REVIEW

Best practice guidelines for molecular analysis in spinal muscular atrophy†

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With a prevalence of approximately 1/10 000, and a carrier frequency of 1/40 – 1/60 the proximal spinal muscular atrophies (SMAs) are among the most frequent autosomal recessive hereditary disorders. Patients can be classified clinically into four groups: acute, intermediate, mild, and adult (SMA types I, II, III, and IV, respectively). The complexity and instability of the genomic region at chromosome 5q13 harbouring the disease-causing survival motor neuron 1 (SMN1) gene hamper molecular diagnosis in SMA. In addition, affected individuals with SMA-like phenotypes not caused by SMN1, and asymptomatic individuals with two mutant alleles exist. The SMN gene is present in at least one telomeric (SMN1) and one centromeric copy (SMN2) per chromosome in normal (non-carrier) individuals, although chromosomes containing more copies of SMN1 and/or SMN2 exist. Moreover, the two SMN genes (SMN1 and SMN2) are highly homologous and contain only five base-pair differences within their 3′ ends. Also, a relatively high *de novo* frequency is present in SMA. Guidelines for molecular analysis in diagnostic applications, carrier detection, and prenatal analysis using direct and indirect approaches are described. Overviews of materials used in the molecular diagnosis as well as Internet resources are included. *European Journal of Human Genetics* (2001) **9**, 484 – 491

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Description of the disease

The spinal muscular atrophies are a clinically and genetically heterogeneous group of neuromuscular disorders caused by degeneration of anterior horn cells. In proximal spinal muscular atrophy (SMA), the muscles of the extremities closest to the trunk are more severely and earlier affected. SMA is clinically classified into four types: acute, intermediate, mild, and adult (SMA types I, II, III, and IV, respectively).[1,2] Used nomenclature is summarised in Table 1. Criteria for clinical classification of SMA I, II, and III patients are summarised in Table 2.[49] With an estimated incidence of ∼1/10 000,[3,4,46 – 48] and a carrier frequency of 1/40 – 1/60[5,6] the SMAs are among the most frequent autosomal recessive hereditary disorders, and apparently the incidence is similar in different populations studied. The SMA 5q13 region shows a complex structure at the genomic level, including a 500-kilobase pair duplication and inversion.[7] The survival of motor neuron (SMN) gene exists as two homologues, at least one telomeric gene (SMN1) and in the vast majority of chromosomes at least one centromeric gene (SMN2) per chromosome in normal (non-carrier) individuals, although also chromosomes with an intact SMN1 gene exist that lack SMN2.[7] The two SMN genes (SMN1 and SMN2) are highly homologous and contain only five base-pair differences that occur within their 3′ regions.[8] These differences in exons 7 and 8 are used to distinguish SMN1 and SMN2 in DNA analysis. Deletions of the SMN1 gene appear to be directly involved in SMA,[7] since exon(s) 7 (and 8) of SMN1 are undetectable in over 95% of patients, irrespective of their clinical type,[7,9 – 12,14] either as a result of homozygous deletions, or because of conversion of sequences of SMN1 into those of the SMN2 gene.[7,11 – 16] The

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Table 1 Nomenclature and gene ID in SMA

OMIM No	OMIM name	Gene name	GDB ID
253300	Spinal muscular atrophy I; (SMA 1, Werdnig-Hoffmann disease)		
253550	Spinal muscular atrophy II; (SMA 2)		
253400	Spinal muscular atrophy III; (SMA 3; Kugelberg-Welander disease)		
*600354		Survival of Motor Neuron 1, telomeric (SMN1)	GDB:120378 (Gene)
			GDB:4405727 (Syndromic region)

The gene nomenclature that has been accepted by the nomenclature committee is SMN1 for the telomeric (pathogenic) SMN gene and SMN2 for the centromeric (copy) gene.

Table 2 Criteria for clinical classification of SMA patients

Clinical subtype	Age of onset (in months)	Motor milestones	Death (in years)
SMA type I	<6	Never sit unaided	<2
SMA type II	<18	Never walk unaided	>2
SMA type III	>18	Stand unaided	adult

A SMA type IV has been described by Pearn[3]. In this subtype the clinical symptoms start after the age of 30 years in the majority of cases.

identification of several small mutations in the SMN1 gene in patients without a deleted or sequence-converted SMN1 allele[7,17–25,29] provide strong evidence that the SMN1 gene is the primary SMA-determining gene. The clinical phenotype of SMA appears to be partly determined by the underlying mutational mechanism. Most cases of SMA type I result from homozygous gene-deletion events, whereas the milder forms of the disease, SMA types II and III, are more likely to result from gene conversion of SMN1 into SMN2; SMA type II from a gene conversion event in one allele in addition to a hemizygous deletion event in the other allele; in SMA type III from gene conversions in both alleles.[7,13,15,16,26,27] As a result, patients suffering from the milder forms of SMA carry more copies of the SMN2 gene. In the Smn1−/−SMN2 mouse models, multiple copies of human SMN2 seem to prevent SMA development in Smn1-deleted animals (almost) completely.[38,39] The absence of SMN1 exon 7 in SMA patients can be used as a diagnostic tool for SMA,[7,28] with a sensitivity of approximately 95% in clinically strictly defined SMA patients in most populations. Most available assays are not quantitative, however. Based on Hardy-Weinberg equilibrium, the remaining patients (SMA patients without a homozygous SMN1 deletion) are virtually all assumed to have a hemizygous SMN1 absence of at least exon 7, either by deletion or by conversion. Several papers including those by

Table 3 Guidelines for the clinical indications for DNA analysis

Clinical presentation	Test
Any reasonable suspicion of SMA	Homozygous SMN1 exon 7 deletion test
Clinical criteria supporting the diagnosis of SMA types I, II, or III should be in agreement with those described,[40] including characteristic abnormalities in a muscle biopsy	Hemizygous SMN1 exon 7 deletion test/SMN1 subtle mutation screening

McAndrew *et al*,[21] Wirth *et al*,[29] Gerard *et al*[41] and Scheffer *et al*[30] have described quantitative assays based on competitive amplification of SMN1 and SMN2 exon 7 to determine their gene-copy number.

Common reasons for referral
Confirmation of the clinical diagnosis or suspicion of SMA by the detection of homozygous SMN1 exons 7 and exon 8 deletions is fast, inexpensive, and reliable. The clinical sensitivity is around 95%, the clinical specificity is over 99%. Therefore, no restricting clinical criteria are warranted before undertaking DNA analysis. This is certainly so in neonatal cases in which a rapid molecular analysis and diagnosis may render more invasive procedures (ie, a muscle biopsy) unnecessary. A different situation exists when hemizygous SMN1 deletion analysis or subtle mutation analysis is asked for. Our guidelines are summarised in Table 3.

Other reasons for referral include carrier detection by direct hemizygous SMN1 exon 7 deletion analysis and/or indirect linkage analysis. Carrier detection by direct analysis is indicated in siblings and close relatives of SMA patients with a homozyous SMN1 exons 7 and 8 deletion, in parents of SMA patients who died, and in partners of ascertained carriers without positive family history for SMA. Indirect analysis can only be applied in family members of a patient, assuming that an adequate number of family members are available for analysis to establish linkage phase. Direct analysis is strongly recommended in these cases.

A third indication for DNA analysis is prenatal analysis. This is often requested by parents of a SMA patient, and may also be indicated for couples who have been shown to be carriers of a mutant SMN1 allele, or for couples of an affected patient with SMA with an identified carrier, which is not a rare situation in SMA type III.

Approaches
General strategy
1 Appropriate patients selected for molecular analysis.
2 Confirmation of clinical diagnosis by analysis of homozygous SMN1 exon 7 deletion.

3 Before embarking on hemizygous SMN1 deletion analysis in cases without homozygous deletion, additional clinical information including muscle biopsy is requested according to criteria summarised in Table 3.

4 Heterozygous deletion analysis by dosimetric SMN1 exon 7 test. If a patient, fulfilling the clinical criteria of SMA, is found heterozygous, there is a 95% chance that (s)he will be compound heterozygous for a deletion and a (subtle) mutation (see below).

5 Subtle mutation analysis (optional; see below).

6 Carrier detection by dosimetric SMN1 exon 7 test and/or linkage analysis in relatives. Prior to dosimetric analysis in relatives testing of parents of the homozygously SMN1 deleted index case is strongly recommended (cf. section SMN1 Exon 7 Dosage Analysis and Carrier Testing).

7 Carrier testing in partners of ascertained carriers by dosimetric SMN1 exon 7 test.

8 Prenatal analysis by homozygous SMN1 exon 7 deletion analysis or by a combination of (subtle) mutation analysis and linkage analysis. Some laboratories confirm the results by linkage analysis using markers flanking SMN1. Exclusion by microsatellite analysis of an interfering degree of admixture of maternal cells in the foetal tissue is obligatory.

Confirmation of clinical diagnosis

The ability to detect deletions in SMN1 means that testing to confirm a diagnosis of SMA in affected individuals is possible. The presence of a homozygous deletion of exons 7 and 8 is consistent with a diagnosis of SMA given that there are clinical grounds for suspecting SMA. The absence of a homozygous SMN1 deletion does not rule out a diagnosis of SMA since approximately 6% of cases are known not to have a homozygous SMN1 deletion, a proportion of those SMA patients will have a subtle mutation in one or extremely rarely in both SMN1 alleles instead.[45] In routine SMN1 testing, a DNA-laboratory often has no or only scarce clinical data on a given patient. In that case, whenever a homozygous SMN1 deletion is not found in a patient, one should doubt the diagnosis of SMA. The presence of a hemizygous SMN1 exon 7 deletion identified by a quantitative determination of the number of SMN1 exon 7 copies may provide further support for the diagnosis of SMA (appr. 95–96% of SMA patients have at least a heterozygous SMN1 deletion). Nevertheless, formal confirmation of the clinical diagnosis in those cases can only be obtained after identification of subtle SMN1 mutation(s).

The clinical subtype of SMA appears to be determined (in part) by the nature of the SMN1 mutations. In general, SMA type I is associated with deletions in both alleles, SMA type 2 with a deletion in one, and a gene conversion in the other allele, and SMA type 3 with gene conversions in both alleles. The molecular identification of absence of exon 7 in both alleles of SMN1 in the presence of one or two copies of SMN1 exon 8 is indicative for such gene conversions. However,

these data, when available, can at present not be used to predict the clinical course of the disease.

Carrier testing

Several papers including those by McAndrew *et al*,[21] Wirth *et al*,[29] Gerard *et al*[41] and Scheffer *et al*[30] have described quantitative assays based on competitive amplification of SMN1 and SMN2 exons 7 to determine their gene-copy number. These techniques have now been implemented in a number of service labs for carrier detection.

SMN1 *de novo* deletions occur at a frequency of approximately 2% of SMA patients, the majority being of paternal origin.[6,32] Testing of parents of homozygously deleted SMA patients may give additional information on *de novo* deletions. It has been shown in the majority of cases that these *de novo* deletions are associated with a meiotic recombination or meiotic gene conversion. Hence the recurrence risk in such particular families would theoretically not be elevated. However, Campbell *et al*[50] reported on possible germinal mosaicism in SMA, which would lead to an elevated recurrence risk. In practice, these data should lead to prenatal diagnosis despite evidence of a *de novo* mutation.

A complication/pitfall in the dosimetric analysis of SMN1 exon 7 is the occurrence of two (or more) SMN1 genes on a single chromosome 5 in about 4%, as observed by McAndrew *et al*,[21] Wirth *et al*[35] and others. This would bear on the sensitivity of this technique for carrier identification, since individuals with an SMN1 [1,1] genotype (a single SMN1 gene on each chromosome; non-carriers) cannot be distinguished from individuals with an SMN1 [0,2] genotype (both SMN1 genes on a single chromosome; carriers). Also, in carrier testing of individuals without a positive family history for SMA, eg, testing of partners of known carriers, it should be taken into account that mutant non-deletion SMN1 alleles (approximately 2.5% of mutant SMN1 alleles) will not be detected. The consequences for the sensitivity and specificity of carrier testing by a dosimetric method have been discussed.[29,30]

Since the frequency of carriers with two SMN1 genes per chromosome is 4%, compared to only 1% of parents being non-carrier due to a *de novo* mutation, no direct conclusions should be drawn from apparently normal results in carrier testing in parents of affected individuals. This problem can be resolved by the identification of a recombination event associated with the *de novo* mutation by indirect analysis, eg, by using the multicopy markers Ag1-CA and C212[32] or other flanking markers. Alternatively, the occurrence of a *de novo* deletion can be confirmed by normal SMN1 dosage in the parents of the apparent non-carrier parent, ie, two of the grandparents of the affected child. When two SMN1 genes on a single chromosome (opposite to a chromosome lacking an intact SMN1 gene) cause the normal SMN1 dosage in the carrier parent, one grandparent will have an SMN1 dosage compatible with a single SMN1 gene. The other grandparent will have an SMN1 dosage compatible with three SMN1

genes. When the normal dosage in the parent is caused by a *de novo* mutational event in the affected child, both grand-parents will have normal SMN1 dosage.

Quantitative SMN1 exon 7 analysis should be restricted to families in which a homozygous SMN1 deletion has been identified in the index case, in order to prevent performing dosage analysis in relatives of patients with a SMA type phenotype not caused by SMN1. An exception could be made in parents of a deceased patient with a clinically quite clear diagnosis of SMA.

Carrier testing in relatives of SMA patients, eg, siblings and/or uncles and aunts could also be performed by indirect analysis using flanking markers. A prerequisite is the availability of sufficient DNA samples from family members for establishing linkage phase and informativity of the markers.

Prenatal testing
Prenatal analysis is carried out preferably by direct mutation detection, eg, by testing for a homozygous SMN1 exon 7 deletion. A prerequisite is the identification of a homozygous deletion in the index patient.

Homozygous deletions of SMN1 exons 7 and 8 in unaffected haplo-identical sibs of SMA patients have been described.[10,11,31] Parents of a SMA patient may actually have a homozygous SMN1 deletion themselves. Although it is hard to predict whether the parental SMN1 deletion allele that had not been transmitted to the affected child may have a pathogenic effect at all, it is our opinion that it cannot be considered simply as a non-risk SMN1 allele. Such an allele should be dealt with as an allele with a potential pathogenic effect.

Prenatal testing based on linkage analysis is useful in families with a clinically ascertained patient with proximal SMA without a homozygous SMN1 deletion. It is recommended that this approach should only be used when a hemizygous SMN1 deletion has been identified in the index case. An exception could be made in special cases with a clinically quite clear diagnosis of SMA and consanguinity of the parents, making homozygosity for a rare nondeletion mutant SMN1 allele in the index case more likely. Prenatal analysis in families with non-deleted patients should generally not be performed. Flanking markers may also be used to confirm the results of deletion analysis in prenatal testing, and to exclude interfering admixture of maternal cells in the foetal sample. Some laboratories prefer the use of markers from other chromosomes for exclusion of maternal admixture from foetal tissue to circumvent unwanted information about the foetal carrier status.

Linkage analysis
Linkage analysis is useful for carrier testing if quantitative deletion analysis is not feasible.

In postnatal applications, linkage-based testing of un-affected siblings, or preferably the grand-parents of the index case, may be useful to identify a possible meiotic recombination associated with a *de novo* deletion or rearrangement involving SMN1. Also, uniparental isodisomy may be identified by the use of flanking markers.

Some laboratories have the policy to include unaffected siblings in a linkage analysis only when necessary, in order to avoid problems with the identification of carriers in young children. However, this depends on the local situation.

Technical protocols
Analysis of homozygous SMN1 deletions
Homozygous deletions of exons 7 and 8 of SMN1 can be detected by several methods including:

1. SSCP – either on polyacrylamide (8% on 12 cm gels works well) or MDE gels.[7] Primer sequences and PCR conditions are summarised in the section 'Primer Sequences'. The deletion can be visualised by silver staining of the gels as well as by radioactivity or fluorescence.[42] SSCP has the advantage that subtle mutations in the gene may be detected as well as deletions. However, the method will also identify neutral polymorphisms. A study from Wang *et al*[31] has shown that 4% of asymptomatic family members carry a polymorphic variant of exon 7 of SMN1 (telomeric) which looks like a homozygous deletion on SSCP gels.

2. Restriction enzyme digestion.[28,29] For exon 7 the enzymes *Dra*I or *Hinf*I could be used to separate SMN1 and SMN2 in combination with specific primers, for exon 8 *Dde*I can be used. Primer sequences and PCR conditions are summarised in the section 'Primer sequences'. These are rapid assays, and have the additional advantage that the pathogenic absence of SMN1 exons 7 and 8 is very specifically tested for.

Gene conversion can be detected by SSCP analysis, dependent on local laboratory conditions used. The restriction digest method does not differentiate between a true deletion and a gene conversion, but correctly determines absence of SMN1 exons 7 and 8 from their normal genomic position.

Some laboratories prefer to confirm exon 7 only deletions by a second, independent test (in the majority of cases absence of both exons 7 and 8 is detected; these test results confirm each other). Testing for SMN1 exon 7 is the most accurate test, and some laboratories consider testing for SMN1 exon 8 being redundant. Testing for SMN1 exon 8 only may lead to a false negative result.

It is recommended that samples from patients known to have a homozygous SMN1 deletion (positive reference samples) as well as samples known to contain intact SMN1 allele(s) (negative reference samples) are always digested and run in parallel on gels to monitor completeness of the

digestion, in addition to a no template control reaction to exclude carry-over contamination.

Dosage analysis of hemizygous SMN1 deletions

A number of quantitative assays based on competitive amplification of SMN1 and SMN2 exons 7 to determine their gene-copy number have been described.[21,29,30,42] Primer sequences and PCR conditions are summarised in the section 'Primer Sequences'. Technical details and pitfalls can be found in the original papers by McAndrew *et al*,[21] Wirth *et al*,[29] Gerard *et al*[41] and Scheffer *et al*.[30] The latter system makes use of fluorescently labelled primers, enabling the analysis to be performed on an automated sequencer. Reference samples with known SMN1 exon 7 copy number should be included on the gels. These assays make use of internal standard plasmids that are added to the reaction mixture for monitoring of the efficiency of the amplification. For availability of these standards and internal controls, the corresponding authors of the respective papers should be contacted.

New PCR technology, eg, based on real time PCR (eg, Taqman or FRET technologies), allows the quantification of the PCR during the course of the reaction. Applications for SMA are currently under development.

Since in clinical DNA service practice the majority of the tests used are currently based on qualitative analyses, it is strongly recommended that implementation of a quantitative SMN1 assay should be preceded by a thorough validation of sensitivity and specificity by the laboratory wishing to undertake this testing.

Linkage analysis

A number of informative microsatellite markers flanking the SMA gene are summarised in Table 4.[33–37] It should be possible to find an informative proximal and distal marker in the majority of cases so that the overall error based on linkage with flanking markers is <1%.

There are also multi-copy markers including C212 and C272 (Ag1-CA), which lie near the 5′ end of SMN1.[6,43,44] These can also be used although the interpretation of alleles requires considerable experience.

SMN1 subtle mutation analysis

SMN1 subtle mutation analysis is beyond the scope of current routine DNA service for SMA. In cases where analysis of subtle mutations is necessary, laboratories offering this facility (eg, Bonn, Groningen) should be contacted. In these cases, the clinical diagnosis should meet the criteria formulated in Table 3.

Primer sequences

The primer sequences are taken from the references indicated. Individuals should consult the appropriate reference for full instructions and information on primer use. While every effort has been made to ensure the accuracy of

Table 4 Genetic markers flanking the SMN1 gene at 5q11.2-q13.3

Locus	Location	Recombination	PIC	Size
D5S679	Proximal	3	0.63	180–222
D5S680	Proximal	3	0.57	141–173
D5S125	Proximal	2	0.38	143 and 147
D5S681	Proximal	2	0.68	142–156
D5S435	Proximal	1	0.68	128–144
D5S629	Proximal	1	0.81	233–253
D5S823	Proximal	1	0.54	128–150
D5S1556/ D5F150	Intragenic/SMN1 promoter region	0	0.98	90–122
D5S149	Intragenic/SMN1 promoter region	0	0.99	166–203
D5S557	Distal	1	0.46	148–172
D5S610	Distal	1	0.80	106–124
D5S351	Distal	1	0.74	196–234
5′-MAP1B	Distal	1	0.76	~100
3′-MAP1B	Distal	1	0.72	212–226
D5S112	Distal	3	0.74	94–120
D5S127	Distal	5	0.84	96–114
D5S39	Distal	7	0.74	212–220

Primer sequences, PCR conditions, etc. can be found in the Genome Database (http://gdbwww.gdb.org).

the primer sequences, individual users should confirm these sequences before use.

SMN1/2 exon 7 and exon 8 amplification for SSCP

Lefebvre et al[7] Exon 7: R111: 5′-AGACTATCAACTTAATTT-CTGATCA-3′ 541C770: 5′-TAAGGAATGTGAGCACCTTCCT-TC-3′ (Modified by Matthijs *et al*.[42] into SMA7B: 5′-TCCTTAATTTAAGGAATGTGAGCA-3′); Exon 8: 541C960: 5′-GTAATAACCAAATGCAATGTGAA-3′; 541C1120: 5′-CTA-CAACACCCTTCTCACAG-3′

SMN1/2 exon 7 homozygous deletion tests using restriction enzymes *Dra*I or *Hin*fI

Van der Steege et al[28] R111 is used as forward primer. X7-Dra: 5′-CCTTCCTTCTTTTTGATTTTGTTT-3′; *Dra*I will cleave SMN2 exon 7 specifically.

Wirth et al[29] SMN7F: 5′-CTTCCTTTTATTTTCCTTACAGG-GATT-3′; SMN7R: 5′-TCCACAAACCATAAAGTTTTAC-3′; The SMN1 exon 7 product will contain two *Hin*fI sites; the SMN2 exon 7 product will contain one *Hin*fI site.

SMN1 exon 8 homozygous deletion test using *Dde*I

541C960 and 541C1120 are used as forward and reverse primers, respectively. *Dde*I will cleave SMN2 exon 8 specifically.

SMN1 exon 7 dosage analysis

McAndrew et al[21] R111 and X7-Dra are used as forward and reverse primers, respectively. The reference PCR product from the CFTR gene is amplified using CF621F: 5′-AGTCAC-CAAAGCAGTACAGC-3′ as forward primer, and CF621R: 5′-

GGGCCTGTGCAAGGAAGTGTTA-3′ as reverse primer. For monitoring the efficiency of the PCR, cloned DNA of a SMN and a CFTR internal standard are added to the multiplex reaction.

Wirth et al[29] SMN7F and SMN7R are used as forward and reverse primers, respectively. The reference PCR product is amplified using CF621F and CF621R. For monitoring the efficiency of the PCR, cloned DNA of a SMN and a CFTR internal standard are added to the multiplex reaction.

Scheffer et al[30] R111 (fluorescently labelled) and X7-Dra are used as forward and reverse primers, respectively. The reference product from the RB1 gene is amplified using Rbex13F: 5′-ATTACACAGTATCCTCGACA-3′; RBex13R (fluorescently labelled): 5′-TATACGAACTGGAAAGATGC-3′. For monitoring the efficiency of the PCR, cloned DNA of a SMN and an RB1 internal standard are added to the multiplex reaction.

Internet resources

http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?600354 (OMIM entry for SMN1)

http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?253300 (OMIM entry for SMA1)

http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?253550 (OMIM entry for SMA2)

http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?253400 (OMIM entry for SMA3)

http://bioinfo.weizmann.ac.il/cards-bin/cardsearch.pl?search=SMA (Genecards for SMA and SMN1 and SMN2)

http://gdbwww.gdb.org/gdb-bin/genera/genera/hgd/Gene?!key=GDB%3A120378&!sub=0 (SMA entry in Genome Database)

ftp://ftp.genethon.fr/pub/Gmap/Nature-1995/data/data_chrom5 (Genethon genetic map of chromosome 5)

http://www.ncbi.nlm.nih.gov/genemap/map.cgi?MAP=GB4&BIN=180&MARK=2660 (Genemap 99 section including SMN1)

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=PubMed (PubMed search page)

http://archive.uwcm.ac.uk/uwcm/mg/search/5215173.html (Human Gene Mutation Database entry for SMN1)

http://www.neuro.wustl.edu/neuromuscular/synmot.html#sma5q

Appendix 1

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References

1 Emery AE: The nosology of spinal muscular atrophies. *J Med Genet* 1971; **8**: 481–495.
2 Dubowitz V: Muscle disorders in childhood. Philadelphia, Saunders Co, 1978.
3 Thieme A, Mitulla B, Schulze F, Spiegler AW: Epidemiological data on Werdnig-Hoffmann disease in Germany (West-Thuringen). *Hum Genet* 1993; **91**: 295–297.
4 Thieme A, Mitulla B, Schulze F, Spiegler AW: Chronic childhood spinal muscular atrophy in Germany (West-Thuringen) – an epidemiological study. *Hum Genet* 1994; **93**: 344–346.
5 Pearn J: Classification of spinal muscular atrophies. *Lancet* 1980; **i**: 919–922.
6 Melki J, Lefebvre S, Bürglen, L *et al*: De novo and inherited deletions of the 5q13 region in spinal muscular atrophies. *Science* 1994; **264**: 1474–1477.
7 Lefebvre S, Bürglen L, Reboullet S *et al*: Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* 1995; **80**: 155–165.
8 Bürglen L, Lefebvre S, Clermont O *et al*: Structure and organization of the human survival motor neurone (SMN) gene. *Genomics* 1996; **32**: 479–482.
9 Bussaglia E, Clermont O, Tizzano E *et al*: A frameshift deletion in the survival motor neuron gene in Spanish spinal muscular atrophy patients. *Nature Genet* 1995; **11**: 335–337.
10 Cobben JM, van der Steege G, Grootscholten P, de Visser M, Scheffer H, Buys CHCM. Deletions of the survival motor neuron gene in unaffected siblings of patients with spinal muscular atrophy. *Am J Hum Genet* 1995; **57**: 805–808.
11 Hahnen E, Forkert R, Marke C *et al*: Molecular analysis of candidate genes on chromosome 5q13 in autosomal recessive spinal muscular atrophy: evidence of homozygous deletions of the SMN gene in unaffected individuals. *Hum Molec Genet* 1995; **4**: 1927–1933.
12 Velasco E, Valero C, Valero A, Moreno F, Hernandez-Chico C: Molecular analysis of the SMN and NAIP genes in Spanish spinal muscular atrophy (SMA) families and correlation between number of copies of cBCD541 and SMA phenotype. *Hum Mol Genet* 1996; **5**: 257–263.
13 Hahnen E, Schonling J, Rudnik-Schoneborn S, Zerres K, Wirth B: Hybrid survival motor neuron genes in patients with autosomal recessive spinal muscular atrophy: new insights into molecular mechanisms responsible for the disease. *Am J Hum Genet* 1996; **59**: 1057–1065.
14 Rodrigues NR, Owen N, Talbot K, Ignatius J, Dubowitz V, Davies KE: Deletions in the survival motor neuron gene on 5q13 in autosomal recessive spinal muscular atrophy. *Hum Mol Genet* 1995; **4**: 631–634.
15 van der Steege G, Grootscholten PM, Cobben JM *et al*: Apparent gene conversions involving the SMN gene in the region of the spinal muscular atrophy locus on chromosome 5. *Am J Hum Genet* 1996; **59**: 834–838.
16 DiDonato CJ, Chen X-N, Noya D, Korenberg JR, Nadeau JH, Simard LR: Cloning, characterization, and copy number of the murine survival motor neuron gene: homolog of the spinal muscular atrophy-determining gene. *Genome Res* 1997; **7**: 339–352.
17 Brahe C, Clermont O, Zappata S, Tiziano F, Melki J, Neri G: Frameshift mutation in the survival motor neuron gene in a severe case of SMA type I. *Hum Mol Genet* 1996; **5**: 1971–1976.
18 Bürglen L, Seroz T, Miniou P *et al*: The gene encoding p44, a subunit of the transcription factor TFIIH, is involved in large-scale deletions associated with Werdnig-Hoffmann disease. *Am J Hum Genet* 1997; **60**: 72–79.
19 Parsons DW, McAndrew PE, Monani UR, Mendell JR, Burghes WHM, Prior TW: An 11 base pair duplication in exon 6 of the SMN gene produces a type I spinal muscular atrophy (SMA) phenotype: further evidence for SMN as the primary SMA-determining gene. *Hum Mol Genet* 1996; **5**: 1727–1732.
20 Hahnen E, Schonling J, Rudnik-Schoneborn S, Zerres K, Wirth B: Missense mutations in exon 6 of the survival motor neuron gene in patients with spinal muscular atrophy. *Hum Mol Genet* 1997; **6**: 821–825.
21 McAndrew PE, Parsons DW, Simard LR *et al*: Identification of proximal spinal muscular atrophy carriers and patients by analysis of SMNT and SMNC gene copy number. *Am J Hum Genet* 1997; **60**: 1411–1422.
22 Rochette CF, Surh LC, Ray PN *et al*: Molecular diagnosis of non-deletion SMA patients using quantitative PCR of SMN exon 7. *Neurogenetics* 1997; **1**: 1: 141–147.

23 Talbot K, Ponting PC, Theodosiou AM *et al*: Missense mutation clustering in the survival motor neuron gene: a role for a conserved tyrosine and glycine rich region of the protein in RNA metabolism? *Hum Mol Genet* 1997; **6**: 497–500.

24 Parsons DW, McAndrew PE, Allison PS, Parker Jr WD, Burghes WHM, Prior TW: Diagnosis of spinal muscular atrophy in an SMN non-deletion patient using a quantitative PCR screen and mutation analysis. *J Med Genet* 1998; **35**: 674–676.

25 Wang CH, Papendick BD, Bruinsma P, Day JK: Identification of a novel missense mutation of the SMNt gene in two siblings with spinal muscular atrophy. *Neurogenetics* 1998; **1**: 273–276.

26 Burghes AHM: When is a deletion not a deletion? When it is converted. *Am J Hum Genet* 1997; **61**: 9–15.

27 Campbell L, Potter A, Ignatius J, Dubowitz V, Davies K: Genomic variation and gene conversion in spinal muscular atrophy: implications for disease process and clinical phenotype. *Am J Hum Genet* 1997; **61**: 40–50.

28 van der Steege G, Grootscholten PM, van der Vlies P *et al*: PCR-based DNA test to confirm clinical diagnosis of autosomal recessive spinal muscular atrophy [letter]. *Lancet* 1995; **345**: 985–986.

29 Wirth B, Herz M, Wetter A *et al*: Quantitative analysis of Survival Motor Neuron Copies: Identification of Subtle SMN1 Mutations in Patients with Spinal Muscular Atrophy, Genotype-Phenotype Correlation, and Implications for Genetic Counseling. *Am J Hum Genet* 1999; **64**: 1340–1356.

30 Scheffer H, Cobben JM, Mensink RGJ, Stulp RP, van der Steege G, Buys CHCM: SMA carrier detection: Validation of hemizygous SMN exon 7 deletion test for the identification of proximal spinal muscular atrophy carriers and patients with a single allele deletion. *Eur J Hum Genet* 2000; **8**: 79–86.

31 Wang CH, Xu Y, Carter TA *et al*: Characterisation of survival motor neuron (SMNT) gene deletions in asymptomatic carriers of spinal muscular atrophy. *Hum Mol Genet* 1996; **5**: 359–365.

32 Wirth B, Schmidt T, Hahnen E *et al*: De novo rearrangements found in 2% of index patients with spinal muscular atrophy: mutational mechanisms, parental origin, mutation rate, and implications for genetic counseling. *Am J Hum Genet* 1997; **61**: 1102–1111.

33 Daniels *et al*: Prenatal prediction of spinal muscular atrophy. *J Med Genet* 1992; **29**: 165–170.

34 Morrison *et al*: Two novel microsatellite markers for prenatal prediction of spinal muscular atrophy. *Hum Genet* 1993; **92**: 133–138.

35 Wirth *et al*: Large linkage analysis in 100 families with autosomal recessive spinal muscular atrophy (SMA) and 11 CEPH families using 15 polymorphic loci in the region 5q11.2-q13.3. *Genomics* 1994; **20**: 84–93.

36 Burghes *et al*: Linkage mapping of the spinal muscular atrophy gene. *Hum Genet* 1994; **93**: 305–312.

37 Brahe *et al*: Mapping of two new markers within the smallest interval harboring the spinal muscular atrophy locus by family and radiation hybrid analysis. *Hum Genet* 1994; **93**: 494–501.

38 Monani UR, Sendtner M, Coovert DD *et al*: The human centromeric survival motor neuron gene (SMN2) rescues embryonic lethality in Smn(−/−) mice and results in a mouse with spinal muscular atrophy. *Hum Mol Genet* 2000; **9**: 333–339.

39 Hsieh-Li HM, Chang JG, Jong YJ *et al*: A mouse model for spinal muscular atrophy. *Nat Genet* 2000; **24**: 66–70.

40 Munsat TL, Davies KE: Meeting report: International SMA Consortium Meeting. *Neuromuscul Disord* 1992; **2**: 423–428.

41 Gerard B, Ginet N, Matthijs G *et al*: Genotype determination at the survival motor neuron locus in a normal population and SMA carriers using competitive PCR and primer extension. *Hum Mutat* 2000; **16**: 253–263.

42 Matthijs G, Schollen E, Legius E *et al*: Unususal molecular findings in autosomal recessive spinal muscular atrophy. *J Med Genet* 1996; **33**: 469–474.

43 DiDonato C, Morgan K, Carpten JD *et al*: Association between Ag1-CA alleles and severity of autosomal recessive proximal spinal muscular atrophy. *Am J Hum Genet* 1994; **55**: 1218–1229.

44 Wirth B, Hahnen E, Morgan K *et al*: Allelic association and deletions in autosomal recessive proximal spinal muscular atrophy. Association of marker genotype with disease severity and candidate cDNAs. *Hum Mol Genet;* **4**: 1273–1284.

45 Wirth B: An update of the mutation spectrum of the survival motor neuron gene (SMN1) in autosomal recessive spinal muscular atrophy (SMA). *Hum Mutat* 2000; **15**: 228–237.

46 Pearn J: The gene frequency of acute Werdnig-Hoffmann disease (SMA type I). A total population survey in North-East England. *J Med Genet* 1973; **10**: 260–265.

47 Pearn J: Incidence, prevalence and gene frequency of chronic childhood spinal muscular atrophy. *J Med Genet* 1978; **15**: 409–413.

48 Czeizel A, Hamula J: A Hungarian study on Werdnig-Hoffmann disease. *J Med Genet* 1989; **26**: 761–763.

49 Munsat TL: Workshop report – International SMA collaboration. *Neuromusc Disord* 1991; **1**: 81.

50 Campbell L, Daniels RJ, Dubowitz V, Davies KE: Maternal mosaicism for a second mutational event in a type I spinal muscular atrophy family. *Am J Human Genet* 1998; **63**: 37–44.