CLINICAL STUDY

Molecular Analysis of SMN1 and NAIP Genes in Egyptian Patients with Spinal Muscular Atrophy

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Abstract

Objectives: The aim of this study is to provide preliminary molecular data on spinal muscular atrophy in Egyptian patients thus facilitating a rapid and conventional molecular assay for accurate diagnosis of SMA.

Background: Childhood spinal muscular atrophy (SMA) is one of the most common autosomal recessive disorders. It is characterized by symmetrical muscle weakness and atrophy of limbs and trunk. At least four SMA related genes have been identified [survival motor neuron (SMN), neuronal apoptosis inhibitory protein (NAIP), the gene encoding the transcriptional factor p44 and H4F5 gene].

Methods: Homozygous absence of exons 7 and 8 of the SMN1 gene was detected using PCR-SSCP analysis, while NAIP gene deletion was detected using multiplex PCR-agarose gel electrophoresis.

Results: Homozygous absence of SMN1 exons 7 and 8, or exon 7 only, was found in 80% of patients. Of those patients, 45% were also deleted for NAIP exon 5.

Conclusion: The molecular basis of SMA in Egyptian patients has a similar pattern to that reported in most populations, but a larger study is recommended for more comprehensive characterization (Tab. 1, Fig. 2, Ref. 33). Full Text (Free, PDF) www.bmj.sk.

Key words: Spinal Muscular Atrophy, SMN, NAIP, molecular diagnosis, PCR-SSCP analysis.

Children spinal muscular atrophy (SMA) represents the second most common autosomal recessive disorder after cystic fibrosis (1). The SMA has an estimated incidence of about 1:6000 live births and has a carrier frequency of approximately 1:50 (2). It is characterized by degeneration of α-motor neurons in the anterior horn of the spinal cord leading to proximal and symmetrical weakness and atrophy of limbs and trunk (3).

According to the age of onset and clinical severity, SMA was subdivided into 3 types (Types I, II and III) (4) all of which were linked to chromosome 5q13 (5). The genes involved in SMA are the survival motor neuron (SMN), the neuronal apoptosis inhibitory protein (NAIP), the gene encoding p44, and the H4F5 genes (6). SMN gene is present in telomeric (SMN1) and centromeric (SMN2) copies. SMN1 and SMN2 genes have been discriminated by a single-base changes in exons 7 and 8. It is now well established that mutations or deletions of the telomeric SMN gene (SMN1) cause the disease. In addition, NAIP gene was found to be more frequently deleted in the severest form of the disease [type I] (7). This study aimed to shed further insight on the molecular basis of the SMA among Egyptian patients.

Patients and methods

Patients

Twenty Egyptian patients from 20 different families were referred to the Department of Medical Molecular Genetics by the Clinic of Inherited Neuromuscular Diseases, National Research Center (NCR), Cairo, Egypt. All patients were subjected to clinical and laboratory investigations including electromyography (EMG) and nerve conduction studies as well as estimation of serum level of creatine kinase (CK).

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Fig. 1. (a) Deletion of SMN1 exons 7 and 8 in Egyptian SMA cases. 12 % polyacrylamide gel presenting SSCP of SMN genes exon 7. Lanes 1, 2, 3 and 5 represent four SMA patients with homozygous absence of SMN1 gene exon 7; lane 4 represents a patient with no homozygous absence of SMN1 gene exon 7, lanes 6 and 7 represent control samples. (b) A 12 % polyacrylamide gel presenting SSCP of SMN genes exon 8. Lanes 1 and 2 represent control samples, lanes 3, 4, 5 and 7 represent four SMA patients with homozygous absence of SMN1 gene exon 8, lane 6 represents a patient with no homozygous absence of SMN1 gene exon 8.

All patients fulfilled the clinical diagnostic criteria for proximal SMA defined by the International SMA Consortium (4) and confirmed by the European Neuromuscular Center (ENMC) International Workshop (8).

Molecular studies

Genomic DNA was extracted from (,.3–7") ml peripheral blood leukocytes of the 20 SMA patients using salting-out procedure (9).

Single-stranded conformational polymorphism (SSCP) assay

PCR amplification of SMN genes exons 7 and 8 was carried out in uniplex reactions in 25 µl total volume containing 500 ng of genomic DNA, 375 µM of each dNTP (DYNAzymeTM II, Finzymes, Finland), 2 pmol each of forward and reverse primers (MWG-Biotech, Germany). 1.25 U Taq Polymerase (DYNAzymeTM II DNA polymerase, Finzymes, Finland) in a PCR buffer comprising 1.5 mM MgCl2. Primer pairs R111 and SMN7-B and SMN8-A and SMN8-C were used to amplify exon 7 and exon 8, respectively (10 and 11). Cycling conditions included the initial denaturation at 94 °C for three minutes; 30 cycles of 94 °C for one minute, 60 °C for one minute, and 72 °C for one minute; and a final extention of 72 °C for 10 minutes (12). Three µl of the amplified PCR product were diluted with 15 µl formamide loading dye. The samples were denatured for 10 minutes at 95 °C before loading onto a 12 % non-denaturing polyacrylamide gel. Gels were run at 500 V at 7 °C in 1 X TBE buffer then visualized by silver staining (SILVER SEQUENCE TM DNA Sequencing System, Promega, USA).

Multiplex polymerase chain reaction assay

Multiplex PCR amplification of NAIP gene exon 5 and 13 was carried out in 25 µl reaction volume containing 200 ng of genomic DNA, 2 pmol each of primers 1863 and 1864, and 5 pmol each of primers 1258 and 1343 to amplify NAIP exon 5 and exon 13, respectively (13). The other PCR constituents and the cycling protocol were as described above. The multiplex PCR products were analyzed on a 2 % agarose gel stained with ethidium bromide, run in 0.5 X TBE buffer and visualized under UV light.

Tab. 1. Deletion analysis of exon 7 and 8 of the SMN1 gene and exon 5 of NAIP gene in Egyptian SMA patients.

<table>
<thead>
<tr>
<th>SMA Type</th>
<th>SMN1 exon 7 only</th>
<th>SMN1 exons 7 and 8</th>
<th>SMN1 exon 7 only and NAIP exon 5</th>
<th>SMN1 exons 7 and 8 and or exon 7 only</th>
<th>Total NAIP</th>
<th>Total of SMN1 and NAIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (n=5)</td>
<td>0/5</td>
<td>0/5</td>
<td>1/5 (20%)</td>
<td>3/5 (60%)</td>
<td>4/5 (80%)</td>
<td>1/5 (20%)</td>
</tr>
<tr>
<td>II (n=9)</td>
<td>1/9 (11.1%)</td>
<td>3/9 (33.3%)</td>
<td>0/9</td>
<td>2/9 (22.2%)</td>
<td>6/9 (67%)</td>
<td>3/9 (33.3%)</td>
</tr>
<tr>
<td>III (n=6)</td>
<td>0/6</td>
<td>3/6 (50%)</td>
<td>1/6 (16.7%)</td>
<td>2/6 (33.3%)</td>
<td>6/6 (100%)</td>
<td>3/6 (50%)</td>
</tr>
<tr>
<td>Total SMA (n=20)</td>
<td>1/20 (5%)</td>
<td>6/20 (30%)</td>
<td>2/20 (10%)</td>
<td>7/20 (35%)</td>
<td>16/20 (80%)</td>
<td>9/20 (45%)</td>
</tr>
</tbody>
</table>

SMN1 – The telomeric copy of the Survival Motor Neuron gene
NAIP – Neuronal Apoptosis Inhibitory Protein
Results

Analysis of SMN genes

Screening of the 20 SMA patients by SSCP of SMN genes exons 7 and 8 (Fig. 1) revealed that 80% of them have either homozygous absence of SMN1 exons 7 and 8 (65%), or of exon 7 only (15%) (Tab. 1). The remaining 20% retained the two exons. Different frequencies of homozygous absence of exons 7 and 8, or exon 7 only were found among the different types: 4/5 type I, 6/9 type II and 6/6 type III (Tab. 1).

Analysis of NAIP gene

Multiplex PCR amplification products of NAIP exons 5 and 13 are shown in Figure 2. Nine out of the 20 SMA patients (45%) showed deletion of NAIP exon 5 [4/5 type I, 2/9 type II and 3/6 type III] (Tab. 1), and absence of SMN1 gene exon 7.

Discussion

SMN1 gene

Molecular diagnosis of SMA by detecting absence of SMN1 exons 7 and 8 has been extensively investigated among various ethnic groups. High frequencies (81-100%) for homozygous absence of either exons 7 and 8 or exon 7 of the SMN1 gene have been reported (10; 11; 14; 15; 16; 17; 18; and 19). Also lower frequencies in type I SMA patients (43% and 58%) have been reported (20 and 21, respectively). A frequency of only 65.5% has been reported in South African Black patients (22). The same study also detected 100% homozygous absence in eleven South African Caucasian SMA patients, thus reflecting a different molecular pattern among South African Black and Caucasian patients with SMA. Our data in the present study is similar to that reported in most countries. It shows that, regardless of disease severity, 80% of studied Egyptian patients have either homozygous absence of SMN1 exons 7 and 8, or of exon 7. It is interesting that results of another published study on Egyptian SMA patients reported a much lower frequency of homozygous absence of exons 7 and 8 or exon 7 only (18/33); thus 54.5% of whom 36.3% had homozygous absence of both exons and 18.2% had homozygous absence of exon 7 only (23). A larger study could resolve this controversy of data thus confirming the true molecular data of Egyptian patients with SMA.

The issue of different frequencies of absence of the SMN1 exons 7 and 8, or exon 7 only, within the different types was also pursued in several studies. In the present study, the frequency of homozygous absence of SMN1 exons 7 and 8, or exon 7 only, was higher in patients with the mildest form of the disease (100%) compared to those with the severest (80%) and the intermediate form (67%). These results are similar to that reported by other studies (14; 18 and 24). In South African Black SMA patients, the frequency of homozygous absence was higher in types II/III than in type I (22). The highest frequency of absence was associated with type II Canadian SMA patients (19). On the other hand, homozygous absence of SMN1 exons 7 and 8, or exon 7 only, was higher in types I and II as compared to type III patients in other studies (15; 25 and 26). An earlier study on Egyptian SMA patients reported a higher frequency of homozygous absence in type I patients than in type II (23). All these results prove that mutations in SMN1 gene do not influence the clinical severity of the disease, if not considering the extent of deletion on the chromosome 5q13.

Another interesting finding in the present study revealed that all patients with absence of exon 8 had also the absence of SMN1 exon 7. The same result was also reported by several studies (11; 14; 15; 16; 23; 26 and 27). Such absence may not necessarily constitute a disease allele (28). On the other hand in two different studies, two patients were reported missing only exon 8 (12 and 22). Also, no patient was found to have homozygous absence of both SMN1 and SMN2 genes; the presence of such a mutation in both the telomeric and the centromeric copies of SMN genes were proposed to be a lethal mutation (3).

NAIP gene

Deletion of exon 5 in the NAIP gene is another mutation related to SMA, and it contributes to the molecular defect and severity of the phenotype in SMA (13; 14; 15; 16; 27; 29; 30 and 31). Deletion of exon 5 has been reported to be associated with a 5-fold increased risk of type I SMA reflecting the direct correlation between the severity of phenotype and the deletion (32). Complete or partial loss of SMN1 gene results in the selective degeneration of alpha motor neuron cells in SMA, but loss of NAIP gene may lead to extensive neuronal cell death at the onset of the disease, thus modifying the clinical presentation of SMA into the severe form (19). Furthermore, in type I patients lacking the NAIP gene, the deterioration in respiratory function was more rapid than in type I patients retaining the NAIP gene (14). The deletion of the NAIP gene in the present study was found in 45% (9/20) of the Egyptian SMA patients. The frequency of the deletion was more frequent in type I patients (80%); 4/5 as compared to types II (22%); 2/9 and III (50%; 3/6). The corre-
lation of the deletion and clinical severity was obvious, thus justifying the use of this information for prognosis and genetic counseling.

The incidence of NAIP exon 5 deletions varies greatly among different ethnic populations. In our study, a relatively high incidence of exon 5 deletions was observed in type I SMA (80%), a result which correlates well with reported NAIP analysis of other populations, including Egyptians, ranging between (82–87.5%) (12; 15 and 30). In Kuwait (33) and Saudi Arabian patients (16) the incidence was even higher (100%), thus higher frequencies of NAIP gene deletions were found in Arab populations compared to populations of other ethnic origins (30). Relatively lower incidence (66–68%) was reported for Spanish (11), Finnish (31), Turkish (27) and Canadian (19) patients; while South African Blacks (22), Chinese (29), Slovak (26) and Japanese (14) patients showed even lower incidence (17–40%). A notable result obtained in the present study was the higher frequency of exon 5 deletions in type III (50%) than in type II (22%) SMA patients. Similar results have also been reported by two studies on Spanish and Finnish SMA patients (11 and 31; respectively). Such variability in the frequency of NAIP exon 5 deletions among different ethnic groups could be attributed to the presence of other modifier genes or yet unknown factors that may influence the phenotype-genotype correlation.

In conclusion, successful implementation of molecular techniques by analyzing for SMN1 and NAIP deletions for the confirmation of clinical diagnosis or suspicion of SMA was pursued at the National Research Center in Egypt. The molecular basis of SMA in Egyptian patients appears to be similar to that reported in most populations, but a larger study is recommended for proper statistical evaluation.

References

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Received January 3, 2007.
Accepted January 26, 2007.

Abbreviations:
CK — creatine kinase
DNA — deoxyribonucleic acid
EMG — electromyography
NAIP — neuronal apoptosis inhibitory protein
PCR-SSCP — polymerase chain reaction-single stranded conformational polymorphism
SMA — spinal muscular atrophy
SMN1 — the telomeric copy of the survival motor neuron gene
SMN2 — the centromeric copy of the survival motor neuron gene
TFIIH — transcription factor IIH