of triploidy and trisomy simultaneously. The proband's parents were examined with the help of DNA analysis, and it was found out that the extra set in triploid clone was of paternal origin.
Only a comprehensive study including DNA-analysis and a cytogenetic analysis allows to determine the karyotype and the abnormality formation mechanism in a complex chromosome pathology.

T. Asadchuk: None. **L. Padliashchuk:** None.

J19.86
Complex chromosome rearrangement, t(1;21;4) in an azoospermic male

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Complex chromosome rearrangements (CCRs) are very rare structural aberrations in human population involving at least three chromosomes with three or more chromosomal breakpoints. Most of the males with CCRs have shown infertility problems and there have been several such reports in the literature of CCRs in males with oligozoospermia or azoospermia. Here we report 54 years old male with normal phenotype who was diagnosed with azoospermia, during routine infertility examinations including clinical history, spermiogram and blood hormone levels. The testicular biopsy which was later performed confirmed the diagnosis showing spermatogenic arrest at the spermatocyte level on histology. Karyotype analysis with standard GTG- banding on peripheral lymphocytes was then performed and revealed a 46, XY, t(1;21;4) (q21; q22; p15) chromosome complement. The cytogenetic investigations showed a complex chromosomal rearrangement involving firstly a translocation between the long arm of chromosome 1 and the long arm of chromosome 21, secondly a translocation between the long arm of the same chromosome 21 and the short arm of chromosome 4 and third a translocation between the short arm of the same chromosome 4 and the long arm of chromosome 1.
We conclude that the complexity of CCRs might affect the severity of spermatogenetic impairment, thus azoospermia of our patient can be explained with apoptosis of produced genetic imbalanced spermatocytes.

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J19.87
High Incidence of Chromosomal Aberrations in Couples with Reproductive Failure in Canakkale

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Numerical and structural abnormalities of chromosomes may be the reasons of infertility and recurrent pregnancy losses (RPL). Patients should include karyotyping of both parents for chromosomal aberrations. Mainly balanced rearrangements are common in couples with reproductive disorders.
In Canakkale On Sekiz Mart University, School of Medicine, Department of Medical Genetics, Cytogenetic Laboratory, from 94 couples diagnosed as infertility and 118 couples diagnosed as recurrent pregnancy loss- total 424 patients- chromosome analysis was done with heparinized peripheral blood samples by applying the G-banding who referred to Clinic of Obstetrics and Gynecology and Clinic of Medical Genetics between May 2011-November 2012.
In RPL group, there was no numerical anomalies but there was 4.26 % balanced structural chromosomal abnormality. There were structural abnormalities in 9 patients: 46,XY,inv(11)(p13q11), 46,XX,inv(11)(p13q11) (no consanguineous marriage), 46,XY,ins(9;22)(9pter-q12::22q11.1-q13.33::9q12-9qter), 46,XX,9qh+ in 2 patients, 46,XY,inv(9)(p11q13), 46,XY,t(4;6)(q13.1;qter)(4pter-4q13;6pter-6qter::4q13-4qter), 46,XX,t(8;12)(p22;p12.3), 46,X,t(X;2)(q27;p25).
In infertility group numerical chromosomal anomaly was 3.72 %, balanced structural chromosomal anomaly was 4.26 %. Our results were 6 numerical and 8 structural abnormalities: 45,X in 3 patients, 47,XXY in 2 patients, 46,X,i(X)(qter-->q10:q10-->qter), mos46,X,i(X)(qter-->q10:q10>qter)[14/45,X[7, mos47,XXY[33/46,XY[17, 46,XX,robt(13;14) in 2 patients, 46,XY,robt(13;14), 46XY,t(1;2)(p36;p14-->16)pat, 46,XY,t(3;8)(q21;q24) and 46,XY,inv(9)(p11q13). In a couple with a history of two failed IVF and first degree cousin marriage, it was detected robt(13;14) in both wife and husband.
Genetic counseling was given to couples about possible outcomes of pregnancies, having healthy children, prenatal diagnosis and preimplantation genetic diagnosis.

F. Silan: None. **S. Yalcintepe:** None. **D. Uysal:** None. **A. Uludag:** None. **E. Cosar:** None. **A.N. Cakir Gungor:** None. **O. Ozdemir:** None.

J19.88
A mosaic Klinefelter Syndrome variant identified with amniocentesis

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Purpose: In 80% of the cases, the karyotype in Klinefelter syndrome is 47 XXY. Remaining 20% chromosomal variants including mosaicism is seen. Here we report a 32-year-old pregnant woman referred to our laboratory due to abnormal sonographic finding.
Methods: The karyotyping and QF-PCR of the fetus was carried out in our department from the sample of amniotic fluid which was sent from department of Gynecology and Obstetrics of Balcali Hospital. This fetus is the sixth pregnancy of consanguineous Turkish parents, after one mol pregnancy, one intrauterine death at last trimester, two female and one male birth. A standart GTG banding procedure was performed on fetal cells and peripheral blood cell of the family. Quantitative fluorescent PCR was used for trisomy detection.
Results: Chromosome analysis of amniotic fluid from two culture flask revealed mos 46,XY[21] / 48,XXXY[6] karyotype. The parents and fetus were normal with QF-PCR analysis. Omphalocele was detected by USG at the fetus as a different feature from Klinefelter syndrome.
Conclusions: A mosaic Klinefelter syndrome variant is compared with other reported cases of the literature and the follow-up of prenatally diagnosed mosaic Klinefelter variant is discussed. Due to presence of omphalocele, this case should be investigated for another abnormality.

H.Ü. Lüleyap: None. **A. Pazarbaşı:** None. **F.T. Özgünen:** None. **S.T. Bozdoğan:** None. **S. Büyükkurt:** None. **D. Alptekin:** None.

J19.89
Association of MTHFR, MTR, MTRR polymorphism with the risk of pregnancy loss: a study on a Russian population

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The objective of study is the genetic association between methylenetetrahydrofolate reductase (MTHFR c.665C>T и c.1286A>C), methyltetrahydrofolate homocysteine methyltransferase (MTR c.2756A>G), 5-methylenetetrahydrofolate-homocysteine methyltransferase reductase (MTRR c.66A>G) polymorphism and recurrent pregnancy loss among women with petrochemical toxic substances. It as done prospective case-control study. A total of 159 recurrent pregnancy loss female workers at the petrochemical plant and 150 healthy control female workers with successful pregnancy were analyzed.
Genotyping was done by tetraprimer ARMS-PCR. Age and professional exposition adjusted odds ratios were calculated by logistic regression analysis. All surveyed women were Russian.
The frequency of rare allele polymorphism MTHFR c.1286A>C among women with recurrent pregnancy loss was 42%, among healthy control 31%. We found that presence of rare allele „C" polymorphism c.1286A> C and heterozygous and rare homozygous genotypes significantly increased the

risk of recurrent pregnancy loss. Association was identified polymorphism c.1286A> C MTHFR gene to the risk of fetal loss in women for the „CC" genotype (OR=2.61 (CI 95% 1.23-5.53); p=0.038) and for the allele „C" OR=1.52 (CI 95% 1.07-2.18); p=0.02. The association of haplotype TC gene MTHFR (c.667C> T and c.1286A> C) with the development of fetal loss (OR = 1.96 (CI 95% 1.05 - 3.68); p = 0.037).
No significant association in MTHFR (c.665C>T) и MTR (c.2756A>G), MTRR (c.66A>G) genotype frequency was observed for the recurrent pregnancy loss.
Conclusion: Our results confirm that polymorphisms in the MTHFR gene are associated with recurrent pregnancy loss susceptibility.

O.V. Kochetova: None. **T.V. Viktorova:** None.

J19.90
Mosaic trisomy 22 after in vitro fertilization

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We report a case of mosaic trisomy 22 after in vitro fertilization (IVF). Assisted reproductive treatment was done in a 36 years old, healthy female with history of sterility because of oligoasthenospermia in her husband, 39 old year male. This was a first pregnancy of noncounsanguinous couple after first IVF.
Biochemical screening was done in 12th week of gestation (gw): free beta hCG was 10.15 MoM, PAPP was 1.17 MoM. Expert ultrasound in 12th GW revealed nuchal transluceny 1.5 mm and crown rump length 69.7mm without pathological findings or congenital anomalies. Expert ultrasound in 17th gw revealed intrauterine growth retardation and in 20th gw, complex heart congenital anomalies, pulmonary atresia and ventricular septal defect were seen. Amniocentesis was done in 18th gw and finding was: 46, $XY_{[15]}$ /47,XY,+$22_{[15]}$, mosaic trisomy 22 (G-banding technique, 30 metaphases). Couple was informed about cytogenetical findings by genetic counselor and pregnancy was terminated. In next pregnancy, also after IVF, amniocentesis was performed in 16 gw and result wasnormal male karyotype. This case emphasizes the importance of genetic counseling and preimplantation diagnostics.

I.I. Kavecan: None. **J. Jovanovic Privrodski:** None. **M. Obrenovic:** None. **M. Kolarski:** None. **D. Radovanov:** None.

J19.91
GAPDHS gene polymorphism in asthenozoospermic men with dysplasia of the sperm fibrous sheath

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Dysplasia of the sperm fibrous sheath is a genetic sperm defect, characterized by dysplastic development of the axonemal and periaxonemal cytoskeleton. Most of the glycolytic enzymes are localised in principal piece of the sperm tail and the sperm-specific isoform of glyceraldehyde-3-phosphate dehydrogenase is tightly associated with the sperm fibrous sheath is an essential enzyme for glycolysis.
In order to investigate possible *GAPDHS* alterations semen samples from 5 asthenozoospermic men with dysplasia of the fibrous sheath and 5 sperm donors were collected. DNA was extracted from each semen sample. Analysis on the possible *GAPDHS* alterations was performed by PCR amplification and sequencing.
Single nucleotide polymorphism rs29381 (660-22 G>A) of *GAPDHS* was revealed in all analyzed samples from asthenozoospermic men with dysplasia of the fibrous sheath, whereas DNA samples extracted from donated sperm correspond to reference *GAPDHS* sequence.
Further studies are currently ongoing in order to validate these findings.

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J19.92
Investigation of relationship between the risk of Down syndrome and 844ins 68 polymorphism of the cystathionine β-synthase gene

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Introduction: Human cystathionine β-synthase is a hemoprotein which catalyzes the condensation of Hcy and serine to form cystathionine The gene encoding cystathionine synthase (CBS) has been localized in chromosome 21 (21q22.3) in a region correlated with Down syndrome phenotype. The aim of the present study was to investigate the effect of 844ins 68 polymorphism in cystathionine β-synthase gene as a maternal risk factor for DS.
Materials and methods: The study was performed on a total number of 72 women split in two groups: one group of 26 women who gave birth to at least 1 DS baby and the other group comprises 46 women, who gave birth only to healthy children.
Human genomic DNA was isolated from peripheral blood samples with peqGOLD blood DNA mini kit. The common CBS polymorphism that causes an insertion of 68 bp at the 844 position was identified using the method PCR.
Results: The frequencies of CBS 844ins68 genotypes, (ins-/ins-, ins+/ins-) among mothers with DS children were 84.6 and 15.4%, respectively. The corresponding frequencies among controls were 91.3 and 8.7%, respectively. There were no significant differences, in genotype frequencies between the two groups (OR 1.91; 95% CI 0.44 - 8.38 P 0.39) and there were no subjects identified with homozygous genotype ins+/ins+ .
Conclusions: Allele frequencies for CBS 844ins68 polymorphism do not point to the association between this particular polymorphism and DS phenotype. Therefore, CBS 844ins68 the polymorphism is not a maternal risk factor for DS.

D. Neagos: None. **L. Bohiltea:** None. **R. Cretu:** None.

J19.93
Repair and cell cycle control systems' genes analysis for miscarriage

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According to current conception miscarriage or spontaneous abortion is the multifactorial disorder. Most spontaneous abortions are occur during the first trimester (from 50 to 70 %). Genetic component of miscarriage multifactorial etiology is much diversified and involves many functional groups of genes which in combination with lifestyles and environmental factors lead to spontaneous abortion development. The goal of our work was to investigation of repair (APEX1, ERCC2 (XPD)) and cell cycle control system (CHEK2) gene variants in blood cells and chorion tissues from women with physiological and miscarriage pregnancies using allele-specific PCR assays. The following polymorphic variants were tested: APEX1 Asp148Glu, ERCC2 Lys751Gln, 2 1100delC. The difference was not between controls and spontaneous abortion groups during analysis of the blood samples genotype and alleles frequency. During analysis of the chorionic samples significant difference in genotype and gene variant frequency for 1100delC polymorphism of 2 gene was determined between controls and undeveloped pregnancy group. The frequency decreasing of normal homozygotes for ERCC2 and 2 polymorphisms and increasing of part of Gln/Gln and del/del occurs in the spontaneous abortion group in comparison with controls. Also significant differences in ERCC2 Lys751Gln and 1100delC 2 gene variants frequency were revealed between control and undeveloped pregnancy groups. Our results suggest that the presence of the polymorphic variants of repair system genes in the genotype lead to the spontaneous abortion susceptibility. This work was supported by the RFBR (project № 12-04-31408).

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J20.01
Validation of a commercially available mRNA reprogramming method for iPS cell generation.

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Since the pioneer work of Takahashi and Yamanaka in 2006, many methods have been reported on the generation of induced pluripotent stem (iPS) cells. Most protocols rely on viral vector-mediated transduction of reprogramming genes involving random insertions of exogenous sequences into the genome with insertional mutagenesis being a serious concern. Recently, a non-integrating system has been reported to efficiently reprogram human BJ foreskin fibroblasts using synthetic mRNA trancripts encoding the Yamanaka factors. The mRNA method requires the use of irradiated human newborn foreskin fibroblasts (NuFF) as feeder cells. We evaluated the commercially available mRNA reprogramming system (Stemgent) on human adult skin fibroblasts (ASF) using human BJ foreskin fibroblasts as positive control. For the required feeder cells, we used either irradiated NuFF or human fetal skin fibroblasts. Five mRNA encoding reprogramming factors (Oct4, Klf4, Sox2, Lin28, c-Myc) were delivered to the target cells via a lipid-based vehicle (RNAiMAX, Invitrogen). Transfection efficiency for both ASF and BJ cells was about 50% positive cells. For the BJ culture, colonies began

to appear as early as 10 days demonstrating a reprogramming efficiency of 1.45%. For the ASF experiment, colonies began to emerge by day 17 with an iPSC conversion efficiency of 0.1%. Transcriptional expressions of Nanog, Oct3/4, Sox2 and Rex1 confirmed the pluripotency of the colonies. In our hands, we obtained iPS-colonies only when using NuFF feeder layer. Our results demonstrate that this mRNA method can efficiently reprogram fibroblasts and it must be further investigated if it is applicable to other cell types.

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J20.02

Mesenchymal derivatives of genetically unstable human embryonic stem cells (hESCs) also show genetic alterations but undergo senescence in culture as do bone marrow derived mesenchymal stem cells.

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Cultured hESCs usually reveal at their undifferentiated stage genomic instability that renders them inappropriate for the application of cell therapy. However, it is not yet clear whether genetic or epigenetic alterations occuring in the pluripotent cells during culture, would exhibit any effect on the growth potential of the specific differentiated derivatives. We report that hESC lines HUES-7 and -9 carrying genetic alterations, produce *in vitro* mesenchymal stem cells (hESC-MSCs) which show progressive growth arrest and enter senescence after 12 and 15 passages, respectively. No difference was observed in their proliferative potential compared to bone marrow derived MSCs. Comparative genomic hybridization analysis (a-CGH) of hESCs and hESC-MSC revealed a significant overlap in chromosomal alterations (Table) suggesting that genetically altered hESCs are not selected out during differentiation. Our findings suggest that hESC-MSC despite their genomic instability, exhibit an *in vitro* growth pattern of normal MSCs and not that of transformed cells not acquiring selective *in vitro* growth advantage.

CHROMOSOMAL ABERRATION	SIZE/ START-END (HG18)	SIZE/ START-END (HG18)	SIZE/ START-END (HG18)	SIZE/ START-END (HG18)
	HUES9		HUES7	
	ESC	MSC	ESC	MSC
DUP 1p34.3-p34.2	4.34Mb; 39022499-43361452	4.173Mb; 39022499-43195864	-	-
DUP 3p22.1	2.30Mb; 39769426-42064909	2.295Mb; 39769426-42064909	-	-
DEL 10q11.22	-	-	990Kb; 46158156-47148546	990Kb; 46158156-47148546
DUP 12p13.33-p11.1	-	-	34.2Mb; 64510-34274228	34.2Mb; 163593-34382961
DUP 12q12-q24.33	-	-	95.5Mb; 36761973-132289149	95.26Mb; 38475706-133732062
DUP 15q11.2	-	-	254.1Kb; 19805960-20060120	414.9Kb; 22143844-22558756
DUP 17p13.3-p11.2	-	-	21.64Mb; 76263-21720142	22.15Mb; 51885-22205821
DUP 17q11.1-q25.3			56.1Mb; 22508198-78653589	55.7Mb; 25403446-81099040
DUP 20q11.21-q11.21	1.82Mb; 28133609-29952965	1.62Mb; 28255567-29875977	-	-

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J20.03

Isodicentric Y chromosomes in Egyptian patients with Disorders of Sex Development (DSD)

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Isodicentric chromosome formation is the most common structural abnormality of the Y chromosome. As dicentrics are mitotically unstable, they are subsequently lost during cell division resulting in mosaicism with a 45,X cell line.
This study reports six patients with variable signs of disorders of sex development (DSD) including ambiguous genitalia, short stature, primary amenorrhea and male infertility with azoospermia.
Cytogenetic studies showed the presence of a sex chromosome marker in all patients; associated with a 45,X cell line in five of them. FISH technique was used to determine the structure and the breakage sites of the markers that all proved to be isodicentric Y chromosomes.
Three patients, were found to have similar breakpoints: idic Y(qter→ p11.32:: p11.32→ qter), two of them presented with ambiguous genitalia and were found to have ovotesticular DSD, while the third presented with short stature and hypomelanosis of Ito.
One female patient presenting with primary amenorrhea, Turner manifestations and ambiguous genitalia revealed the breakpoint: idic Y (pter→q11.1::q11.1→pter). The same breakpoint was detected in a male with azoospermia but in non-mosaic form.
An infant with ambiguous genitalia and mixed gonadal dysgenesis had the breakpoint at Yq11.2: idic Y(pter→q11.2::q11.2→pter).
SRY signals were detected in all patients.
Sequencing of the *SRY* gene was carried out for three patients with normal results.
This study emphasizes the importance of FISH analysis in the diagnosis of patients with DSD as well as the establishment of the relationship between phenotype and karyotype.

M.K. Mekkawy: None.

J20.04

Promyelocytic leukemia (PML) expression during mouse embryonic stem cells (mESCs) neural differentiation indicates a role for PML nuclear bodies (PML-NBs) in cellular pluripotency than neural differentiation

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Promyelocytic leukemia nuclear bodies (PML-NBs) have been known as one of the major cellular features, containing several proteins which are involved in cell growth and differentiation. Promyelocytic leukemia protein (PML) is one of the major proteins in the PML-NBs. In somatic cells there are numerous PML-NBs while in human embryonic stem cells (hESCs) there are few large PML-NB structures with two different "rod" and "rosette" morphologies and different contents. In hESCs prior to or concomitant with the onset of differentiation, different PML-NB structures form and the nuclear size increases. This results in alteration in cell state, pluripotency and self-renewal property. In addition, an antiproliferative activity has been reported for PML. This operates either directly or indirectly by means of interaction with the neural differentiation machinery. This suggested a role yet to be known for PML in neural differentiation and nervous system development. To understand the actual role of PML, the expression pattern of this protein was investigated in embryonic stem cells (mESCs) at different stages of neural differentiation. Our data, for the first time, showed that PML protein expression decreased in neural precursor cells relative to ESCs, but this expression slightly reappeared in neural cells. These data suggested that the PML expression may have a more important role in cellular pluripotency than neural differentiation.

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J20.05

Inherited mosaic 16p11.2 microduplication in patient with cognitive impairment

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Gene copy number variations (CNVs) at the 16p11.2 locus have been associated with cognitive disorders including autism (deletions), schizophrenia (duplication) and more recently with severe obesity and being underweight. Even if these chromosomal imbalances were de novo in the majority of cases, some of them were inherited from a parent to a child in an autosomal dominant manner. Chromosomal abnormalities can be homogeneous or in mosaic form. Mosaicism is the presence in an individual of more than one genetically distinct cell line developed from one zygote. Mosaicism can be

observed in both somatic and germ line cells and assumes that individual could transmit the specific affected cell line to his or her offspring homogeneously.
We report clinical, cytogenetic and molecular investigations of a 14 years-old girl with cognitive impairment and mildly dysmorphic appearance. We carried out detailed clinical phenotyping of this patient and investigated the genetic basis using Agilent 180K array comparative genomic hybridization (array-CGH).
We identified by array-CGH and confirmed by Fluorescence In Situ Hybridization a mosaic 16p11.2 microduplication. Family investigations reveal the same abnormality in the mother, also in mosaic form. Most cases of inherited mosaic chromosomal abnormalities represented supernumerary markers, and only few cases of inherited mosaic interstitial microduplications have been described.

S. Kemeny: None. **C. Pebrel-Richard:** None. **E. Pierre-Eymard:** None. **A. Tchirkov:** None. **C. Francannet:** None. **P. Vago:** None.

J20.06
Report of a child with a pure de novo 17p13.2pter duplication localized to the terminal region of the short arm of chromosome 14

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Chromosomal duplications including 17p13.3 has been recently defined as a new distinctive syndrome (MIM#613215) with several diagnosed patients. Some variation is known to occur in the breakpoints of the duplicated region and consequently in the genes content as well. This variation is reflected by the slight variability in phenotype. When the duplication includes all four genes strongly correlated with the phenotype (*PAFAH1B1*, *RPA1*, *CRK*, *YWHAE*), clinical picture seems to be complex. Most of these aberrations are relatively small interstitial duplications and only 4 involved terminal regions.
Here, we report on another, fifth patient, a 4-year-old girl with a pure *de novo* subtelomeric duplication 17p13.2pter identified by MLPA. This finding was confirmed and further characterized by FISH and aCGH studies. As it turned out a 5.77 Mb duplicated region of 17p13.2pter is resided on the non-deleted terminal region of chromosome 14p and involves all genes important for defining the phenotype.
Our patient with moderate psychomotor delay presents all facial features described so far in duplication 17p13.3 comprising marked hypotonic and long face, downslanting palpebral fissures, low-set ears, small nose with round tip and small mouth. In addition, unilateral palmar transversal crease and oculocutaneous albinism are present.
Our case indicates that the facial features observed in individuals with psychomotor delay and *de novo* duplication encompassing the whole 17p13.3 region are specific enough to allow delineating of a clinically recognizable phenotype.
The study was supported by MNiSW Grant No. 0605/B/P01/2009/37. Acquiring of the Roche NimbleGen microarray platform was co-financed by ERDF project POIG.02.01.00-14-059/09.

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J20.07
Characterization of 42 small supernumerary marker chromosomes by FISH methods

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We report 42 cases with small supernumerary marker chromosomes (sSMCs) that were not identified unambiguously by conventional chromosome-banding alone. In order to determine the origin of sSMCs, we used a variety of FISH methods with different DNA-probes as well as multicolor FISH techniques (mFISH, mBAND, cen-mFISH). A total of 12 sSMCs (28,6%) originated from chromosome 15: inv dup(15)(q11.1) markers were detected in 9 cases (1 mosaic) with normal phenotype; inv dup(15)(q13) sSMCs , contained PWS/AS critical region– in 3 cases with abnormal phenotype. sSMCs derived from chromosome 22 were detected in 13 cases (31%): inv dup(22)(q11.1) markers were detected in 8 cases (2 mosaic) with normal phenotype; inv dup(22)(q11.2) – in 3 cases (2 mosaic) (cat eye syndrome); der(22)t(11;22)(q23;q11.2) – in 1 case (Emanuel syndrome); inv dup (22)(:p11.1→qter)– in 1 case. sSMC originating from chromosome 21 - inv dup(21)(q11.1) was detected in one case. sSMCs as the product of 3:1 segregation of a reciprocal translocation were characterized in 2 cases. sSMCs associated with Pallister-Killian syndrome were detected in 3cases, with i(18)(p10) syndrome – in 4 cases. Small marker ring chromosomes were identified in 3 cases: one case - r(20) and two cases - r(16). Neocentromere- sSMCs were revealed in 2 cases: unbalanced inv dup(8)(p23) and balanced r(1)(p13p21). One case was mosaic marker chromosome derived from chromosome Y – i(Y)(p10). Unusual case with sSMCs mosaicism - 47,XY,+ i(18)(p10)/47,XY,+der(18)t(18;21)(q21.1;q11.2)- was identified in proband with abnormal phenotype.Thus FISH methods are highly suited to define the chromosomal origin and the composition of sSMCs.

N.V. Shilova: None. **M.E. Minzhenkova:** None. **Z.G. Markova:** None. **Y.O. Kozlova:** None. **V.G. Antonenko:** None. **T.G. Tsvetkova:** None. **T.V. Zolotukhina:** None.

J20.08
Deletions at 18q21.1-q21.2 associated with an apparently balanced translocation t(6;18)(q13;q22.3) in a patient with developmental delay, agenesis of the corpus callosum and craniofacial dysmorphisms

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We report an apparently balanced de novo translocation t(6;18)(q13;q22.3) in an 3-year-old boy with developmental delay, agenesis of the corpus callosum (ACC) and craniofacial dimorphisms. The chromosome 6 breakpoint was mapped by FISH to a 762 kb segment at 6q13 (chr6:70,302,791-71-,064,771; hg19). *LMBRD1, COL19A1, COL9A1* genes that might have been disrupted by the breakpoint were discarded as candidates for the phenotype based on their function. The chromosome 18 breakpoint was mapped to a 962 kb gene negative region at 18q22.3 (chr18:68,777,622-69,739,381). After a-CGH (180K platform; Agilent) and FISH, two deletions were detected on the der(18): a 2.7 Mb segment at 18q21.1-q21.2 (chr18:47,555,744-50-,276,170), and a 375 kb segment at 18q21.2 (chr18:50,600,596-50,976,024). The deletions encompass 12 genes (*MYO5B, CCDC11, MBD1, CXXC1, SKA1, MAPK4, MRO, ME2, ELAC1, SMAD4, MEX3C, DCC*), three of them (*ME2, DCC* and *MBD1*) with a function in the central nervous system. The *DCC* encodes a netrin-1 receptor. Netrin-DCC signaling regulates corpus callosum formation (Fothergill et al. Cereb Cortex 2013; Epub), and ACC is documented in null *DCC* or null netrin-1 mice (Fazeli et al. Nature 1997; 386:796). ACC, however, was not associated with a heterozygous *DCC* deletion in humans (Kato et al. Birth Defects Res A Clin Mol Teratol 2010; 88:132). In our patient, the deletion on chromosome 18 might have unmasked a *DCC* mutation on the normal chromosome 18. The imbalances were located more than 22 Mb from the chromosome 18 breakpoint demonstrating the importance of array analysis of apparently balanced translocations.
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J20.09
New mutation in the FBN1 gene in a patient bilateral ectopia lentis

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Background: FBN1 gene mutations increase the susceptibility of fibrillin-1 to proteolysis, resulting in fragmentation of microfibrils. The alteration of fibrillin also interferes with signaling pathways between the different cells. The penetrance is high and the phenotypic expression is variable.
The known mutations of the gene FBN1 are more than 500. In 75% of cases mutations are inherited from one parent (autosomal dominant); in 25% of cases they occur de novo.
Materials and methods: We describe the case of a 4 years-old girl affected by bilateral ectopia lentis. Analysis of the coding sequence of the FBN1 gene (MIM #126900) was performed in the proband and her parents.
Results: We found the heterozygous mutation p.Cys154Tyr, corresponding to a nucleotide substitution G>A in exon 6, only in the proband. This variant has not been previously described in literature. The analysis extended to the parents was negative for this mutation.
Conclusions: The FBN1 gene mutations are associated with different pathologies presenting ectopia lentis as major clinical sign. These include the Marfan syndrome and Weill-Marchesani syndrome. However, they may also be associated with isolated ectopia lentis.

The loss of a cysteine residue at position 154 of the protein FBN1 causes deletion of a disulfide bridge necessary for correct folding and for the definition of the tertiary structure.
The follow up of the patient will be useful to characterize the pathogenicity of this allelic form.

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J20.10
9q34.4 deletion and large inversion of chromosome 9 - a new case report of Kleefstra syndrome

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Kleefstra syndrome is caused by a rare constitutional microdeletion of the subtelomeric region of the long arm of chromosome 9. This disease is characterized by developmental delay, moderate to severe MR, hypotonia, congenital heart defects, seizures and facial dysmorphism. The characteristic phenotype observed for these individuals is mainly caused by the deletion or loss of function mutations of EHMT1 (euchromatic histone methyltransferase 1) gene.
Here we report the case of a 9 month old female presenting a *de novo* del(9)(q34.4) detected by aCGH and confirmed by telomeric FISH, presenting also an unusual inversion on the other chromosome 9. The deletion spans 1,513 kb and encompasses some 86 genes, including EHMT1 and CACNA1B. The phenotypic features where mildly suggestive for Kleefstra syndrome, with dysmorphic facies, congenital heart defect, axial hypotonia, growth retardation and no clinical evidence for epileptic seizures.
Submicroscopic deletion of 9q34.3 (OMIM 610253) is a relatively newly characterized genomic disorder. The phenotypic and genetic features of our patient are compared with those of patients previously reported, along with a short review of the literature.

D.A. Ochiana: None. **V. Plaiasu:** None. **G. Motei:** None. **D. Galetzka:** None. **J. Schroder:** None. **O. Bartsch:** None.

J20.11
Functional analysis of Midnolin and Taube Nuss genes: Potential approach for identification of cardiac specific physiological processes

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Differential gene expression has importance in the regulation of tissue specific physiological processes. In our previous studies,
heart specific subtractive hybridization cDNA library was constructed in order to identify heart specific novel genes and related biological processes. In this study, we first performed bioinformatics analyses regarding the genes that have increased expression levels in the cardiac tissue and we then concentrated on two genes, Midnolin (Midn) and Taube Nuss (Tbn). Midn is a novel gene with unknown function other than having an ubiquitin-like domain. Human homolog of Tbn gene is Taf8 which encodes a subunit of TFIID. Briefly, Midn gene was silenced by siRNA transfection in the BALB/c neonatal primer cardiomyoblast cells whereas Tbn gene was silenced in rat cardiyomyoblast cell line (H9C2). RNA isolation was performed at the 24th and 48th hours of transfection. Target genes were selected after bioinformatics analysis and gene expressions were analyzed by qRT-PCR. Gapdh, Actb and MAPK were the control genes used in experiments. When compared to the untreated controls, the expression of the Midnolin target genes Bat3, Nedd8 and Ubc were 3.7, 2 and 2.4 fold increased espectively. These genes involve in the regulation of stability and degradation processes of the proteins. Additionally, the expression of the Tbn target gene PAPR-gamma was 3.75 fold increased whereas expression of MYOCD was significantly decreased (-0.04-fold). Both Midn and Tbn genes express in early stages of embryogenesis and might have important roles in the regulation of proper cardiac development and function.

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J20.12
Cytogenetic findings in patients with myelodysplastic syndromes in the Presov region (Slovakia)

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Myelodysplastic syndromes (MDS) are a heterogenous group of clonal disorders of haematopoetic stem cells characterized by dysplasia and ineffective haematopoiesis in one or more myeloid cell lines and peripheral cytopenias with high risk of progression to acute myeloid leukemia. MDS is characterized by non-random chromosomal abnormalities. The aim of the study was to analyze the results of cytogenetic examinations of 180 patients with MDS in the Prešov region (1995-2012). Chromosome analyses were performed from unstimulated bone marrow cell cultures, karyotypes were analysed by G-banding techniques. The median onset age in analyzed survey of patients was 69.7 years. Chromosomal analyses declosed structural and numerical chromosomal abnormalities in 28.9% of examined samples. Among 52 chromosomally abnormal patients, 13 patients (25%) showed numerical aberration, 26 patients (50%) showed structural aberration, and the other 13 patients (25%) showed both numerical and structural aberration. In 61.5% of cases only one chromosomal anomaly, in 15.4% two chromosomal anomalies and in 23.1% a complex karyotype was identified. The atypical chromosomal findings, that have not been described in literature yet, were detected also. Chromosomal analysis provides important diagnostic and prognostic informations which are helpful in determination of survival and risk of leukemic transformation. The risk of leukemic transformation is evaluated using the International Prognostic Scoring System for MDS, which is the sum of the scores of bone marrow blasts, peripheral cytopenias and cytogenetic characteristics. The results of cytogenetic methods are complemented by molecular genetics, the combination of these methods could improve the sensitivity of chromosome aberrations detection.

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J20.13
CYP2C9 gene and the metabolism of diclofenac

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INTRODUCTION: Several studies have analyzed the relationship between the CYP2C9 genotype and its influence in the metabolism of diclofenac. CYP2C9 catalyzes the 4´-hydroxylation of diclofenac. The presence between several polymorphisms and pharmacokinetics parameters is controversial. OBJECTIVE: The aim of the present study is to analyze the wild type CYP2C9 gene and its effect in the metabolism of diclofenac in a sample of healthy Mexican subjects. METHODS: We conducted a genotyping study in 25 female healthy volunteers which previously had been submitted to a pharmacological study with one-single 50mg dose of diclofenac using High Performance Liquid Chromatography and measuring AUC (0-t), AUC (0-∞), C(max), T(max) and T1/2. Genotyping of the CYP2C9 gene was performed through DNA automatic sequencing. RESULTS: Mean (SD) AUC (0-t) was 3214.062 (1009.1 ng*h/ml) and AUC (0-∞) 3334.559 (998.895 ng*h/ml), with minima and maxima values of 1399.983-6329.641 ng*h/ml and 1995.008-6390.294 ng*h/ml, respectively. CONCLUSION: These results show that bioavailability of diclofenac does not only depend on the function of CYP2C9. It is important to consider the effect of other CYPs in the metabolism of diclofenac.

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J20.14
Chromosomal microarray analysis(CMA) in a boy with ring chromosome 22.

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Introduction: Ring 22 chromosome is a rare chromosomal aberration with only ~60 cases reported. The clinical phenotype is variable and includes: severe developmental delay, speech disturbance, hypotonia and microcephaly. No clear correlation between deletion size and phenotype has been reported.
Case presentation: We present a 2.5 year-old boy with global developmental delay, hypotonia, and dysmorphic features including coarse facies, broad nasal bridge, long eyelashes, large hands and feet, small umbilical hernia,

bilateral inguinal hernia scars. Brain MRI showed a thickened corpus callosum and mild changes in the white matter that may represent periventricular leukomalacia. Chromosomal analysis from blood leukocytes revealed an abnormal karyotype: 46,XY,r(22)(pq).
Chromosomal microarray analysis (CMA) was performed by Affymetrix CytoScan HD Array that contain ~ 2.7 millions genetic markers revealed copy number loss of about 4.8Mb, arr22q13.31(46,386,970-51,197,724)x1 including more than 60 genes. Both parents had a normal karyotype.
Discussion: The deleted region in 22q13.31 found by CMA includes many genes, some of them found to be responsible for clinical significant disorders include *ARSA* (metachromatic leukodystrophy), *TYMP* (mitochondrial nero-gastrointestinal encephalomyopathy), *MLC1* (megalencephalic leukoencephalopathy with subcortical cysts), *ALG12* (congenital disorders of glycosylation) and *SHANK3* which is known to be associated with autistic spectrum disorders.
Up to date, very few CMAs were performed in case of ring chromosome 22. Our report emphasizes the potential contribution of CMA in order to better understand the spectrum of abnormalities in each case, and assess genotype-phenotype correlation.

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J20.15
Molecular cytogenetic analysis in patients with Turner syndrome

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Context. It has been suggested that most of the cases with Turner syndrome are mosaics that conventional cytogenetic techniques cannot diagnose and homogeneous monosomy would be incompatible with life, knowing that 99% of the 45,X conceptuses abort spontaneously. FISH techniques show a higher frequency of the mosaics, because it allows the analysis of much more cells. The aim was to determine sex chromosomes mosaicism using FISH in patients diagnosed with homogeneous X monosomy by conventional cytogenetic analysis. **Material and method.** We studied patients with Turner syndrome who were selected from the database records for monitoring patients treated with GH in France. The karyotype made at diagnosis was established by the analysis of 4 to 100 metaphases. The mosaics were investigated applying FISH technique on lymphocytes of peripheral blood. **Results.** Using standard cytogenetics we observed that 738(51%) patients presented homogeneous X monosomy, the others cases being represented by karyotypes in mosaic(45,X/46,XX, 45,X/47,XXX, 45,X/46,XX/47,XXX) in 203(14%) patients or by others structural abnormalities of sex chromosomes, homogeneous or in mosaic, in 496(35%) patients. FISH techniques used in patients with homogeneous X monosomy show the results summarized in the next table.

Karyotype	Patients n,(%)	Level of mosaicism
45,X	178(93%)	0%
45,X/46,XX	9(5%)	2-32%
45,X/47,XXX	1(0,5%)	19%
45,X/46,XX/47,XXX	3(1,5%)	7-16%
Y chromosome	0(0%)	-

Conclusions. FISH technique is useful to better characterize the karyotype but the results obtained on this large study group does not support the hypothesis that in order to be viable a 45,X conceptus should possess another cell line.

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J20.16
De novo duplication of chromosome(5)(p13p15)

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Duplication of the short arm of chromosome 5 can be seen in the literature, which is a rare abnormality. We report a two months girl who was born from unrelated parents. The mother was diabetic, father was healthy. Physical examination of the patient showed a facial dysmorphism which were brachycephaly, frontal bossing, bitemporal narrowing, short palpebral fissures, low-set ears, micrognathia, microphthalmia, bulbous nasal tip, sparse brows and eyelashes. These findings are specific for 5p duplication syndrome. The cytogenetic and FISH studies showed 46,XX(5)(p13p15) karyotype and the telomeres belong to 5p and 5q. Both parents have normal karyotypes. Theuse of conventional and molecular cytogenetics in this case confirms diagnosis and phenotype/genotype correlation.

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J20.17
Evaluation of the in-vitro protective effect of plant extract (Astaxanthin) on chromosomal breakage in Fanconi anemia cell culture Maha M Eid1, Sami A Temtamy2, Engy S Soliman1 , Marwa I Shehab1, Sami H Abd Alaziz3, Dina H Baraka3 , Mona Hamdy4 1-Human Cytogenetics Department National research Center - Cairo-Egypt 2-Clinical Genetic Department- National Research Center-Cairo- Egypt 3-Faculty of science- Banha University Banha- Egypt 4-Faculty of Medicine - Cairo University- Egypt

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Background Fanconi anemia is an inherited bone marrow failure syndrome associated with congenital abnormalities. The high frequency of chromosomal breaks in Fanconi anemia (FA) lymphocytes has been related to the increased oxidative damage. Reactive oxygen species (ROS) are derived from the metabolism of molecular oxygen. Antioxidants have the ability to transform ROS into stable and harmless compounds. Astaxanthin is a natural and safe source of antioxidant; its effect might protect cells from oxidative damage.
Aim of work This study was designed to compare between the antioxidant effect of both astaxanthin and vit E as measured by their ability to reduce the frequency of chromosomal breakage.
Subjects and methods The study included 15 Fanconi anemia (FA) patients 9 females and 6 males, their age ranged from 4 to 21 years. The diagnosis of FA was confirmed by DEB. Astaxanthin and vit E were added at the start of the peripheral blood lymphocyte cultures then caffeine was added at last 6 hrs of culture to induce chromosomal breakage.
Results and conclusion The level of breakage was markedly reduced by using astaxanthin and vitamin E however, there was no significant difference between the effect of both substances. Astaxanthin was found in wide diversity of natural sources also it is 10 times potent than vitamin A and much safer than vitamin E. Our study is the first to investigate the effect of astaxanthin on chromosomal breakage in-vitro. We conclude that admistration could be beneficial for patients with FA to improve their hematopoietic state.

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J20.18
Arginine vasopressin receptor genes (AVPR1A, AVPR1B) and their involvement in personality traits variation

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Arginine vasopressin and its receptors affect many social and behavioral traits in sexually-specific manner. We aimed to assess the main, haplotypic and GxG effect of AVPR1A (rs11174811, microsatellite RS1) and AVPR1B gene (rs28632197, rs33911258) on personality traits variation.
We recruited 1018 healthy individuals (68% women) of Caucasian origin (Russians-357, Tatars-549, Udmurts-112) from Russia (mean age: 19.53±2.24 years) without any history of psychopathologies subjected to personality assessment (EPI, TCI-125). Genotyping of SNPs was performed using PCR, PCR-RFLP. AVPR1A RS1 alleles were designated as S-allele (≤10 repeats) and L-allele (>11 repeats). Statistical analysis was conducted with PLINK v.1.07, Haploview 4.1.
The main effect of AVPR1B rs28632197 and rs33911258 on Self-transcendence (ST) was observed in total group (P=0,013 and P=0,003 for rs28632197 and rs33911258, respectively) and in females (P=0,024 and P=0,028). Haplotype analysis revealed an association of AVPR1A C*S-haplotype (rs11174811, RS1, respectively, D'=0,546) and increased Extraversion (P=0,015) and A*S-haplotype and decreased Reward Dependence in total group (P=0,024). AVPR1B A*G- and G*A-haplotypes (rs28632197, rs33911258, D'=0.87) were associated with higher (P=0,012) and lower

ST (P=0,002), respectively, in total group and in females. Epistatic effect of rs33911258 and RS1 on Neuroticism (P=0,045 in total group and P=0,019 in females) and on ST (P=0,018 and P=0,019 for total group and females, respectively) was demonstrated.
Our findings indicate gender-mediated main, haplotypic (AVPR1B) and epistatic effect of studied genes on Self-transcendence - trait reflecting spirituality, self-awareness, creativity; while AVPR1A haplotypic effect on sociability-related traits (Extraversion, Reward Dependence) was observed.
Study was supported by Russian Foundation for Basic Research (№11-04-97032-r_povolzhye_a).

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J20.19

Conventional Cytogenetic assays and the recent CBMN Cyt assay: could they be used as biomarkers to predict the genetic instability in the high risk groups?

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The genetic instability in the diseased individuals was estimated by the application of conventional cytogenetic techniques such as chromosomal aberration (CAs) and the frequency of micronuclei (MN) analysis in peripheral blood lymphocytes (PBLs). However, the recent CBMN Cyt assay endpoints provide valuable information about the gene expression, measure of the chromosomal damage events and individual susceptibility. For this study, PBLs of healthy individuals (n=50) and the patients with Dilated Cardiomyopathy (n=50), Diabetes and complications (n=50), Head and Neck Cancers (n=25), Aplastic Anemia (n=10), Down syndrome (n=25) were subjected to the conventional cytogenetic assays and CBMN Cyt assay. Their demographic characteristics were also analyzed. It was observed that there was a significantly increased frequency of CAs and MN in the patients and the use of CBMN Cyt assay could give information about the chromosome damage events such as nuclear buds (NBUDs) and nucleoplasmic bridges (NPBs) which were significantly higher in the patient group in comparison to the control group (p<0.001). Thus these findings provide preliminary evidence that CBMN Cyt assay could measure the genetic damage events in the etiology of the diseases and used as biomarker. The identification of individual susceptibility and high risk groups by CBMN Cyt assay could serve as a potential tool for public health screening.

R. Saraswathy: None.

J20.20

In silico personal omics profiling on the basis of whole genome array CGH analysis uncovers disease pathways

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Regardless of significant efforts made towards definition of personified disease pathways in clinical populations using whole genome array CGH analysis, there is still a need for developing new tools to process whole genome scan data for definition of causative genome variations. To succeed, we have proposed an original bioinformatic strategy of CNV/gene prioritization by rating data acquired from clinical, cytogenetic and genomic variation databases and by means of bioinformatic tools for genome, epigenome and pathway analysis. To test the strategy, we have evaluated the Russian cohort of children with intellectual disability, autism, epilepsy and congenital anomalies (116 individuals; partially described in Iourov I.Y. et al. Mol. Cytogenet. 2012; 5:46.). Causative CNVs were deletions of 1q42.13, 2q31.1, 2q35, 2q37.3, 3p22.1, 3p11.1, 7q31.1, 9q34.3, 10q21.3, 13q32.2, 17q25.3, 19p12, 20p12.2, Xq12 and duplications of 2q24.1, 7p14.3, 10p13, 12p13.31, 16p13.3, 19p13.3, 19q13.2, Xp22.12. Accordingly, alterations to such pathways as apoptosis, axon guidance, brain (fetal) development, transcriptional regulation, cell-cycle regulation, Fanconi anemia, histone acetylation, mitotic checkpoint, o-glycan biosynthesis, p53-pathway, V(D)J recombination as well as calcium, carbohydrates and zinc metabolism or transport were found to be associated with phenotypic outcome. Moreover, we have determined a Y chromosome locus associated with autism and intellectual disability. Our results demonstrate that a bioinformatic strategy encompassing all the “omics” approaches, which are performed in silico on the basis of data obtained by whole genome array CGH analysis, is a valuable tool for uncovering disease pathways in personalized genomic medicine.Supported by the President of the Russian Federation Grant MD-4401.2013.7

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J20.21

Paternal Uniparental Isodisomy 11p mosaicism in a patient with Beckwith Wiedemann

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We present the genetic molecular characterization of a male with clinically diagnosis of Beckwith-Wiedemann syndrome (BWS, MIM#130650) seeking reproductive genetic counselling.
BWS is the most common genetic overgrowth disorder, characterized by somatic overgrowth, macroglosia and abdominal wall defects. BWS is a multigenetic disorder associated, up to 90% of patients, with dysregulation of the expression or function of one or more gene encompassing the imprinted 11p15 chromosomal region. This region is organized into two imprinting centers or domains, telomeric (IC1) and proximal or centromeric (IC2). The phenotypic and genotypic heterogeneity makes reproductive genetic counseling a challenging situation in many cases. The main genetic causes of BWS are: a) loss of methylation at the IC2 (40-50% of cases), b) paternal UDP of 11p15 (10-20%), c) gain of methylation at the IC1 (10%) and d) mutations in the maternal CDKN1C allele. Chromosomal rearrangements are relative rare (2-3% of cases).
We have analyzed the methylation pattern of the IC1 and IC2 imprinting centers by methylation-specific restriction enzyme digestion and STRs segregation of a panel of 6 six microsatellites mapping to short arm of chromosome 11 has been performed in patient and their parents.
Methylation analyses are compatible with IC1 hipermethylation and IC2 hipomethylation patterns. STR analyses show a dosage increase of microsatellites encompassing the 11p15 region. Both results suggest the presence of a paternal uniparental isodisomy 11p mosaicism in the patient. The identification of the genetic cause of the disease makes easier a subsequent reproductive genetic counseling.

M. Garcia-Hoyos: A. Employment (full or part-time); Significant; Instituto de Medicina Genomica. **M. Molero:** A. Employment (full or part-time); Significant; Instituto de Medicina Genomica. **L. Rausell:** A. Employment (full or part-time); Significant; Instituto de Medicina Genomica. **M. Lazaro:** A. Employment (full or part-time); Significant; Instituto de Medicina Genomica. **M. Bermejo:** A. Employment (full or part-time); Significant; Instituto de Medicina Genomica. **C. Ruiz-Lafora:** A. Employment (full or part-time); Significant; Instituto de Medicina Genomica. **M. Perez-Alonso:** F. Consultant/Advisory Board; Significant; Instituto de Medicina Genomica. **A. Lopez-Arizteguí:** None. **J. Garcia-Planells:** A. Employment (full or part-time); Significant; Instituto de Medicina Genomica. F. Consultant/Advisory Board; Significant; Genagen.

J20.22

Evaluation of stem cell markers expression in breast cancer cells in relation to other disease prognostic factors.

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Cancer stem cells are considered to be responsible for tumor formation. CD44, CD24 and CD133 antigens have been reported as markers of cancer stem-like cells in breast cancer. The aim was to investigate any correlation between expression of these antigens and the accompanying clinicopathological features. For this purpose 39 paraffin embedded breast cancer samples were analysed by IHC. In all cases grading, hormonal receptor status, c-erbB-2 expression, Ki-67 index and p53 gene expression were also analyzed. Lymph node status was known only for a subset of the studied samples.
No significant relation was found between expression of these markers and the differentiation of the tumor, lymph node status and p53 expression. Nevertheless, studying the estrogen receptor status, CD24 expression was detected in the 80% of the negative cases vs 25% of the positive cases (p=0,02), while CD44 and CD133 had no expression differences. Moreover, CD44 was expressed in the 39,2% of cerbB-2 negative cases vs 72,7% positive cases(p=0,060). The Ki-67 index showed statistically significant correlation with CD24 expression (68,1% for >15% and 35,2%<15%).According to these results, tumors with high CD24 expressing breast cancer cell populations tend to have poorer prognosis as they are related to negative ER status and high Ki-67 index. CD44 expression is found to be more common in cerbB-2 positive cases, possibly rendering these cancer cells more tumorigenic. Such information may prove supporting towards planning more effective treatments, as a small fraction of cells can determine the overall

course of the disease.

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J20.23

Constitutive heterochromatin regions of chromosome from human chorionic villi with properties euchromatin regions

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Higher eukaryote genomes contain satellite DNAs, often concentrated in transcriptionally inactive constitutive heterochromatin regions (**CHRs**).) CHRs of the autosomes 1, 9, 16 from chorion villi are characterized by decondensation, early replication, hypomethylation and DNAse 1 hypersensitivity.

We studied peculiarities of CHRs in direct and semi-direct slides from chorionic villi dyed by Acridine orange (**AO**). AO is nucleic acid selective metachromatic stain that discriminates single-stranded from double stranded nuclei acids as orange-red and yellow-green fluorescence respectively. After standard AO staining of untreated direct chromosomal preparations CHRs manifests unusually bright red fluorescence in 1qh, 9qh, 16qh. The nature of this fluorescence was studied on direct chromosomal preparations pretreated with different enzymes (RNase A, RNase H, DNase I, DNA ligase T4) followed by AO staining. Results of these work show that ssRNA and DNA*RNA hybrids are present in CHR of chromosome 1 (1q12) on fixed chromosomes.

It is known that CHRs are associated with nuclear periphery and form chromocenter. Our 3D-FISH results showed a significant repositioning of 1q12 towards the centre of the nucleus and near of chromocenter in chorionic villi sample from early pregnancy (4-5 week). We found no change in the position of 1q12 in chorionic villi from 5-6 week to 36 week pregnancy. Almost all of FISH-signals were closer to the nuclear periphery and in chromocenter.

Our results confirm for unusual properties of CHRs which could be attributed to the feasible transcriptional activity of pericentromeric satellite DNA in chorionic cells during embryogenesis.

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J20.24

A novel 1p32.1p31.1 deletion: evidences for a 1p32p31 deletion syndrome subtype

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Interstitial deletions of 1p32p31 are rare. Currently, no fewer than 8 cases (including a case of prenatal diagnosis) of deletions involving this chromosomal region have been reported. Here, we describe a boy showing severe intellectual disability, hypoplasia of the corpus callosum, vantriculomegaly, dismorphic features, hypospadias, congenital heart defect, dolichocephaly, polydactyly. Cytogenetic analysis lead to a suggestion that a deletion within the short arm of chromosome 1 is present, i.e. 46,XY,?del(1)(pter->p32.3::p32.1->qter). Array CGH using a set of platforms (resolution from 1 Mb to 1 kb) has demonstrated the presence of a deletion (size: 12 Mb) that lead to a loss of 39 OMIM genes. Among the latter, there was NFIA, suggested to be the main contributor to the phenotype outcome of 1p32p31 microdeletion syndrome. Despite of reproducibility of phenotypic findings in the index case, we have suggested that congenital heart defects, polydactyly and hypospadias are likely to be produced by losses of genes, which are more proximal, in contrast to those typically deleted in the microdeletion syndrome [OMIM: 613735]. Furthermore, one can propose that a deletion affecting genomic loci within the interval between 59 and 72 Mb on chromosome 1 is more likely to lead to a condition, arbitrarily designed as a 1p32p31 deletion syndrome subtype. In addition to typical phenotypic findings, this subtype is also featured by congenital heart defects, hypospadias and polydactyly, which are atypical for the microdeletion syndrome with a critical region 1p32.1-p31.3.

I.A. Demidova: None. **S.G. Vorsanova:** None. **Y.B. Yurov:** None. **I.Y. Iourov:** None.

J20.25

Molecular variability of delF508 mutations in CFTR gene

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Cystic fibrosis (CF MIM 219700) is the most common genetic disease among Caucasians. The frequencies of CF are 1:4000 - 1:7000 newborns in Western-Siberian and North Western regions respectively. Molecular-genetic analysis DNA of CF patients from Novosibirsk and St. Petersburg has shown that frequencies of delF508 among all mutations of CFTR gene are 0,425 and 0,520 respectively. The main element of molecular diagnosis for cystic fibrosis is based on the direct identification of this mutation in the CFTR gene.

Two alternative methods were used to identify mutation delF508 in patients with CF: allele-specific Real-Time PCR and classical PCR of exon 10 CFTR gene with electrophoresis of the PCR-product in PAAG. One sample which appeared to be disputable was sequenced. The mutation c.1522_1524delTTT(delF508) have been found in this sample by direct sequencing of exon 10 CFTR gene, instead of c.1521_1523delCTT(delF508) mutation described in literature. We have developed ACRS RFLP methodology, allowing to identify and distinguish these delF508 mutations. The molecular testing of DNA samples (62 patients, 94 alleles with delF508) from St. Petersburg and (32 patients, 29 alleles with delF508) from Novosibirsk have found c.1522_1524delTTT mutation in two and one samples respectively.

Our data have shown the molecular variability of delF508 and high frequency of c.1522_1524delTTT mutation among patients of CF in the Russian population.

A.B. Maslennikov: None. **Y.A. Nasykhova:** None. **L.F. Mitrohina:** None. **A.R. Shorina:** None. **E.V. Maslova:** None.

J20.26

Cytogenetic analysis of Human dermal fibroblasts (HDF) cells using karyotyping test

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Human dermal fibroblasts (HDFs) are a common source of somatic cells for generation of induced pluripotent stem cells (iPSCs). The confirmation of HDFs cytogenetic stability is an essential step for generation of a suitable and functional iPS line. In this study, HDF cells were isolated from foreskin samples and then were cultured for 10-15 passages. The HDF's cytogenetic stability was evaluated during their first 10 to 15 passages using karyotype test. Our results showed that HDF cells have normal karyotype during their first 10-15 passages and they maintain satisfactory proliferation rate up to 10 passages. Our findings reinforce the interest to generate iPS cells from HDFs because in addition to their availability and convenient isolation, they showed good proliferation kinetic and kept cytogenetic stability for 10 to 15 passages as essential prerequisite factors before iPS generation.

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J20.27

Emanuel Syndrome: breakpoints determination by NGS.

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The recurrent constitutional translocation between chromosomes 11 and 22 is the most common non- Robertsonian translocation in humans. Clustered breakpoints involving chromosome regions 11q23 and 22q11 have been reported in unrelated families. Balanced translocation carriers are clinically normal. Their offspring have a risk to herit a supernumerary der(22) t(11;22) chromosome, which results in a rather constant phenotype. This very rare genomic syndrome was named Emanuel syndrome.
The diagnostic of our patient with Emanuel syndrome was done by aCGH. By NGS we determined that the breakpoints lie within palindromic AT-rich repeats (PATRRs) on both chromosomes 11 and 22. We used primers in reference to Kurahashi et al. 2000, who were the first to describe the exact breakpoints of this recurrent constitutional translocation.
The breakpoints 11q23 and 22q11 lie in the similar region with AT repeats and differ in size only tens to hundreds bp variable from patient to patient. The properties of this palindromic region are the explanation for recurrent translocations,
Patients with Emanuel syndrome have a reliable correlation between genotype and phenotye. This predictible phenotype is determined by a very constant size and region of the duplication of chromosomes 11 and 22. Both breakpoints of the constitutional translocation t(11;22) vary only few between the studied patients. The breakpoints on 11q23 and 22q11 are both situated in a palindromic AT-rich repeats (PATRRs) region predicted to induce genomic instability, which mediates the translocation. This confirms the the hypothesis that numerous constitutional translocationst (11;22) arise by the same mechnism.

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J20.28

Evaluation of genotoxic damage in patients undergoing hemodialysis, before and after vitamin supplementation

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Haemodialysis is a most common treatment for chronic kidney disease (CKD), however it increases oxidative stress and the risk to cancer. It is supposed that the use of vitamins could decrease this risk. In this study, genotoxic effects in patients with CKD undergoing regular haemodialysis have been investigated by the cytokinesis-blocked micronucleus assay and buccal mucosa cells assay, with and without supplementation by folic acid and vitamins C, E and B_{12}. Sixteen volunteer patients provided the necessary samples for each test, before supplementation and after one or two months of vitamins therapy. Micronucleus test was effective for evaluation of genotoxic effects, showing a significant reduction of nuclear and cell damages after vitamins supplementation. It was observed a significant reduction in the cytotoxicity in lymphocytes, more evident in women. Also, there was a gradual decrease in the frequency of micronuclei in lymphocytes during the months of supplementation. The frequency of micronuclei in exfoliated cells from the oral cavity in patients undergoing haemodialysis with supplementation was inferior to that of patients undergoing haemodialysis without the use of vitamins, in both sexes, with a higher damage reduction in the first month of supplementation. The frequency of binucleated cells and apoptosis was significantly reduced after the first month of supplementation, remained decreased until the end of treatment. Additionally, different response to the vitamins therapy was observed among the genders of patients. These results may contribute to improve health of patients and prevention of possible diseases that affect individuals with CKD undergoing haemodialysis.

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J20.29

Genotoxicity associated with the hydroxyurea use in sickle cell anemia

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Sickle cell anemia (SCA) provides to the carrier diversified and complexes manifestations. Hemoglobin S polymerization results in generation of oxygen reactive species that promotes lipids, proteins and also DNA oxidation. This event modifies cells mechanisms and provokes intensive cells and tissues damages. In the top of the mains therapies used in SCA there is the hydroxyurea (HU) administration. In short periods of administration, hydroxyurea doesn't present significant toxicity, but it is controversial considering long periods of treatment. The present study aimed evaluate the genotoxicity and citotoxicity markers using the comet assay and Howell-Jolly bodies (micronucleus) count. The data was associated with the use of HU. Were evaluated 84 individuals with SCA, 39 with HU (SCA + HU), taking for ± 930 days; 45 without HU use (SCA - HU) and 53 individuals without hemoglobinopathies (control group). Comet assay analysis demonstrated increase of DNA lesion in the both test group (SCA + HU) and (SCA - HU), in comparison with the control group ($p < 0.001$). The comparison between the two test groups doesn't showed significant difference. Micronucleus analysis demonstrated increase in DNA lesion rate in the SCA + HU group, when compared to the other two groups ($p < 0.001$). These results suggests a possible genotoxic and mutagenic effect associated to hydroxyurea use in SCA.
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J20.30

Comparison of genotoxic effect of dioxidine and methyl methanesulfonate measured by comet-assay and cytogenetic method

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The aim of this study was to assess the effect of mutagens with different mechanisms on DNA and chromosome levels and compare Comet assay and chromosome aberration assay.
The levels of spontaneous and *in vitro* induced DNA damage and chromosome aberrations in peripheral blood lymphocytes of 14 healthy donors were analyzed. Whole blood samples were cultivated during 54 hours using the standard procedure. One of two mutagens: prooxidant dioxidine (0.01 and 0.1 mg/ml) and alkylating agent methyl methanesulfonate (MMS) (0.0025 and 0.01 mg/ml) was added to culture in 24 hours of cultivation.
Spontaneous level of chromosome aberrations was 2.25±0.17% in the experiment with dioxidine and 2.58±0.28% in the experiment with MMS (with the same donors). Dioxidine at concentration 0.01 mg/ml increased the level of aberrant cells to 7.63±0.36% and at concentration 0.1 mg/ml - to 15.38±0.63%. The %DNA in tail was 5.59±1.1% without dioxidine and 9.64±0.84% and 14.95±2.02% with low and high doses of dioxidine respectively. MMS at low dose (0.0025 mg/ml) increased the level of aberrant cells to 5.14±0.37% and at high dose (0.01 mg/ml) - to 6.78±0.43%. Before adding of MMS the %DNA in tail was 4.81±0.54%. MMS increased this parameter to 9.6±1.42% and to 22.7±1.33% at low and high concentration respectively. In conclusion, both mutagens significantly and dose-dependently increased level of DNA damage as well as chromosome aberrations. We revealed the correlation between these two parameters. These results may be applied in research of mechanisms of realization of primary DNA damage to chromosome aberrations.

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J20.31

A rare case of Prader Willi and Jacobs syndrome

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Background and aims: The association of two or more chromosomal defects is rarely reported. New molecular techniques made possible the identification of the many genetic conditions that were undiagnosed untill now. In this paper we report a case of a child diagnosed with Prader Willi syndrome and a 47,XYY karyotype.
Method: We will present the child's evolution from birth until age 14.
Results: The child exhibits rapid weight accumulation between 1 and 6 years, hyperphagia, narrow bifrontal diameter, almond shaped eyes, down slanting palpebral fissure of the eyes, thin upper lip, down turned corners of the mouth, hipogonadism, short hands, mild mental retardation, behavioural disturbances. Based on the clinical manifestations the diagnosis of Prader Willi syndrome was suspected. The cytogenetic analysis revealed a 47,XYY karyotype in all the 30 metaphase evaluated. FISH analysis showed

no deletion of the 15q11-q13 region. The DNA methylation analysis of the Prader Willi locus on the chromosome 15q11-q13 confirmed the disomy. To the best of our knowledge, the co-existence of Prader Willi syndrome and 47,XYY karyotyope has been previously reported in two cases. From these cases, only one presented disomy in a newborn. Child development till the puberty age presented several differences as compared with the Prader Willi patients or those with Jacobs syndrome.
Conclusion: We consider important to report this case as it presents the evolution of the patient having a rare association of two chromosomal aberrations and because it shows how those anomalies can modulate the phenotypic effect of each other.

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