Articles

Molecular analysis of the spinal muscular atrophy and neuronal apoptosis inhibitory protein genes in Saudi patients with spinal muscular atrophy

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ABSTRACT

Objective: Spinal muscular atrophy (SMA) is a common, often fatal, autosomal recessive disease leading to progressive muscle wasting and paralysis as a result of degeneration of anterior horn cells of the spinal cord. The prevalence of SMA cases in the Kingdom of Saudi Arabia (KSA) is much higher than the European and North American population. Deletions or mutations in 2 genes, telomeric form of the survival motor neuron (SMN1) and the neuronal apoptosis inhibitory protein (NAIP), are known to be associated with SMA. The aim of this study is to examine the deletions or interruptions of the SMN1 and NAIP genes in Saudi patients.

Methods: The study included 121 Saudi SMA patients [type I (60 patients); type II (26 patients); and type III (35 patients)]. The deletions or interruptions of the SMN1 and NAIP genes were detected by using polymerase chain reaction. The study was carried out at the King Fahad National Guard Hospital, Riyadh, KSA between 2000 and 2002.

Results: The homozygous deletions of exons 7 and 8 of the SMN1 gene were found in 94% and 87% of the patients. Exon 5 of the NAIP gene was deleted in 70%, but its deletion was more frequent in SMA type I (93%) as compared to type II (54%) and type III (43%). Seven patients with SMA diagnosis did not show any of the above homozygous deletions. All 230 control subjects had at least one copy of both SMN1 and NAIP genes, as expected.

Conclusion: Our results demonstrate that the deletion rate (94%) of the SMN1 gene in Saudi SMA patients is similar, irrespective of types, compared with patients of other ethnic groups. We also show that the incidence of NAIP deletion is higher in the more severe SMA cases and the dual deletion of the SMN1 and NAIP genes are more common in Saudi SMA type I patients compared with patients of other ethnic groups.

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Proximal spinal muscular atrophy (SMA) is a neuromuscular disorder, which results in the loss of motor neurons in the spinal cord and lower brainstem leading to symmetrical progressive paralysis with muscle atrophy. Spinal muscular atrophy is classified into 3 clinical groups based on age at onset, achievement of developmental milestones and lifespan.[1] Type I SMA, known as Werdnig-Hoffmann disease (the most severe form of SMA), is characterized by severe generalized muscle weakness and hypotonia with onset between birth and 6 months. Death from respiratory failure usually occurs within the first 2 years. In type II

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(intermediate form) and type III (Kugelberg-Welander disease), patients learn to walk, but suffer from proximal muscle weakness with onset after the age of 2. The candidate region for SMA locus has been mapped to chromosome 5q11.2-5q13.3 by linkage analysis.[2,3] Two candidate genes are known to be involved in SMA. The survival motor neuron gene (SMN) is present in 2 highly homologous copies, telomeric SMN (SMN1) and centromeric SMN (SMN2) within the SMN region.[4] Both copies are composed of 9 exons, which encode identical amino acid sequences. The 2 genes differ in their exons by only 2 base pairs, one in exon 7 and one in exon 8, which allows distinction of SMN1 from SMN2 by single strand conformation polymorphism analysis (SSCP) and restriction site assay.[4,5] Independent of the clinical severity, homozygous deletions or interruptions of the SMN1 gene that affect exon 7 or exons 7 and 8 were found in 90-98% of SMA patients in many ethnic groups.[4-7] The SMN2 gene is not interrupted in 95% normal and SMA chromosomes.[7] Thus, there is a strong correlation between deletion of the SMN1 gene and SMA and this deletion has been suggested to be the most reliable means of diagnosis of the disease. Other candidate gene is the neuronal apoptosis inhibitory protein gene (NAIP), which contains more than 16 exons.[8] The NAIP gene shows homozygous deletions in 45-67% of type I and 20-42% of type II and type III patients.[6-9] Spinal muscular atrophy is reported to have an overall worldwide incidence of one in 10,000 live births; however, due to the prevailing custom of consanguineous marriage, incidence, prevalence, and carrier frequency of SMA in Saudi population are much higher than the European and the North American population.[10] In this report we present gene deletions analysis in 121 SMA cases belonging to 97 families.

Methods.

Patient selection and blood collection. The study included 121 SMA Saudi patients. All patients were evaluated by neurologists and fulfilled diagnostic criteria for proximal SMA defined by the International SMA Consortium.[1] The patients were classified into SMA type I (60 patients), type II (25 patients) and type III (35 patients). One hundred and ten healthy family members, 90 unrelated normal individuals and 30 patients with other neuromuscular diseases were used as controls. The local ethics committee reviewed the study and an informed consent was obtained from the parents of the patients. The study was carried out at the King Fahad National Guard Hospital, Riyadh, Kingdom of Saudi Arabia between 2000 and 2002. Genomic DNA was extracted from 2-5 ml of peripheral blood collected from each individual following the method by QIAamp™ DNA blood midi kit according to manufacturer (QIAGEN, Germany) protocol.

Deletion analysis of the survival motor neuron gene. Deletions in the SMN1 gene were identified by restriction site analysis after amplification of exon 7 and exon 8.[5] This method allows exons 7 and 8 of SMN1 to be distinguished from the corresponding exons of SMN2. The polymerase chain reaction (PCR) protocol is similar to the method described previously.[5] The amplification was carried out with a hot start for 7 minutes at 94°C and subsequently with 35 cycles (denaturation for one minute at 94°C, annealing for one minute at 55°C [for exon 7] or one minute at 57°C [for exon 8], and extension for one minute at 72°C). The amplified products are further digested with either Dra1 (for exon 7) or Dde1 (for exon 8) for 4 hours at 37°C. The digested products are run on 2% agarose gel and subsequently visualized under UV light.

Deletion analysis of the neuronal apoptosis inhibitory protein gene. Neuronal apoptosis inhibitory protein gene analysis was performed by PCR amplification of exon 5 and exon 13 following the method described earlier.[8] All reactions were carried out with a hot start for 5 minute at 94°C and subsequently with 35 cycles (denaturation for one minute at 94°C, annealing for one minute at 57°C, and extension for one

Table 1 - Percentage distribution of SMN1 (survival motor neuron) and NAIP (neuronal apoptosis inhibitory protein) deletions in different groups.

SMA Type (n of patients)	SMN1 exon 7 n of patients had deletion n (%)	SMN1 exon 8 n of patients had deletion n (%)	NAIP exon 5 n of patients had deletion n (%)
Type I 60	57 (95)	52 (87)	56 (93)
Type II 26	25 (96)	22 (85)	14 (54)
Type III 35	32 (91)	31 (88)	15 (43)
Total 121	**114 (94)**	**105 (87)**	**85 (70)**

SMA - spinal muscular atrophy, SMN1 - survival motor neuron, NAIP - neuronal apoptosis inhibitory protein

Table 2 - Percentage of consanguinity and family history.

SMA Type (n of patients)	Parents related (%)	First degree (%)	Positive family history (%)
Type I 60	(69)	(56)	(67)
Type II 26	(55)	(35)	(75)
Type III 35	(52)	(40)	(61)
Total 121	**(59)**	**(44)**	**(68)**

SMA - spinal muscular atrophy

minute at 72°C). The PCR products were analyzed for the presence and absence of exon 5 and exon 13. Amplification of NAIP exon 13 served as control for the presence of the NAIP gene.

Results. As shown in **Table 1**, exon 7 of the SMN1 gene was absent or interrupted in 57 out of 60 SMA type I patients, 25 out of 26 type II patients and 32 out of 35 type-III patients. Exon 8 of the SMN1 gene was absent or interrupted in 105 out of 121 patients (87%). Seventy percent of the patients showed homozygous deletion of NAIP exon 5 although the deletions of NAIP exon 5 were more common in type I SMA patients (93%). All control subjects (n=230) had at least one copy of both SMN1 and NAIP genes, as expected. Positive family history of SMA in the siblings or other first-degree relatives was seen in 82 out of 121 SMA patients (**Table 2**). Fifty-nine percent of the SMA patients were born of consanguineous marriages, but the parents who are first degree cousins were more common in SMA type I (56%) as shown in **Table 2**.

Discussion. Our study showed homozygous deletion of exons 7 of the SMN1 gene in 94% of Saudi SMA patients with severe or milder form, which is in agreement with previous reports dealing with different ethnic groups.[4,6,7,9,11-13] Neuronal apoptosis inhibitory protein gene exon 5 was mostly deleted in type I patients. We also demonstrated that most patients who lacked the NAIP exon 5 also lacked SMN1 exons 7 and 8. Seven of our SMA patients (3 each from SMA type I and III and one from type II) did not show homozygosity for a deletion in the SMN1 gene in exon 7. These patients have been diagnosed based on the International SMA Consortium criteria. Several studies have reported that a small minority of SMA patients lack homozygous SMN1 deletions.[4,7,11] For some of the SMA patients, smaller mutations in the SMN gene have been described.[4,14] In some SMA patients gene conversion and rearrangement events leading to formation of hybrid SMN1 or SMN2 genes have been reported.[13,15] Studies are underway to determine, which of the above events are playing a role in causing the disease in minority of Saudi patients without homozygous deletion of SMN1.

Our results are in agreement with the general consensus that there is no correlation between genotype and phenotypic expression of the disease. The data also suggest that the incidence of NAIP deletion is higher in the more severe SMA cases, and the dual deletion of the SMN1 and NAIP genes are more common in the Saudi SMA type I patients compared with patients of other ethnic groups.[7-9] Akutusu et al[16] have suggested that NAIP deletion may be a prognostic indicator of SMA. Presence of positive family history is more common in Saudi SMA patients comparing with other ethnic groups. Furthermore, although consanguinity in Saudi SMA parents is similar to the Saudi general population, first degree consanguinity related to SMA type I cases is higher than the general population (56% versus 35%).

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