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Hybrid Survival Motor Neuron Genes in Patients with Autosomal Recessive Spinal Muscular Atrophy: New Insights into Molecular Mechanisms Responsible for the Disease

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Summary

Spinal muscular atrophy (SMA) is a frequent autosomal recessive neurodegenerative disorder leading to weakness and atrophy of voluntary muscles. The survival motor-neuron gene (SMN), a strong candidate for SMA, is present in two highly homologous copies (telSMN and cenSMN) within the SMA region. Only five nucleotide differences within the region between intron 6 and exon 8 distinguish these homologues. Independent of the severity of the disease, 90%–98% of all SMA patients carry homozygous deletions in telSMN, affecting either exon 7 or both exons 7 and 8. We present the molecular analysis of 42 SMA patients who carry homozygous deletions of telSMN exon 7 but not of exon 8. The question arises whether in these cases the telSMN is truncated upstream of exon 8 or whether hybrid SMN genes exist that are composed of centromeric and telomeric sequences. By a simple PCR-based assay we demonstrate that in each case the remaining telSMN exon 8 is part of a hybrid SMN gene. Sequencing of cloned hybrid SMN genes from seven patients, as well as direct sequencing and single-strand conformation analysis of all patients, revealed the same composition in all but two patients: the base-pair differences in introns 6 and 7 and exon 7 are of centromeric origin whereas exon 8 is of telomeric origin. Nonetheless, haplotype analysis with polymorphic multicopy markers, Ag1-CA and C212, localized at the 5′ end of the SMN genes suggests different mechanisms of occurrence, unequal rearrangements, and gene conversion involving both copies of the SMN genes. In approximately half of all patients, we identified a consensus haplotype, suggesting a common origin. Interestingly, we identified a putative recombination hot spot represented by recombination-stimulating elements (TGGGG and TGAGGT) in exon 8 that is homologous to the human deletion–hot spot consensus sequence in the immunoglobulin switch region, the α-globin cluster, and the polymerase α arrest sites. This may explain why independent hybrid SMN genes show identical sequences.

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Introduction

Proximal spinal muscular atrophy (SMA) is a frequent autosomal recessive neuromuscular disorder characterized by the degeneration of α-motor neurons in the spinal cord, resulting in weakness and wasting of voluntary muscles. Because of the extremely variable clinical picture, affected individuals have been classified into three types depending on the age at onset, achieved motor milestones, and life span (International SMA Consortium 1992).

Two candidate genes for childhood-onset SMA have been reported: the survival motor-neuron gene (SMN) (Lefebvre et al. 1995) is present in two highly homologous copies (telSMN and cenSMN) within the SMA candidate region (5q11.2-q13.3). Both copies are composed of nine exons (1–2a and 2b–8), which encode identical amino acid sequences and span ~20 kb on genomic level. Only because of two base differences in exons 7 and 8 are the telomeric copy, telSMN, and the centromeric copy, cenSMN, distinguishable by single-strand conformation analysis (SSCA) (Lefebvre et al. 1995) and restriction-site assay (van der Steege et al. 1995). Recently, three base-pair differences in introns 6 and 7 (Bürglen et al. 1996) and a DNA variant in exon 2a (Hahnen and Wirth 1996) were reported. Independent of the clinical severity, homozygous deletions of telSMN that affect exon 7 or exons 7 and 8 were found in 90%–98% of SMA patients (Cobben et al. 1995; Bussaglia et al. 1995; Chang et al. 1995; Hahnen et al. 1995; Lefebvre et al. 1995; Rodrigues et al. 1995; Velasco et al. 1996), whereas homozygous deletions of cenSMN (found in ~4%–5% of carriers and control persons) seem to have no clinical consequences. However, in nine SMA families, homozygous deletions of telSMN exons 7 and 8 were found in asymptomatic haploidentical sibs and parents of SMA patients (Cobben et al. 1995; Hahnen et al. 1995; Wang et al. 1996). The neuronal apoptosis-inhibitory protein (NAIP) gene (Roy et al. 1995) shows homozygous deletions in ~45% of type I and <20% of type II and type III SMA patients but also in ~2% of carriers with no phenotypic evidence of SMA (Hahnen et al. 1995; Roy et al. 1995; Rodrigues et al. 1996).

The SMA region shows a highly polymorphic and

dynamic character, which is the consequence of its complex genomic structure containing a large duplicated fragment (Lefebvre et al. 1995), several polymorphic multicopy microsatellites (Kleyn et al. 1993; Brahe et al. 1994; Burghes et al. 1994; DiDonato et al. 1994; Melki et al. 1994; Wirth et al. 1995*a*), and multicopy genes (Theodosiou et al. 1994; Lefebvre et al. 1995; Roy et al. 1995). Such repeated regions are often prone to unequal rearrangements between highly homologous elements, resulting in deletions and/or duplications or gene-conversion events (see the Discussion). Within this dynamic structure of the region we have to search for the underlying mechanisms producing different SMA phenotypes. Several studies have shown a correlation between the extent of deletions including homozygous deletions of the SMN/NAIP genes, as well as the copy number of two polymorphic markers (Ag1-CA and C212, both localized at the 5′ end of the SMN genes), and the severity of the disease (DiDonato et al. 1994; Melki et al. 1994; Wirth et al. 1995*b;* Rodrigues et al. 1996; Velasco et al. 1996). Although the frequency of deletions found in the NAIP gene and the Ag1-CA and C212 copy number correlate with the severity of the disease, there is almost no such correlation found for the telSMN gene.

In this paper, we present the molecular analysis of 42 patients who are homozygously deleted for the telSMN exon 7 but not for the telSMN exon 8. Two of these patients show additionally homozygous deletions of cenSMN exon 8. We postulated three events that may explain the lack of the telSMN exon 7 but not of exon 8: (i) deletion that leads to disruption of telSMN upstream of exon 8; (ii) unequal rearrangement between the telSMN and cenSMN, resulting in a hybrid SMN gene and reduction of the SMN copy number on SMA chromosomes; and (iii) gene conversion in which a sequence block of cenSMN is copied into the highly homologous telSMN counterpart and the copy number of SMN genes is not changed on these chromosomes. Distinguishing between these three possibilities provides new insights into the molecular mechanisms responsible for the disease.

Patients and Methods

Patients

All 42 SMA patients fulfilled the diagnostic criteria for proximal SMA, as defined by the International SMA Consortium (1992). Most patients and their families previously have been analyzed with polymorphic multicopy markers (Ag1-CA and C212; Wirth et al. 1995*b*) and have been tested for homozygous deletions in the SMN and NAIP genes (Hahnen et al. 1995). The other patients were collected from cases analyzed because of diagnostic reasons.

DNA Isolation

DNA was isolated from peripheral venous blood samples by the salting-out method (Miller at al. 1988) or from frozen muscle by use of the standard phenol-chloroform extraction method (Sambrook et al. 1989).

PCR Amplification and SSCA

Multiplex PCR of NAIP exons 5, 6, and 13 (Roy et al. 1995) was performed as described by Wirth et al. (1995*b*). Exons 7 and 8 of the SMN genes were amplified and analyzed by SSCP (Lefebvre et al. 1995) and restriction-site assay (van der Steege et al. 1995), as described in detail by Hahnen et al. (1995). The region including exons 7 and 8 was amplified with the primers R111, localized in intron 6, and 541C1120, localized in exon 8 (Lefebvre et al. 1995). Thirty nanograms of genomic DNA from patients was amplified in 25 μl containing 10 pmol of each primer, 120 μM dNTPs, 1 × Cetus PCR buffer, and 1 U of *Taq* polymerase (Gibco-BRL). Cycling conditions included a 7-min initial denaturation at 94°C followed by 30 cycles of 45 s at 94°C, 45 s at 56°C, and 1 min at 72°C and by a final extension for 10 min at 72°C, in a Perkin Elmer Cetus Cycler 9600.

Cloning of PCR Products and Sequencing

The 1-kb intron 6–exon 8 PCR fragments were cloned into pUC18 vector by means of the SURE-Clone-kit (Pharmacia). Sequencing was performed with the USB sequencing kit (Amersham), with vector and several internal primers.

Detection of Intronic Base-Pair Differences by SSCA and Direct Sequencing

Three intronic base-pair differences have been described by Bürglen et al. (1996):

1. in intron 6 at position −45 bp/exon 7, atgt (telomeric) and atat (centromeric);
2. in intron 7 at position +100 bp/exon 7, ttaa (telomeric) and ttag (centromeric);
3. in intron 7 at position +214 bp/exon 7, ttat (telomeric) and ttgt (centromeric).

The difference in intron 6 was tested by SSCA, by use of the primers R111 (Lefebvre et al. 1995) and X7-Dra (van der Steege et al. 1995). PCR and SSCA were performed as elsewhere described for the SMN analysis of exons 7 and 8 (Hahnen et al. 1995). We were not able to detect the two differences in intron 7 by SSCA. Therefore, direct sequencing was performed by use of Dynabeads (Dynal) according to the supplier's protocol. PCR of the 6–8 SMN fragment was performed as described above, except that the reverse primer was biotinylated. After single-strand separation, internal primers were used for direct sequencing.

Genotype and Haplotype Analysis

The multicopy markers Ag1-CA and C212 amplify 0–3 alleles or copies/chromosome. Segregation and hap-

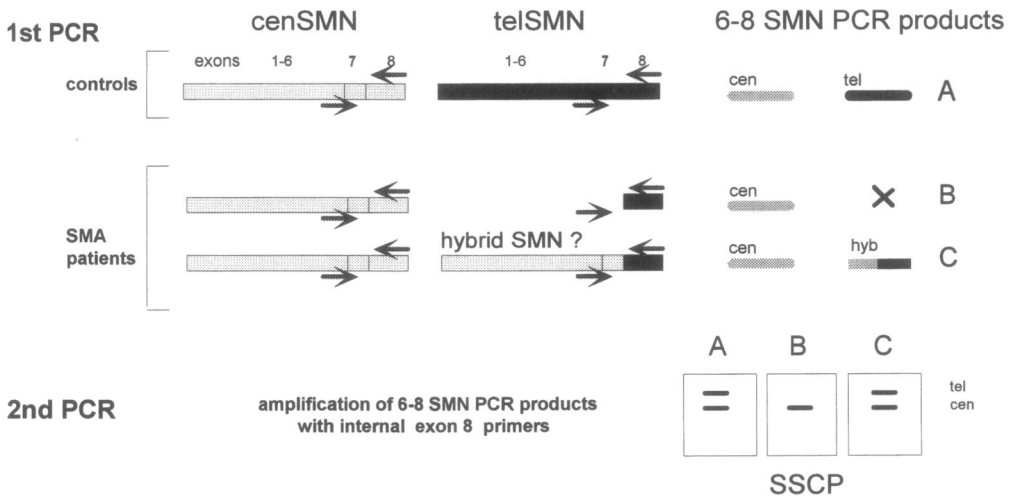

Figure 1 Detection of hybrid SMN genes in SMA patients by a two-step PCR-based assay. The arrows indicate the position of the primers (R111 and 541C1120) in the first PCR. Two hundred fifty picograms of the first PCR product and the primers 541C960 and 541C1120 were used in the second PCR.

lotype analysis was performed as described in detail elsewhere (DiDonato et al. 1994; Wirth et al. 1995*b*). After segregation of the Ag1-CA and C212 alleles, more than one arrangement of these alleles was possible in three cases (ambiguous haplotypes). In the remaining patients, *un*ambiguous haplotypes were constructed. The haplotype designation refers to the combination of C212 and Ag1-CA alleles on a particular chromosome, whereas the genotype designation refers to the pairs of alleles, Ag1-CA and C212; for example, genotype 1,2 refers to one Ag1-CA and one C212 allele on one parental chromosome and two Ag1-CA and two C212 alleles on the second parental chromosome.

Results

We found homozygous deletions of the telSMN exon 7 but not of telSMN exon 8 in 42 independent SMA patients (14 type I, 13 type II, and 15 type III) but in none of >450 carriers. Twenty-three of these patients have been reported previously, in a study including 191 SMA patients mainly of German origin (Hahnen et al. 1995). Interestingly, 2 of the 42 patients (i.e., patients TW and DS), both with a severe type I SMA, are homozygously deleted not only for the telSMN exon 7 but also for cenSMN exon 8. In all but patients TW and DS the cenSMN exons 7 and 8 were present. In all cases genotype analysis of the SMN copies was performed by nonradioactive SSCA and was confirmed by restriction-site assay (see Patients and Methods). All 42 patients retained at least one copy of NAIP exons 5 and 6.

Detection of Hybrid SMN Genes and Sequence Analysis

We developed a simple PCR-based assay composed of two successive amplification steps that allowed us to distinguish disrupted from hybrid SMN genes (fig. 1). First we performed a PCR using SMN intron 6 forward and exon 8 reverse primers. A PCR product of ~1 kb was obtained in all 42 SMA patients. These 6–8 SMN PCR products can be the result of exponential amplification of either cenSMN only, in case the telSMN gene is truncated upstream of exon 8, or, additionally, of a hybrid SMN gene, when telSMN exon 8 is joined to cenSMN sequences. The hybrid SMN gene may either occur alone, as expected for the two patients TW and DS, or include amplified sequence from the cenSMN copy as well.

To distinguish between these possibilities, a second PCR was performed using exon 8 forward and reverse primers and <250 pg of DNA of the first PCR product. In case of a truncated telSMN gene, no exponential amplification between the first set of primers including telomeric sequences can be obtained, and consequently no second PCR product for the telomeric exon 8 will result. If a hybrid SMN gene is present, the first amplification step will produce a 6–8 SMN PCR product, and, in the second PCR, telomeric sequences will be amplified and could be detected by SSCA or restriction-site assay. To exclude false results due to either traces of initial DNA template or linear amplification of telomeric sequences, we performed a PCR using control DNA and the exon 8 reverse primer only in the first step. The product was diluted and was followed by the second PCR, as described above; no amplification product was obtained. In contrast, all 42 patients show a telSMN exon 8 product by SSCA.

To confirm these data, we cloned the 1-kb 6–8 SMN PCR products of patients TW and DS, who showed homozygous deletions of telSMN exon 7 and cenSMN exon 8, and of four patients who showed homozygous

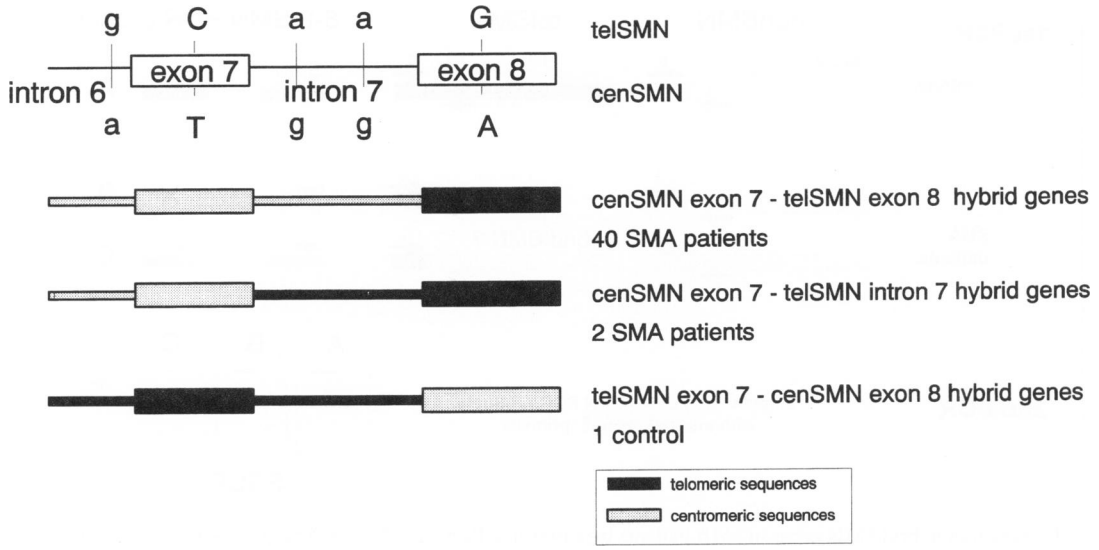

Figure 2 Graphic illustration of the three types of hybrid SMN genes found in SMA patients and in one control. The exact positions of the base-pair differences are noted in the text.

deletions of telSMN exon 7 only. Approximately 20 independent clones from each individual were PCR amplified at exons 7 and 8 and were analyzed by SSCA, in order to assign the clones to one of the two categories, cenSMN or hybrid SMN. As expected, patients TW and DS showed only hybrid SMN clones whereas the other four patients revealed cenSMN and hybrid SMN clones. Complete sequence analysis of the hybrid SMN clones showed cenSMN sequences in intron 6 (position −45 bp/exon 7), exon 7 (nucleotide [nt] 842), and intron 7 (positions +100 bp/exon 7 and +214 bp/exon 7) and the telSMN sequence in exon 8 (nt 1155). No further base pairs were missed or duplicated.

The DNA differences of introns 6 and 7 in all 42 SMA patients were analyzed by SSCA or direct sequencing. In all but two cases the intronic differences corresponded to the cenSMN copy, producing cenSMN exon 7–telSMN exon 8 hybrid genes (fig. 2). In two cases both differences in intron 7 were of telomeric origin; from one patient the hybrid gene was subsequently cloned, and the order of the differences was confirmed as cenSMN exon 7–telSMN intron 7 (fig. 2).

Additionally, we identified an unaffected individual who carries a homozygous deletion of cenSMN exon 7 only. Cloning and sequencing of the hybrid SMN gene revealed the reciprocal state on a non-SMA chromosome. All base-pair differences in introns 6 and 7 and exon 7 were of telomeric origin, whereas that in exon 8 was of centromeric origin, resulting in a telSMN exon 7–cenSMN exon 8 hybrid gene (fig. 2).

Furthermore, we identified three differences from the sequence previously published by Lefebvre et al. (1995) and Bürglen et al. (1996), in all clones as well as by direct sequencing: intron 6 at position −17 bp/exon 7 CTTTA instead of CTTTTA; intron 7 at position +86 bp/exon 7 ATT instead of AGT; and exon 8 position nt 1128 TCTATT instead of TCTT.

Haplotype Analysis in Patients Carrying Hybrid SMN Genes

To clarify the extent and the mechanism of deletions in SMA patients with hybrid SMN genes, we performed haplotype analysis with the multicopy markers Ag1-CA and C212. Both of these markers are located near the 5′ end of the SMN genes. The number of alleles per chromosome roughly reflects the number of SMN copies localized on each chromosome. For 32 of 42 patients, DNA of parents and additional sibs were available for haplotype analysis. In 29 of 32 patients the construction of *un*ambiguous haplotypes was possible (table 1).

Patients TW and DS showed a loss of inheritance of all Ag1-CA and C212 marker alleles on one parental chromosome whereas the second chromosome carried only one copy of Ag1-CA and C212 (genotype 0,1) that is associated with the hybrid SMN gene. This is in accordance with the fact that both patients reveal homozygous deletions of telSMN exon 7 and cenSMN exon 8 (fig. 3). In five patients, one allele of Ag1-CA and C212 per chromosome (genotype 1,1) was identified, which suggests, with regard to the SMN gene, that one parental chromosome carries the SMN hybrid gene and that the second one carries a cenSMN copy. The genotypes in these patients are consistent with an extensive loss of genomic DNA, such as that resulting from unequal crossing-over or intrachromosomal deletion between the highly homologous copies. No consensus haplotype was identified in these patients, which suggests that each hybrid gene represents an independent event.

Table 1

Genotype and Haplotype Analysis with Multicopy Markers, Ag1-CA and C212, in Patients Carrying Hybrid Genes

Group and Genotype of Ag1-CA and C212[a]	No. of Individuals with SMA				No. of Individuals with Consensus Haplotype 100/106-31/33
	Type I	Type II	Type III	Total	
Patients, with complete family analysis:					
*Un*ambigous genotypes:					
0,1	2	...	...	2	...
1,1	3	2	...	5	...
1,2	5	8	2	15	10
0,2	1	...	...	1	...
2,2	1	...	5	6	4
Ambigous genotypes	2	...	1	3	...
Patients only, no family members available	...	3	7	10	7 (?)
Total	14	13	15	42	14 + 7 (?)

[a] Haplotype designations refer to the exact Ag1-CA and C212 alleles segregating on each parental chromosome, whereas genotype designations refer to the number of copies or alleles of Ag1-CA and C212 per chromosome. For example, genotype 1,2 refers to one Ag1-CA and one C212 allele on one parental chromosome and two Ag1-CA and two C212 alleles on the second parental chromosome. For more details, see Patients and Methods.

In 24 patients, three or four alleles of Ag1-CA and C212 (genotype 1,2 or 2,2) were found (table 1). In 21 of 24 cases the construction of *un*ambiguous haplotypes was possible. Fourteen of these patients revealed a consensus haplotype, 100/106 and 31/33 of Ag1-CA and C212; in 2 of these 14 cases it occurred homozygously. Furthermore, 7 of 10 patients whose parents were not available for analysis have, most probably, the same consensus haplotype. Altogether, the consensus haplotype is associated with half of all chromosomes carrying hybrid genes. Considering that all patients had a deletion in the telSMN gene, the remaining copies must be cenSMN or hybrid SMN copies. For those 7 patients who revealed two alleles per chromosome (genotype 2,2 and 2,0) and for those 10 of 15 showing a consensus haplotype (genotype 1,2), we can assume that the hybrid gene occurs in addition to a cenSMN copy per chromosome. The presence of a cenSMN in addition to a hybrid SMN gene on one chromosome suggests gene conversion as the most likely mechanism.

Discussion

By a simple PCR-based test we were able to demonstrate the existence of hybrid SMN genes in 42 independent patients with SMA who show homozygous deletions of exon 7 but not of exon 8 of the telSMN copy. This phenomenon has recently been described in ~12% of 191 SMA patients mainly of German origin (Hahnen et al. 1995). Nine of the 42 patients presented here are of Polish or Czech origin, whereas only 32 patients who participated in this study were from these two countries. This indicates a high frequency (~30%) of hybrid SMN genes in SMA patients with Czech or Polish background. There have been reported various frequencies (3%–28%) of homozygous deletions of telSMN exon 7, depending on the ethnic origin of the SMA patients (Bussaglia et al. 1995; Chang et al. 1995; Cobben et al. 1995; Hahnen et al. 1995; Lefebvre et al. 1995; Rodrigues et al. 1995). Although we found a roughly equal distribution in all three types of SMA (Hahnen et al. 1995), all other studies except one (Cobben et al. 1995) found this phenomenon in type II and type III SMA patients only.

Besides the SMN gene, which is usually present in at least two copies per chromosome, all other genes and markers localized within the SMA region are also present in several copies. It is known from other duplicated genes and regions that these are prone to unequal rearrangements resulting in deletions and duplications or gene-conversion events. Examples include the α-globin gene cluster (Hill et al. 1985; Nicholls et al. 1987), immunoglobulin gene cluster (for review, see Gritzmacher 1989), Charcot-Marie-Tooth neuropathy type 1A (CMT1A) and hereditary neuropathy with liability to pressure palsies (HNPP) (Pentao et al. 1992; Chance et al. 1993), red- and green-opsin genes (Reyniers et al. 1995), the cytochrome P450 CYP2D cluster (Steen et al. 1995), steroid 21-hydroxylase genes CYP21A and CYP21-B (Urabe et al. 1990), and juvenile nephronophthisis (Konrad et al. 1996).

In general it is assumed that large deletions observed within duplicated regions may occur either interchromosomally, because of misalignment of nonsister chromatids during meiosis (Pentao et al. 1992; Chance et al. 1993), or intrachromosomally, because of either sister-chromatid exchange during mitosis or DNA slippage during replication (for review, see Krawczak and Cooper 1991). In many cases deletion hot spots were de-

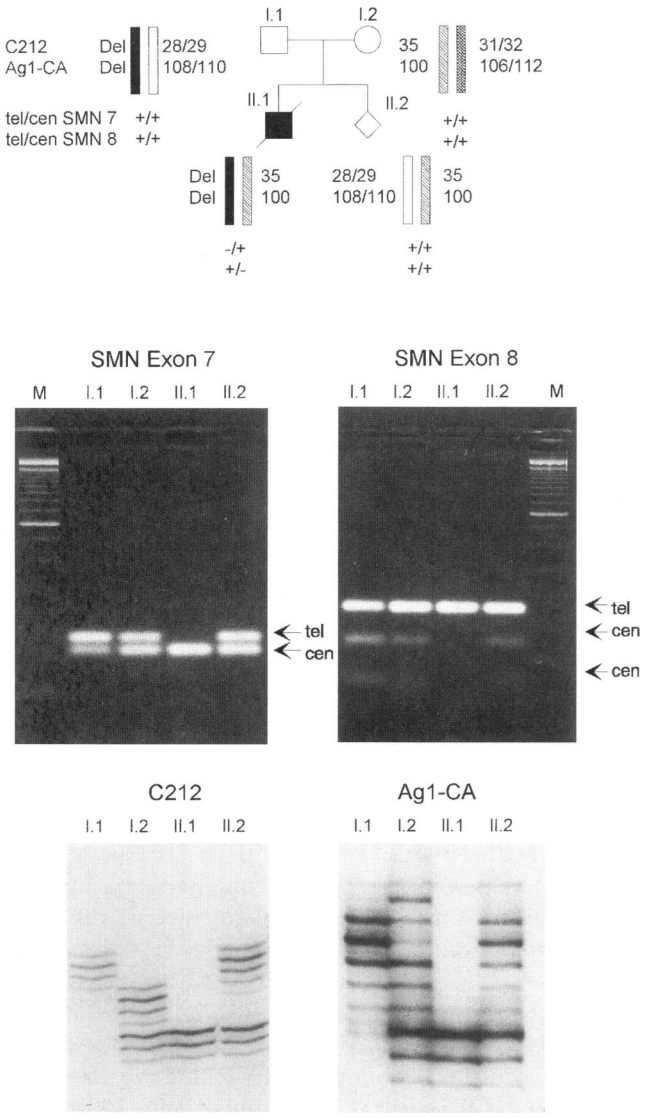

Figure 3 Molecular analysis of SMN gene exons 7 and 8, by restriction-site assay, and of the multicopy flanking markers C212 and Ag1-CA, by radioactive PCR, in an SMA family. Patient DS (II.1) shows a homozygous deletion of telSMN exon 7 and cenSMN exon 8. Additionally, he shows a paternally inherited deletion of marker alleles of Ag1-CA and C212. Lanes M, 100-bp ladder (Gibco-BRL).

scribed that are correlated with typical consensus sequences such as TGGGG and TGAGGT, found in the tandemly repeated immunoglobulin switch (Sμ) regions (for review, see Gritzmacher 1989) in the α-globin gene cluster (Nicholls et al. 1987) and to putative arrest sites for polymerase α, which often contain a GAG motif (Weaver and DePamphilis 1982). We identified the same motifs in the SMN gene exon 8, starting at position nt 1153 (TGG_{tel}/A_{cen}GGTGGGGGTGGGAGG). This sequence might represent the distal breakpoint, since all but two hybrid genes contain sequences of centromeric origin proximal to the motif and distal to the motif of telomeric origin, as shown by the few specific base-pair differences. Thus we assume that the repeated motifs may act as recombination hot spots. The arrest of DNA synthesis at the replication fork may increase the probability of either the occurrence of a slipped-mispairing event or the formation of secondary-structure intermediates potentiated by the presence of inverted repeats or symmetrical elements.

Although we found the same DNA sequence in all but two hybrid genes, the haplotype analysis with the polymorphic multicopy markers, Ag1-CA and C212, suggests that different mechanisms are responsible for their occurrence. In the following section, we will discuss the most probable mechanisms responsible for hybrid SMN genes: unequal crossing-over, intrachromosomal deletions, and gene-conversion events.

Unequal Recombination and Intrachromosomal Deletions

In two patients, TW and DS, telSMN exon 7 and cenSMN exon 8 were homozygously deleted. Since both patients inherited large-scale deletions including all alleles of both markers Ag1-CA and C212 from one parent, as well as the single Ag1-CA/C212 allele derived from the other parent, the hybrid gene must be associated with this only existing copy. Both patients had a severe form of SMA type I, with prenatal onset and death at 2 and 8 mo of age. Five patients reveal only one allele of Ag1-CA and C212 per chromosome (genotype 1,1), which suggests that one chromosome contains a hybrid SMN copy and that the other chromosome contains a cenSMN copy as the only one. No consensus haplotype was identified for these hybrid genes, a finding that suggests different origins. The most likely mechanisms of occurrence for these cases are either unequal crossing-over between the homologous copies (fig. 4*A*) or intrachromosomal deletions (fig. 4*B*).

Several further observations support these models: (i) the finding of de novo deletions of only one copy of Ag1-CA, C212, and telSMN in SMA patients in families with haploidentical affected and unaffected sibs (Hahnen et al. 1995; Rodrigues et al. 1995; Wirth et al. 1995*b*) points toward intrachromosomal deletion events; (ii) the finding of de novo deletions of one copy each of Ag1-CA, C212, and telSMN in SMA patients, as a result of a recombination event (Wirth et al. 1995*b*; B.W., unpublished data), suggests misalignment and unequal crossing-over events between nonsister chromatids; (iii) the presence of 0–3 alleles of Ag1-CA and C212 per chromosome (DiDonato et al. 1994; Wirth et al. 1995*b*) suggests that all or part of the 500-kb element (Lefebvre et al. 1995) is also present in 0–3 copies, which facilitates the process of unequal recombination; and (iv) the finding of the reciprocal counterpart, telSMN exon 7–cenSMN exon 8 hybrid genes in healthy individuals (Velasco et al. 1996; present study). The telSMN exon 7–cenSMN exon 8 hybrid genes may oc-

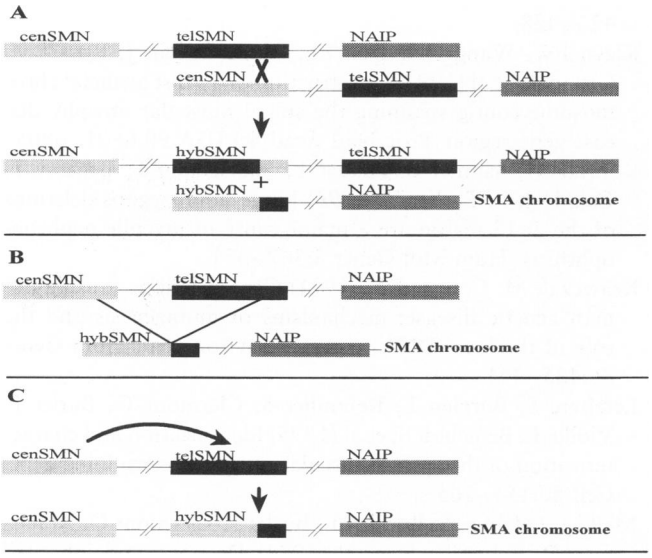

Figure 4 Hypothetical mechanisms responsible for hybrid SMN genes: unequal recombination (*A*), intrachromosomal deletion (*B*), and gene conversion (*C*). The telomeric location of the NAIP gene with respect to the SMN gene is supported by fact that it was present in all 42 patients and thus was not involved in the rearrangements.

cur more often in the normal population but may be masked by the presence of cenSMN copies. Only in individuals who carry deletions of cenSMN copies (4%–5% of the control population) can the cenSMN exon 7–cenSMN exon 8 hybrid gene be detected by SSCA or restriction-site assay.

Therefore, we assume that in these patients the hybrid SMN copy is the result of a fusion between the cenSMN copy and the telSMN copy, with deletion of the intervening region. Most patients belonging to this category reveal a more severe phenotype (five cases of type I SMA and two cases of type II SMA). This is also in accordance with the previously reported genotype-phenotype correlation analysis regarding the extent of deletion including multicopy markers (DiDonato et al. 1994; Melki et al. 1994; Wirth et al. 1995*b*).

Gene Conversion

One patient has the genotype 2,0 and six patients have the genotype 2,2—as reflected by the number of Ag1-CA and C212 alleles per chromosome. In all seven cases the hybrid gene occurs in addition to a cenSMN copy, whereas a complete telSMN is absent. Fifteen patients showed the genotype 1,2, in which the hybrid SMN gene can be present either on the one-allele chromosome or on the two-allele chromosome. However, 10 of 15 cases presented the consensus haplotype 31/33-100/106, which suggests that the hybrid SMN copy is associated with this chromosome and, consequently, with the two-allele chromosome. In conclusion, in $\geq$16 independent patients the hybrid gene occurs in addition to a cenSMN copy, on one chromosome. This points toward another mechanism, such as gene conversion, in which part of the cenSMN is copied into its telSMN counterpart—that is, the donor remains unaffected whereas the acceptor becomes a hybrid SMN gene (fig. 4*C*). The proximal border of the transferred segment is not yet known. Since no further base-pair differences upstream of intron 6 have been described so far, the identification of the exact proximal breakpoint will be quite difficult. However, the role of this new construct for the pathogenesis of the disease remains to be determined. Nonetheless, it can be excluded that cenSMN exon 7–telSMN exon 8 hybrid genes are benign DNA variants, by the fact that this phenomenon has never been found in >2,000 carriers or controls reported by different groups. Lefebvre et al. (1995) reported a type III SMA patient with exon 7 of centromeric and exon 8 of telomeric origin, whereas Bussaglia et al. (1995) reported a case of SMN rearrangement in which only exon 7 is of centromeric origin whereas the flanking introns are of telomeric origin. Both studies suggest the existence of gene-conversion events.

In a previous study we have shown that certain Ag1-CA/C212 haplotypes are strongly associated with SMA chromosomes (Wirth et al. 1995*b*). Thus, we identified the haplotype 100/106 and 31/33 nine times, only on SMA chromosomes and never on normal chromosomes ($P < .0036$). In the present study we have been able to show that all those nine cases are associated with hybrid SMN genes. In addition, we found the same haplotype in 12 additional patients with hybrid SMN genes. This suggests a common origin for the mutation in these 21 independent patients of German, Austrian, Czech, and Polish origin. In two type III SMA patients (one of consanguineous parents) the consensus haplotype occurred homozygously, associated with a mild phenotype. However, in compound situations all three types of SMA were seen. In four additional patients the same Ag1-CA haplotype 100/106 was found, but there was a slightly modified one for C212 (30/32-33/34-32/33). In the remaining patients no common haplotype could be identified, which points toward different origins. Nonetheless, identical sequences were identified in all but two of these hybrid genes, suggesting a recombination hot spot, as discussed above.

In conclusion, we have presented the existence of hybrid SMN genes in 42 SMA patients and the most likely mechanism of occurrence. We have identified a putative recombination hot spot given by a consensus recombination sequence in exon 8. Furthermore, we have identified a single haplotype for half of the hybrid genes analyzed, suggesting that these SMA chromosomes share a common origin.

Acknowledgments

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