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Indication Criteria for Genetic Testing
Evaluation of validity and clinical utility

Indication criteria for disease:
Duchenne muscular dystrophy (DMD) [Dystrophin]

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2. Disease characteristics

2.1 Name of the Disease (Synonyms): *Duchenne muscular dystrophy, DMD*

2.2 OMIM# of the Disease: *#310200*

2.3 Name of the Analysed Genes or DNA/Chromosome Segments: *Dystrophin*

2.4 OMIM# of the Gene(s): **300377*

2.5 Mutational Spectrum:
In about 65% of the patients: deletions of one or more exons;
in about 7% of the patients: duplications of one or more exons;
in about 21% of the patients: point mutations (nonsense, splice, missense).
In up to 7% none of these mutations is found.
[ref. 1]

2.6 Analytical Methods:
Method of choice is a stepwise approach:
Step 1: For exon deletions: multiplex PCR for selected exons (at least 18) –
alternatively or in addition: for exon deletions and duplications: MLPA for all 79
exons. Deletions of a single exon must be confirmed by an independent
procedure.

Step 2: If a deletion/duplication has been excluded: screening and/or
sequencing of the coding regions and splice sites for detection of point
mutations.

2.7 Analytical Validation
Follow-up examination of patient samples that have already been analysed
with other methods (cDNA Southern blot; multiplex PCR); in exchange with
other labs if necessary.

2.8 Estimated Frequency of the Disease in Germany
(Incidence at birth ("birth prevalence") or population prevalence):
Prevalence at birth 1:3,000 male newborns

2.9 If applicable, prevalence in the ethnic group of investigated person:
The prevalence at birth is about the same in all studied populations.

2.10 Diagnostic Setting:

	Yes.	No.
A. (Differential)diagnostics	☒	☐
B. Predictive Testing	☐	☒
C. Risk assessment in Relatives	☒	☐
D. Prenatal	☒	☐

Comment: *Predictive diagnosis in the narrower sense has practically no role in*
DMD. A very high CK level discoverd by chance in a very small boy with no
additional problems must be considered a clinical symptom and deserves
clarification.
Five to 10% of female carriers manifest clinical signs of a myopathy which
may extend til the full picture of a DMD. A "differential-diagnostic setting" could
be applied for such girls too. However, the following informations on test
characteristics relate to boys only, because none are available yet for "DMD
girls".

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3. Test characteristics

	genotype or disease	
	present	absent
test pos.	A	B
test neg.	C	D

A: true positives   C: false negatives
B: false positives   D: true negatives

sensitivity:	A/(A+C)
specificity:	D/(D+B)
pos. predict. value:	A/(A+B)
neg. predict. value:	D/(C+D)

3.1 **Analytical Sensitivity**
(proportion of positive tests if the genotype is present)
(Information relates to the kind of analysed mutation)
Multiplex-PCR (for exon deletions only) = 0.98
MLPA (for exon deletions and duplications) = 0.99
Direct sequencing of coding and splice regions (point mutations) = 0.97 [ref. 2]

3.2 **Analytical Specificity**
(proportion of negative tests if the genotype is not present)
(Information relates to the kind of analysed mutation)
multiplex PCR = 0.99
MLPA = 0.99
Sequencing = 0.97

3.3 **Clinical Sensitivity**
(proportion of positive tests if the disease is present)
The clinical sensitivity can be dependent on variable factors such as age or family history. In such cases a general statement should be given, even if a quantification can only be made case by case.
(Information relates to all potentially pathogenic mutations)
With stepwise diagnostics as described in 2.6 and considering the analytical sensitivities given in 3.1, the following values - depending on method - are obtained:
Multiplex PCR (for exon deletions only) = 0.64
MLPA (for exon deletions and duplications) = 0.71
In cases where no exon deletion or duplication was found: Direct sequencing of coding and splice regions (point mutations) = 0.20.

3.4 **Clinical Specificity**
(proportion of negative tests if the disease is not present)
The clinical specificity can be dependent on variable factors such as age or family history. In such cases a general statement should be given, even if a quantification can only be made case by case.
(Information relates to the kind of analysed mutation)
Multiplex PCR = 0.99
MLPA = 0.99
Sequencing = 0.97

3.5 **Positive clinical predictive value**
(life time risk to develop the disease if the test is positive).
Multiplex PCR = 0.98
MLPA = 0.99
Sequencing = 0.97

3.6 **Negative clinical predictive value**
(Probability not to develop the disease if the test is negative).
Assume an increased risk based on family history for a non-affected person.
Allelic and locus heterogeneity may need to be considered.
General remark to 3.6: A (predictive) molecular genetic analysis of a (presently) unaffected family member with increased risk for DMD appears applicable and useful only in the following situations:
-   prenatal diagnosis (with male fetus)
-   heterozygote test in female relatives of a patient.

Index case in that family had been tested:
With all three methods mentioned, presence of the known mutation can be excluded in the family with practically 100% reliability if the same method is used.
When the family specific mutation has been excluded during prenatal diagnosis, the child nevertheless may develop DMD later. This is possible because of the high rate of new mutations (about 1/3 of cases, or about 1:9,000 male newborns).

Multiplex PCR = >0.99
MLPA = >0.99
Sequencing = >0.99

Index case in that family had not been tested:
As a rule, no predictive tests are performed in such cases.
No generally applicable information can be given for this situation. The probability of not developing the disease for a relative of an untested patient in whom the test (irrespective of the method used) turned out negative depends on the family constellation, i.e., on the a-priori risk. The a-priori risk is determined by the relatedness of a person to the index patient as well as by the probability that the index case carries a new mutation. These numbers must be obtained individually for each person. Depending on the result, it must be decided individually whether molecular genetic diagnostics are useful at all.

4. Clinical Utility

4.1 (Differential)diagnosis: The tested person ist clinically affected
(To be answered if in 2.10 "A" was marked)

4.1.1 Can a diagnosis be made other than through a genetic test?

No.   ☐ (continue with 4.1.4)
Yes,   ☒
      clinically.   ☐
      imaging.   ☐
      endoscopy.   ☐
      biochemistry. ☐
      electrophysiology. ☒
      other (please describe)
      Immuno-histology and immuno-blot after muscle biopsy

4.1.2 Describe the burden of alternative diagnostic methods to the patient
A muscle biopsy in mostly small children is an invasive surgical intervention and associated with the risks of general anesthesia and wound healing. Also, formation of scars is possible.

4.1.3 How ist the cost effectiveness of alternative diagnostic methods to be judged?
Only a few centers can perform muscle biopsy as outpatient procedure by using needle biopsy. This will be less expensive than molecular genetic diagnostics. In most cases, with reception on ward and open biopsy the costs are above step1 and below step2 genetic diagnostics.

4.1.4 Will disease management be influenced by the result of a genetic test?

No.   ☐

Yes.   ☒

Therapy (please describe)	*In the great majority of cases, the genetic diagnostics allows a prognosis of clinical course (muscle dystrophy Duchenne vs. Becker). This determines possible therapeutic interventions like physical therapy or corticosteroid therapy, if necessary.*
	Also new therapeutic concepts (exon skipping, read-through of stop mutations) depend on the kind of genetic defect.
Prognosis (please describe)	*For exon deletions the 'reading frame hypothesis' allows a prognostic differentiation between Duchenne and Becker muscular dystrophy. It directly determines life span, modes of therapy, quality of life, choice of schooling and occupation, and family planning.*
Management (please describe)	*Beside the factors mentioned above, the differentiation Duchenne vs. Becker has also practical implications, for provision of articles of support, for insurance and legal matters, and for designing the private home.*

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4.2 Predictive Setting: The tested person is clinically unaffected but carries an increased risk based on family history
(To be answered if in 2.10 "B" was marked)

4.2.1 Will the result of a genetic test influence lifestyle and prevention?

If the test result is positive (please describe)

If the test result is negative (please describe)

4.2.2 Which options in view of lifestyle and prevention does a person at-risk have if no genetic test has been done (please describe)?

4.3 Genetic risk assessment in family members of a diseased person
(To be answered if in 2.10 "C" was marked)

4.3.1 Does the result of a genetic test resolve the genetic situation in that family?
Initially, only the X-linked inheritance is proven. Because of the high mutation rate of the dystrophin gene (1:10,000), the situation of the family can only be clarified through additional investigations.

4.3.2 Can a genetic test in the index patient save genetic or other tests in family members?
With limitations only (see 4.3.1). In families with definite X-linked inheritance, a genetic analysis of obligate heterozygous women may eventually be omitted.

4.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?
Yes.

4.4 Prenatal diagnosis
(To be answered if in 2.10 "D" was marked)

4.4.1 Does a positive genetic test result in the index patient enable a prenatal diagnostic?
Yes.

5. If applicable, further consequences of testing
Please assume that the result of a genetic test has no immediate medical consequences. Is there any evidence that a genetic test is nevertheless useful for the patient or his/her relatives? (Please describe)
The genetic test helps avoid invasive and/or lengthy diagnostic meanders. It takes away the psychological burden of a missing diagnosis.
It facilitates early care by experienced specialists (e.g. definition of the right time for orthopedic interventions).
It is the base for the genetic counseling of relatives and for diagnosing female carriers, with the option of prenatal diagnosis. It enables – on the demand of carriers – prevention of additional cases in the family.

References:
[1] White SJ et al. (2006) Hum Mutat 27: 938-945
[2] Dent KM et al. (2005) Am J Med Genet A 134: 295-298