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Deletion analysis of the SMN and NAIP genes in Kuwaiti patients with spinal muscular atrophy

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Abstract Two genes are known to be involved in spinal muscular atrophy (SMA), namely, SMN (survival motor neuron) and NAIP (neuronal apoptosis inhibitory protein). Deletion analysis of these genes has been reported for many ethnic groups. We have extended this analysis to include 15 Arabic patients (11 unrelated cases of type I, which represent practically all of the patients diagnosed within the last 2 years in Kuwait, and 4 type-II cases from a single kinship). Also, 41 healthy relatives (parents and sibs) and 44 control individuals of Arabic origin were analyzed. The homozygous deletions of exons 7 and 8 of the SMN gene were found in all SMA patients studied. Exon 5 of NAIP was homozygously absent in all type-I patients, but was retained in type-II cases. Among members of SMA families, one mother was found to be homozygously deleted for NAIP. All of the control individuals had both normal SMN and NAIP. Our results are in agreement with the general consensus that the incidence of NAIP deletion is higher in the more severe SMA cases. Furthermore, they suggest that SMA type-I chromosomes, with the dual deletion of the SMN and NAIP genes, are more common in Arabs than in patients of other ethnic origin.

Introduction

The spinal muscular atrophies (SMAs) are a clinically heterogeneous group of genetic disorders caused by degeneration of anterior horn neurons. Depending on the severity and time of onset, SMAs are classified into three childhood forms (type I/Werdnig-Hoffmann disease, type II and type III/Kugelberg-Welander disease) and one adult-onset form (Munsat 1991). All these forms have been mapped to chromosomal region 5q13 (Brzustowicz et al. 1990; Gilliam et al. 1990; Melki et al. 1990a, b). Recently, two candidate genes, namely SMN (survival motor neuron) and NAIP (neuronal apoptosis inhibitory protein), have been suggested as SMA-determining and SMA-modifying genes, respectively. The SMN gene has been found to be homozygously absent or interrupted in 98.6% of childhood SMAs (Lefebvre et al. 1995) and in at least some patients with the adult form (Brahe et al. 1995a). The frequency of homozygous deletion of the intact NAIP gene was found to be different in SMA type I and type II/III (45% versus 18%) (Roy et al. 1995), thus leading to the suggestion that the severity of the disease may depend on the deletion of the NAIP gene.

Analysis of both the SMN and NAIP genes is significantly complicated by the existence of highly homologous genes that limit the ability of the currently used tests to detect only homozygous deletion of these genes. The SMN gene, compared with its centromeric homolog, the ^{c}BCD541 gene, has a few mismatches, particularly in exons 7 and 8. These nucleotide differences have been used in polymerase chain reaction(PCR)/single strand conformation polymorphism (Lefebvre et al. 1995) and PCR/restriction fragment length polymorphism (van der Steege et al. 1995) analyses to distinguish between SMN and ^{c}BCD541, thus allowing for the identification of homozygous deletions of SMN exons 7 or 8. In the case of NAIP, in addition to the intact gene, several truncated and internally deleted variants exist, and these are present in a variable number of copies. The identification of the intact NAIP gene is based on the amplification of exon 5 or 6 (the first two coding exons), which are believed to be deleted in the other forms (Roy et al. 1995).

Within the last year, there have been several reports of SMN and NAIP gene deletions in SMA patients of different ethnic origin: French (Lefebvre et al. 1995), Italian (Brahe et al. 1995b), Hispanic (Bussaglia et al. 1995), Dutch (Cobben et al. 1995), German (Wirth et al. 1995), Polish (Brzustowicz et al. 1995), American (Kant et al. 1995; Wang et al. 1995), Canadian (Aubry et al. 1995) and Chinese (Lin et al. 1995). However, information pertaining to the analysis of these genes in Arabic patients is

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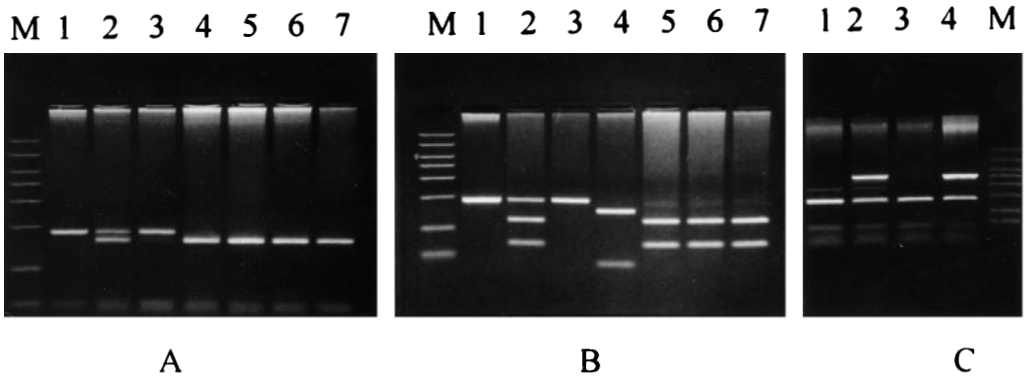

Fig. 1A–C Polymerase chain reaction (PCR) analysis of spinal muscular atrophy (SMN) and neuronal apoptosis inhibitory protein (NAIP) genes. (*M* molecular weight markers: 50, 100, 200, 300 bp) **A** SMN exon 7 (*upper band* SMN, *lower band* cBCD541). *Lane 1* undigested PCR product, *lanes 2–7 Dra*I digests. *Lane 2* healthy individual with both the SMN and ᶜBCD541 genes, *lane 3* the father of an SMA type-II patient with a deletion of ᶜBCD541, *lanes 4–7* SMA probands with a deletion of the SMN gene. **B** SMN exon 8 (*upper band* SMN, *two lower bands* ᶜBCD541). *Lane 1* undigested PCR product, *lanes 2, 3, 5–7 Dde*I digest, *lane 4*:l *Hin*fI digest, *lane 2* healthy individual with both the SMN and ᶜBCD541 genes, *lane 3* the father of a SMA type-II patient with a deletion of ᶜBCD541, *lane 4 Hin*fI digest of PCR product from the individual of *lane 3*, to confirm that the deletion of ᶜBCD541 was not due to a failure of restriction digestion, *lanes 5–7* SMA probands with a deletion of the SMN gene. **C** Multiplex PCR of exon 5 of NAIP gene (*upper band*) and SMN exon 8 (*lower band*). *Lanes 1, 3* SMA probands showing the absence of amplification of NAIP exon 5, *lanes 2, 4* healthy individuals showing both NAIP exon 5 and SMN exon 8 bands

lacking in the literature. Here, we report the results of the deletion analysis of the SMN and NAIP genes in Arabic SMA patients from Kuwait.

Materials and methods

Fifteen SMA patients (11 unrelated cases of type I and 4 type-II cases from a single kinship), 41 healthy relatives (parents and sibs) and 44 control individuals were analyzed for deletions of exons 7 and 8 of the SMN gene and exon 5 of the NAIP gene. At least five families with SMA type I and the type-II kinship are known to be consanguineous. In the other cases it was possible to trace the origin of both parents to the same tribe or tiny village from which their ancestors migrated to Kuwait.

The analysis of DNA samples was performed as reported elsewhere (Roy et al. 1995; van der Steege et al. 1995), except that a different control for amplification was used in the PCR for NAIP exon 5 (i.e., instead of exon 13 of the NAIP gene, exon 8 of SMN was simultaneously amplified). After PCR amplification of exons 7 and 8, the products were digested with *Dra*I or *Dde*I, respectively and analyzed by agarose gel electrophoresis.

Results

In the analysis of exon 7 of SMN, *Dra*I digestion of the PCR product yields two fragments: the one with a higher molecular weight corresponds to the SMN gene and the other to ᶜBCD541. In the case of exon 8, *Dde*I digestion results in three fragments: the higher molecular weight fragment corresponds to SMN, while the other two correspond to ᶜBCD541. As for the NAIP gene, the presence of only the control band (SMA exon 8 fragment) indicates a deletion of NAIP exon 5. Representative results of the deletion analysis are shown in Fig. 1.

All patients with SMA type I were found to be deleted for exons 7 and 8 of the SMN gene and also for exon 5 of the NAIP gene. Analysis of their parents and sibs as well as control individuals revealed the band pattern corresponding to the presence of the intact NAIP gene and both the SMN and ᶜBCD541 genes on at least a single chromosome in all cases except for one. This individual, the mother of a SMA proband, was found to be homozygously deleted for exon 5 of the NAIP gene.

The type-II patients analyzed belong to a single kinship (Fig. 2) with all members known to originate from the same tribe. They retained the intact NAIP, but were deleted for both exons 7 and 8 of the SMN gene. The mothers of these patients were found to have the normal band pattern for NAIP, SMN and ᶜBCD541. However, both fathers, while having the NAIP and SMN genes, seemed to lack the centromeric homolog, ᶜBCD541, since the corresponding bands were not revealed in the exon 7/*Dra*I and the exon 8/*Dde*I digests. The failure of restriction digestion due to the presence of enzyme inhibitors was excluded, since the digestion of the PCR product of exon 8 with another enzyme, *Hin*fI, having a recognition site in both SMN and ᶜBCD541, was successful. The paternal homozygosity for the ᶜBCD541 deletion led to the recognition that two different SMA chromosomes are segregating in this consanguineous kinship: the maternal SMA chromosome is deleted for only SMN and not for ᶜBCD541 (which is proved by the presence of ᶜBCD541 in affected children), while the paternal SMA chromosome carries neither ᶜBCD541 nor SMN (both fathers are homozygously deleted for ᶜBCD541).

Discussion

Our results are in agreement with data reported for patients of other ethnic origin insofar as the SMN gene is concerned. However, the deletion of the NAIP gene in each of our type-I patients was surprising, since in previ-

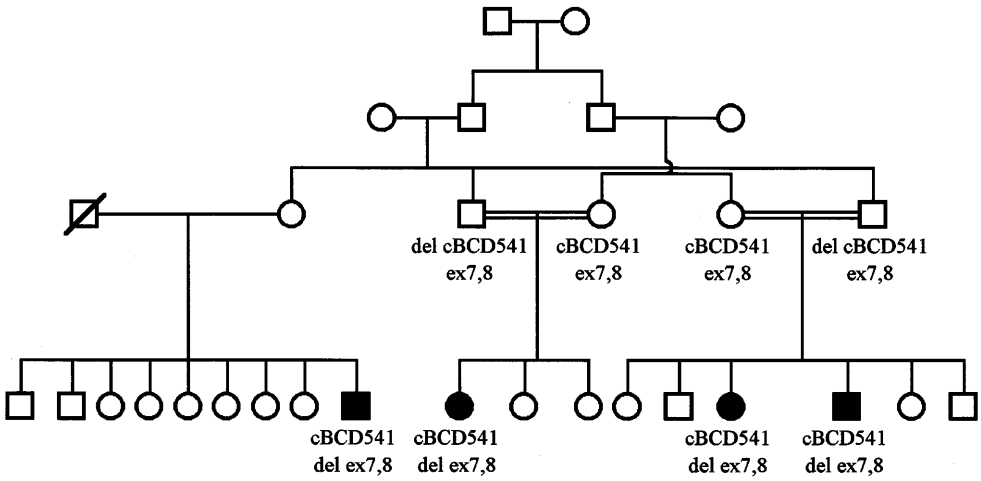

Fig. 2 Pedigree of kinship with SMA type-II patients with the result of SMN gene analysis. The deletions of the SMN and ᶜBCD541 genes are shown as *del ex7,8* and *del cBCD541*, respectively

ous studies, the frequency of homozygous deletion was found to be less than 50% in type-I patients from Europe and North America (Aubry et al. 1995; Lefebvre et al. 1995; Wirth et al. 1995) and 62–66% in ethnically diverse groups (Burlet et al. 1996; Rodrigues et al. 1996). Our data would suggest that the SMA type-I chromosomes with the dual deletion of the SMN and NAIP genes are more common in Arabs than in patients of other ethnic origin. It is desirable to extend this study by including a larger number of SMA cases of Arabic background. However, in a small country such as Kuwait, despite the relatively high incidence of SMA type I, the overall number of cases is low. In fact, the present study reports practically all cases diagnosed for SMA type I in Kuwait within the last 2 years. Additional evidence for "ethnic impact" on the SMA chromosome can be obtained from Chinese patients, where a pattern opposite to that seen with Arabic SMA chromosomes is observed. The Chinese patients, while being homozygously deleted for exon 7 of the SMN gene, have retained exons 5 and 6 of the NAIP gene (Lin et al. 1995).

What can account for the differences in the frequency of the homozygous deletion of NAIP among various ethnic groups? It can be suggested that there are chromosomes with a deleted NAIP variant that has actually retained exons 5 and 6, and the frequency of such chromosomes can vary among different populations. Although this view contradicts the existing belief that the absence of exons 5 and 6 is characteristic of all deleted NAIP forms, it cannot be completely ignored. Indeed, a truncated variant (CC20.3), which consists of exons 5–7 and intron 7 was identified among the cDNAs isolated from a human fetal brain library (Roy et al. 1995). If the hypothesis is true, then the actual number of SMA cases with the deletion of the intact NAIP gene may be higher than detected by PCR amplification of NAIP exons 5 and 6.

Most of the families studied here are either consanguineous or both parents originate from the same tribe. Thus, the homozygosity by descent would be expected. However, in the SMA type-II kinship, despite the consanguinity, two different SMA chromosomes were found to segregate: exons 7 and 8 of SMN were deleted on both SMA chromosomes, but the centromeric ᶜBCD541 gene was present only on one of these. The absence of ᶜBCD541 represents a normal polymorphism: the homozygous deletion of ᶜBCD541 has been reported in 4.4% of healthy individuals (Lefebvre et al. 1995).

In agreement with the general consensus that the incidence of NAIP deletion is much higher in the clinically more severe SMA cases than in the milder forms, all our patients with type I were homozygously deleted for NAIP, while the type-II cases had at least one copy of the intact NAIP gene. A recent study, based on the assumption that the SMN gene is flanked by the C212/C217 markers (upstream) and NAIP (downstream), has shown that type I is characterized by deletions involving not only SMN and NAIP but also C212/C217. Such large-scale deletions are virtually absent in type-II and type-III patients (Burlet et al. 1996).

In spite of the overwhelming evidence for the involvement of the SMN and NAIP genes in the genesis of SMA, there are some data that could challenge their role in the disease. Among them is the identification of homozygous deletion of SMN or NAIP in healthy individuals from SMA families. The homozygous deletion of the intact NAIP gene was observed in at least 20 healthy carriers reported from Canada (Roy et al. 1995), Germany (Wirth et al. 1995), the UK (Rodrigues et al. 1996) and from Kuwait (this study). Also, the deletion of SMN exons 7 or 8 was identified in at least 15 healthy persons (sibs and parents of SMA probands) from Holland (Cobben et al. 1995), Germany (Wirth et al. 1995), and North America (Gilliam et al. 1995). In one of these cases, the asymptomatic mother revealed mild neurogenic abnormalities when muscle biopsy and electromyography were performed (Gilliam et al. 1995). Interestingly, all asymptomatic cases with homozygous SMN deletion, for which the clinical description of the proband was given, belong to families with the milder forms of SMA (type II or III). To explain the healthy status in individuals homozygously deleted for SMN or NAIP, different mechanisms have been proposed such as a gene dosage effect, incomplete penetrance, previously undetected extreme clinical variability (at least in

the families with mild SMA forms), as well as the existence of yet another SMA-determining or SMA-modifying gene (Gilliam et al. 1995; Somerville et al. 1995; Theodosiou et al. 1995; Wirth et al. 1995). Further studies of the mechanisms that can influence the genotype-phenotype relationship in SMA are obviously required.

The currently available molecular tests seem to be extremely useful for SMA diagnosis, despite the above-mentioned controversy regarding the etiological role of SMN and NAIP. However, in Kuwait, the implementation of diagnostic tests for many genetic diseases is limited by the scarcity of data on the mutations common in the local population. The direct extrapolation of data from studies of North-American and west-European patients is often worthless because the mutations frequent among these patients are less common or even absent in patients of Arabic origin. The results presented here show that, fortunately, this is not the case with SMA and the diagnostic tests based on the detection of the SMN and NAIP deletions can be implemented in this region.

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