

## CLINICAL UTILITY GENE CARD

# Clinical utility gene card for: proximal spinal muscular atrophy

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### 1. DISEASE CHARACTERISTICS

#### 1.1 Name of the disease (synonyms)

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

#### 1.2 OMIM# of the disease

253300, 253550, 271150.

#### 1.3 Name of the analysed genes or DNA/chromosome segments

*SMN1* and *SMN2*<sup>2</sup>/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 mutations of the *SMN1* gene. *SMN2* gene copy number varies in the normal population from 0 to 3, with approximately 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. As 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. Mutations of *SMN1* include whole gene deletions, single exon deletions, point mutations and genomic rearrangements. For frequency of mutations see 2.3.

#### 1.6 Analytical methods

PCR (restriction digest),<sup>3</sup> Competitive PCR,<sup>4</sup> real-time PCR on lightCycler<sup>5</sup> or TaqMan basis.<sup>6</sup> Microarray typing for detection of large deletions. Multiplex ligation-dependent probe amplification (MLPA).<sup>7</sup> Sanger sequencing of the total coding region and the exon–intron boundaries of the *SMN1* gene. If an intragenic mutation is detected, it is necessary to verify that the mutation 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, for example, by long-range PCR protocol<sup>8</sup> or subcloning.<sup>9,10</sup>

#### 1.7 Analytical validation

All mutations identified should be confirmed by a second, independent test (PCR, quantitative PCR, sequencing, 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.<sup>11</sup>

#### 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	<input checked="" type="checkbox"/>	<input type="checkbox"/>
B. Predictive testing	<input checked="" type="checkbox"/>	<input type="checkbox"/>
C. Risk assessment in relatives	<input checked="" type="checkbox"/>	<input type="checkbox"/>
D. Prenatal	<input checked="" type="checkbox"/>	<input type="checkbox"/>

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, as 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	C: False negative
				B: False positives	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.<sup>12</sup>

*SMN1* compound heterozygous deletion exon 7/8 and subtle mutation (intragenic deletion or duplication or point mutation): 2–5%.<sup>10,13</sup>

*SMN1* homozygous subtle mutation: exceptionally, restricted to consanguineous families.<sup>14</sup>

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, whereas 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:

>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) diagnosis: 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	<input checked="" type="checkbox"/> (continue with 3.1.4)	<input type="checkbox"/>
Yes	Only for subdivision of a primary myopathy, neuropathy or anterior horn cell disease.	<input type="checkbox"/>
	Clinically	<input type="checkbox"/>
	Imaging	<input type="checkbox"/>
	Endoscopy	<input type="checkbox"/>
	Biochemistry	<input type="checkbox"/>
	Electrophysiology	<input checked="" type="checkbox"/>
	Other (please describe): muscle biopsy	<input type="checkbox"/>

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	<input type="checkbox"/>	
Yes	<input checked="" type="checkbox"/>	
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 <i>SMN1</i> mutation. The <i>SMN2</i> gene copy number shows an inverse correlation with severity, but cannot be used as a predictive measure due to broad overlaps between SMA types. <sup>5</sup> Point mutations in <i>SMN1</i> or <i>SMN2</i> 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, 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:

The test sensitivity for heterozygous carriers does not exceed 95% because two or more SMN1 copies are present on about 5% of normal Caucasian chromosomes.<sup>15</sup> Alleles containing more than one SMN1 gene copy mask a deletion of SMN1 on the other allele. The frequency of these alleles varies significantly by ethnicity and is highest in African Americans (27%) and lowest in Caucasians.<sup>15</sup> 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%.<sup>15</sup> If a parent shows two SMN1 copies in the quantitative test, further segregation tests including the grandparents are feasible to determine whether the deletion found in the patient occurred as a *de novo* event.

New mutations in SMA occur with a frequency of 0.84% of chromosomes,<sup>11,16</sup> and subtle SMN1 gene mutations have been observed in about 2–5% of patients, that is, ~2% of disease alleles.<sup>10,13</sup> 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.<sup>17</sup>

#### 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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