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INVITED EDITORIAL
When Is a Deletion Not a Deletion? When It Is Converted

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Spinal Muscular Atrophy (SMA)

Proximal SMA is an autosomal recessive disorder that results in destruction of the motor neurons in the anterior horn of the spinal cord. SMA has an estimated incidence of 1/10,000 live births, with a carrier frequency of 1/40 (Pearn 1980). The childhood SMAs can be classified into three groups based on age at onset and clinical course. Type I SMA is the most severe form, with onset of symptoms before the age of 6 mo and with death occurring within the first 2 years of life. Type II SMA patients have an intermediate severity, with onset before age 18 mo and with patients never gaining the ability to walk. Type III SMA is the mildest form of this disease, with onset after the age of 18 mo and with patients achieving the ability to walk.

All three forms of SMA have been mapped to 5q12-13 (Brzustowicz et al. 1990; Melki et al. 1990; Simard et al. 1992; Francis et al. 1993; Brahe et al. 1994; Burghes et al. 1994*a;* Wirth et al. 1994, 1995*a*). In 1995, three papers reported different cDNAs (neuronal apoptosis inhibitory protein [NAIP] [Roy et al. 1995], survival motor neuron [SMN] [Lefebvre et al. 1995], and XS2G3 [XS2G3 is a segment of the NAIP gene] [Thompson et al. 1995]) that detect deletions in SMA patients. The NAIP and SMN genes are duplicated with a telomeric SMN (SMN^T) and a centromeric SMN (SMN^C), and NAIP is duplicated either with exon 5 ($NAIP^5$) or without exon 5 ($NAIP^D$). The $NAIP^5$ gene was deleted in 50% of type I SMA patients, whereas the telomeric SMN^T gene was deleted in 95% of patients of all severities (Cobben et al. 1995; Hahnen et al. 1995; Lefebvre et al. 1995; Rodrigues et al. 1995; Matthijs et al. 1996; Velasco et al. 1996; DiDonato et al. 1997). These reports gave optimism that the molecular nature of SMA could be clarified but resulted in confusion as to which gene was the SMA gene and what determined phenotype. To determine which of these two candidate genes was the SMA gene, it was important to identify nondeletion mutations within the genes in SMA patients. During the past 2 years, a series of missense and frameshift mutations in the SMN^T gene has been identified in patients with SMA who have an intact $NAIP^5$ gene (table 1). This strongly indicates that the SMN^T gene is the causative SMA gene.

Since 95% of SMA patients have no detectable SMN^T, regardless of clinical phenotype the genotypic difference between a type I SMA patient with no SMN^T and a type II or type III SMA patient with no SMN^T has required clarification. Previous studies suggested that the loss of the SMN^T gene occurs by two different mechanisms: deletion or conversion of SMN^T to SMN^C, in which case conversion could produce a mild-SMA allele and deletion could produce a severe-SMA allele. However, physical evidence for conversion was lacking. In this issue of the *Journal,* Campbell et al. (1997) present physical evidence for conversion of SMN^T to SMN^C on mild-SMA chromosomes, which, when combined with data from assays that measure SMN^C and SMN^T gene copy number, does provide a much needed vision of the molecular basis of genotype/phenotype relationship in SMA.

The SMA Duplicated Region and the Genes That It Contains

Markers and genes that lie within the SMA region are represented multiple times on a chromosome and vary in copy number in different individuals (Kleyn et al. 1993; Burghes et al. 1994*b;* DiDonato et al. 1994; Melki et al. 1994; Lefebvre et al. 1995; Roy et al. 1995; Thompson et al. 1995). Physical maps of the region have been constructed, but there is no consensus map, because of the instability of the YACs, the repeated nature of the region, and its variation on different chromosomes (Francis et al. 1993; Kleyn et al. 1993; Carpten et al. 1994; Melki et al. 1994; Lefebvre et al. 1995). On normal chromosomes the SMN gene is duplicated, and the size of the restriction fragments that contain the SMN genes varies in different individuals (Lefebvre et al. 1995).

The SMN cDNA is encoded by two nearly identical genes, SMN^T and SMN^C, which can be distinguished by base changes in exons 7 and 8 (Lefebvre et al. 1995;

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Table 1

Small Mutations of SMN^T

Mutation	SMA Type(s)	Source
4-bp deletion exon 3[a]	I–III	Bussaglia et al. (1995)
5-bp deletion exon 3	I	Brahe et al. (1996)
11-bp duplication in exon 6[b]	I	Parsons et al. (1996)
Codon 279 G→V	I	Talbot et al. (1997)
Codon 272 Y→C	I[c]	Lefebvre et al. (1995), Rochette et al. (in press)
Codon 245 P→L	III	Rochette et al (in press)
Codon 274 T→I	III	Hahnen et al. (1997)
Codon 262 S→I	III	Hahnen et al. (1997), McAndrew et al. (1997)
Codon 275 G→S	III	Burglen et al. (1996*b*)

[a] Found in the Spanish population; the type II case was consanguineous.

[b] Has now been found in two additional unrelated patients with type I SMA (D. W. Parsons and T. W. Prior, personal communication).

[c] Phenotype reported for one patient (Rochette et al., in press).

van der Steege et al. 1995). The NAIP gene is present in multiple copies, but the copy that is associated with deletions in SMA patients can be distinguished because only this copy contains exon 5 (referred to here as "$NAIP^5$") (Roy et al. 1995). Another gene in the region, BTF2p44, also exists as multiple copies, but only one copy, $BTF2p44^T$, is associated with SMA deletions (Bürglen et al. 1997; Carter et al. 1997). The SMA locus and the position of these genes, as well as polymorphic markers associated with the SMN^T and SMN^C gene, are diagrammed in figure 1 and listed in table 2. Because these markers are highly informative, they can be used to determine the copy number of the combined SMN^T and SMN^C genes on chromosomes.

Evidence for Conversion and Deletion of SMN^T in SMA

A number of observations indicate that the variation in phenotypic severity results from alterations at the

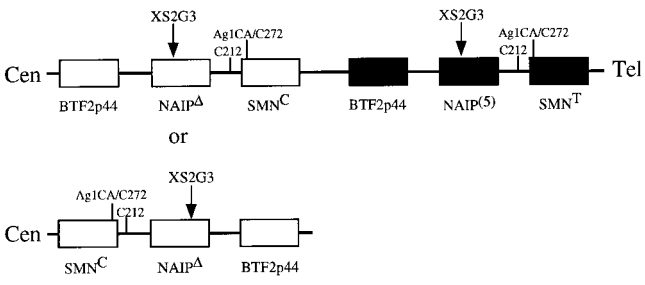

Figure 1 Diagrammatic representation of the SMN^T locus, showing positions of markers and genes. The SMN^C locus is similar but lacks the NAIP gene containing exon 5. It is most likely that the telomeric locus and centromeric locus can exist in either orientation, depending on the particular chromosome. The marker Ag1-CA (C272) lies at the 5′ end of the SMN genes (Bürglen et al. 1996*a*).

Table 2

Distribution of SMN^c Copy Number

Group (No. of Individuals)	No. (%) of Individuals for SMN^c Copy Number				
	0	1	2	3	4
Normal (53)	4 (7.5)	25 (47.2)	23 (43.4)	1 (1.9)	0
Type I SMA (21)	0	8 (38.1)	9 (42.9)	4 (19.0)	0
Type II and type III SMA (58)	0	7 (12.0)	28 (48.3)	20 (34.5)	3 (5.2)

SMA locus, rather than being an epigenetic effect. First, the $NAIP^5$ gene is deleted in ~50% of type I SMA patients but is much less frequently deleted in type II and type III SMA patients (Cobben et al. 1995; Hahnen et al. 1995; Roy et al. 1995; Thompson et al. 1995; Rodrigues et al. 1996; Velasco et al. 1996). Second, the polymorphic markers adjacent to SMN^T and SMN^C can be used as an indicator of the copy number of these genes. These marker studies demonstrate an association of SMN^C copy number with phenotype, in the following manner. Approximately 50% of type I SMA patients have a single copy of SMN^C on each chromosome, so that the SMN^T gene is deleted and SMN^C copy number is unaffected (DiDonato et al. 1994; Melki et al. 1994; Lefebvre et al. 1995; Wirth et al. 1995*b*). The genotype (1,1) of one copy of SMN^C on each chromosome, together with the loss of $NAIP^5$, occurs only on type I SMA chromosomes and implies a large deletion. (Wirth et al. 1995*b;* Burlet et al. 1996; Rodrigues et al. 1996; Simard et al., in press). In type II and type III SMA, although they lack SMN^T, they most often have one chromosome with one copy of SMN^C and have the other chromosome with two copies of SMN^C (DiDonato et al. 1994; Wirth et al. 1995*b*). In type II and type III SMA, the SMN^T gene is missing, but the $NAIP^5$ gene is present, as are the markers that lie in the 5′ end of the SMN^T gene. Because the SMN^T gene is not detected but the markers reveal that the locus is still present, another mechanism besides deletion of SMN^T must be operating; the most likely mechanism is conversion of SMN^T to SMN^C.

Further insight into the role of SMN^C-gene copy number was gained by the use of carrier parent DNA samples and scanning densitometry to measure SMN^T:SMN^C ratios. Analysis of SMN^C copy number by use of the SMN^T:SMN^C ratio in obligate carriers of type I, type II, or type III SMA indicated that type II and type III SMA carriers have more copies of SMN^C than do type I SMA carriers (Velasco et al. 1996). Because the SMN^T gene is absent in all SMA types, the increase in SMN^C copy number indicates that a large number of type II and type III SMA chromosomes contain a converted allele, rather

than a deleted allele. The critical element in these studies is an assay that measures the number of copies of SMN^C independently of the SMN^T gene, because the number of SMN^C and SMN^T genes varies in different chromosomes. Such an assay is also useful for detection of nondeletion patients and carriers. In the June and July issues of the *Journal*, two independent papers report methods for determining the copy number of the SMN^C and SMN^T genes. The strategies used differ: McAndrew et al. (1997) used multiplex PCR and compared the copy number of SMN^T and SMN^C to that of an exon of the CFTR gene, whereas Campbell et al. (1997) used pulsed-field gel electrophoresis to assay the number of SMN^T and SMN^C genes. The $NAIP^5$ gene lies 3′ of the SMN^T gene (see fig. 1), and the two are in a single *Eag*I or *Bss*HII fragment. Using probes specific for the $NAIP^5$ gene and probes that detect both of the SMN genes, Campbell et al. (1997) demonstrate that there is a remarkable variability in the size of the fragments containing the SMN^C gene or the SMN^T gene. In most cases the copy number of the SMN^C gene is obtained by counting the number of bands that are detected with the SMN probes but not with the $NAIP^5$ gene probe, and the number of SMN^T genes is determined by counting the number of bands that are detected with both $NAIP^5$ and SMN. The fragment-size variability between individuals explains some of the difficulty in assembling physical maps of this region. As expected, in normal individuals there are usually two copies of SMN^C and two copies of SMN^T, indicating that there is one copy of each gene on a chromosome.

The importance of an assay that measures the SMN^C and SMN^T copy number is that it can distinguish between the loss of SMN^T by deletion and the loss of SMN^T by conversion. In conversion of SMN^T to SMN^C, there is gain of a copy of SMN^C, which will alter the distribution of SMN^C alleles; deletion of SMN^T will not alter the distribution of SMN^C alleles. Indeed, if conversion is a common mechanism in the generation of mild-SMA alleles, we would expect the whole distribution to shift one copy number along the *x*-axis (see fig. 2) as each allele group gains an SMN^C allele. Examination of the data in the report by McAndrew et al. (1997), for the SMA carriers of the different phenotypic classes, clearly shows this shift in the distribution. In normal individuals, 1/53 had three copies of SMN^C, 4/21 type I SMA carriers had three copies of SMN^C, and 20/58 type II and type III SMA carriers had three copies of SMN^C. The chromosomes with two copies of SMN^C were associated with the SMA chromosome in these carrier individuals (P. E. McAndrew, personal communication). This indicates that chromosomes with more than one copy of SMN^C are more common in type II and type III SMA, which is consistent with gene conversion giving rise to mild-SMA alleles. If gene conversion is a common event in mild-SMA chromosomes, then the distribution of copies in the population should shift by one copy number along the *x*-axis, and one would predict a corresponding decrease in the number of individuals with only a single copy of SMN^C. Indeed, this is the case, as can be seen in figure 2, which shows 25/53 normals having a single copy of SMN^C and only 7/58 type II and type III SMA carriers having a single copy of SMN^C. This is consistent with a mild-SMA chromosome containing a conversion event. Correlation of SMN^C copy number with marker data will allow a more complete analysis of the frequency and extent of deletion and conversion events that occur in the different SMA types.

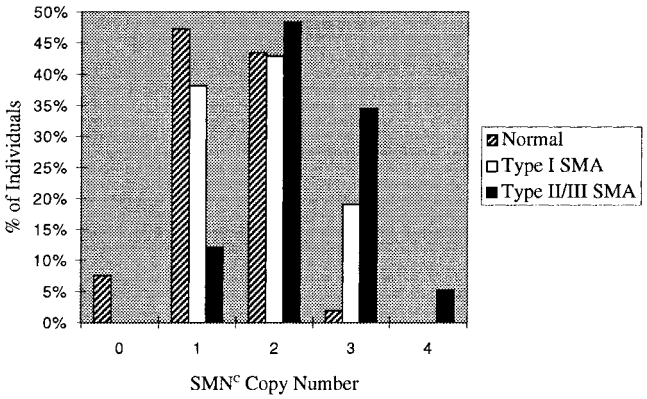

Figure 2 Distribution of SMN^C in the SMA-carrier population and in the normal population. The two-copy SMN^C chromosomes are associated with the SMA chromosomes.

Chimeric genes have been identified as SMA alleles (Lefebvre et al. 1995; Devriendt et al. 1996; Hahnen et al. 1996; van der Steege et al. 1996; DiDonato et al. 1997) and can arise by one of two mechanisms: (1) a deletion that removes the material between the SMN^T and SMN^C gene and fuses the 5′ end of the SMN^C gene to the 3′ end of the SMN^T gene or (2) a conversion event that effects exon 7, but not exon 8, of the SMN^T gene (Hahnen et al. 1996). The chimeric genes formed by deletion and joining of SMN^C to SMN^T are severe-SMA alleles. Hahnen et al. (1996) showed that chimeric SMN genes can occur in type I SMA chromosomes such that there is one chimeric gene and one SMN^C gene on a chromosome (evidence is based on marker studies). Given that there are chromosomes in the normal population that have two copies of SMN^C and one copy of SMN^T, the chimeric gene in these SMA individuals could have arisen by either a deletion event or a conversion event. If conversion can give rise to a severe-SMA allele (note that three copies of SMN^C does occur slightly more frequently in type I SMA than in the normal population), then the extent of conversion might be different in severe-SMA and mild-SMA alleles. Interestingly, Bussaglia et al. (1995) reported a mild-SMA patient who had a conversion event confined to exon 7, which would indi-

cate that the change in exon 7 critically affects SMN although it does not alter the encoded amino acids.

Although the studies described above indicate that gene conversion is the likely mechanism in SMA, it is the experiments of Campbell et al. (1997) that provide the physical evidence that conversion, and not deletion, has occurred in mild-SMA chromosomes. They analyzed DNA from type I SMA patients who lack the NAIP^5 gene and from type II and type III SMA patients who have the NAIP^5 gene. None has a detectable SMN^T gene. In the type I SMA patients, it is not surprising that there were (*a*) no fragments detectable with the NAIP^5 probes and (*b*) not a change in the number of SMN^C copies compared with that in the normal individuals, indicating that a deletion that removed SMN^T had occurred in these cases. In the type II and type III SMA cases which had the NAIP^5 gene, a single band cohybridized with the SMN and NAIP^5 probes. Since the NAIP^5-gene probes detects the telomeric locus, this indicated that these mild-SMA individuals have one copy of the SMN^T locus but that at least exons 7 and 8 of this SMN^T gene contain the sequences usually associated with the SMN^C gene. This demonstrated that one chromosome of the mild-SMA individual contained a conversion event, rather than a deletion event, providing physical evidence of a conversion event.

As Campbell et al. indicate, one interesting group of patients to study with this assay would be type I SMA patients with a detectable NAIP^5 gene. Would these patients show a deletion of SMN^T so that there were no NAIP exon 5–SMN cohybridizing bands, or would some of the alleles in type I SMA patients be conversion alleles? Specifically, do type I SMA patients who have three copies of SMN^C or patients with chimeric genes associated with two copies of SMN^C conversions or deletions? If conversion does occur in type I SMA, then is it the extent of conversion 5′ of exon 7 that distinguishes the mild-SMA SMN^C alleles from the severe-SMA SMN^C alleles? If this is the case, what are the critical elements 5′ of exon 7 that are altered in severe-SMA conversions? One difficulty that should be kept in mind is the possibility that on some chromosomes the orientation of the gene clusters is flipped so that the NAIP^5 gene does not lie between the SMN^C and SMN^T genes and the SMN^C and SMN^T lie adjacent to each other. In the case of a deletion occurring on such a chromosome, it is possible that the NAIP^5 gene gets placed adjacent to the SMN^C gene, but then the allele would not have arisen by conversion.

In the case of mild-SMA conversion events, do type II and type III SMA patients who have a single copy of SMN^C have a conversion allele? Although this is most likely the case, further studies will be necessary to delineate these events. In particular, it will be very useful to use the SMN dosage analysis, in combination with marker analysis, to identify type I, type II, and type III SMA patients who have one, two, or three copies of SMN^C and then to study representative members of each group, by use of the pulsed-field gel electrophoresis assay. There are a number of reports of families in which two sibs have remarkably discordant phenotypes (Muller et al. 1992; Burghes et al. 1994*a;* Cobben et al. 1995; Hahnen et al. 1995; Wang et al. 1996; DiDonato et al. 1997), with one individual being asymptomatic and the other being an SMA phenotype but with both of them lacking the SMN^T gene. It is most likely that conversion—and not deletion—has occurred in these patients, but the copy number of SMN^C does not explain the phenotypic variation (McAndrew et al. 1997). It is now clear that absence of SMN^T is caused by both conversion events and deletion events, with the conversion events predominating on mild-SMA chromosomes and with deletion predominating on severe-SMA chromosomes.

Models of SMA and What SMN^C Alleles Do

It is now clear that loss of the SMN^T alleles on both chromosomes is the first determinant of the SMA phenotype but that the mechanism of loss, deletion, or conversion is important in the determination of phenotypic severity. We have previously suggested a model for SMA in which type I SMA patients contain two severe-SMA alleles, type II SMA patients contain a mild-SMA allele and a severe-SMA allele, and type III SMA patients contain two mild-SMA alleles (DiDonato et al. 1994, 1997; Wirth et al. 1995*b*). It has now become clear that conversion predominates on mild-SMA chromosomes and that deletions predominate on severe-SMA chromosomes. But what modifies the phenotypic severity of SMA? Analysis of the copy number of the SMN^C and SMN^T genes demonstrates that in the different SMN^C-copy-number classes there are SMA patients of all clinical types. Thus a type I SMA patient can have two copies of SMN^C, and so can a type II or type III SMA patient. This indicates that the copy number of SMN does not directly influence the phenotype. However, what can be said on the basis of the present data is that there are two types of SMN^C alleles—(1) one that is generated by conversion and that lies close to the NAIP^5 and (2) the normal SMN^C gene. Indeed, analysis of copy number of SMN^C, combined with protein analysis, indicates that not all SMN^C alleles are equivalent and that those in type II and type III SMA patients are capable of producing proteins that form gems (punctate nuclear structures containing SMN protein [Liu and Dreyfuss 1996]) whereas those in type I SMA patients are not (Coovert et al., in press). What is the molecular difference between an SMN^C gene that can partially complement the loss of SMN^T and an SMN^C gene that cannot complement the loss of SMN^T? There are two possibilities that come to mind: (*a*) position effect and (*b*) other sequence

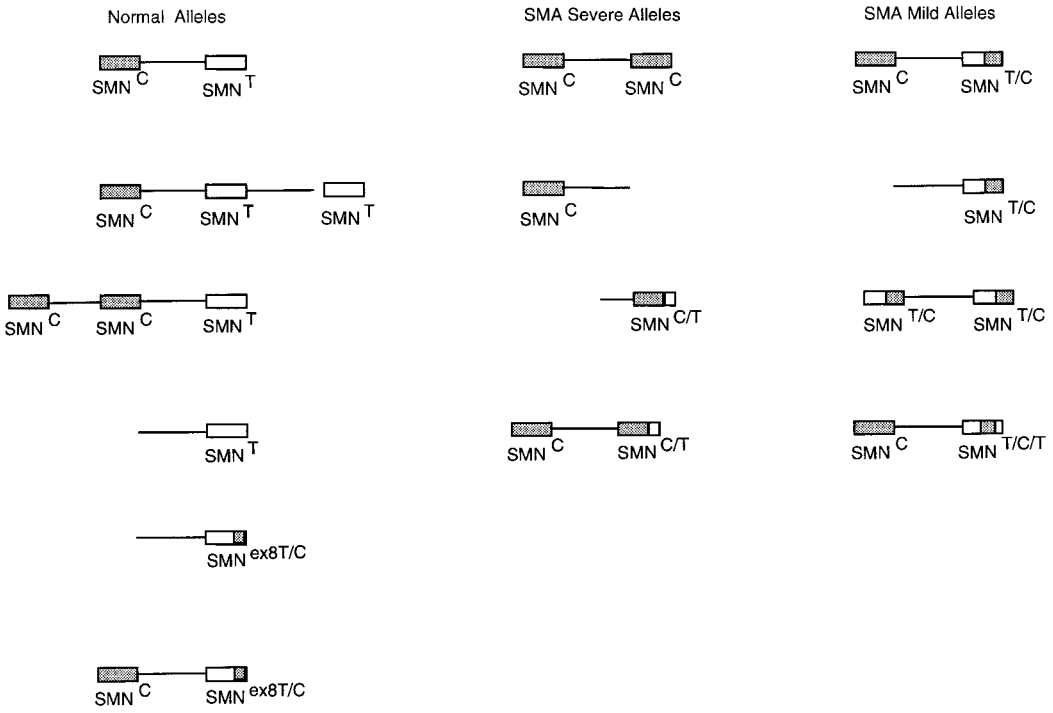

Figure 3 Model of alleles present in the normal population and in the SMA population. A combination of two severe-SMA alleles results in type I SMA; a combination that results in one copy of $SMN^{T/C}$ or $SMN^{T/C/T}$ results in type II SMA; and a combination that results in two copies of the $SMN^{T/C}$ or $SMN^{T/C/T}$ results in type III SMA. $SMN^{T/C}$ are alleles that retain the 5′ end of SMN^{T} but not the 3′ end (exons 7 and 8 contain the base changes associated with SMN^{C}); $SMN^{T/C/T}$ is a rare conversion allele, in which exon 7 of SMN^{T} is converted to SMN^{T}); and $SMN^{ex8T/C}$ is an allele in the normal population and contains the SMN^{C} exon 8 but the rest of the gene is SMN^{T}. The chromosome containing two copies of the modifying $SMN^{T/C}$ should always give rise to a mild-SMA phenotype. The exact difference between $SMN^{T/C}$s that allows it to modify the phenotype, whereas SMN^{C} cannot, is unknown at present.

changes between the SMN gene that affect either expression or the type of SMN protein produced. The complete genomic sequence of the SMN^{C} and SMN^{T} genes is nearing completion, and comparison of these sequences clearly shows that there are only minor changes between SMN^{C} and SMN^{T} genes, with some of these minor changes probably representing polymorphic variants (J. McPherson, personal communication). The availability of the genomic sequence should allow differentiation of these two possibilities.

In the future the study of SMN knockout mice and transgenes that express the human SMN^{C} and SMN^{T} genes separately should determine the contribution of the SMN^{C} gene to severity of the SMA phenotype. The models that have been presented elsewhere (Wirth et al. 1995*b;* Campbell et al. 1997; Didonato et al. 1997) can now be elaborated to account for the possibility of two different types of SMN^{C} genes (fig. 3). One type of SMN^{C} gene ($SMN^{T/C}$ and $SMN^{T/C/T}$, which are created by a conversion that does not extend through the 5′ end of the SMN^{T}) is capable of modifying the phenotype, whereas the other (SMN^{C} or a conversion extending through the entire SMN^{T} gene) is not. Thus zero copies of the modifying SMN^{C} gene results in type I SMA, one copy results in type II SMA, and two copies results in type III SMA. A converted allele would, in most instances, be equivalent to the modifying SMN allele. A model of the normal and SMA alleles is shown in figure 3. This model in some ways recalls the original suggestions of Becker (1964), who suggested the possibility of a modifying gene.

In conclusion, these are exciting times in SMA research. The gene has been cloned, various mutations have been identified, and the distinction between conversion events and deletion events has revealed a correlation of phenotype with genotype. Further work is required to clearly define the mechanism by which the converted alleles modify phenotype, and it is possible that deletion of adjacent genes, such as NAIP, could influence the exact severity of the phenotype. However, it appears most likely that the deletion of NAIP marks the extent of the deletion and that different forms of SMN^{C} modify the SMA phenotype. This is not to say that NAIP cannot protect motor neurons from cell death and that it can be explored as a target for therapeutic intervention, but perhaps the most intriguing target is the SMN^{C} gene. Can the SMN^{C} gene be activated to compensate for SMA, as suggested by Campbell et al. (1997)? Can the SMN^{C} gene be activated to make different forms of SMN? And what differences exist between SMN^{C} genes?

The SMN protein is located in nuclear structures called "gems" and is thought to have a role in RNA processing (Liu and Dreyfuss 1996), but its function(s) is yet to be defined. In SMA research there are now many avenues to follow: unraveling of the function of the SMN protein, determination of the feasibility of therapeutic approaches that activate SMN^C or replace SMN^T, and studies of the genetics of this complex locus.

Research in SMA has often felt like a religious experience firmly based in the Old Testament, with pain inflicted frequently and with only glimpses of the Promised Land. Despite the complexities of the genomic region containing the SMA gene, the Promised Land is becoming visible, and it is now clear that gene conversion—not religious conversion—has been one key to clarity.

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