

Deletions in the Survival Motor Neuron Gene in Iranian Patients with Spinal Muscular Atrophy

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Abstract

Introduction: Spinal muscular atrophy (SMA) is a common neuromuscular disorder with progressive paralysis caused by the loss of α -motor neurons in the spinal cord. The survival motor neuron (SMN) protein is encoded by 2 genes, SMN1 and SMN2. The most frequent mutation is the biallelic deletion of exon 7 of the SMN1 gene. In SMA, SMN2 cannot compensate for the loss of SMN1, due to the exclusion of exon 7. The aim of our study was to estimate the frequency of the common SMN1 exon 7 deletion in patients referred to our centre for carrier detection and prenatal diagnosis. **Materials and Methods:** We performed the detection of exon 7 deletion of the SMN1 gene for the affected patients and fetuses suspected to have SMA. **Results:** Of 243 families, 195 were classified as SMA type I, 30 as type II, and 18 as type III according to their family histories. The analysis of exon 7 deletion among living affected children showed that 94% of the patients with SMA type I, 95% with type II families and 100% with type III had homozygous deletions. Of the prenatal diagnoses, 21 (22.8%) of the 92 fetuses were found to be affected and these pregnancies were terminated. **Conclusions:** The homozygosity frequency for the deletion of SMN1 exon 7 for all 3 types was (94%), similar to those of Western Europe, China, Japan and Kuwait.

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Key words: Iranian patients, SMN1

Introduction

Spinal muscular atrophy (SMA) is a group of autosomal recessive neuromuscular disorders characterised by the degeneration of the anterior horn motor neurons of the spinal cord, leading to symmetrical muscle weakness and atrophy.^{1,2} SMA represents the second most common fatal autosomal recessive disorder after cystic fibrosis, with an incidence of 1/6000 to 1/10,000 in newborns and has an estimated carrier frequency of 1:50.^{1,3-5}

Affected individuals are typically classified into 3 groups depending on the age of onset and disease progression. Type I (acute) SMA is the most severe form. These children usually have symptoms of SMA before 6 months of age and usually do not live past the age of 2 years. Types II and III (chronic) SMA are milder, with the onset of symptoms occurring between 6 months and 17 years of age.^{1,6} All 3 types of childhood SMA have been mapped to an 850 kb

interval on 5q13, using genetic linkage studies.⁷⁻⁹ The 3 genes associated with SMA, SMN, NAIP and P44 have been identified in this region. Survival motor neuron (SMN), the first SMA-associated gene, contains 9 exons and encodes a 294-amino acid protein. There are 2 copies of the SMN gene within the SMA-candidate region. Single base changes in exon 7 and 8 allow us to distinguish telomeric and centromeric SMN copies. The absence of the telomeric copy is generally interpreted as a deletion, but it can also be a gene conversion event. Several groups have shown that at least the SMNt exon 7 is deleted in the majority of SMA patients.^{2,10-15} The SMN1 exon 7 deletion has been reported to be more prevalent among Western Europeans, the Chinese, Japanese and Kuwaiti populations, with frequencies of more than 90%, although lower frequencies of approximately 80% have been found in Malaysia and India. In addition, a rare point mutation and/or small

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deletion has been identified in the telomeric copy of SMN, which provides convincing evidence that telomeric SMN is indeed the SMA-determining gene.¹⁵⁻¹⁹

In the present study, we analyse the prevalence of SMN1 exon 7 deletions in Iranian patients and summarise our prenatal diagnosis results for the Iranian population since the inception of this service in March 1999 through March 2007.

Materials and Methods

Between March 1999 and March 2007, a total of 243 families fulfilled the diagnostic criteria of SMA according to the International SMA Consortium, and were included in this study. Of these 243 families, 96 families had a live affected child, each of whom we tested for the deletion of exon 7. Informed consent for DNA analysis was obtained from the patients' parents. 10 mL of peripheral blood were collected from each patient, and genomic DNA was extracted using the salting-out method.²⁰

All subjects were analysed for deletion of exon 7 in the SMN1 gene, using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method described previously by Van der Steege et al.²¹

In the second step, prenatal testing was performed for fetuses who had at least 1 affected sibling. The fetal samples were obtained from chorionic villus sampling (CVS) and amniotic fluid (AF). DNA was extracted from 77 CVS samples by the salting-out method and from 15 AF samples using a DNA kit (ViennaLab Diagnostics GmbH, Vienna, Austria). We investigated the presence of SMN1 exon 7 in fetal samples as described above.

Results

In this study, 243 families were examined for SMA, most of whom were from central and northern Iran, with a frequency of consanguineous marriage of 65.7%. Of these families, 195 were classified as SMA type I, 30 as type II and the remaining 18 individuals had type III disease, according to their medical history. All the families as well as 96 live affected children and 92 fetuses were screened for the deletion of exon 7. Among the live affected children, the homozygous deletion of exon 7 was found in 94% of the families with SMA type I, 95% with type II and all the individuals with type III showed the deletion (Table 1). For prenatal diagnosis, a total of 92 samples (77 CVS and 15 amnio), were analysed for exon 7 deletion. Fifty-two (56.5%) had a heterozygous deletion, 21 (22.8%) were homozygous for the deletion and 19 (20.6%) were normal.

Discussion

Many different ethnic groups made up the population of Iran. The consanguinity rate among married couples is very

Table 1. Deletion Frequencies of SMN1 exon 7 Deletion in Families with Affected Children

SMA types	Number of SMN1 exon 7 deletions
I (n = 66)	62 (94%)
II (n = 20)	19 (95%)
III (n = 10)	10 (100%)

high and in some regions exceeds more than 50%, resulting in a high incidence and prevalence of recessive genetic disorders.

The majority of SMA patients are characterised by homozygous deletions in exon 7 and 8 of the SMN1 gene.^{2,10-13} Van der Steege et al²⁹ identified a gene conversion event that changed the sequence of the SMN1 gene into that of an SMN2 gene in some SMA patients in which the SMN exon 7 had been deleted but exon 8 was retained. Since the deletion of exon 8 alone is very rare, we did not include in our study the analysis of exon 8, and focused on the importance of exon 7 in SMN1.

In the present study, we demonstrate that the homozygosity frequency for the deletion of SMN1 exon 7 for all 3 types of SMA was approximately 94%, similar to those reported in China, Japan, Kuwait, Tunis, Western Europe and Canada.^{10-16,22-28} A lower frequency of this deletion was observed among SMA patients in Malaysia and India.^{30,31} A recent publication from Iran by Derakhshandeh-Peykar³² showed exon 7 deletion in SMA families with 100% type I, in 66% with type II and in 50% with type III in our population. Surprisingly, our findings do not support this finding and the difference between our results could be explained by the fact that they have analysed a smaller group and had very few of type II and type III patients (type II only 2 and type III 3 individuals). As there were too few samples, they were not statistically valid to report prevalence. Our result shows 94% deletion in exon 7 of SMA patients which is in accordance with other findings in our neighbouring countries.

There were several reports for homozygous deletions of exon 7 SMN1 among other countries including Singaporean patients which were reported to be at 87.5%, 92.3% and 100% in type I, II and III respectively, and among Turkish patients, the figures stood at 93%, 96% and 92% respectively.^{26,33}

A similar homozygous SMN1 deletion rate was observed in our patients when compared to the other populations from China,¹⁰ Japan,¹¹ Netherlands,²² Germany,²³ France,^{16,24} Belgium, Turkey,^{25,26} Finland, UK,¹³ Kuwait,²⁷ Spain,¹⁵ Tunis²⁸ and Canada.¹⁴

Of the 92 fetal samples analysed, 22.8% showed the homozygous deletion of exon 7 and these pregnancies were

terminated. All fetuses predicted to be unaffected by deletion testing are healthy after birth, therefore the molecular analysis has been reconfirmed. Thus, with the observed distribution in full accordance with Mendelian law, reliable prenatal diagnoses and genetic counselling has been achieved. This is essential in a developing country with a high rate of consanguineous marriage.

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