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Homozygous deletion of exon 7 in SMN1 gene without phenotypic features of spinal muscular atrophy

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Abstract

Spinal muscular atrophy (SMA)(OMIM#:253300) is an autosomal recessive disorder, resulting in symmetrical progressive weakness of skeletal and respiratory muscles and atrophy. The corresponding gene for the disease is the survival motor neuron 1 (*SMN1*) and *SMN2* genes. Homozygous deletion of *SMN1* exons is the most common underlying cause of the disease, and *SMN2* copy numbers modify the disease phenotype. However, homozygous deletion of exon 7 of *SMN1* in a completely asymptomatic individual is an extremely rare finding. The present report discusses a case of homozygote deletion of exon 7 of *SMN1* in a healthy female. A healthy couple with a family history of affected family members with SMA was referred for genetic counseling. Genomic DNA was extracted from the peripheral blood of the couple and the copy number of exon 7 of the *SMN1* gene was assessed for using real-time polymerase chain reaction (PCR) and PCR-Restriction fragment length polymorphism (RFLP). Assessment of *SMN1*-related *ct* in the female compared with control samples showed that the female had a homozygous deletion in the *SMN1* gene. PCR-RFLP and gel electrophoresis results also confirmed the homozygous deletion of exon 7 in the female *SMN1* gene.

Conclusion: According to the results of this study and also other findings in previous studies, the lack of symptoms in the female with biallelic deletion of SMN1 may be related to the presence of *SMN2* copies or other modifier genes.

Keywords: **S**pinal muscular atrophy, SMA, SMN1, homozygous deletion, biallelic deletion

INTRODUCTION

Chromosome 5q-related spinal muscular atrophy (SMA) (OMIM#:253300) is a fatal autosomal recessive disorder after cystic fibrosis, characterized by degeneration of the anterior horn cells of the spinal cord, resulting in progressive weakness of skeletal and respiratory muscles atrophy.[1-3] The corresponding gene for the disease is the survival motor neuron 1 (*SMN1*) gene, positioned in the telomeric side of 5q13.[4] Another gene known as *SMN2* is located in the centromeric of the same region and is almost genetically identical with *SMN1*.[4-9] There are only five single nucleotide variations (SNV) between *SMN1* and *SMN2* which none of them cause change the amino acidic product (Figure 1).[4,10,11] Of the variations, c.840C>T results in inappropriate splicing and leads to deletion of exon 7.[12] This phenomenon monopolizes SMN1 synthesis to *SMN2*, a reduced amount of full-length protein and a variable amount of truncated and unstable protein results from a deletion in exon 7 (10-50 %).[5] Approximately 95 % of affected individuals with SMA have a homozygous deletion in *SMN1* or conversion of *SMN1* to *SMN2*.[1] Nearly 3 % of patients have a deletion of exon 7 of one allele and a delicate conversion in another.[13] According to the International SMA Consortium, SMA is classified into three groups based on the age of onset and clinical outcome, which mainly depends on the copy number of *SMN2*.[14-16] SMA type I (Werdnig-Hoffmann), the most common and severe type, accounts for approximately 50 % of SMA cases.[17,18] Patients

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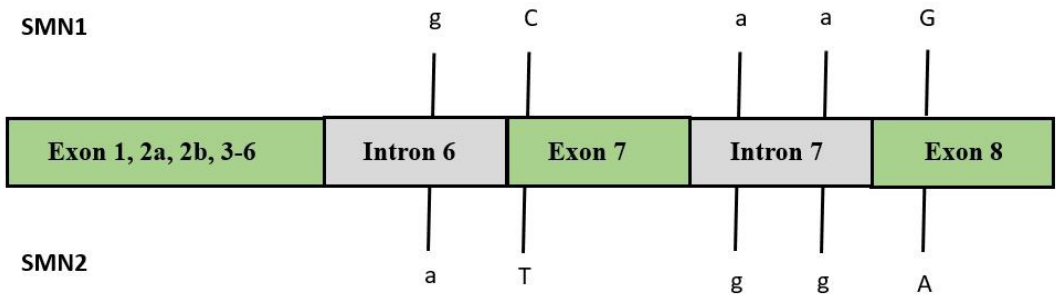

Figure 1. Schematic representation of single nucleotide variations between SMN1 and SMN2

affected by the disease represent the onset of clinical signs at birth or before six months and can never sit and walk unsupported. These patients do not survive beyond they are two years old and usually die from respiratory problems.[19] SMA type II (intermediate form) develops between 7-18 months. These patients can sit but cannot stand or walk independently. Death usually occurs within the first four years.[20,21] SMA type III (Kugelberg–Welander) is a milder form, develop after 18 months. These patients can sit and walk unassisted; nevertheless, they often need a wheelchair during their youth or adulthood. SMA type III is usually subdivided into two groups based on the age of onset; SMA IIIa with the age of onset before three years and SMA IIIb with an age of onset ≥ 3 years.[22] Adult-onset SMA, which is also known as type IIIb and type IV, has been added to SMA classification for the description of patients with adult-onset (>18 years) and mildest clinical course, typically without life-threatening events, including respiratory or nutritional difficulties before adulthood.[22-28]

The copy number of *SMN2* in healthy individuals is about 1-2; however, in SMA patients, the copy number of *SMN2* increases up to four.[13] Although SMA occurs due to the disruption in *SMN1*; however, the severity depends on the *SMN2* copies.[29-33] Some studies have claimed that the existence of five copies of *SMN2* reimburses the absence of two *SMN1* alleles and may be justifiable for exclusively rare asymptomatic homozygous deletion of *SMN1* in unaffected patients.[34] However, asymptomatic individuals with deletion of two SMN1 alleles have also been reported to have less than five copies of SMN2, suggesting a role for other factors in determining disease phenotype.[35] The present study presented an asymptomatic female with a homozygous deletion in SMN1, which was identified after referring to ACECR Vakilabad Genetics Laboratory for the prenatal diagnostic test.

METHODS

Patients

A couple with consanguineous marriage was referred for the diagnostic workup of SMA because of multiple affected family members with clinically confirmed SMA. (Figure 2) Informed written consent was obtained from them the couple. Prenatal diagnosis was performed for the female patient in 12th week of pregnancy.

DNA extraction

Genomic DNA was extracted from peripheral blood leucocytes of the couple following the manufacture's protocol (Favorgen Biotech, Cat-No.: FABGK001, Taiwan). Next, the quality and concentration of extracted DNA were evaluated by agarose gel electrophoresis (Sigma Aldrich, Cat-No.: A9539, Germany) and NanoDrop (Applied Biosystems, USA), respectively. In the end, DNA was stored at -20°C for further use in the following stages of the experiment.

Real-time polymerase chain reaction (PCR)

Due to the test's quantitative nature and to reduce experimental error, the same concentration of DNA samples was prepared. 2X SYBR Green Real-Time PCR and intercalating dye were used to perform the real-time PCR. The albumin (*Alb*) gene was considered a normalizer with the same number of copies in all subjects (healthy, carrier, and infected) to determine the number of copies of the *SMN1* gene. To increase the accuracy of the experiment and a more straightforward interpretation, the DNA of individuals with two alleles (calibrator), one allele (carrier), and no allele (infected) was used as a control. The test was

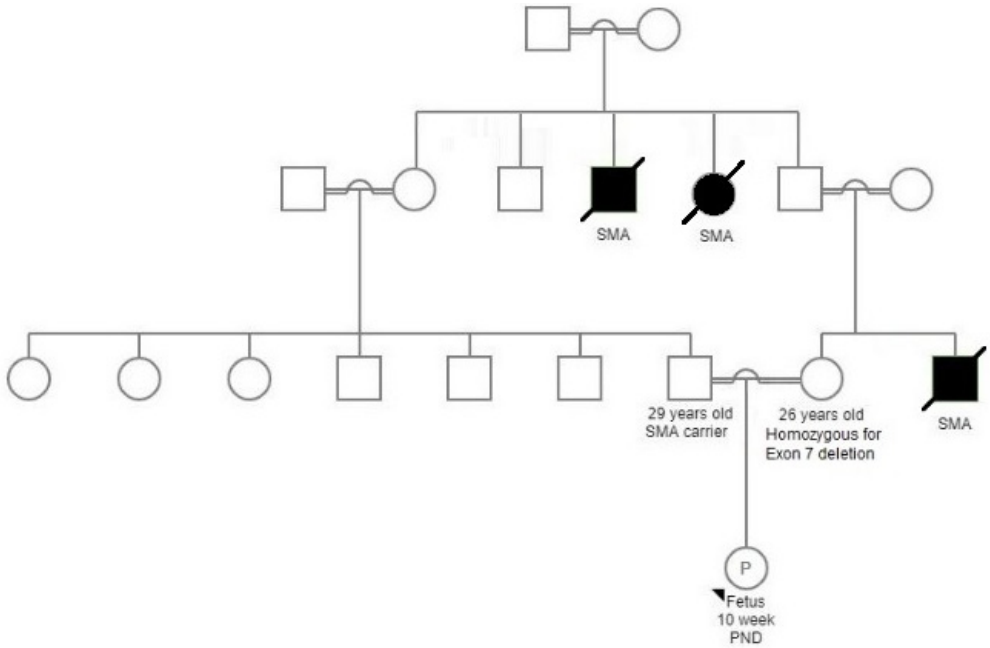

Figure 2. Pedigree of the suspected couple of SMA.

done as a triplicate for all samples. Appropriate primers were designed to determine the exon 7 and 8 of *SMN1* and gene dosage. The SMN2 copy numbers were checked by Real-Time PCR method as described by Anhuf *et al*. [1,36]

PCR-Restriction fragment length polymorphism (RFLP)

To further confirm the results of the real-time PCR, the PCR-RFLP technique was used. The RFPL method for deletion of exone 7 and 8 was the same as described by Bagheri *et al*.[37] Briefly, different primers were used, and PCR products were electrophoresed on 2% agarose gel. The PCR products were enzymatically digested with DraI for 16 hours at 37 °C, and the products of enzymatic digestion were electrophoresed on 3.5% agarose gel. The technique was designed so that the DNA of individuals with one or two copies of *SMN1* showed two bands, and individuals with both deleted copies of *SMN1* showed one band on the agarose gel.

RESULTS

The extracted DNA of all samples had suitable integrity based on the existence of a band on 1% agarose gel electrophoresis and had concentrations between 25 to 70 ng/μl, which was acceptable for performing real-time PCR and RFLP.

The real-time PCR results: The *ct* values related to *Alb* are considered as a normalizer gene in different samples. All samples had a relatively similar *ct* for *Alb*, which confirms the correctness of its selection as a normalizer gene and the correct dilution of DNA (Figure 3). Examination of *SMN1*-related *ct* in the studied samples compared with control samples showed that the male patient had a heterozygous deletion and the female had a homozygous deletion in the *SMN1* gene (Figure 3). The Melt curve analysis also confirmed the non-proliferation of exon 7 of the *SMN1* in the female. The TaqMan Real Time PCR showed 4 copies of *SMN2* gene.

PCR-RFLP and gel electrophoresis results showed the formation of one band for the female and two bands for the male, which confirms the homozygous deletion of exon 7 and 8 in the female *SMN1* gene and the presence of at least one copy in the male. (Figure 4).

The female patient had uneventful pregnancy until the 30th week of gestation.

DISCUSSION

SMA is an autosomal recessive neuromuscular disorder that results from decreasing in SMN proteins. The corresponding region to SMN proteins was mapped to 5q11.2-q13.3, including *SMN1* and *SMN2*, two almost identical genes.[1,4] However, almost all SMN proteins are encoded by *SMN1*, in which *SMN2* plays a minimal role. There are usually one or two copies of

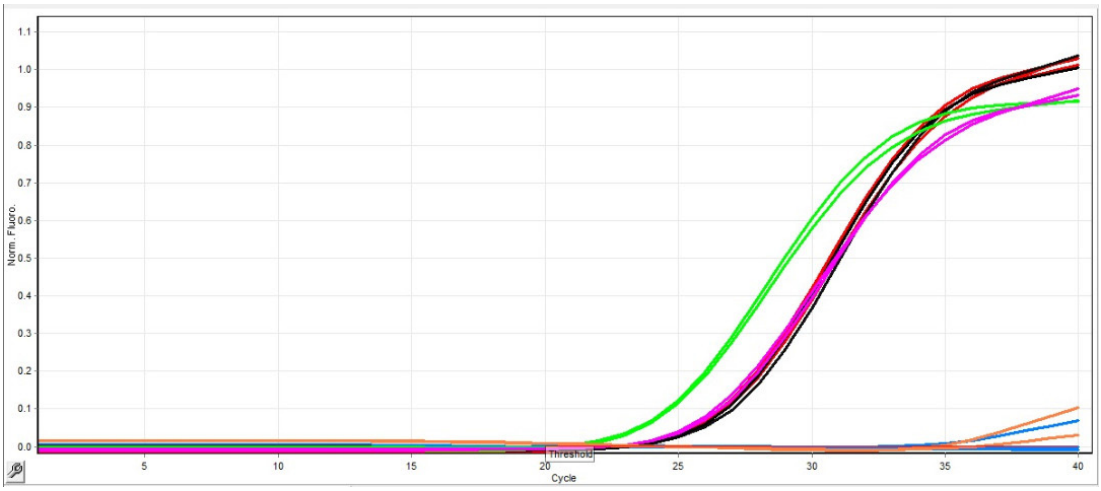

Figure 3. Real-time results for the SMN1 gene, all the samples were assessed in triplicate. Green curves represent a normal homozygous sample, black, pink, and red curves represent father, fetus CVS and a carrier heterozygous of exon 7 deletion respectively, orange and blue curves represent mother sample and a homozygous exon 7 deletion sample respectively.

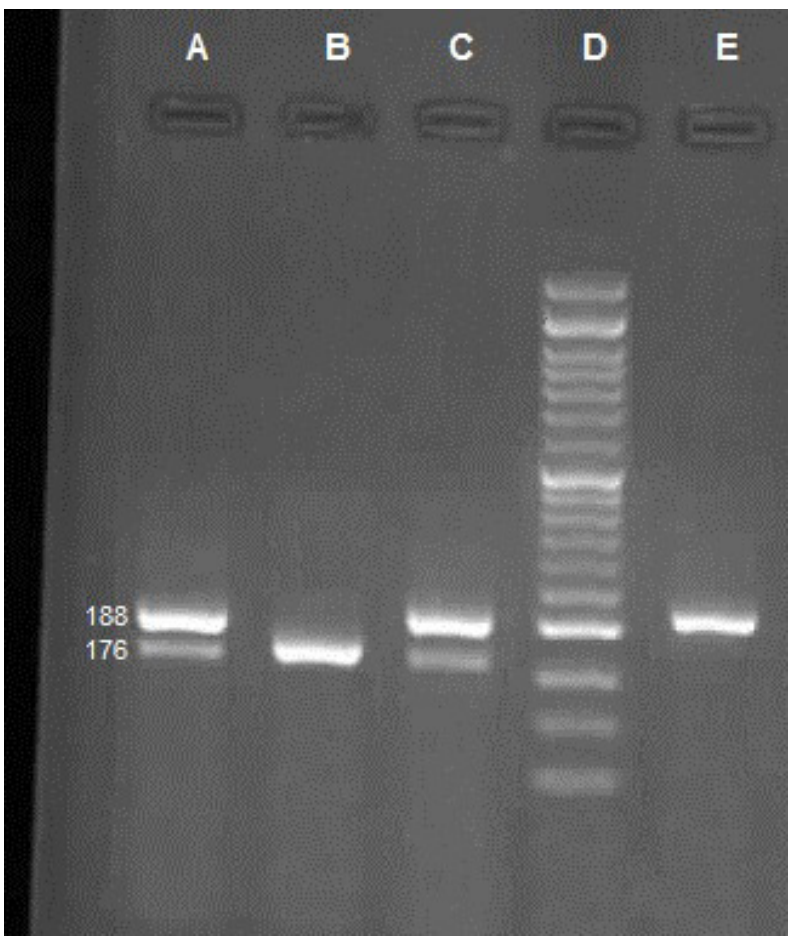

Figure 4. PCR-RFLP results by electrophoresis on the agarose gel (3.5%). DraI digestion makes 188 bp and 176 bp products for SMN1 and SMN2 genes respectively. Lane A: father, Lane B: mother, Lane C: fetus, Lane D: 50bp size standard DNA ladder, Lane E: untreated sample

SMN2 in most healthy individuals; however, the number of *SMN2* copies consists of a more extensive range in SMA patients, from 1 to 6.[39-42] Unlike homozygous deletion of exon 7 in the *SMN1*, the number of copies of the *SMN2* is the primary determinant of the disease phenotype.[4] Given that the homozygous deletion of exon 7 in the *SMN1* is the cause of the disease in more than 95% of patients detecting the number of copies of *SMN1* containing exon 7 is the leading diagnostic solution for patients with suggestive clinical features.[1,13]

Table 1 summaries the studies reporting asymptomatic or mildly symptomatic individuals with homozygous deletion of *SMN1* exons. Most of these studies demonstrated that more than three copies of *SMN2* gene have alleviative effects of individual's clinical symptoms with homozygous deletion of *SMN1* gene. Opera *et al*. reported eight asymptomatic females who had inherited *SMN1* and *SMN2* alleles from their affected siblings and introduced PLS3 protein as a gender-specific modifier of the SMA phenotype.[46] PLS3 is a protein highly expressed in the human spinal cord and involved in the rescues of axon length and axonogenesis in SMA patients.[46] In the same vein, Riessland *et al*. reported five cases of asymptomatic individuals with deleted SMN1 gene who had four copies of *SMN2* and reduced neuronal calcium sensor Neurocalcin delta (NCALD).[50] The protein is a neuronal Ca^{2+} sensor acting as a positive regulator of PLS3. They demonstrated that NCALD acts as a protective modifier of SMA, knocking down gene-induced endocytosis in different animal models.[50]

In the present study, we examined the copy number of *SMN1* in a male and his wife who had a family history of SMA in their relatives. As mentioned above, we found monoallelic and biallelic deletion of *SMN1* in the male and female, respectively. Due to the global expression of SMN proteins and their essential role in RNA splicing, its non-expression is uncommon in healthy individuals.[51-53] Some studies have justified this phenomenon by the copy number of *SMN2*, considering that more copies of *SMN2* lead to

Table 1: Studies reporting asymptomatic or mildly symptomatic patients with *SMN1* gene deletion

Author (year)	Number of individuals	Symptom status	*SMN1* gene status	*SMN2* gene status	Comments
Cobben *et al*. 1995[43]	4	Asymptomatic	Deletion of exone 7 and 8	Not studied	Not studied
Hahnen *et al*. 1995[44]	6	Asymptomatic	Deletion of exone 7 and 8	Not studied	Not studied
Prior *et al*. 2004[45]	3	Asymptomatic	Deletion of exone 7 and 8	5 copies	Not studied
Opera *et al*. 2008[46]	8	Asymptomatic	Deletion of exone 7 and 8	3 to 4 copies	High PLS3 expression. PLS3 is a gender-specific SMA modifier.
Jędrzejowska *et al*.2009[47]	48-year-old male	Mildly symptomatic	Deletion of *SMN1* and *NAIP* genes	4 copies	The number of SMN2 copies alleviates the patient's symptoms
Jędrzejowska *et al*. 2008[48]	3	Asymptomatic	Deletion of exons 7 and 8	Two patients had four copies of *SMN2*, and one patient had 5 copies.	The number of *SMN2* copies alleviates the patient's symptoms
Wang *et al*. 2012[49]	2	Asymptomatic	Deletion of exons 7 and 8	Not studied	Not studied
Riessland *et al*. 2017[50]	5	Asymptomatic	Deletion of exons 7 and 8	4 copies	NCALD is the positive regulator of PLS3

milder forms of the disease.[53] Other modifier genes that diminish the clinical outcomes in SMA patients, including plastin-3 and neurocalcin delta, have also been suggested. These modifiers can explain some differences in clinical manifestations of patients with similar copy numbers of *SMN2*.[34,54] Some studies have suggested that the existence of five or more copies of *SMN2* could completely compensate for the lack of biallelic *SMN1* and might account for the extremely rare asymptomatic biallelic deletion of *SMN1*.[34] Other studies have also reported less than five copies of SMN2 in some asymptomatic individuals with SMN1 homozygous deletion, possibly suggesting a role for other factors in the disease phenotype.[54] According to the literature, biallelic deletion of *SMN1* in asymptomatic individuals is very rare, estimated to be about 0.5 to 0.7 % in first-degree relatives of SMA patients.[55]

In conclusion, although interpreting these results poses a significant challenge to geneticists, it adds to the importance of genetic counseling and genetic testing in such families. In this study, we reported the first asymptomatic patient with a biallelic deletion in *SMN1* in Iran. The results of real-time PCR and PCR-RFLP studies showed that our patient had a homozygous deletion in the *SMN1* gene. As stated in previous studies, the best explanation for this situation seems to be the increase in the number of *SMN2* copies. Another possible factor is the role of modifier genes in determining disease phenotype. These two factors alone or in combination with each other can reduce the symptoms of the disease or even prevent the disease phenotype.

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DISCLOSURE

Conflicts of interest: None

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