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

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Spinal muscular atrophy is an autosomal recessive disorder which affects about 1 in 10,000 individuals. The three clinical forms of SMA were mapped to the 5q13 region. Three candidate genes have been isolated and shown to be deleted in SMA patients: the Survival Motor Neuron gene (SMN), the Neuronal Apoptosis Inhibitory Protein gene (NAIP) and the XS2G3 cDNA. In this report we present the molecular analysis of the SMN exons 7 and 8 and NAIP exon 5 in 65 Spanish SMA families. NAIP was mostly deleted in type I patients (67.9%) and SMN was deleted in 92.3% of patients with severe and milder forms. Most patients who lacked the NAIP gene also lacked the SMN gene, but we identified one type II patient deleted for NAIP exon 5 but not for SMN exons 7 and 8. Two other patients carried deletions of NAIP exon 5 and SMN exon 7 but retained the SMN exon 8. Three polymorphic variants from the SMN gene, showing changes on the sequence of the centromeric (cBCD541) and telomeric copies of the SMN gene, were found. In addition, we show several genetic rearrangements of the telomeric SMN gene, which include duplication of this gene in one normal chromosome, and putative gene conversion events in affected and normal chromosomes. Altogether these results corroborate the high genetic variability of the SMA region. Finally, we have determined the ratio between the number of centromeric and telomeric copies of the SMN gene in parents of SMA patients, showing that the majority of parents of types II and III patients carried three or more copies of the cBCD541 gene; we suggest a relationship between the number of copies of cBCD541 and the disease phenotype.

INTRODUCTION

Spinal muscular atrophy (SMA) is an autosomal recessive disorder with an overall incidence of 1 in 10,000 and a carrier frequency of about 1/40 (1). This neuromuscular disease is characterized by the degeneration of the α motor neurons of the spinal cord, which leads to progressive symmetrical weakness and wasting in the proximal muscles. Three types of SMA have been distinguished on the basis of clinical severity and age of onset. Type I is the most severe form, affected individuals cannot sit unaided and death occurs before 2 years; type II is the intermediate form, with early onset and inability to walk; type III corresponds to the mild form and patients achieve the ability to walk (2).

A locus for the three clinical forms of SMA was mapped to chromosome 5q11.2-q13.3 by linkage analysis (3–6). Characterization of recombinational events with closer polymorphic markers contributed to narrow the SMA locus genetic interval (7–10).

Five YAC contigs spanning the SMA region have been constructed (8,11–14) but physical mapping has been hampered by the instability of this chromosomal region probably due to the presence of many repeated sequences (8,11,15,16). Furthermore, it has been found to contain a large inverted duplication (17) and several classes of expressed pseudogenes (18,19). This complex genomic organization complicated the identification of the SMA gene. Recently, three SMA candidate cDNAs have been reported, the Survival Motor Neuron (SMN) gene (17), the Neuronal Apoptosis Inhibitory Protein (NAIP) gene (20) and the XS2G3 cDNA (21).

Two copies of the SMN gene, centromeric (cBCD541) and telomeric (SMN) have been identified. They are virtually identical at their genomic sequence (only five nucleotide changes along 20 kb) and both are transcribed (17). It has been reported that most SMA patients (98.6%) were homozygous for deletions of the SMN telomeric copy, but no specific mutations explaining the three SMA phenotypes have been found (17,22).

The NAIP gene shows similarity with baculoviral genes involved in inhibition of apoptosis in infected insect cells (20). One NAIP full copy and several truncated copies are present in the SMA region. Specific exons of NAIP were deleted in 45% of type I patients and 18% of types II and III, but also in 2% of unaffected carrier individuals. Thus, loss of NAIP is not sufficient to cause the disease.

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Table 1. Analysis of NAIP and SMN genes in Spanish SMA patients

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
<th>Total</th>
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<tr>
<td>SMN Exon 7</td>
<td>NAIP Exon 8</td>
<td>NAIP Exon 5</td>
<td></td>
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<tr>
<td>Total</td>
<td>28</td>
<td>28</td>
<td>9</td>
<td>65</td>
</tr>
</tbody>
</table>

(–) and (+) denote absence or presence of exon, respectively

As NAIP, the XS2G3 cDNA detects homozygous deletions in more than 50% of type I patients but also in parents of SMA individuals (21). In fact, the XS2G3 sequence is complementary to NAIP exon 7 and flanking intronic sequences (23).

In this study we have screened our SMA patients for deletions of NAIP and SMN genes. We have also characterized several SMN polymorphic variants and other genetic rearrangements that further illustrate the variability of this region. Finally, we have determined the $^4$BCD541/SMN dose ratio in our set of parents of SMA patients, and we have correlated it with the SMA phenotype.

RESULTS

Analysis of NAIP gene

To detect individuals carrying deletions of NAIP, a multiplex PCR reaction to amplify exon 5 (specific of NAIP), exon 13 (existing in both NAIP and in a truncated form of NAIP) and microsatellite D5S1416 (as internal control) was carried out as described in Materials and Methods. Thus 65 SMA affected individuals were analyzed for deletion of NAIP exon 5 (Table 1). The deletion frequency was 38.5% (67.9% in type I patients, 14.3% in type II and 22.2% in type III). Analysis of parents was also performed and we found four of 123 carrier individuals (3.2%) homozygously deleted for NAIP exon 5.

Analysis of SMN exons 7 and 8

SSCP analysis of PCR products was carried out to differentiate between centromeric and telomeric copies of SMN exons 7 and 8 (Fig. 1a, b). Table 1 summarizes the result of the screening of the 65 SMA individuals. Fifty-seven (87.7%) of total SMA patients (96.4% of type I, 78.5% of type II and 88.9% of type III) lacked the telomeric bands of SMN exons 7 and 8, and the telomeric exon 7 was absent in three patients who retained at least one copy of telomeric exon 8. Consequently the telomeric SMN gene was altered in 92.3% of patients. However, all patients carried centromeric copies of both exons.

Five type II patients retained at least one copy of the SMN telomeric gene. One of them carried the normal telomeric exon 7 and the BsmAI telomeric variant described below; another one only retained the BsmAI variant (lane 7 of Fig. 1a), and another patient lacked NAIP exon 5. The remaining two patients retained at least one copy of an apparently normal telomeric SMN gene.

SMN exons 7 and 8 analysis of 120 healthy parents of SMA patients (54 of type I, 50 of type II and 16 of type III) did not show any individual homozygous for deletion of telomeric exon 7, but two showed no telomeric copies of exon 8. In addition, four parents (3.3%; two of type I and two of type II) lacked the centromeric exons 7 and 8. Parents lacking telomeric exon 8 were further investigated. We amplified SMN exon 8 with another pair
of flanking primers (SMN8-D and SMN8-E) and then PCR products were digested with DdeI which only cleaves the centromeric copy. Once more, no telomeric PCR products were obtained; therefore, the non-detection of telomeric exon 8 was not a PCR amplification failure. Furthermore, in one of these parents, amplification from exon 7 to exon 8 (primers SMN78-F at intron 6 and SMN78-R at exon 8) and sequencing analysis of these products, revealed a hybrid SMN gene composed of telomeric exon 7 and centromeric exon 8 on his non-affected chromosome. This hybrid gene might result from a gene conversion event.

**SSCP polymorphic variants**

We have detected several polymorphic variants of the SMN gene by SSCP analysis. The exon 7 PCR product of one SMA patient showed an altered band and no telomeric one. His unaffected father presented an identical SSCP pattern except that he was probably homozygous for this variant (lanes 5 and 7 of Fig. 1a).

Two haploidentical unaffected siblings who inherited the paternal affected chromosome also showed the variant (lanes 8 and 9). A similar SSCP variant of exon 7 was detected on another unaffected father, in this case the altered band did not segregate with the disease phenotype as the affected sib was homozygously deleted for SMN exon 7 (result not shown). Sequence analysis of exon 7 PCR products from two individuals carrying this SSCP variant (Fig. 2a; SMN-2*BsmAI) showed an A→G transition on the telomeric copy, 96 bp upstream of the 3′ end of intron 6, which created a novel BsmAI restriction site. BsmAI restriction digestion of exon 7 PCR products allowed us to estimate the frequency of the SMN-2*BsmAI polymorphism as 2% in normal population.

One variant at exon 7 (cBCD541–2892g→c) was detected on the normal chromosome of an unaffected carrier. This SSCP band moved near the normal exon 7 telomeric one (result not shown). It was a centromeric copy carrying a G→C transition at the 892 position of the cDNA (Fig. 2a), changing a glycine to arginine. This polymorphism was found only once after analyzing 30 control and 120 carrier individuals.

Sequence analysis of PCR products from exon 7 revealed a non-polymorphic intronic difference between centromeric and telomeric copies, a C→G change, 44 bp upstream of the 3′ end of intron 6 (arrows in intron 6 of Fig. 2a). Probably, this is one of the three intronic differences between SMN and cBCD541 reported by Lefebvre et al. (17).

For exon 8, we detected a novel SSCP variant in a female carrier (Fig. 1b). Our sequence analysis of exon 8 PCR products from this woman and control individuals (Fig. 2b) revealed that the normal centromeric and telomeric SSCP bands had two nucleotide changes with respect to the previously published sequence (17) resulting in an AluI restriction site (ATGAGCCT instead of the predicted HaeIII site (ATGGCCCT). The presence of the AluI and the absence of the HaeIII sites were confirmed in 50 control individuals. The sequences obtained in this work are called cBCD541-2 (centromeric copy) and SMN-2 (telomeric copy) in Fig. 2b. To clarify the nomenclature, we have renamed the previously published sequences (17) SMN-1 and cBCD541-1 as the previously published sequences (17), and SMN-2 and cBCD541-2 as the sequences from the telomeric and centromeric copies of SMN, respectively, obtained in this work.

Finally, the polymorphic SSCP band detected in the female carrier matches the reported sequence of SMN exon 8 (SMN-1), except that it also includes the AluI change. So, this variant can be considered as an AT deletion of SMN-2, and we called it SMN-2*AT. In fact, when PCR products of exon 8 from control individuals and other ones carrying the SMN-2*AT variant were loaded on 6% native polyacrylamide gels, two bands of different molecular weight were visualized, the variant corresponding to the band of lower molecular weight (result not shown). This polymorphism was found in one of 120 carrier individuals and one of 30 control individuals.

The SMN-2*AT polymorphism was detected in a woman requesting prenatal diagnosis (lane 3 of Fig. 1b). Her affected type II SMA son lacked telomeric copies of exons 7 and 8 (lane 4). Mother and fetus had the bands corresponding to SMN, cBCD541 and the exon 8 variant (lanes 3 and 5). Genotyping of this family with 5q13 markers indicated that the fetus carried the affected paternal chromosome and the normal maternal one (see haplotypes in Fig. 1b). Thus, the extra-SSCP band segregated with the telomeric one. Amplification from exon 7 to exon 8 and sequencing analysis showed that the SMN-2*AT variant of exon 8 was linked to a telomeric exon 7. So the mother and the fetus normal chromosomes seem to carry two telomeric SMN genes.
The pregnancy proceeded at term and in the immediate postnatal period the baby appeared clinically unaffected.

**Densitometric analysis of SMN exon 7 in parents of SMA patients**

In the screening of SMN exons 7 and 8 by SSCP analysis of parents of SMA patients we detected remarkably strong signals corresponding to the centromeric copies on several individuals. Since we use the same primer set and the same template for amplifying centromeric and telomeric exons, and since the sizes of both products are identical, then it is possible to quantitate the relative amounts of cBCD541 and SMN exon 7 by densitometric analysis of the SSCP bands. All of the parents of affected individuals who lacked the telomeric exon 7 were analyzed. We assumed that these parents had only one copy of SMN and thus the cen/tel dose ratio could reflect the number of copies of cBCD541. A set of control individuals (Fig. 3a) was also analyzed, but it must be stressed that these individuals probably carry two copies of SMN and so, the same cen/tel dose ratio of exon 7 in both cases (control and carrier individuals) does not represent the same number of cBCD541 copies.

Results of this analysis are shown in Figure 3b. Two observations should be pointed out. First, 18.5% of parents of type I patients had a cen/tel dose ratio of 1:1. Given that their affected sibs were homoyzogously deleted for telomeric exon 7, this result suggests that parents carried a large deletion spanning cBCD541 and SMN in one chromosome. Also, parents with a dose ratio of 1:1 could carry two copies of each gene, and then the loss of SMN in their affected children could be due to de novo deletions. These families were further investigated by genotyping the multiloci polymorphic marker C272 (12) which lies in the 5′ end of the SMN gene. Segregation analysis did not demonstrate any de novo rearrangement. Furthermore, only two amplification products of C272 were detected in nine out of 10 parents with a dose ratio of 1:1 (data not shown). Second, in parents of types II and III patients, the cen/tel dose ratio is clearly biased towards higher values being significantly different from parents of type I (χ² = 26.74, d.f. = 6, p < 0.0002). Furthermore, parents of types II and III who had an increased dose of centromeric exon 7 showed a similar dose of centromeric exon 8. Although parents of type III seemed to have more copies than parents of type II, the small size of our type III sample did not permit us to establish significant differences between them (χ² = 4.30, d.f. = 6, p = 0.63). Altogether these results indicate that the number of copies of cBCD541 is quite variable (0 to 4 or more copies per diploid genome) and suggest that there would be a higher number of cBCD541 copies in patients of types II and III than in those of type I.

**DISCUSSION**

Two different genes (NAIP and SMN) have been recently reported to be associated with the SMA disease (17,20). We have performed a genetic study of these genes in our SMA families to try to understand their respective roles in SMA.

The similarity found between NAIP and the baculoviruses inhibitory apoptosis proteins (IAPs) implicates the NAIP gene in the pathological mechanism which causes motor neuron depletion, probably by alteration of the neuronal apoptotic pathway (24). We have found that NAIP exon 5 is mostly deleted in type I patients at a frequency slightly higher than that previously reported (67.9% versus 45%) (20). The majority (96%) of our patients who lacked NAIP exon 5 also lacked SMN. However we found one type II patient who lacked NAIP exon 5...
but retained the SMN exons 7 and 8. Also, four parents of SMA individuals were homozygously deleted for NAIP exon 5 but retained SMN. Thus, NAIP deletions are not always associated with SMN deletions as has been suggested (17).

NAIP deletions are not a general finding in types II and III SMA patients, but the existence of other kind of mutations cannot be excluded as they have not been tested for. It is conceivable that mutations on both genes (NAIP and SMN) must account for SMA disease. Indeed, it has been reported that genes involved in the apoptosis pathway of Drosophila melanogaster are functionally redundant, with one sometimes compensating for the loss of the other (25). So, mutations in two genes (reaper and hid) which lie in the same genomic region are required to obtain apoptosis-defective mutant flies.

The majority of our SMA patients lacked SMN (92.3%) thus corroborating that it is a SMA-determining gene (17,22). The high genetic variability of this gene is also shown in this work. We found a putative gene conversion event in two normal chromosomes from parents of SMA patients who had a hybrid SMN gene containing an exon 7cen-exon 8cen instead of the normal structure. Since the exon 8 is untranslated, it is plausible that this hybrid gene might be fully functional. Also, a putative SMN duplication was found in one normal chromosome which had a normal SMN copy and another polymorphic copy (SMN-2ΔAT polymorphism). Furthermore, we have characterized three polymorphic variants of this gene. One of them, the SMN-2Δhom polymorphism at intron 6, segregates with the disease in two patients. This change would create a putative new 5′ splice site in intron 6 (GTGAG; consensus sequence gGTRAG) (26). Whether this change affects the splicing of SMN can be determined only by mRNA studies. However, hemizygous or homozygous parents for this variant are phenotypically normal, therefore we can conclude that it is not a mutation causing disease and patients harboring this polymorphism must carry another mutation in their SMN gene. The remaining polymorphisms, the SMN-2ΔAT polymorphism at exon 8 and the ΔAT at exon 7, migrate very close to the normal telomeric bands. Therefore, a careful SMN analysis must be conducted to detect all the possible variants. Our analysis has permitted us to distinguish these variants and it has avoided false conclusions in the genetic analysis of our SMA families.

There are two patients (one type I, one type II) carrying deletions in SMN exon 7 and NAIP exon 5 but not in SMN exon 8. Taking into account the relative positions of these two genes (SMN exon7-SMN exon 8-NAIP exon 5) reported by Lefebvre et al. (17) and Wirth et al. (27), there are three possible explanations for the genetic rearrangements detected in these patients. First, the position of NAIP is wrong, being proximal to SMN. Second, deletions of NAIP and SMN are two unrelated rearrangements, but the physical proximity of the two genes argues against this possibility. Third, the presence of exon 8cen in these patients may be equivocal. These patients might have both NAIP and SMN deleted, and have a ‘c BCD541’ gene containing an exon 7cen-exon 8tel, generated by a gene conversion event.

The role of ‘BCD541’ is still unknown. Occasionally, unaffected individuals lacking the centromeric copies of SMN have been identified (17,22). We also found four parents of SMA individuals without ‘BCD541. However, homozygous deletions of the ‘BCD541 gene were never detected in SMA patients lacking SMN, due presumably to the nonviability of these individuals as has been previously suggested (17).

Our densitometric analysis of the SMN gene carried out in parents of SMA patients, indicated that the number of the centromeric copies is variable (0–4 or more copies per diploid genome) and higher in parents of types II and III patients than in parents of type I. In this analysis we assumed that SMN deletions in SMA patients were inherited from their parents. However, de novo deletions can also occur in this chromosomal region, but we consider that this parameter does not affect the global results of our analysis because of the low frequency of this rearrangement (a total of seven de novo deletions in 461 families (12,27,28)). It should be noted that the proportion of parents of type I with a 8BCD541/SMN dose ratio of 1:1 was 18.5%, which could represent one or two copies of each gene. In the case of parents with two copies of SMN, the loss of this gene in their affected children could be due to de novo deletions involving SMN. However, by segregation analysis of the multiloci microsatellite C272 in these families we have not detected such de novo deletions. Moreover, in nine out of 10 parents we have found only two amplification products of C272, and consequently they probably carry a large deletion spanning 8BCD541 and SMN in the affected chromosome. Also, we found 50% of parents of type I SMA patients who had two copies of 8BCD541. These individuals would carry an affected chromosome with a deletion which only involves the SMN gene. All these findings are correlated with a high frequency of type I patients who lacked NAIP exon 5 (67.9%; frequency of deletion 82.4%). Therefore, deletions would be the most frequent phenomenon in type I patients, as has been indicated by several authors (12,27,28). It has been reported that 50% of type I patients carried only one copy of the C212 and Ag1-CA (C272) multiloci markers on each chromosome (27). The C212 marker maps proximal and close to the SMN gene and the Ag1-CA lies at the 5′ end of the SMN gene. Taking into account that SMN is deleted in these patients, it could be inferred that the number of copies of these markers reflects the gene dose of 8BCD541. This analysis is in agreement with the high frequency of parents of type I in our sample who retained two copies of 8BCD541.

Our study shows that 66.5% of parents of type II and 75% of type III appeared to have at least three copies of 8BCD541. Once again, this result is in agreement with the analysis of the multiloci markers cited above (27) which indicated that majority of types II and III patients would carry one copy of these markers in one chromosome, and two copies in the other one.

Several pieces of evidence support the finding that deletions in the SMA region are much more frequent in type I SMA patients than in types II and III. Therefore, other different mutational mechanisms could operate in types II and III SMA chromosomes. We have described putative gene conversion events in affected and normal chromosomes, and a duplication of the telomeric copy. Therefore, the loss of SMN and the gain of one 8BCD541 copy in one chromosome might be caused by a gene conversion event. This mechanism could explain the increased dose of 8BCD541 mainly detected in parents of types II and III SMA patients. Besides, we have detected parents carrying four or more 8BCD541 copies per diploid genome, suggesting that other rearrangements such as duplications might occur in the SMA region.

In conclusion, our genetic data provide evidence for an inverse relationship between the number of 8BCD541 copies and severity of the SMA phenotype as previously suggested by Lefebvre et al.
Further studies are necessary to understand the putative role of *BCD541 and other genes in the disease.

**MATERIALS AND METHODS**

**SMA families**

We analyzed 65 SMA families, 28 of type I, 28 of type II and nine of type III. Patients fulfilled the diagnostic criteria defined by the International SMA Consortium (29). DNA was extracted from peripheral blood by the salting out method (30).

**NAIP analysis**

Deletion of the NAIP gene was analyzed by using exon 5 primers (1863 and 1864) and exon 13 primers (1258 and 1343) in a multiplex PCR reaction performed as previously described (20). As amplification of exon 13 was less effective, another primer pair (116CA1R and 116CA1F) was included to amplify the microsatellite DSS1416 as an additional internal control (10). Three fragments of 435 (exon 5), 213 (exon 13) and 140 bp (DSS1416) were resolved on 1.4% agarose gels.

**SSCP analysis of SMN exons 7 and 8**

In order to amplify exon 7 we selected primers R111 (17) and SMN7-B (5′ TTA ATT TAA GGA ATG TGA GCA C 3′). Exon 8 was amplified by using primers SMN8-A (5′ TAT GTA ATA ACC AAA TGC AAT G 3′) and SMN8-C (5′ ATA AAC TAC AAC ACC CTT CTC A 3′).

The primers SMN8-A or SMN7-B were 5′ end labeled with γ[32P]-dATP (5000 Ci/mmol; Amersham) by using T4 polynucleotide kinase (31). PCR reactions were performed in 20 μl and contained 200 ng of genomic DNA, 15 pmol of labeled primer, 18 pmol of cold primer (SMN8-C or R111), 200 μM of each dNTP, standard Perkin Elmer PCR 1 × buffer, 1.5 mM MgCl2 and 1 U Taq DNA polymerase. Samples were processed through 25 cycles consisting each one of 45 s at 94°C, 1 min at 55°C (exon 8) or 57°C (exon 7) and 1 min at 72°C. Samples were mixed with 20 μl of formamide loading buffer (95% formamide, 10 mM NaOH, 0.05% xylene cyanol and 0.05% bromophenol blue) and denatured for 10 min at 94°C; then 2–5 μl were loaded on a 0.5 × MDE gel and electrophoresed in the running conditions described elsewhere (17). Gels were dried and autoradiographed. The use of a 5′ end-labeled primer makes the resulting band pattern simpler because only two single strand bands are revealed.

Densitometric analysis of the telomeric and centromeric SSCP bands was carried out on a Desktop plus 3008 densitometer with the Diversity One v1.0 software. The *BCD541/SMN dose ratios were calculated, and final values were the average of at least two independent PCR and SSCP experiments.

**DdeI digestion analysis of SMN exon 8**

PCR amplification of SMN exon 8 was performed by using either the primers SMN8-A and SMN8-C, detailed above, or the external primers SMN8-D (5′ TGG GTA ACT CTT CTT GAT TAA A 3′) and SMN8-E (5′ AAG TAT GCA AAT TAA GAC AGT C 3′).

PCR reactions were carried out in a final volume of 25 μl containing 75 ng of genomic DNA, 20 pmol of each primer, 200 μM each dNTP, standard Perkin Elmer PCR 1 × buffer, 1.5 mM MgCl2 and 1 U Taq DNA polymerase (Perkin Elmer). Samples were subjected to 30 cycles of amplification consisting each one of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C.

For differentiating the centromeric exon 8 from the telomeric one, PCR products were digested with DdeI (Boehringer Mannheim) because only the centromeric copy is cleaved by this enzyme (32).

**Genomic PCR amplification from exon 7 to exon 8**

Amplification of the SMN gene from exon 7 to exon 8 was carried out by using the Expand High Fidelity enzyme system (Boehringer Mannheim) and the primers SMN78-F (5′ TTT AAC TTC CTT TAT TT TCT TAC AGG G 3′) and SMN78-R (5′ CAC ATA CGC CTC ACA TAC ATT TTG TT 3′) located at the 3′ end of intron 6 and at the exon 8, respectively. These primers amplified a fragment of about 1 kb.

The final volume was 50 μl and the reaction contained: 300 ng of genomic DNA, 40 pmol of each primer, 200 μM of each dNTP, 1 × buffer supplied by the manufacturer and 2 U of the enzyme mix. Thirty-five PCR cycles were run, consisting each one of: 30 s at 94°C, 30 s at 67°C and 2 min at 72°C increasing this last step by 6 s per cycle. A final extension of 15 min at 72°C was performed.

**Cloning and sequence**

PCR products were cloned into the T-vector pMosBlue (Amersham). Recombinant clones were reamplified and analyzed by SSCP as above indicated. Selected clones carrying centromeric, telomeric or polymorphic fragments were sequenced by using the dyeodeoxy chain termination method and Sequenase v2.0 (USB).

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**ABBREVIATIONS**

SMA: spinal muscular atrophy; SMN: Survival Motor Neuron gene; *BCD541: centromeric copy of the SMN gene; NAIP: Neuronal Apoptosis Inhibitory Protein gene.

**REFERENCES**


