SMN1 and NAIP genes deletions in different types of spinal muscular atrophy in Khuzestan province, Iran

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Received: 28 October 2011       Revised: 9 January 2012     Accepted: 16 January 2012

Abstract

Background: Spinal muscular atrophy (SMA) is the second most common lethal autosomal recessive disease. It is a neuromuscular disorder caused by degenerative of lower motor neurons and occasionally bulbar neurons leading to progressive limb paralysis and muscular atrophy. The SMN1 gene is recognized as a SMA causing gene while NAIP has been characterized as a modifying factor for the clinical severity and age at disease onset in SMA patients (SMA subtypes). The relationship between NAIP deletion and type of SMA remains to be clarified; we investigated this gene alteration in all types of SMA patients.

Methods: Molecular analysis was performed on fifty patients with a clinical diagnosis of SMA in Khuzestan province. In addition to common PCR-RFLP analysis for exon 7 and 8 of SMN1 gene, as an internal control we analysed NAIP deletion with PCR of exon 5 of this gene in a multiplex PCR with exon 13 of it.

Results: Homozygous-deletion frequency rate for the telomeric copy of SMN (SMN1) exon 7 in all types (type I, II, III) of SMA was approximately 90% and the frequency of deletion in exon 7 and 8 together in all types estimated about 70%. Moreover NAIP gene was deleted in about 60% of these patients and this shows deletion in 91% of type I SMA patients. The correlation between NAIP-deletion and SMN1 mutation showed a high frequency rate.

Conclusion: In this study, high frequency of NAIP gene deletion in all type of disease shows the importance role of it in disease pathogenesis. High frequency of NAIP deletion in SMA type I, also shows the importance of the gene in type and severity of disease so it may be a modifier factor in severity of disease.

Keywords: Spinal Muscular Atrophy (SMA), Survival Motor Neuron (SMN) gene, Neuronal Apoptosis Inhibitory Protein (NAIP) gene.

Introduction

Spinal muscular atrophy is clinically and genetically a heterogeneous disease. This disorder is characterized by degeneration of anterior horn cells (motor neurons) of the spinal cord and brain stem cells that lead to progressive symmetrical muscle weakness and atrophy with highly variable age and clinical severity [1,2]. It has an overall incidence frequency from 1 out of 6000 to 10000 live births and a carrier frequency as high as 1 in 40 [3-5]. SMA classified in three types based on disease severity and the age of onset. SMA type I (MIM 253300) or Werdnig-Hoffmann disease is the most severe with onset of symptoms before six months of age. The death is expected in majority of cases before two years and the afflicted

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infants are never able to sit. The onset age in type II or intermediate type (MIM 253550) is different from six to eighteen month and the patients are never able to walk unaided. If the symptoms manifest after eighteen month and the patients achieve walk ability unaided, this is type III of SMA (MIM 253400) [2]. The survival motor neuron gene (SMN1) is disease-causing. By molecular analysis, all three forms of SMA were mapped to chromosom 5q11.2-13.3. The SMN gene occurs in two copies of SMN1 or telomeric copy (located at telomeric end) and SMN2 or centromeric copy (located at centromeric end). SMN2 is not associated with the disease but SMN1 is a determining gene and is deleted or interrupted in 90-98% of SMA patients. Any point mutation in this gene makes SMN1 gene responsible for SMA [6-9]. SMN1 has a highly homologous copy with SMN2 and this copy differs from SMN1 coding sequence of exon 7 and 8 by a single nucleotide in each which alters a restriction enzyme site and allows one to distinguish SMN1 from SMN2 using a PCR-RFLP assay [6,7]. The neighboring gene, neuronal apoptosis inhibitory (NAIP) gene that located on the same chromosomal region is the other candidate gene. Several investigations suggested the possibility of NAIP involvement in the development of SMA [10]. It has been hypothesized that NAIP may be an SMA modifying gene and related to the severity and type of the disease [11,12]. In some studies, a higher frequency of NAIP homozygous deletion in SMA type I rather than type II and III is reported [13-26]. Deletion in NAIP appears to be associated with increase severity of disease but it is not completely clarified and need more studies in different population to understand this gene role in SMA severity and type.

Methods
We investigated 50 SMA (23 children with SMA type I and 27 patients with type II/III) after clinical diagnosis, EMG and NCV. Clinical manifestations included reduced muscle tension, absent tendon reflexes and different degrees of muscle atrophy. Peripheral blood samples were collected and genomic DNA was extracted using Diatom DNA prep kit protocols (Izogen Ltd., Moscow, Russia). This DNA amplified by primers X7 Dra and R111 for exon 7 SMN gene [6,27] and 541C960, 541C1120 primers for

Table 1. Primer sequences used for gene and position amplification.

<table>
<thead>
<tr>
<th>Gene and position</th>
<th>Primer name</th>
<th>Primer sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMN exon 7</td>
<td>X7 Dra</td>
<td>5’-CCT TCC TTC TTT TTG ATT TTG TTT-3’</td>
</tr>
<tr>
<td></td>
<td>R111</td>
<td>5’-AGA CTA TCA ACT TAA TTT CTG ATC A-3’</td>
</tr>
<tr>
<td>SMN exon 8</td>
<td>541C960</td>
<td>5’-CTA CAA CAC CCT TCT CAC AG-3’</td>
</tr>
<tr>
<td></td>
<td>541C1120</td>
<td>5’-GTA ATA ACC AAA TGC AAT GTG AA-3’</td>
</tr>
<tr>
<td>NAIP exon 5</td>
<td>1863</td>
<td>5’-CTC TCA GCC TGC TCT TCA GAT-3’</td>
</tr>
<tr>
<td></td>
<td>1864</td>
<td>5’-AAA GCC TCT GAC GAG AGG ATC-3’</td>
</tr>
<tr>
<td>NAIP exon 13</td>
<td>1258</td>
<td>5’-ATG CTT GGA TCT CTA GAA TGG-3’</td>
</tr>
<tr>
<td></td>
<td>1343</td>
<td>5’-CCA GCT CCT AGA GAA AGA AGG A-3’</td>
</tr>
</tbody>
</table>

Table 2. Molecular analysis of 50 SMA patients in Khuzestan province. The number of cases with absence of investigated exons and disease type were shown.

<table>
<thead>
<tr>
<th>Gene Region</th>
<th>Deletion of exon 7 SMN1</th>
<th>Deletion of exon 8 SMN1</th>
<th>deletion of exon 5 NAIP</th>
<th>Patients number</th>
</tr>
</thead>
<tbody>
<tr>
<td>TYPE I</td>
<td></td>
<td></td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>percent</td>
<td>23/23</td>
<td>21/23</td>
<td>21/23</td>
<td></td>
</tr>
<tr>
<td>Type II, III</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>percent</td>
<td>100%</td>
<td>91%</td>
<td>91%</td>
<td>27</td>
</tr>
<tr>
<td>Patient number</td>
<td></td>
<td></td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>percent</td>
<td>45/50</td>
<td>35/50</td>
<td>30/50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90%</td>
<td>70%</td>
<td>60%</td>
<td>100%</td>
</tr>
</tbody>
</table>
exon 8 of this gene for molecular analysis PCR-RFLP (Table 1). PCR was performed on an Bio-Rad thermal cycler and for exon 7 \textit{SMN} gene amplification consisted of an initial denaturation step at 94°C for 3 min, then 33 cycle at 94°C for 30 sec, 58°C for 45 sec and 72°C for 45 sec and the final extension step at 72°C for 7 min. PCR produced 188bp fragments for exon 7 and 189bp for exon 8 \textit{SMN} gene, the amplification of exon 8 was similar to exon 7 by a difference at annealing temperature that was at 60°C.

\textit{Restriction enzyme digestion for SMN and gel electrophoresis}

The PCR products of \textit{SMN} exons 7 and 8 were subsequently digested with restriction enzyme \textit{DraI} and \textit{DdeI} respectively. And the resultant bands were visualized in 2/5% agarose gel that stained with ethidium bromide under UV light. Digestion of exon 7 with \textit{DraI} cut \textit{SMN2} copy into 168bp and 20bp fragments and \textit{SMN1} remains as 187bp band. For exon 8, \textit{DdeI} digests \textit{SMN2} into 122bp and 67bp fragments while \textit{SMN1} remains as 189bp band without any cut [6,27]. Exons 5 and 13 (1863/1864bp and
Table 3. SMN1 and NAIP deletions found in different types of SMA.

<table>
<thead>
<tr>
<th>Disease type</th>
<th>Only deletion of exon 7</th>
<th>Deletion of exon 7, 8 SMN1 without deletion of NAIP</th>
<th>Deletion of SMN1 exon 7 and NAIP exon 5 with no SMN1 exon 8 deletion</th>
<th>Deletion of three exons (exons 7, 8 of SMN1 and exon 5 of NAIP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>1/23</td>
<td>1/23</td>
<td>3/23</td>
<td>18/23</td>
</tr>
<tr>
<td></td>
<td>4 %</td>
<td>4%</td>
<td>13%</td>
<td>78%</td>
</tr>
<tr>
<td>Type II and III</td>
<td>5/27</td>
<td>8/27</td>
<td>1/27</td>
<td>8/27</td>
</tr>
<tr>
<td></td>
<td>18%</td>
<td>30%</td>
<td>3%</td>
<td>30%</td>
</tr>
<tr>
<td>Type I, II and III</td>
<td>6/50</td>
<td>9/50</td>
<td>4/50</td>
<td>26/50</td>
</tr>
<tr>
<td></td>
<td>12%</td>
<td>18%</td>
<td>8%</td>
<td>52%</td>
</tr>
</tbody>
</table>

1258/1343bp, respectively) of NAIP gene were also amplified utilizing a multiplex PCR (with annealing temperature of 57°C for 45 sec) to get. The produced fragments were two 435bp (exon 5) and 241bp (exon 13) bands. Exon 13 was taken as positive internal control and exon 5 existed only in active copy of NAIP gene, so deletion of it was expected in patients that carry deletion in this gene [10].

Results

Molecular analysis of fifty patients assay revealed forty five patients with homozygous deletion of exon 7 SMN1 while thirty five patients out of them had deletion of exon 8 too. In all type I patients, this homozygous deletion of exon 7 SMN1 was detected but five patients of type II/III do not show this mutation (Fig. 1a & 1b). The PCR-RFLP assay of thirty normal controls did not show homozygous deletion of exons 7 and 8 SMN1 and all of them had also exon 5 NAIP gene. No patient was found to have homozygous absence of both SMN1 and SMN2 genes and this mutation proposed to be lethal. All patients with the absence of exon 8 had also the lack of SMN1 exon 7 (Fig. 1c & 1d). NAIP homozygous deletion was found in thirty cases of them (30/50) including 21 and 9 patients from all 23 and 27 patients involved in SMA type I and type II/III respectively (Fig. 2).

Discussion

According to the newest survey, SMA is the second most common inherited lethal disease. In Iran as a large country with many different ethnic groups and very high rate of consanguinity (especially in Khuzestan province), homozygosity for SMA is also high. This disorder is one of the important genetic causes for infants and children mortality in Iran [28]. Because of similarity of SMA manifestation to many other neuromuscular disorders, upon clinical diagnosis of disease it confirmed through a blood DNA test including PCR-RFLP method. Utilizing this molecular analysis, homozygous deletions of exon 7 and/or 8 of SMN1 were generally considered to be diagnostic for SMA. We concentrated on the deletion in these two exons and NAIP exon 5 in fifty patients (from western south of Iran) who were suspected to have SMA. In this study, homozygous deletion of exon 7 was 90% while synchronous exons 7 and 8 deletions account 70%. We found homozygous deletion of NAIP in 60% of patients, this gene was deleted in 91% of type I patients while 33% of type II and III (Table 2). A high percentage of NAIP deletion in all types of SMA reveals that it has an important role in molecular pathogenesis of this disorder. On the other hand, NAIP frequent deletion in SMA type I, proposes the important role it may play in severity and type of disease as a modifier factor in different presentation of the disease. We found the lack of three exons of the two genes (exons 7 and 8 of SMN1 and exon 5 of NAIP) in 78% (18/23) of SMA type I. An overlap was seen between extension of deletion regions and the type of SMA or disease severity, as the extension of deletion regions can cause disease severity (Table 3).

Acknowledgements

We thank the SMA families involved in this study. This work was supported by a grant from Shahid Chamran University of Ahvaz, Iran. The authors declare that they
have no conflicts of interest in relation to this article.

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