Application of DNA-based tests for diagnosis of spinal muscular atrophy in Saudi Arabia

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SUMMARY We examined the deletion of the survival motor neuron (*SMN*) and neuronal apoptosis inhibitory protein (*NAIP*) genes in patients with spinal muscular atrophy (SMA) using polymerase chain reaction followed by restriction site assay methods. The study included 16 Saudi patients (9 SMA type I and 7 SMA type II) and 6 healthy Saudi volunteers. The homozygous deletions of exons 7 and 8 of the telomeric *SMN* gene, and exon 5 of the *NAIP* gene were found in all SMA type I patients. Exons 7 and 8 of telomeric *SMN* were deleted in all SMA type II patients. However, exon 5 of *NAIP* was deleted in three of the seven cases. All control volunteers and all family members of the patients had normal *SMN* and *NAIP*. The incidence of *NAIP* deletion was higher in the more severe SMA cases and the dual deletion of the *SMN* and *NAIP* genes was more common in Saudi SMA type I patients compared with patients of other ethnic groups.

Introduction

Proximal spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder which results in the loss of motor neurons in the spinal cord, leading to symmetrical progressive paralysis with muscle atrophy [*1*]. On the basis of clinical criteria, patients can be classified into three types [*2*]. Werdnig–Hoffman disease, or type I SMA, is the most severe form, with onset either *in utero* or during the first few months of life. Affected children cannot sit unsupported and death usually occurs at less than 2 years of age. Type II SMA usually manifests within the first year of life, and although affected children may sit unaided, they do not achieve the ability to stand or walk independently. Kugelberg–Welander disease, or type III SMA, is a less severe form, characterized by a later age of onset. Affected individuals have variable severity, walk independently, and may have a normal life expectancy.

The candidate region for SMA locus has been mapped to chromosome 5q11.2–5q13.3 by linkage analysis [*3,4*]. Recent studies by several investigators have shown that the SMA region is highly polymorphic and has a complex genomic structure containing several genes and pseudogenes [*5–7*]. Two candidate genes are known to be involved in SMA. The survival motor neuron gene (*SMN*) exists in two nearly identical copies, telomeric *SMN* (tel*SMN*) and centromeric *SMN* (cen*SMN*), within the

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SMN region [*1,5*]. Both copies are composed of eight exons which encode identical amino acid sequences. The two genes differ in their exons by only two base pairs, one in exon 7 and one in exon 8, that allow the distinction of the tel*SMN* gene from cen*SMN* by single-strand conformation analysis (SSCA) and restriction site assay [*1,8*]. Another candidate gene is the neuronal apoptosis inhibitory protein (*NAIP*) gene which contains more than sixteen exons [*9*]. The *NAIP* gene is duplicated either with exon 5 ($NAIP^5$) or without exon 5 ($NAIP^D$).

SMA has a worldwide incidence of 1 in 10 000 live births with a carrier frequency of 1 in 40. Because of the prevailing custom of consanguineous marriage, however, the incidence of SMA type I in the Saudi population is 15–60 times higher than that seen in populations of western Europe and North America [*10*]. Although the deletions of the *SMN* and *NAIP* genes have been reported for many ethnic groups, until now mutations in the DNA of Saudi SMA patients have not been thoroughly investigated. Here we describe the outcome of our initial effort to identify mutations in the *SMN* and *NAIP* genes in a representative group of Saudi patients with SMA.

Materials and methods

Patient selection and DNA resources

We studied 16 SMA Saudi patients, all of whom were evaluated by neurologists and found to fulfill the diagnostic criteria for proximal SMA defined by the International SMA Consortium [*2*]. For molecular studies, 5–10 mL of blood were collected in vacutainer tubes (acid citrate-dextrose, yellow top) from patients and their unaffected relatives. Blood was generally processed within 24 hours of collection. Human genomic DNA was prepared from peripheral blood by a conventional lysis method [*11*]. Briefly, anticoagulated blood was treated with a hypotonic solution (155 mmol/L ammonium chloride, 10 mmol/L ammonium bicarbonate). From the remaining lymphocytes, DNA was liberated with sodium dodecyl sulfate and proteinase K digestion, and then extracted with phenol and chloroform.

Deletion analysis of the *SMN* gene

Deletions in the tel*SMN* gene were identified by restriction site analysis after amplification of exon 7 and exon 8 [*8*]. The amplification was carried out with a hot start for 7 minutes at 94 °C and subsequently with 35 cycles [1 minute at 94 °C, 1 minute at 55 °C (for exon 7) or 1 minute at 57 °C (for exon 8), and 1 minute at 72 °C], followed by an extension at 72 °C for 10 minutes. The amplified products were further digested with either *Dra* I (for exon 7) or *Dde* I (for exon 8) for 4 hours at 37 °C. The digested products were run on 2% agarose gel and subsequently visualized under ultraviolet light. The digested products corresponded to 190 bp for exon 7 of tel*SMN* and 165 bp and 25 bp for exon 7 of cen*SMN*. For exon 8, the products are 192 bp for tel*SMN* and 122 bp and 70 bp for cen*SMN*. Positive deletion control samples were gifts from Dr Judith Melki, Paris.

Deletion analysis of the *NAIP* gene

NAIP gene analysis was performed by polymerase chain reaction (PCR) amplification of exon 5 (primers 1863 and 1864) and exon 13 (primers 1258 and 1343), according to a method described previously [*9*]. All reactions were carried out with a hot start for 5 minutes at 94 °C and subsequently with 35 cycles (1 minute at 94 °C, 1 minute at 57 °C and 1 minute at 72 °C) followed by an extension at 72 °C for 7 min-

utes. The PCR products were analysed for the presence and absence of exon 5 and exon 13.

Results

The deletion analysis of exons 7 and 8 of the *SMN* gene and exon 5 of the *NAIP* gene are shown in Tables 1 and 2. DNA analysis showed that the tel*SMN* gene was absent or interrupted in all 9 SMA type I patients, including 2 SMA variants (these patients had additional neurological symptoms), and in all SMA type II patients. There were 13 patients who showed homozygous deletion of *NAIP* exon 5, although the deletions of *NAIP* exon 5 were more common in type I SMA patients (Figure 1). All control Saudi volunteers and all family members of the patients had both normal *SMN* and *NAIP* genes (data not shown).

Table 1 **Confirmation of clinical diagnosis of the SMA patients by DNA deletion in the *SMN* and *NAIP* genes**

SMA	tel*SMN* deletion	*NAIP* deletion	Clinical diagnosis
SMA type I			
Patient 1	+	+	Affected
Patient 2	+	+	Affected
Patient 3	+	+	Affected
Patient 4	+	+	Affected
Patient 5	+	+	Affected
Patient 6	+	+	Affected
Patient 7	+	+	Affected
Patient 8[a]	+	+	Affected
Patient 9[a]	+	+	Affected
SMA type II			
Patient 1	+	+	Affected
Patient 2	+	–	Affected
Patient 3	+	–	Affected
Patient 4	+	–	Affected
Patient 5	+	+	Affected
Patient 6	+	+	Affected
Patient 7	+	+	Affected

[a]*Indicates SMA type I variant*
SMA = spinal muscular atrophy
SMN = *survival motor neurone*
NAIP = *neuronal apoptosis inhibitory protein*

Discussion

Previous studies have shown that 90%–95% of SMA patients of different ethnic groups carry homozygous deletions in tel*SMN*, affecting exon 7 only, or both exon 7 and 8, independent of the clinical severity of the disease [*1,5,6,12*]. The cen*SMN* gene was not interrupted in 95% of normal and SMA chromosomes. Thus, a strong correlation exists between deletion of the tel*SMN* gene and SMA. It has been suggested that this deletion is the most reliable means of diagnosis of the disease [*5,12,13*].

The objective of our study was to apply the PCR-based DNA restriction site assays to confirm the clinical diagnosis of SMA in Saudi patients. Our study showed homozygous deletion of exons 7 and 8 of the tel*SMN* gene in all (100%) Saudi SMA patients with severe or milder forms of the disease. This accords with previous reports on different ethnic groups [*1,5,6,8, 9,14,15*]. *NAIP* exon 5 was 100% deleted in type I patients and in 13 (81%) of the total group of 16 patients, irrespective of the severity of the disease. The detection of homozygous deletion in *NAIP* exon 5 in Saudi patients is higher than that reported in previous studies conducted among several different ethnic groups [*1,5,9*]. Further studies involving large numbers of Saudi SMA patients are necessary to substantiate the current observation. Furthermore, we have also demonstrated that most patients who lacked the *NAIP* exon 5 also lacked tel*SMN* exons 7 and 8.

Table 2 **Deletion analysis of exons 7 and 8 of the telomeric *SMN* gene and exon 5 of the *NAIP* gene in 16 SMA patients**

SMA	tel*SMN* exon 7	tel*SMN* exon 8	*NAIP* exon 5
Type 1	●●●● ●●●	●●●● ●●●	●●●● ●●●
Type II	●●●● ●●●	●●●● ●●●	●○○○ ●●●
Variant (type 1)	●●	●●	●●

● *homozygously deleted* ○ *not deleted*
SMN = *survival motor neurone*
NAIP = *neuronal apoptosis inhibitory protein*
SMA = spinal muscular atrophy

Since approximately 95% of SMA patients homozygously lack exon 7 of the tel*SMN* gene, regardless of phenotype [*5,6,7,12,16*], genotype–phenotype correlation for SMA has not been straightforward. However, additional research has shown that losses of tel*SMN* exon 7 are not all equal. Deletions of tel*SMN* are common in severely affected patients with type I SMA, whereas gene conversions between the tel*SMN* and cen*SMN* genes are associated with milder disease phenotypes [*5,6,7, 12,15*].

Recent studies have provided additional information regarding the potential role of the cen*SMN* gene in modifying *SMN* phenotypes [*6.7.12.16*]. An increased cen*SMN* copy number (which can result from sequence conversion of tel*SMN* to cen*SMN*) has been reported to be correlated with a milder disease phenotype in patients who homozygously lack the tel*SMN* gene [*6.7.12.16*]. In light of these observations, further studies are necessary to draw a conclusion on genotype–phenotype correlation in Saudi SMA patients.

We conclude that the incidence of *NAIP* deletion is higher in more severe SMA cases, and that dual deletion of the *SMN* and *NAIP* genes is more common in Saudi SMA type I patients compared with those of other ethnic groups.

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