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CLINICAL UTILITY GENE CARD UPDATE

Clinical utility gene card for: Proximal spinal muscular atrophy (SMA) – update 2015

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1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)

Spinal muscular atrophy (SMA) type I–IV,[1] SMA 5q, proximal SMA, infantile SMA, Werdnig–Hoffmann disease (SMA I), intermediate SMA (SMA II), juvenile spinal muscular atrophy type Kugelberg–Welander (SMA III) and adult onset SMA (SMA IV).

1.2 OMIM# of the disease

253300 (SMA I), 253550 (SMA II), 253400 (SMA III) and 271150 (SMA IV).

1.3 Name of the analyzed genes or DNA/chromosome segments

SMN1 and *SMN2*[2]/5q11.2-13.3.

1.4 OMIM# of the gene(s)

600354 (SMN1)/601627 (SMN2).

1.5 Mutational spectrum

Comment: The following information is limited to variants of the *SMN1* gene. *SMN2* gene copy number varies in the normal population from 0 to 3, with ~5–10% of normal individuals having no *SMN2* copy. In the presence of at least one *SMN1* copy, *SMN2* copies do not contribute much to protein expression. Since a homozygous loss of both *SMN* genes is believed to result in embryonic lethality, the presence of a *SMN2* deletion excludes SMA 5q based on a homozygous *SMN1* loss of function mutation. Genetic variants of *SMN1* include whole-gene deletions, single exon deletions, point mutations, genomic rearrangements (http://www.hgmd.cf.ac.uk/ac/index.php), see also databases for muscular dystrophy and motor neuron diseases including *SMN1* mutations (http://grenada.lumc.nl/LSDB_list/lsdbs/SMN1 and http://www.dmd.nl/nmdb2/home.php?select_db=SMN1). For polymorphisms see NCBI accession number NM_000344.3. For SNPs see http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?geneId=6606. For frequency of genotypes in patients see 2.3.

1.6 Analytical methods

PCR (restriction digest).[3] Competitive PCR,[4] real-time PCR on lightCycler[5] or TaqMan basis.[6] Microarray typing for detection of large deletions. Multiplex ligation-dependent probe amplification (MLPA).[7] Sanger sequencing of the total coding region and the exon–intron boundaries of the *SMN1* gene. If an intragenic variant is detected, it is necessary to verify that the variant has occurred in *SMN1* and not *SMN2*. This requires additional testing by a method that facilitates *SMN1*-specific and *SMN2*-specific amplification and sequence analysis, e.g., by long-range PCR protocol[8] or subcloning.[9,10]

1.7 Analytical validation

All mutations identified should be confirmed by a second, independent test (PCR, quantitative PCR, sequencing and MLPA). It is recommended to confirm the segregation of the mutation in the parents. For intragenic missense mutations to be considered pathogenic, they should not be described in the literature in control alleles, should be in evolutionary conserved regions and should be predicted by applicable software to be probably pathogenic. If feasible, patient tissue might be investigated for SMN protein staining.

1.8 Estimated frequency of the disease (Incidence at birth ('birth prevalence') or population prevalence)

Birth prevalence among Caucasians: 1:10.000[11]

1.9 If applicable, prevalence in the ethnic group of investigated person

Birth prevalence is much higher in certain inbred populations.

1.10 Diagnostic setting

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

Comment:

Predictive testing in SMA should be considered on an individual case basis only, as long as no preventive treatment is available. Children at risk should not be tested according to the European guidelines for genetic testing in minors. In addition, it has to be offered with caution, since a small proportion of subjects with homozygous SMN1 deletions/mutations will not develop the disease.

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2. TEST CHARACTERISTICS

	Genotype or disease		A: True positives B: False positives	C: False negative D: True negative
	Present	Absent		
Test				
Positive	A	B	Sensitivity:	A/(A+C)
			Specificity:	D/(D+B)
Negative	C	D	Positive predictive value:	A/(A+B)
			Negative predictive value:	D/(C+D)

2.1 Analytical sensitivity
(**proportion of positive tests if the genotype is present**)

Depending on the ethnic origin and the applied methods:

PCR, restriction digest: >90% (only homozygous *SMN1* exon 7 and 8 deletions)

MLPA/quantitative PCR: 100% (homozygous *SMN1* deletion), heterozygous *SMN1* deletion

MLPA/quantitative PCR plus *SMN1* sequencing: >99% (compound heterozygous *SMN1* deletion/mutation)

Comment: Screening does not detect the extremely rare homozygous point mutations.

2.2 Analytical specificity
(**proportion of negative tests if the genotype is not present**)

PCR, restriction digest: >90%

MLPA/quantitative PCR: >99% for homozygous/heterozygous *SMN1* (and *SMN2)* deletions, no information about rare point mutations

2.3 Clinical sensitivity
(**proportion of positive tests if the disease is present**) Proportions of genotypes among patients with mutations of the SMN1 gene (SMA 5q):

SMN1 homozygous deletion exon 7 and 8: 85–90%

SMN1 homozygous deletion exon 7 only: 5–10%

SMN1 homozygous deletion exon 8 only: exceptionally[12]

SMN1 compound heterozygous deletion exon 7/8 and subtle mutation (intragenic deletion or duplication or point mutation): 2–5%[10,13]

SMN1 homozygous subtle mutation: exceptionally, restricted to consanguineous families[14]

Proportion of patients with the clinical picture of proximal SMA displaying mutations of the SMN1 gene:

The sensitivity for *SMN1* deletion and sequence analysis for the detection of SMA types I–III is probably almost 100% in a clinically well-defined patient group. Those patients with the clinical picture of SMA I–III not showing *SMN1* mutations (non 5q-SMA) represent other genetic and non-genetic entities. The proportion of non 5q-SMA is <2% in SMA I+II, <10% in SMA III, while only few (<10%) of SMA IV patients are caused by *SMN1* mutations.

2.4 Clinical specificity
(**proportion of negative tests if the disease is not present**)

>99% for *SMN1* homozygous deletion/mutation.

90–95% for *SMN2* homozygous deletion.

2.5 Positive clinical predictive value
(**life time risk to develop the disease if the test is positive**)

>99% for homozygous *SMN1* deletions, compound heterozygous *SMN1* deletion/subtle mutation or homozygous subtle mutations. A small fraction (<1%) of individuals with homozygous *SMN1* deletion/mutation will not develop clinical features.

0% for homozygous *SMN2* deletions (according to current knowledge, see 1.5)

2.6 Negative clinical predictive value
(**Probability not to develop the disease if the test is negative**)

Index case in that family had been tested and was positive for a homozygous *SMN1* deletion/mutation:

>99% (for a homozygous *SMN1* deletion/mutation)

Index case in that family had not been tested for *SMN1* deletion/mutation:

>95% for SMA I+II, >90% for SMA III, <10% for SMA IV (for a homozygous *SMN1* deletion/mutation)

3. CLINICAL UTILITY

3.1 (Differential) diagnostics: The tested person is clinically affected
(To be answered if in 1.10 'A' was marked)

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

No	(continue with 3.1.4)	☐
Yes	Only for subdivision of a primary myopathy, neuropathy or anterior horn cell disease.	
	Clinically	☐
	Imaging	☐
	Endoscopy	☐
	Biochemistry	☐
	Electrophysiology	☒
	Other (please describe)	Muscle biopsy

Comment: electrophysiology (electromyography, electroneurography) and muscle biopsy are generally applicable to diagnose anterior horn cell disease.

3.1.2 Describe the burden of alternative diagnostic methods to the patient

Electrophysiology (electromyography, electroneurography) and muscle biopsy are painful and invasive means and will not specify the underlying genetic defect.

3.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?

Genetic testing is much cheaper than clinical neurological work-up including electrophysiology and muscle biopsy.

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

No ☐
Yes ☒

Therapy (please describe)	Prevention of invasive diagnostic procedures or unnecessary interventions/treatments. If a causal treatment becomes available, early genetic diagnosis will be mandatory.
Prognosis (please describe)	SMA I and to a lesser extent SMA II and III have a predictable clinical course on which medical literature data are available, and which can be communicated to the parents at the time of DNA-diagnosis of a homozygous *SMN1* mutation. *SMN2* gene copy number shows an inverse correlation with severity but cannot be used as a predictive measure due to broad overlaps between SMA types.[5] Point mutations in *SMN1* or *SMN2* may have a variable effect on protein expression.
Management (please describe)	Symptomatic management following international recommendations.

3.2 Predictive Setting: The tested person is clinically unaffected but carries an increased risk based on family history
(To be answered if in 1.10 'B' was marked)

3.2.1 Will the result of a genetic test influence lifestyle and prevention?
If the test result is **positive** (please describe) No.
If the test result is **negative** (please describe) No.

3.2.2 Which options in view of lifestyle and prevention does a person at-risk have if no genetic test has been done (please describe)?
None.

3.3 Genetic risk assessment in family members of a diseased person
(To be answered if in 1.10 'C' was marked)

3.3.1 Does the result of a genetic test resolve the genetic situation in that family?
Yes, it confirms the mode of inheritance and is the prerequisite for genetic risk assessment in relatives.

3.3.2 Can a genetic test in the index patient save genetic or other tests in family members?
Yes, in order to clarify the diagnosis and the mode of inheritance. No, as regards genetic risk calculation for unaffected relatives.

Proportion of heterozygous carriers for infantile SMA displaying mutations of the SMN1 gene:

Carrier rates are highest in Caucasian populations (1 in 47) and lowest in African Americans (1 in 72) with a pan-ethnic carrier frequency of 1 in 54.[15] The test sensitivity for heterozygous carriers does not exceed 92–97% because two or more *SMN1* copies are present on about 3–8% of normal chromosomes in most populations apart from African Americans.[15] Alleles containing more than one *SMN1* gene copy mask a deletion of *SMN1* on the other allele. The frequency of these 2-copy *SMN1* alleles varies significantly by ethnicity and is highest in African Americans (27%) and lowest (3–4%) in Caucasians.[15] The risk reduction of a quantitative carrier screening test also depends on the gene frequency in the ethnic group. Altogether all but African Americans have a carrier detection rate exceeding 90%.[15] If a parent shows two *SMN1* copies in the quantitative test, further segregation tests including the grandparents are feasible to determine whether the deletion occurred as a de novo event.

New mutations in SMA occur with a frequency of 0.84% of chromosomes,[11,16] and subtle *SMN1* gene mutations have been observed in about 2–5% of patients, that is, ~2% of disease alleles.[10,13] Given the low incidence of subtle mutations in the general population, it is not advised to exclude those for carrier-risk estimation unless the family history is positive for such a mutation.

For indications of carrier testing, *SMN2* copy number determination does not provide useful additional information. The *SMN2* copy number indicates the total copy number for both alleles, therefore, it is not possible to determine the *SMN2* phase in unaffected individuals.[17]

3.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?
Yes, but it is only exceptionally requested. For limitations see 1.10.

3.4 Prenatal diagnosis
(To be answered if in 1.10 'D' was marked)

3.4.1 Does a positive genetic test result in the index patient enable a prenatal diagnosis?
Yes. It is advised to confirm SMA carrier status in both parents before prenatal diagnosis.

4. 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)

Yes, genetic testing is the gold standard for confirmation of the diagnosis and the mode of inheritance, helps to avoid unnecessary and invasive diagnostic procedures. It allows prognostic evaluations and is the prerequisite for prenatal testing, preimplantation genetic diagnosis and genetic risk estimation of relatives.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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