An update of the mutation spectrum of the Survival Motor Neuron gene (SMN1) in autosomal recessive spinal muscular atrophy...

Article *in* Human Mutation · March 2000

DOI: 10.1002/(SICI)1098-1004(200003)15:3<228::AID-HUMU3>3.0.CO;2-9 · Source: PubMed

CITATIONS
369

READS
80

1 author:

Brunhilde Wirth
University of Cologne
199 PUBLICATIONS **8,699** CITATIONS

SEE PROFILE

HUMAN MUTATION 15:228–237 (2000)

MUTATION UPDATE

An Update of the Mutation Spectrum of the Survival Motor Neuron Gene (SMN1) in Autosomal Recessive Spinal Muscular Atrophy (SMA)

Brunhilde Wirth*

Institute of Human Genetics, Bonn, Germany

Communicated by Richard G.H. Cotton

Spinal muscular atrophy (SMA) is characterized by degeneration of motor neurons in the spinal cord, causing progressive weakness of the limbs and trunk, followed by muscle atrophy. SMA is one of the most frequent autosomal recessive diseases, with a carrier frequency of 1 in 50 and the most common genetic cause of childhood mortality. The phenotype is extremely variable, and patients have been classified in type I–III SMA based on age at onset and clinical course. All three types of SMA are caused by mutations in the survival motor neuron gene (SMN1). There are two almost identical copies, SMN1 and SMN2, present on chromosome 5q13. Only homozygous absence of SMN1 is responsible for SMA, while homozygous absence of SMN2, found in about 5% of controls, has no clinical phenotype. Ninety-six percent of SMA patients display mutations in SMN1, while 4% are unlinked to 5q13. Of the 5q13-linked SMA patients, 96.4% show homozygous absence of SMN1 exons 7 and 8 or exon 7 only, whereas 3.6% present a compound heterozygosity with a subtle mutation on one chromosome and a deletion/gene conversion on the other chromosome. Among the 23 different subtle mutations described so far, the Y272C missense mutation is the most frequent one, at 20%. Given this uniform mutation spectrum, direct molecular genetic testing is an easy and rapid analysis for most of the SMA patients. Direct testing of heterozygotes, while not trivial, is compromised by the presence of two SMN1 copies per chromosome in about 4% of individuals. The number of SMN2 copies modulates the SMA phenotype. Nevertheless, it should not be used for prediction of severity of the SMA. Hum Mutat 15:228–237, 2000.

KEY WORDS: **mutation spectrum; survival motor neuron genes; SMN1; SMN2; spinal muscular atrophy; SMA**

INTRODUCTION

Spinal muscular atrophy (SMA) is a group of autosomal recessive neuromuscular disorders characterized by degeneration of the anterior horn cells of the spinal cord, leading to symmetrical muscle weakness and atrophy. SMA represents the second most common fetal autosomal recessive disorder after cystic fibrosis. The disorder is subdivided into three clinical groups (types I–III SMA) on the basis of clinical onset and maximum motor function [International SMA Consortium, 1992; Zerres and Rudnik-Schöneborn, 1995]. Patients with type I SMA disease (Werdnig-Hoffmann, MIM# 253300) show onset at birth or before six months, and usually die of respiratory insufficiency within two years. Type I SMA patients are never able to sit or walk. Patients with type II SMA (intermediate form, MIM# 253550) show onset after 6 months. They can sit but are never able to walk unaided, and life expectancy is significantly reduced. Type III SMA (Kugelberg-Wlander disease, MIM# 253400) patients show first symptoms after 18 months and are able to stand and walk, but often become wheelchair-bound during youth or adulthood. The gene involved in type I–III SMA has been mapped to 5q12-q13 by linkage analysis, and refined to a region of about 750 kb [Brzustowicz et al., 1990; Melki

Received 27 September 1999; accepted revised manuscript 16 November 1999.

*Correspondence to: PD Dr. Brunhilde Wirth, Institute of Human Genetics, Wilhelmstr. 31, D-53111 Bonn, Germany. E-mail: bwirth@uni-bonn.de

Contract grant sponsor: Deutsche Forschungsgemeinschaft.

et al., 1990a, b; Gilliam et al., 1990; Wirth et al., 1995a]. The region contains a large inverted duplication consisting of at least four genes, which are present in a telomeric (t) and a centromeric (c) copy: survival motor neuron gene (SMN1 or SMNt and SMN2 or SMNc); neuronal apoptosis inhibitory protein gene (NAIP and ψNAIP); basal transcription factor subunit p44 (BTFp44t and BTFp44c); and a novel protein with unknown function H4F5t and H4F5c [Lefebvre et al., 1995; Roy et al., 1995; Bürglen et al., 1997; Carter et al., 1997; Scharf et al., 1998] (Fig. 1A). Although homozygous deletions encompassing all these genes are found in SMA patients, it is now well established that mutations or deletions of SMN1 (MIM# 600354) cause the disease, while the number of SMN2 (MIM# 601627) copies modulates the SMA phenotype [Lefebvre et al., 1998].

Type IV SMA (the adult form) seems to be heterogeneous: in few cases homozygous absence of SMN1 has been described, whereas most cases were 5q13-unlinked [Brahe et al., 1995; Clermont et al., 1995; Zerres et al., 1995; Wirth, unpublished results].

SMN GENES

There are two almost identical copies, SMN1 and SMN2, within the 5q13 region [Lefebvre et al., 1995]. Each SMN gene encompasses 27 kb including nine exons (1, 2a, 2b, 3–8) [Bürglen et al., 1996a; Chen et al., 1997]. The ubiquitously expressed SMN transcript is 1.7 kb and codes for a protein consisting of 294 amino acids [Lefebvre et al., 1995]. Sequencing of the complete genomic region containing SMN1 and SMN2, respectively, revealed a 99% homology between the two copies [Monani et al., 1999]. SMN1 can be distinguished from SMN2 by only five nucleotide differences: one in intron 6, one in exon 7, two in intron 7 and one in exon 8. The C-to-T transition in exon 7 is a translationally silent nucleotide exchange, and the one in exon 8 lies within the 3′ untranslated region (Fig. 1B, Table 3) [Lefebvre et al., 1995; Bürglen et al.,1996a; Monani et al., 1999]. The full length cDNAs of the two genes are identical except for the two nucleotide exchanges in exons 7 and 8, yet SMN1 produces a majority of full-length SMN1 while SMN2 produces primarily transcripts lacking exon 7 (~60%) and minor amounts of full-length transcripts (~40%) [Lefebvre et al., 1995; Gennarelli et al., 1995]. It has been recently demonstrated that the C-to-T transition in exon 7 disrupts a putative exonic splicing enhancer (ESE), causes alternative splicing of SMN2 exon 7, and is thus the molecular defect responsible for SMA [Lorson et al., 1999]. Although the two SMN copies encode identical pro-

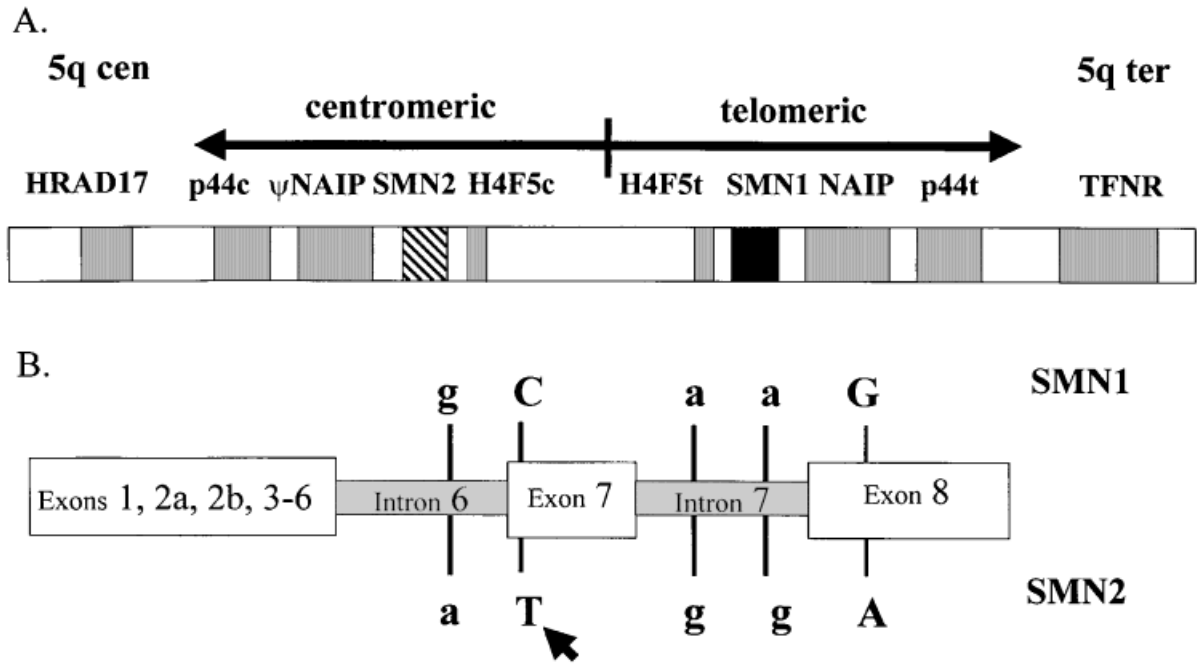

FIGURE 1. **A:** Schematic representation of the inverted and duplicated SMA region (5q13), including four duplicated genes: H4F5, SMN, NAIP, and BTFp44. The region is flanked by HRAD17 proximal [von Deimling et al., 1999] and TFNR (Kelter and Wirth, unpublished results). **B:** Localization of the nucleotides by which SMN1 can be distinguished from SMN2.

teins, SMN2 mainly produces a protein with a different carboxy terminus due to exon 7 skipping. Exons 6 and 7 of the SMN gene contain a functional relevant domain responsible for the self-oligomerization of the SMN protein, and thus exon skipping or mutations within this region lead to reduced self-association [Lorson et al., 1998]. This explains why the SMA phenotype correlates with the copy number of SMN2. The more SMN2 copies a patient has, the more full-length SMN2 protein is present and the milder is the SMA phenotype [Lefebvre et al., 1995; Burghes 1997; Campbell et al., 1997; Wirth et al., 1999]. However, other unknown factors must modify the SMA phenotype, since affected and unaffected sibs with homozygous deletions of SMN1 and identical haplotypes have been described in rare cases [Cobben et al., 1995; Hahnen et al., 1995; Wang et al., 1996; Wirth et al., 1999].

The SMN protein is a 38 kDa polypeptide which is ubiquitously expressed, but is found in especially high levels in the spinal motor neurons (Lefebvre et al., 1997; Coovert et al., 1997). The SMN protein level is significantly reduced in tissues derived from type I SMA patients, and moderately reduced in tissues derived from type II and III SMA patients compared to controls [Lefebvre et al., 1997; Coovert et al., 1997]. SMN plays an essential role in spliceosomal snRNP biogenesis and pre-mRNA splicing [Liu et al., 1997; Fischer et al., 1997; Pellizzoni et al., 1998]. Together with SIP1 (an SMN-interacting protein), SMN1 is involved in the assembly of the snRNP components [Liu et al., 1997; Fischer et al., 1997]. Both SMN and SIP1 are expressed in the cytoplasm and in distinct compartments of the nucleus called gems [Liu and Dreyfuss, 1996]. In accordance with the important function within the cell, SMN knock-out mice are early embryonic lethal [Schrank et al., 1997]. Given the important function of SMN as a housekeeping gene, it is still questionable why the absence of SMN1 causes only degeneration of spinal motor neurons but not of other cells. A possible answer is an interaction of SMN with a neuron-specific protein. Recently, Giesemann et al. [1999] described such an interaction between SMN and the neuron specific profilin II (PFNII), a protein which binds to proline-rich regions.

MUTATIONS AND POLYMORPHISMS IN SMN1/SMN2

The nucleotide differences between the SMN copies in exons 7 and 8 are used for the molecular diagnosis of SMA. The homozygous absence of SMN1 can be easily determined by PCR of exon 7 followed by DraI digest, which cuts SMN2 [van der Steege et al., 1995], or Hinf I digest, which cuts SMN1 [Wirth et al., 1999], and by PCR of exon 8 followed by DdeI digest, which cuts SMN2 [van der Steege et al., 1995]. Alternatively, the SMN copies can be distinguished by SSCA [Lefebvre et al., 1995].

Exon 7 and 8, or only exon 7, of the SMN1 gene are homozygously absent in 94% of SMA patients (Table 1). There is a slightly higher frequency of homozygous absence of SMN1 in types I and II (96% and 94%, respectively) as compared to type III SMA (86%). Nevertheless, the disease mechanism responsible for the homozygous absence of SMN1 in types I–III SMA is different (see below).

In rare cases subtle mutations in SMN1 are responsible for the disease (Table 2, Fig. 2). It is difficult to calculate an overall frequency of rare mutations from the literature data, because the number of identified mutations was not given relative to the total number of clinically well characterized SMA patients. From a large study carried out in 525 typical SMA patients, we identified homozygous absence of SMN1 in 92%, subtle mutations in 3.4%, and no mutation in 4.6% [Wirth et al., 1999].

Based on the high frequency of homozygous absence of SMN1 found in SMA patients, and according to Hardy-Weinberg equilibrium, 99.7% of all SMA patients must carry at least one SMN1 deletion on one chromosome. This means that the non-deletion, non-consanguineous SMA patients should be compound heterozygous, carrying a deletion on one chromosome and one subtle mutation on the other one. Consequently, quantitative tests have been developed which enable the determination of the SMN1 copy number in non-deletion SMA patients [McAndrew et al., 1997; Wirth et al., 1999]. Non-deletion SMA patients should show only one SMN1 copy in the quantitative tests. The same tests allow the identification of carriers of SMA, except for 4% of carriers who have two SMN1 copies per chromosome and thus cannot be detected [McAndrew et al., 1997; Wirth et al., 1999].

The identification of subtle mutations is complicated by the presence of SMN2. Our strategy to identify subtle mutations in non-consanguineous families is: 1) Quantitative SMN1 testing of the patient and the parents. The patient and one parent should carry only one SMN1 copy. 2) Haplotype studies with Ag1-CA and C212, two multicopy polymorphic markers localized at the 5′-end

TABLE 1. Frequency of Hmozygous Absence of the SMN1 Gene Found in Type I-III SMA Patients

Reference	Type of SMA	Homozygous absence of SMN1 exons (%)		
		Exons 7 and 8	Exon 7 only	Non-deleted
Lefebvre et al., 1995	I-III	93 (213/229)[a]	6(13/229)	1 (3/229)
Rodrigues et al., 1995	I	96 (49/51)	2(1/51)	2 (1/51)
	II	93 (54/58)	5(3/58)	2 (1/58)
	III	84 (26/31)	13(4/31)	3 (1/31)
Cobben et al., 1995	I	92 (45/49)	0 (0/49)	8 (4/49)
	II	88 (30/34)	6 (2/34)	6 (2/34)
	III	90 (18/20)	5 (1/20)	5 (1/20)
Hahnen et al., 1995;	I	91 (247/270)	7 (18/270)	7 (11/270)
Wirth et al., 1999;	II	83 (103/124)	10 (13/124)	7 (8/124)
Wirth, unpublished results	III	68 (89/131)	15 (19/131)	18 (23/131)
Velasco et al., 1996	I	96 (27/28)	4 (1/28)	0 (0/28)
	II	79 (22/28)	4 (1/28)	18 (5/28)
	III	89 (8/9)	11 (1/19)	0 (0/9)
Simard et al., 1997	I	68 (19/22)	18 (1/22)	9 (2/22)
	II	100 (13/13)	0 (0/13)	0 (0/13)
	III	72 (18/25)	4 (1/25)	24 (6/25)
Total for each type of SMA	I[b]	91 (379/418)	5 (21/418)	4 (18/418)
	II[b]	87 (222/257)	7 (19/257)	6 (16/257)
	III[b]	74 (159/216)	12 (26/216)	14 (31/216)
Total for all types of SMA	I, II and III	87 (975/1122)	7 (79/1122)	6 (68/1122)

[a]213/229 means 213 of 229 cases.
[b]These sums exclude the data reported by Lefebvre et al., [1995] since no frequencies have been given for each type of SMA.

of the SMN genes. Identical mutations were found on identical haplotypes (founder effect), and can thus give a hint to the particular mutation. 3) Sequencing of the complete SMN1 coding region from cloned RT-PCR products [Wirth et al., 1999].

Direct sequencing of genomic DNA is inappropriate due to unequally higher amounts of SMN2 copies. Direct sequencing of cDNA is inadequate not only because of unequal amounts of SMN1/SMN2, but also due to the lack of exons 3, 5, and 7 in alternatively spliced transcripts. Furthermore, any mutation found must be assigned to the correct SMN copy, which makes subcloning and sequencing of SMN1 absolutely necessary. SSCP analysis of each exon is more time-consuming; not all mutations can be identified; and sequencing for the exact mutation and assignment to the correct SMN copy must be carried out anyway.

There are 23 different subtle mutations published so far, which include non-sense, frameshift and missense mutations, deletions, inversions and splice site mutations (Table 2, Fig. 2). The Y272C and 813ins/dup11 mutations are the most frequently occurring mutations (17% (9/53) and 13% (7/53), respectively) among all subtle mutations. In the German population the Y272C missense mutation accounts for 37% (7/19) of all rare mutations. It has been shown that most of the missense mutations are localized within a highly conserved region of exons 6 and 7 [Talbot et al., 1997; Hahnen et al., 1997; Wirth et al., 1999], and that these mutations reduce the self-oligomerization capacity of the SMN protein [Lorson et al.,1998].

Homozygous absence of SMN2, a condition found in about 3–5 % of control individuals, has no clinical consequences [Lefebvre et al.,1995]. Each viable individual has at least an SMN1 or an SMN2 copy. Due to its function in the snRNP biogenesis and pre-mRNA splicing, SMN is an important housekeeping gene, and its absence is lethal [Schrank et al., 1996].

There are 31 polymorphisms reported so far: twelve in the promotor, one in intron 1 [Monani et al., 1999], one in exon 2a [Hahnen and Wirth, 1996], one in exon 3 [Brahe et al., 1996], and sixteen in intron 6 [Monani et al., 1999] (Table 3).

DISEASE MECHANISMS

The SMA region contains a 500 kb duplication and inversion in the majority of control individuals. This repeated unit can vary from 0–4 copies per chromosome, however, which confers high instability and leads to deletions and gene conversion within the region. The high de novo mutation frequency found in 2% of SMA patients is caused mainly by unequal crossing-over between the repeated units during paternal meiosis. This usually leads to deletions of variable sizes, including at least SMN1, Ag1-CA, C212, and in some cases NAIP [Wirth et al., 1997]. Gene conversion of SMN1 into SMN2, a second mechanism responsible for the absence of SMN1, has been described as a de novo event in an SMA patient [Wirth et al., 1997,

TABLE 2. Subtle Mutations Within the SMN1 Gene Described in SMA Patients

Type of mutation	Exon (E)/ Intron (I)	Mutations of SMN1	Nucloetide change	Type of SMA	No. of patients	Reference
Deletions	E8	Deletion of exon 8 only		II, III	2	Gambardella et al., 1998
	I4-I6	6.4 kb Alu-mediated deletion including exons 5 and 6		I	2	Wirth et al., 1999
Nonsense mutations	E1	Q15X	78 C→T	I, III	2	Wirth et al., 1999
Frameshift mutations	E2a	124insT	ATTCT→ATTTCT	I	1	Wirth et al., 1999
	E2b	241-242in4	ACCT→ACGTGTCT	III	1	Wirth et al., 1999
	E3	430del4	ATAGAGAG→ATAG	I-III	4	Bussaglia et al., 1995
	E3	472del5	GATGAAGTG→GATG	I	1	Brahe et al., 1996
	E4	542delGT	AAGTGA→AAGA	I-III	3	Parsons et al., 1998a, 1998b
	E4	591delA	CAAAT→CAAT	II	1	Wirth et al., 1999
	E4	618insT	CCTCCA→CCTTCCA	I	1	Clermont et al., 1997; Lefebvre, personal communication
	E6	813ins/dup11	ATGCTGATGCTTG→A TGCTGATGCTTTGCTG ATGCTTG	I, II	7	Parsons et al., 1996; Parsons et al., 1998b; Clermont et al., 1997; Lefebvre, personal communication
Splice site mutations	I6	c.868-11del7	aacttccttattttccttacagGG →aactttntccttacagGG	I	1	Lefebvre et al., 1995, and personal communication
	I7	c.922+3del4	GAgtaagtct→GAgtat	II	1	Lefebvre et al., 1995, and personal communication
	I7	c.922+6T→G	GAgtaagtct→GAgtaaggct	III	1	Wirth et al., 1999
Missense mutations	E1	A2G	38 C→G	II, III	3	Parsons et al., 1998b
	E3	E134K	433 G→A	I	1	Clermont et al., 1997; Lefebvre, personal communication
	E6	P245L	767 C→T	III	1	Rochette et al., 1997
	E6	S262I	818 G→T	III	2	Hahnen et al., 1997; McAndrew et al., 1997
	E6	Y272C	848 A→G	I-III	9	Lefebvre et al., 1995; Rochette et al., 1997; Wirth et al., 1999; Herchenbach and Wirth, unpublished results
	E6	T274I	854 C→T	II, III	4	Hahnen et al., 1997; Wirth et al., 1999; Parsons et al., 1998
	E6	G275S	856 G→A	III	1	Bürglen et al., 1996b
	E7	G279C	868 G→T	II, III	2	Wang et al., 1998
	E7	G279V	869 G→T	I	2	Talbot et al., 1997

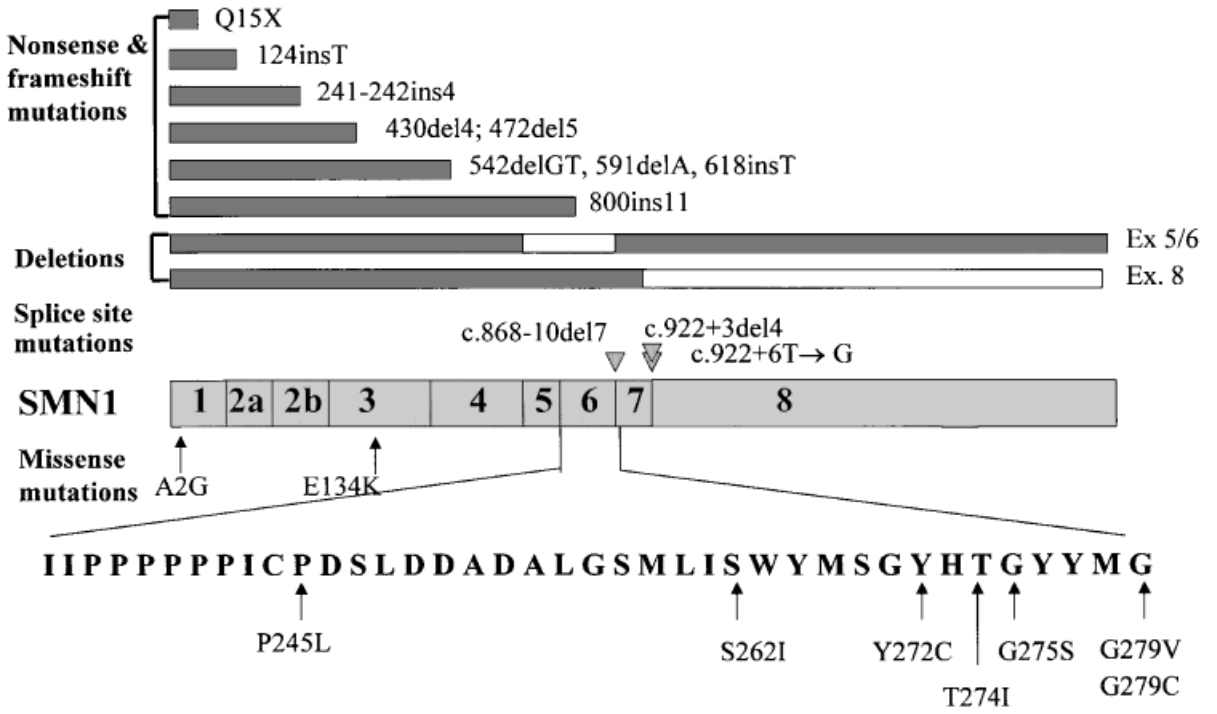

FIGURE 2. **Distribution of subtle mutations showing an effect on SMN1 cDNA in SMA patients. Polymorphisms are listed for both SMN1 and SMN2 in Table 3.**

1999]. This encompasses either the complete SMN1 gene or part of it, resulting in hybrid SMN genes which usually have origin in exon 7 of SMN2 and exon 8 of SMN1 [Lefebvre et al.,1995; Bussaglia et al., 1995; Hahnen et al., 1996; van der Steege et al., 1996; Wirth et al., 1999]. Gene conversion leads to an increase of SMN2 copies, as determined by quantitative analysis and pulse field gel analysis [Campbell et al.,1997; McAndrew et al., 1997; Burghes, 1997; Wirth et al., 1999].

While the majority of type I SMA patients carry real homozygous deletions of SMN1, the majority of type II and III SMA patients show homozygous absence of SMN1 as a result of gene conversion of SMN1 into SMN2, leading to an increase of 3–4 copies of SMN2 (Fig. 3). A strong correlation between SMN2 copy number, also reflected by the Ag1-CA and C212 copy number/alleles and the severity of SMA, has been shown by various studies using different methods [Melki et al., 1994; DiDonato et al.,1994; Wirth et al., 1995b, 1999; Campbell et al., 1997; McAndrew et al., 1997; Burghes, 1997]. But since this is not a strict correlation, it should not be used for predictions of the severity of SMA.

DIAGNOSTIC RELEVANCE

SMA can be quickly and reliably diagnosed due both to identification of SMN1 as the determinant gene for SMA, and discovery of the various mutations responsible for SMA (which in 94% of cases are homozygous absence of SMN1). In most cases, therefore, invasive muscle biopsy can be avoided. Furthermore, the SMN1 deletion test allows identification of SMA patients who would not otherwise be diagnosed on the basis of clinical and pathological examination [Bürglen et al., 1995; 1996c; Devriendt et al., 1996; Rudnik-Schöneborn et al., 1996; Korinthenberg et al., 1997; Omran et al., 1998]. Quantitative testing of SMN1 copies can be used to identify SMA patients heterozygous for the deletion of SMN1 who are likely to carry a subtle mutation on the other chromosome. Furthermore, the quantitative SMN1 deletion tests allow identification of 96% of SMA carriers. No reliable prediction of the type of SMA can be made by means of molecular genetic analysis.

Future Prospects

As mentioned above, recent findings revealed that alternative splicing of exon 7 of SMN2 is caused by a single nucleotide exchange that disrupts an exonic splicing enhancer within exon 7. Since each SMA patient has at least one SMN2 copy, one can envisage a therapy based on up-regulation of the SMN2 full-length transcript. Compared to other inherited diseases where an

TABLE 3. Polymorphisms Identified in Both SMN1 and SMN2

Position	Nucleotide position	Polymorphism	Reference
Promotor	–3366	–/G	Monani et al., 1999
	–2052	C/A	Monani et al., 1999
	–2020	C/T	Monani et al., 1999
	–1990	C/T	Monani et al., 1999
	–1805	C/G	Monani et al., 1999
	–1438	A/T	Monani et al., 1999
	–1427	C/G	Monani et al., 1999
	–1317	C/G	Monani et al., 1999
	–1155	G/A	Monani et al., 1999
	–893	A/G	Monani et al., 1999
	–769	GAG/–	Monani et al., 1999
	–318	GCC/–	Monani et al., 1999
Intron 1	+8451	T/C	Monani et al., 1999
Exon 2a	+14035 (codon 28)[a]	C/T	Hahnen and Wirth, 1996
Exon 3	+17739 (codon 154)	A/G	Brahe et al., 1996
Intron 6	+21851	G/T	Monani et al., 1999
	+22872	A/G	Monani et al., 1999
	+23117	G/A	Monani et al., 1999
	+23505	A/G	Monani et al., 1999
	+25239	T/C	Monani et al., 1999
	+25379	G/A	Monani et al., 1999
	+25381	T/C	Monani et al., 1999
	+25519	G/A	Monani et al., 1999
	+25683	G/A	Monani et al., 1999
	+25729	C/G	Monani et al., 1999
	+26156	G/A	Monani et al., 1999
	+26236	–/AGGCA	Monani et al., 1999
	+26287	A/C	Monani et al., 1999
	+26587	G/A	Monani et al., 1999
	+26658	T/C	Monani et al., 1999
	+26769	C/A	Monani et al., 1999
	+27092[b]	G/A	Bürglen et al., 1996a
Exon 7	+27141 (codon 280)[b]	C/T	Lefebvre et al., 1995
Intron 7	+27289[b]	A/G	Bürglen et al., 1996a
	+27404[b]	A/G	Bürglen et al., 1996a
Exon 8	+27869[b]	G/A	Lefebvre et al., 1995

[a]This polymorphism has been described only for SMN2.
[b]Nucleotide difference (no polymorphism) which distinguishes SMN1 from SMN2 (see also Fig. 1).

incorrect or no protein is produced and scientists are eagerly trying to reintroduce the gene by gene therapy, the gene is present in the case of SMA but is inefficiently expressed. Therapy directed towards up-regulation of full-length SMN2 might therefore be a useful strategy.

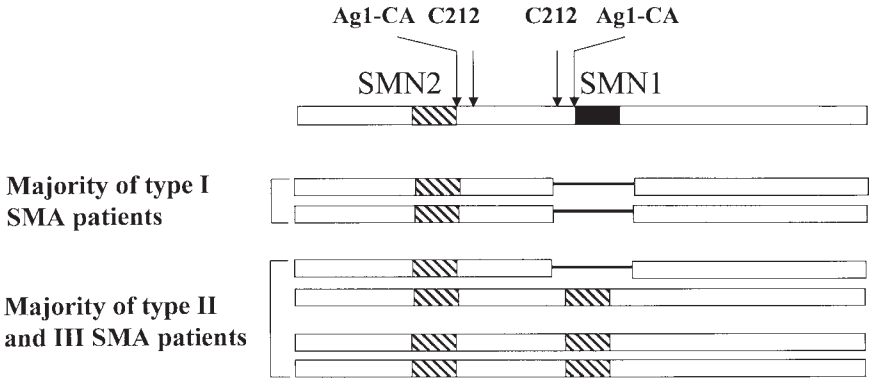

FIGURE 3. Schematic representation of the most frequently observed SMA chromosomes in acute SMA (type I) and mild SMA (type II/III). While type I SMA chromosomes usually carry true SMN1 deletions on both 5q13 homologs, type II and III SMA chromosomes carry either on one or on both 5q13-homologs an increased SMN2 copy number caused by gene conversion of SMN1 into SMN2.

ACKNOWLEDGMENTS

I would like to thank all members of my lab and all collaborators for their great help and hard work. I am grateful to all members of the International SMA Consortium for helpful discussions, and to the European Neuromuscular Center, Families of SMA, and Muscular Dystrophy Association of America for organizing and supporting scientific SMA meetings.

REFERENCES

Brahe C, Servidei S, Zappata S, Ricci E, Tonali P, Neri G. 1995. Genetic homogeneity between childhood-onset and adult-onset autosomal recessive spinal muscular atrophy. Lancet 346:741–742.

Brahe C, Clermont O, Zappata S, Tiziano F, Melki J, Neri G. 1996. Frameshift mutation in the survival motor neuron gene in a severe case of SMA type I. Hum Mol Genet 5:1971–1976.

Brzustowicz LM, Lehner T, Castilla LH, Penchaszadeh GK, Wilhelmsen KC, Daniels R, Davies KE, Leppert M, Ziter F, Wood D, Dubowitz V, Zerres K, Hausmanowa-Petrusewicz I, Ott J, Munsat TL, Gilliam TC. 1990. Genetic mapping of chronic childhood-onset spinal muscular atrophy to chromosome 5q11.2-13.3. Nature 344:540–541.

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

Bürglen L, Spiegel R, Ignatius J, Cobben JM, Landrieu P, Lefebvre S, Munnich A, Melki J. 1995. SMN gene deletion in variant of infantile spinal muscular atrophy. Lancet 1995 346:316–317.

Bürglen L, Lefebvre S, Clermont O, Burlet P, Viollet L, Cruaud C, Munnich A, Melki J. 1996a. Structure and organization of the human survival motor neurone (SMN) gene. Genomics 32:479–482.

Bürglen L, Patel S, Dubowitz V, Melki J, Muntoni F. 1996b. A novel point mutation in the SMN gene in a patient with type III spinal muscular atrophy. First Congress of the World Muscle Society 1996, Elsevier, S39.

Bürglen L, Amiel J, Viollet L, Lefebvre S, Burlet P, Clermont O, Raclin V, Landrieu P, Verloes A, Munnich A, Melki J. 1996c. Survival motor neuron gene deletion in the arthrogryposis multiplex congenita-spinal muscular atrophy association. J Clin Invest 98:1130–1132.

Bürglen L, Seroz T, Minou P, Lefebvre S, Burlet P, Munnich A, Pequignot EV, Egly JM, Melki J. 1997. 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 60:72–79.

Bussaglia E, Clermont O, Tizzano E, Lefevbre S, Bürglen L, Cruaud C, Urtizberea JA, Colomer J, Munnich A, Baiget M, Melki J. 1995. A frame-shift deletion in the survival motor neuron gene in Spanish spinal muscular atrophy patients. Nature Genet 11:335–337.

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

Carter TA, Bönnemann CG, Wang CH, Obici S, Parano E, Fatima Bonaldo de M, Ross BM, Penchaszadeh GK, Mackenzie A, Soares MB, Kunkel LM, Gilliam TC. 1997. A multicopy transcription-repair gene, BTF2p44, maps to the region and demonstrates SMA associated deletions. Hum Mol Genet 6:229–236.

Chen Q, Baird SD, Mahadevan M, Besner-Johnston A, Farahani R, Xuan JY, Kang X, Lefebvre C, Jkeda JE, Korueluk RG, Mackenzie AE. 1998. Sequence of a 131-kb region of 5q13.1 containing the spinal muscular atrophy candidate genes SMN and NAIP. Genomics 48:121–127.

Clermont O, Burlet P, Lefebvre S, Burglen L, Munnich A, Melki J. 1995. SMN gene deletions in adult-onset spinal muscular atrophy. Lancet 346:1712–1713.

Clermont O, Burlet P, Cruaud C, Bertrandy S, Melki J, Munnich A, Lefebvre S. 1997. Mutation analysis of the SMN gene in undeleted SMA patients. Annual meeting of the American Society of Human Genetics. Am J Hum Genet 61:A329.

Cobben JM, van der Steege G, Grootscholten P, Visser de M, Scheffer H, Buys CHCM. 1995. Deletions of the survival motor neuron gene in unaffected siblings of patients with spinal muscular atrophy. Am J Hum Genet 57:805–808.

Coovert DD, Le TT, McAndrew PE, Strasswimmer J, Crawford TO, Mendell JR, Coulson SE, Androphy EJ, Prior TW, Burghes AH. 1997. The survival motor neuron protein in spinal muscular atrophy. Hum Mol Genet 6:1205–1214.

Devriendt K, Lammens M, Schollen E, Van Hole C, Dom R, Devlieger H, Cassiman JJ, Fryns JP, Matthijs G. 1996. Clinical and molecular genetic features of congenital spinal muscular atrophy. Ann Neurol 40:731–738.

DiDonato CJ, Morgan K, Carpten JD, Fuerst P, Ingraham SE, Prescott G, McPherson JD, Wirth B, Zerres K, Hurko O, Wasmuth JJ, Mendell JR, Burghes AHM, Simard LR. 1994. Association between Ag1-CA alleles and severity of autosomal recessive proximal spinal muscular atrophy. Am J Hum Genet 55:1218–1229.

Fischer U, Liu Q, Dreyfuss G. 1997. The SMN-SIP1 complex has an essential role in spliceosomal snRNP biogenesis. Cell 90:1023–1029.

Gambardella A, Mazzei R, Toscano A, Annesi G, Pasqua A, Annesi I, Oliveri RL, Valentino P, Bono F, Aguglia U, Zappia M, Vita G, Quatlroue A. 1998. Spinal muscular atrophy due to an isolated deletion of exon 8 of the telomeric survival motor neuron gene. Ann Neurol 44:836–839.

Gennarelli M, Lucarelli M, Capon F, Pizzuti A, Merlini L, Angelini C, Novelli G, Dallapiccola B. 1995. Survival motor neuron gene transcript analysis in muscles from spinal muscular atrophy patients. Biochem Biophys Res Commun 213:342–348.

Giesemann T, Rathke-Hartlieb S, Rothkegel M, Bartsch JW, Buchmeier S, Jockusch BM, Jockusch H. 1999. A role for polyproline motifs in the spinal muscular atrophy SMN: Profilins bind to and colocalize with SMN in nuclear gems. J Biol Chem 32:37908–37914.

Gilliam TC, Brzustowicz LM, Castilla LH, Lehner T, Penchaszadeh GK, Daniels RJ, Byth BC, Knowles J, Hislop JE, Shapira Y, Dubowitz V, Munsat TL, Ott J, Davies KE. 1990. Genetic homogeneity between acute and chronic forms of spinal muscular atrophy. Nature 345:823–825.

Hahnen E, Forkert R, Marke C, Rudnik-Schöneborn S, Zerres K, Wirth B. 1995. 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 Mol Genet 4:1927–1933.

Hahnen E, Wirth B. 1996. Frequent DNA variant in exon 2a of the survival motor neuron gene (SMN): a further possibility for distinguishing the two copies of the gene. Hum Genet 98:122–123.

Hahnen E, Schönling J, Rudnik-Schöneborn S, Zerres K, Wirth B. 1996. 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 59:1057–1065.

Hahnen E, Schönling J, Rudnik-Schöneborn S, Raschke H, Zerres K, Wirth B. 1997. Missense mutations in exon 6 of the sur-

vival motor neuron gene in patients with spinal muscular atrophy (SMA). Hum Mol Genet 6:821–825.

International SMA Consortium. 1992. Meeting report: International SMA Consortium Meeting. Neuromusc Disord 2:423–428.

Korinthenberg R, Sauer M, Ketelsen UP, Haneman CO, Stoll G, Graf M, Baborie A, Volk B, Wirth B, Rudnik-Schöneborn S, Zerres K. 1997. Congenital axonal neurophathy caused by deletions in the SMA region. Ann Neurol 42:364–368.

Lefebvre S, Bürglen L, Reboullet S, Clermont O, Burlet P, Viollet L, Benichou B, Cruaud M, Millasseau P, Zeviani M, LePaslier D, Frezal J, Cohen D, Weissenbach J, Munnich A, Melki J. 1995. Identification and characterization of the spinal muscular atrophy determining gene. Cell 80:155–165.

Lefebvre S, Burlet P, Liu Q, Bertrandy S, Clermont O, Munnich A, Dreyfuss G, Melki J. 1997. Correlation between severity and SMN protein level in spinal muscular atrophy. Nat Genet 16:265–269

Lefebvre S, Burglen L, Frezal J, Munnich A, Melki J. 1998. The role of the SMN gene in proximal spinal muscular atrophy. Hum Mol Genet 7:1531–1536.

Liu Q, Dreyfuss G. 1996. A novel nuclear structure containing the survival of motor neuron protein. EMBO J 15:3555–3565.

Liu Q, Fischer U, Wang F, Dreyfuss G. 1997. The spinal muscular atrophy disease gene product, SMN, and its associated protein SIP1 are in a complex with spliceosomal snRNP proteins. Cell 90:1013–1021.

Lorson CL, Strasswimmer J, Yao L-M, Baleja J, Hahnen E, Wirth B, Le T, Burghes A, Androphy EJ. 1998. SMN oligomerization defect correlates with spinal muscular atrophy severity. Nature Genet 19:63–67.

Lorson CL, Hahnen E, Androphy EJ, Wirth B. 1999. A single nucleotide in the SMN gene regulates splicing and is responsible for spinal muscular atrophy. Proc Natl Acad Sci USA 96(11):6307–6311.

McAndrew PE, Parsons DW, Simard LR, Rochette C, Ray PN, Mendell J, Prior TW, Burghes AH. 1997. Identification of proximal spinal muscular atrophy carriers and patients by analysis of SMNt and SMNc gene copy number. Am J Hum Genet 60:1411–1422.

Melki J, Abdelhak S, Sheth P, Bachelot MF, Burlet P, Marcadet A, Aicardi J, Barois A, Carriere JP, Fardeau M, Fontan D, Ponsot G, Billette T, Angelini C, Barbosa C, Ferriere G, Lanzi G, Ottolini A, Babton MC, Cohen D, Hanauer A, Clerget-Darpoux F, Lathrop M, Munnich A, Frezal J. 1990a. Gene for chronic proximal spinal muscular atrophies maps to chromosomes 5q. Nature 344:767–768.

Melki J, Sheth P, Abdelhak S, Burlet P, Bachelot MF, Lathrop MG, Frezal J, Munnich A. 1990b. Mapping of acute (type 1) spinal muscular atrophy to chromosome 5q12-q14. Lancet 336:271–273.

Melki J, Lefebvre S, Burglen L, Burlet P, Clermont O, Millasseau P, Reboullet S, Benichou B, Zeviani M, Le Paslier D, Cohen D, Weissenbach J, Munnich A. 1994. De novo and inherited deletions of the 5q13 region in spinal muscular atrophies. Science 264:1474–1477.

Monani UR, Lorson CL, Parsons DW, Prior TW, Androphy EJ, Burghes AHM, McPherson JD. 1999. A single nucleotide difference that alters splicing patterns distinguishes the SMA gene SMN1 from the copy gene SMN2. Hum Mol Genet 8:1177–1183.

Omran H, Ketelsen U-P, Heinen F, Sauer M, Rudnik-Schöneborn S, Wirth B, Zerres K, Kratzer W, Korinthenberg R. 1998. Axonal neurophathy and predominance of type II myofibres in SMA I. J Child Neurol 13:327–331.

Parsons DW, McAndrew PE, Monani UR, Mendell JR, Burghes AHM, Prior TW. 1996. An 11 base pair duplication in exon 6 of the SMN gene produces a type I spinal muscular atrophy (SMA) phenotype: further evidence for the SMN as the primary SMA-determining gene. Hum Mol Genet 11:1727–1732.

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

Parsons DW, McAndrew PE, Iannaccoue ST, Mendell JR, Burghes AHM, Prior TW. 1998b. Intragenic telSMN mutations: frequency, distribution, evidence of a founder effect, and modification of the spinal muscular atrophy phenotype by ceuSMN copy number. Am J Hum Genet 63:1712–1723.

Pellizzoni L, Kataoka N, Charroux B, Dreyfuss G. 1998. A novel function for SMN, the spinal muscular atrophy disease gene product, in pre-mRNA splicing. Cell 95:615–624.

Rochette CF, Surh LC, Ray PN, McAndrew PE, Prior TW, Burghes AHM, Vanasse M, Simard LR. 1997. Molecular diagnosis of non-deletion SMA patients using quantitative PCR of SMN exon 7. Neurogenet 1:141–147.

Rodrigues NR, Owen N, Talbot K, Ignatius J, Dubowitz V, Davies KE. 1995. Deletions in the survival motor neuron gene on 5q13 in autosomal recessive spinal muscular atrophy. Hum Mol Genet 4:631–634.

Roy N, Mahadevan MS, McLean M, Shutler G, Yaraghi Z, Farahani R, Baird S, Besner-Johnston A, Lefebvre C, Kang X, Salih M, Aubry H, Tamai K, Guan X, Jannou P, Crawford TO, de Joug PJ, Surh L, Jkeda JE, Korueluk RG, MacKenzie A. 1995. The gene for neuronal apoptosis inhibitory protein (NAIP), a novel protein with homology to baculoviral inhibitors of apoptosis, is partially deleted in individuals with type 1, 2 and 3 spinal muscular atrophy (SMA). Cell 80:167–178.

Rudnik-Schöneborn S, Forkert R, Hahnen E, Wirth B, Zerres K. 1996. Clinical spectrum and diagnostic criteria of spinal muscular atrophy: further delineation on the basis of SMN gene deletion findings. Neuropediatrics 27:8–15.

Scharf JM, Endrizzi MG, Wetter A, Huang S, Thompson TG, Zerres K, Dietrich WF, Wirth B, Kunkel LM. 1998. Identification of a candidate modifying gene for spinal muscular atrophy by comparative genomics. Nature Genet 20:83–86.

Schrank B, Götz R, Gunnersen JM, Ure JM, Toyka KV, Schmith AG, Sendtner M. 1997. Inactivation of the survival motor neuron gene, a candidate gene for human spinal muscular atrophy, leads to massive cell death in early mouse embryos. Proc Natl Acad Sci USA 94:9920-9925.

Simard LR, Rochette C, Semionov A, Morgan K, Vanasse M. 1997. SMN(T) and NAIP mutations in Canadian families with spinal muscular atrophy (SMA): genotype/phenotype correlations with disease severity. Am J Med Genet 72:51–58.

Talbot K, Ponting CP, Theodosiou AM, Rodrigues NR, Surtees R, Mountford R, Davies KE. 1997. 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 6:497–500.

Velasco E, Valero C, Valero A, Moreno F, Hernandez-Chico C. 1996. 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 5:257–263.

van der Steege G, Grootscholten PM, van der Vlies P, Draaijers TG, Osinga J, Cobben JM, Scheffer H, et al. 1995. PCR-based DNA test to confirm clinical diagnosis of autosomal recessive spinal muscular atrophy. Lancet 345:985–986.

van der Steege G, Grootscholten PM, Cobben M, Zappata S, Scheffer H, den Dunneu JT, van Ommen GJB, Brahe C, Buys CHCM. 1996. Apparent gene conversion involving the SMN

gene in the region of the spinal muscular atrophy locus on chromosome 5. Am J Hum Genet 58:834–838.

von Deimling F, Scharf J, Liehr T, Rothe M, Kelter A-R, Albers P, Dietrich W, Kunkel L, Wernert N, Wirth B. 1999. Human and mouse *RAD17* genes: identification, localization, genomic structure and histological expression pattern in normal testis and seminoma. Hum Genet 105:17–27.

Wang CH, Xu J, Carter TA, Ross BM, Dominski MK, Bellcross CA, Penchaszadeh GK, Munsat TL, Gilliam TC. 1996. Characterization of survival motor neuron (SMNt) gene deletions in asymptomatic carriers of spinal muscular atrophy. Hum Mol Genet 5:359–365.

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

Wirth B, El-Agwany A, Baasner A, Burghes A, Koch A, Dadze A, Piechaczek-Wappenschmidt B, Rudnik-Schöneborn S, Zerres K, Schönling J. 1995a. Mapping of the spinal muscular atrophy (SMA) gene to a 750 kb interval flanked by two new microsatellites. Eur J Hum Genet 3:56–60.

Wirth B, Hahnen E, Morgan K, DiDonato CJ, Dadze A, Rudnik-Schöneborn S, Simard LR, Zerres K, Burghes AHM. 1995b. Allelic association and deletions in autosomal recessive proximal spinal muscular atrophy: association of marker genotype with disease severity and candidate cDNAs. Hum Mol Genet 4:273–1284.

Wirth B, Schmidt T, Hahnen E, Rudnik-Schöneborn S, Krawczak M, Müller-Myhsok B, Schönling J, Zerres K. 1997. De novo rearrangements found in 2% index patients with spinal muscular atrophy (SMA): mutational mechanisms, parental origin, mutation rate and implications for prenatal diagnosis. Am J Hum Genet 61:1102–1111.

Wirth B, Herz M, Wetter A, Moskau S, Hahnen E, Rudnik-Schöneborn S, Wienker T, Zerres K. 1999. Quantitative analysis of survival motor neuron copies: identification of subtle SMN1 mutations in patients with spinal muscular atrophy, genotype-phenotype correlation, and implication for genetic counseling. Am J Hum Genet 64:1340–1356.

Zerres K, Rudnik-Schöneborn S, Forkert R, Wirth B. 1995. Genetic basis of adult onset spinal muscular atrophy. Lancet 346:1162.

Zerres K, Rudnik-Schoneborn S. 1995. Natural history in proximal spinal muscular atrophy. Clinical analysis of 445 patients and suggestions for a modification of existing classifications. Arch Neurol 52:518–523.