Deletion analysis of *SMN* and *NAIP* genes in Tunisian patients with spinal muscular atrophy

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Abstract

Background: Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder involving degeneration of anterior horn cells of spinal cord, resulting in progressive muscle weakness and atrophy. **Aims:** The purpose of our study was to determine the frequency of SMN and NAIP deletions in Tunisian SMA patients. **Materials and Methods:** Polymerase chain reaction (PCR) combined with restriction fragment length polymorphism (RFLP) was used to detect the deletion of exon 7 and exon 8 of SMN1 gene, as well as multiplex PCR for exon 5 and 13 of NAIP gene. **Results:** Fifteen (45.4%) out of 33 SMA patients were homozygously deleted for exons 7 and/or 8 of SMN1. Homozygous deletion of NAIP gene was observed in 20% (3 / 15) of patients. **Conclusions:** The molecular diagnosis system based on PCR-RFLP analysis can conveniently be applied in the clinical testing, genetic counseling, prenatal diagnosis, and pre-implantation genetic diagnosis of SMA.

Key Words

Neuronal apoptosis inhibitory protein (*NAIP*) gene, spinal muscular atrophy, survival motor neuron (*SMN*) gene

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Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive (AR) neuromuscular disorder characterized by symmetrical muscle weakness and atrophy caused by degeneration of the anterior horn cells in the spinal cord.[1]

The International SMA Consortium classification defines several types of SMA depending on the age of onset and clinical severity (Type I - IV).[2]

The majority of SMA cases are caused by homozygous deletion or mutation in the *SMN1* gene. *SMN* is contained in a 500-kb sequence on chromosome 5q12.2-q13.3, which consists of 9 exons and is present in two copies: Atelomeric one (*SMN1*) and a centromeric one (*SMN2*). *SMN1* gene has a highly homologous copy with *SMN2*. This copy is present in 90%~95% of normal controls and hampers detection of deletions and mutations within the *SMN1* gene.[3,4] The coding sequence of *SMN2* exon 7 differs from that of *SMN1* by a single nucleotide (840C < T), which alters a restriction enzyme site and allows one to easily distinguish *SMN1* from *SMN2* using a polymerase chain reaction (PCR)-based assay.[5,6] The neuronal apoptosis inhibitory protein (*NAIP*) gene located on 5q12.2-q13.3 has been hypothesized to be an SMA modifying gene.[7,8]

The purpose of our study was to determine the percentage of *SMN* and *NAIP*genes deletions in Tunisian SMA patients.

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Materials and Methods

Patient's selection

We selected 33 patients from 14 different families originating from southern Tunisia that fulfilled the diagnostic criteria of SMA according to the International SMA Consortium. The diagnoses were confirmed by clinical symptoms, electroneuromyographic (ENMG) evaluation, and some time by muscle biopsy.

Clinically, all patients could be classified as SMA type III according to the classification of Pearn *et al*.[9] and Serratrice.[10]

After written and informed consent had been obtained, blood was sampled and DNA was extracted using a standard protocol.

Detection of *SMN* and *NAIP* gene deletions by PCR RFLP method

SMN exon 7 and exon 8 deletions were detected by PCR

amplification and restriction-enzyme digestion as described elsewhere.[11]

The 33 patients included in the study were studied for *SMN1* and *NAIP* gene deletions. Eight samples from non-SMA patients were also run as controls.

Polymerase chain reaction (PCR) amplification of *SMN* exons 7 and 8 was carried out based on the method described by Wirth *et al*.[12] With slight modifications. The exon 7 PCR products (20 μL) were digested with 1.5 u HinfI (Fermentas life sciences) for 2 hours at 37°C and run on a 4% agarose gel at 150 V in 1X tris-borate-EDTA (TBE) (Amresco Inc., Solon, Ohio, USA) during 30 minutes. PCR products of SMN exon 8 (20 μl) were subsequently digested with restriction enzyme Dde I (1.5 units).

For *NAIP*, exon 5 and 13 (exon 13 taken as positive control), a multiplex reaction was carried out for 30 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min using primers 1864 and 1863 for exon 5 and primers 1258 and 1343 for exon 13.[7,13]

Results

The clinical and paraclinical features of 15 patients from 14 families with SMN and/or NAIP deletion are summarized in Table 1.

Molecular analysis using PCR-RFLP assay revealed 15 patients (n = 15/33, 45.4%) with homozygous deletions in SMN1 or/and NAIP genes [Table 1].

Deletion analysis of *SMN* and *NAIP* genes in adult SMA patients using PCR/RFLP are shown in [Figures 1a, b and c].

A PCR product of exon 7 of *SMN* gene (135 bp) was obtained at the end of PCR. SMN/exon 7/HinfI digestion result in 4fragments corresponding to the *SMN1* (101 bp and 34 bp) and *SMN2* (78 bp, 34 bp and 23 bp). All patients (n = 15/15, 100%) have a deletion on exon 7 (missing of 78 pb and 23 pb bands) [Figure 1a]. For exon 8, the nucleotide, which differs between *SMN1* and *SMN2* genes, is G>A. The nucleotide A in the *SMN2* gene (located at position 1155 of the cDNA) creates a Dde I site, which will digest the amplified DNA into two products of 122 bp and 78 bp.[11,14] The exon 8 of *SMN1* gene does not contain any Dde I site, hence it will not be cut and will remain as 200 bp.Exon 8 deletions were indicated by the absence of a 200 bp band [Figure 1b]. Twelvepatients (n = 12/15, 80%) showed a homozygous deletion of exon 7 and exon 8, while 3 (n = 3/15, 20%) lacked the *SMN* exon 7, but retained the exon 8.

In *NAIP* gene analysis, only control band presentation indicates deletions of exon 5 (435 pb), which is specific for the functional gene. The 241 bp (exon 13) bands appeared (exon 13 taken as positive control) [Figure 1c]. Exon 5 of *NAIP* gene was homozygously deleted in 20% (n = 3/15). No patient had a deletion in *NAIP* gene without a deletion in the *SMN1* gene. No homozygous deletion of *SMN* and *NAIP* exon 5 genes was detected in the 8 control individuals.

Discussion

Spinal muscular atrophies are hereditary disorders characterized by degeneration of spinal cord motor neurons. The majority of SMA cases showed AR inheritance and are caused by homozygous deletion or mutation of the *SMN1* gene on 5q (OMIM 253300, 253550, 253400, and 271150). Non-5q SMA is rare, clinically diverse, and genetically heterogeneous.[15,16]

Among all the candidate genes, *SMN1* is believed to be the primary SMA disease-causing gene. The finding of homozygous deletions of exons 7 and/or 8 of SMA patients with consistent clinical features is generally considered to be diagnostic of SMA.[17]

In the present study, we demonstrate that the percentage of homozygosity for the deletion of SMN1 exon 7 for all Tunisian patients was 45.4%, similar to those reported in Iran (100% in type I, 66% in type II, and 50% in type III).[18]

A higher frequency was observed in other populations, especially among SMA patients from China,[19] Netherlands,[20] Finland, UK,[21] and Tunis.[22]

The majority of SMA patients are characterized by homozygous deletions in exon 7 and 8 of the SMN1 gene.[14,19,21] Van der Steege *et al*.[23] 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. The deletion of exon 8 alone is very rare. Deletions involving both exons 7 and 8 (80%) were much more frequent than deletions of only exon 7 (20%) or only exon 8 (0%).

In addition, there was no correlation between deletions in the SMN gene and phenotype severity in accordance with previous reports in the literature.[24]

Deletions in exon 5 of NAIP (20% in our study), which is specific for the functional NAIP gene, showed wide frequency variation (0 to 67%) in different population studies.[19,21] NAIP deletion alone, without SMN1 is being deleted, was not seen in any of our patients. This is contrary to the results of Japanese patients where two unaffected mothers of patients with NAIP and SMN1 deletions showed deletions of only NAIP gene.[25]

The role of NAIP gene in the pathogenesis of SMA remains controversial. However, several studies suggest that NAIP gene can be a predictive marker of SMA prognosis and acts a cofactor for the differentiation and survival of neuronal cells including the motor neurons.Further studies will be necessary to clarify its exact functional role.

Conclusion

With the advent of molecular biology techniques, SMN gene deletion study represents nowadays a useful and reliable tool to confirm the diagnosis of SMA suspected clinically. Demonstrate of homozygous deletions of exons 7 and/or 8

Table 1: Clinical characteristics of patients of Tunisian family TUN34

	TUN7	TUN8	TUN10	TUN11	TUN15	TUN16	TUN17
Individual N°(sex)	113/07(Female)	114/07(Female)	116/07(Male)	117/07(Female)	02/08(Male)	03/08(Female)	14/08(Male)
Age at examination, years	15	18	18	21	40	32	26
Age at onset, years	02	02	15	02	16	4	15
Disease duration, years	13	16	3	20	24	28	11
AMS type	Type III	Type III	Type III	Type III	Type III	Type III	Type III
Consanguinity	Yes (3)	No	Yes (1)	No	No	Yes (1)	Yes (1)
Walking handicap	Without help	Bedridden	Without help	Bedridden	Bedridden	Bilateral help	Without help
Echelle MFM D1/D2/D3 or Hammersmith (/40)	14/28/19	4/40	07/28/17	09/25/14	0/12/9	6/40	27/32/15
LL reflexes	Abolished	Abolished	Abolished	Normal	Abolished	Abolished	Abolished
LL weakness	Moderate	Severe	Moderate	Severe	Severe	Severe	Moderate
LL amyotrophy	Moderate	Severe	No	No	Severe	No	No
UL reflexes	Abolished	Abolished	Abolished	Abolished	Abolished	Normal	Abolished
UL weakness	Moderate	Severe	Moderate	moderate	Moderate	Moderate	Mild
UL amyotrophy	No	Severe	Moderate	No	Severe	No	No
Dysmorphic signs	Pescavus	No	No	Pescavus	Scoliosis	No	Pescavus
EMG	neurogene	neurogene	neurogene	neurogene	neurogene	Neurogene	neurogene
Muscle biopsy	Atrophieneurogene	Atrophieneurogene	Atrophieneurogene	Atrophieneurogene	Atrophieneurogene	Normal	Atrophieneurogene
CPK	450	150	847	200	2600/1370	82	2061
Deletion analysis SMN Exon 7	Del	Del	Del	Del	Del	Del	Del
Deletion analysis SMN Exon 8	Del	Del	Del	Del	Non-del	Del	Del
NAIP Exon5	Non-del	Del	Non-del	Non-del	Non-del	Non-del	Non-del

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(Cont...)

Table 1: Clinical characteristics of patients of Tunisian family TUN34

	TUN17	TUN21	TUN22	TUN26	TUN28	TUN30	TUN32	TUN34
Individual N° (sex)	15/08 (Female)	73/08(Male)	165/08 (Male)	350/08 (Male)	352/08 (Male)	02/09 (Male)	224/08 (Female)	207/09 (Male)
Age at examination, years	27	46	33	19	27	32	20	33
Age at onset, years	8	14	14	2	9	10	10	12
Disease duration, years	19	32	24	17	18	22	10	21
AMS type	Type III	Type III	Type III	Type III	Type III	Type III	Type III	Type III
Consanguinity	Yes (1)	No	No	Yes (1)	Yes (4)	Yes (1)	Yes (1)	Yes (1)
Walking handicap	Bilateral help	Bilateral help	Bedridden	Without help	Without help	Without help	Without help	Without help
Echelle MFM D1/D2/D3 or Hammersmith (/40)	3/23/08	7/40	4/40	8/33/19	17/34/20	15/40	10/29/20	1440
LL reflexes	Abolished	Abolished	Normal	Abolished	Abolished	Abolished	Abolished	Abolished
LL weakness	Severe	Severe	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
LL amyotrophy	Severe	Severe	Moderate	Moderate	No	Moderate	Moderate	Moderate
UL reflexes	Abolished	Abolished	Abolished	Abolished	Abolished	Normal	Abolished	Normal
UL weakness	Severe	Severe	Severe	Moderate	Moderate	Mild	Moderate	Mild
UL amyotrophy	Moderate	Severe	Severe	Moderate	No	No	Moderate	No
Dysmorphic signs	Pescavus, scoliosis	No	No	Pescavus, Scoliosis	Pescavus, Scoliosis	No	Scoliosis	No
EMG	neurogene	Neurogene	Neurogene	Neurogene	Neurogene	Neurogene	Neurogene	Neurogene
Muscle biopsy	ND	Atrophieneurogene	Atrophieneurogene	Normal	Atrophiefasciculaire	Atrophiefasciculaire	Atrophiefasciculaire	Atrophiefasciculaire
CPK	174	454	201	320	656	524	1370	2350
Deletion analysis	Del	Del	Del	Del	Del	Del	Del	Del
	Del	Del	Non-del	Del	Del	Non-del	Del	Del
	Non-del	Del	Non-del	Del	Non-del	Non-del	Non-del	Non-del

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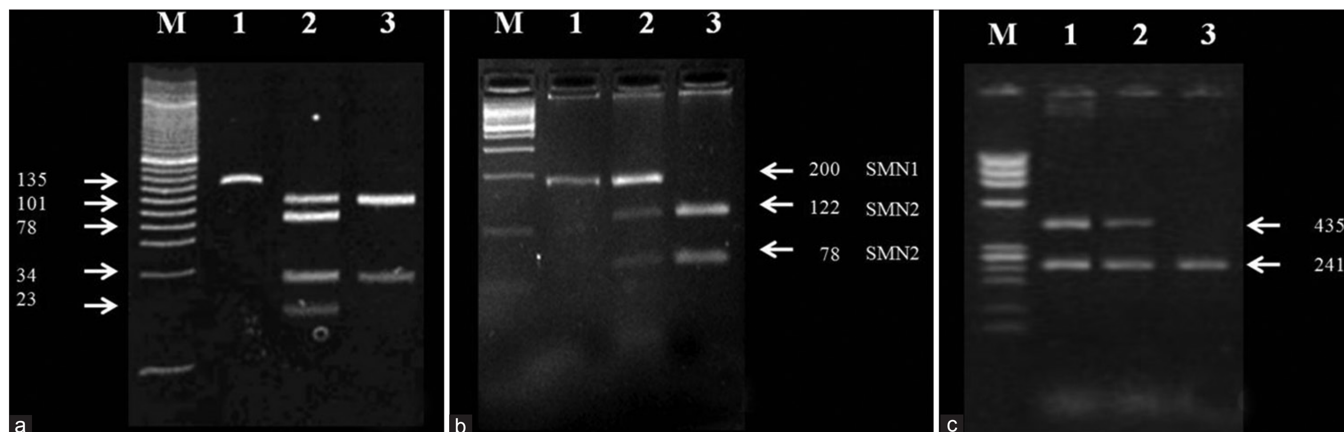

Figure 1: (a) Detection of deletions in the *SMN* gene exon 7. Column 1: *SMN* gene exon 7; Column 2: *SMN* gene exon 7 cleaved with *HinfI* from an unaffected subject; Column 3: *SMN* gene exon7 cleaved with *HinfI* from SMA patients; Column M: 20 pb DNA Ladder (b) DdeI digestion of SMA patients for *SMN* exon 8. Column 1: *SMN* exon 8; Column 2: *SMN* exon 8 control; Column 3: *SMN* gene exon8 cleaved with *DdeI* from SMA patients; Column M: 100 pb DNA Ladder (c) The PCR analysis of the *NAIP* gene. Column 1: normal control; Column 2: normal pattern; Column 3: homozygous deletion of exon 5

of *SMN* gene confirm the diagnosis of SMA, even though the clinical features are incomplete or atypical. Deletions of *NAIP* gene were mainly seen in severely affected patients, hence is useful to predict prognosis.

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