

# EVALUATING THE DELETION AND POINT MUTATIONS OF THE SMN1 GENE IN PATIENTS WITH SPINAL MUSCULAR ATROPHY (SMA) IN WEST AZERBAIJAN PROVINCE OF IRAN

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## Abstract

**Background & Aims:** Spinal muscular atrophy (SMA) is a common disorder with autosomal recessive inheritance pattern. The frequency of carriers of this disease is one in forty to one in sixty. SMA occurs in 98% of cases due to the homozygous deletion of SMN1 exons 7 and 8. The purpose of this study was to evaluating the deletion and point mutations of the SMN1 gene in patients with SMA in west Azerbaijan province of Iran.

**Materials & Methods:** A total of 50 patients with SMA were referred to the Genetic Department after clinical diagnosis for molecular evaluation and genetic counseling. Genomic DNA was extracted from blood samples. The exclusion rate of exons 7 and 8 in the neuronal survival gene 1 was determined by using the PCR-RFLP. **Results:** Deletion of exons 7 and 8 were observed in 98% of the studied cases (49 out of 50 cases). In one patient, the sequencing of exon 5 showed homozygote mutation c.549 del C (p.Lys184ser fs 29) (point mutation).

**Conclusion:** The evaluation of the presence or absence of exons 7 and 8 of the SMN1 gene, as well as point mutations in SMN1 gene in patients suspected of musculoskeletal atrophy, is effective in confirming the clinical diagnosis and subsequent genetic counseling.

**Keywords:** SMA, SMN1, PCR-RFLP

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## Introduction

Spinal muscular atrophy (SMA) as an autosomal recessive disorder is recognized with loss of motor neurons in the ventral horn of the spinal cord, weakness and muscle atrophy(1). The disease first was described by Werding in 1893 and Hoffman in 1890. In human, this gene is located on the long arm of chromosome 5. The survival of motor neurons (SMN1) gene was

recognized as the cause of SMA in 1995 (2). There are two almost identical SMN genes on chromosome 5q13: telomeric gene or SMN1, a gene that determines the SMA and centromeric gene or SMN2 (3). Due to a nucleotide, sequence encoding of SMN2 differs from SMN1 (840C> T). It does not change the amino acid sequence of nucleotides, but leads to different splicing of exon 7 (3). Homozygous deletion of exons 7 and 8

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can be seen in almost 98 percent of patients. All patients had at least one copy of the gene SMN2 and Most SMA patients have 2-4 copies (2-4). SMA is heterogeneous in terms of clinical and genetic observations (5). SMA is the second most common lethal disorder after Cystic Fibrosis (CF) and the prevalence is one in 6000 to 10000 live births (6). SMA is divided to four Class III, II, I and IV based on phenotype and emergence age (7).

Werding Hoffmann disease or SMA class type I is the most severe and most common form of the disease in terms of clinical signs and symptoms can be emphasized in the first six months of life. The fetus with spinal muscular atrophy disease inside the mother's womb have relatively less movement compared to normal embryos. Infants with SMA are not able to drag themselves and control the movement of their head and can never sit without help. Also, babies with SMA usually die before two years of age due to hypotonia, limb paralysis and respiratory failure due to choking and swallowing (9, 8). Class Type II SMA is known in patients between 7 and 18 months with muscle weakness. The infants are able to sit alone, but need help to walk and stand. Deep tendon reflexes are absent in spinal muscular atrophy in patients with type II class. The biggest problem is the weakness of the respiratory muscles, which can cause recurrent infections in these patients (10). Kugelberg-Welander or Class III SMA is heterogeneous in terms of clinical symptoms and the disease starts after 18 months. Spinal muscular atrophy patients with class III disease can walk independently. In this group of patients, muscle weaknesses are evident especially leg muscles while walking, going up and down the stairs at ages 2 to 3 years of age (11). The results of recent studies show patients with Class III SMA is likely to lose the ability to walk by the age of 40 (12).

Class IV SMA is the mildest type associated with muscle weakness to an adult (over 18 years). This group of patients are able to adulthood without particular

problems in walking, breathing and feeding them (13, 14). Genes associated with disease include: survival motor neuron gene (SMN), neuronal cell death inhibitor protein gene (NAIP), p44, H4F5, GTF2H2 (15, 16). SMN1 gene sequence is part of a 500 kV with 9 exons and 8 introns (17). The survival motor neuron gene protein is made of 294 amino acids and its molecular weight is 38 kDa. Telomeric version of the gene (SMN1) has homology with its centromeric copy (SMN2) and their difference is in exons 7 and 8. These differences create restriction sites for restriction enzymes (17). Lack of homozygous SMN2 in 4.5% of healthy individuals indicates that the SMN2 is not associated directly with SMA disease (18).

While SMN1 in 98 per cent of SMA patients has deletions (especially the exons 7 and 8) (9). SMA diagnosis is carried out by a specialist physician in the first phase of clinical trials, perform physical testing, and an electromyogram as well as family history. A definitive diagnosis is through genetic tests for deletion and point mutations of the SMN1 gene (19). Given that it is not already possible to cure the disease completely, the prenatal diagnosis is one of the ways to reduce costs and manage the patients and families about the disease. The aim of this study is the molecular analyses of the SMN1 gene in patients referred to the martyr Motahari hospital using the PCR-RFLP method (Urmia, West Azerbaijan province).

## Materials and Methods

A total of 50 patients with SMA referred to the Genetic Department of Martyr Motahari Hospital (West Azerbaijan, Urmia) were evaluated in order to identify mutations. Patients were selected on the basis of international SMA consortium by pediatricians and by neurologists and diagnosis is done by examining the symptoms confirmed by electromyography (EMG). The parents' informed consent was obtained for the study

and the study sample was taken after completing the relevant forms required from participants.

2-3 ml of peripheral blood samples were collected by a nurse in genetic section. These samples are collected in 15 ml Falcon tubes containing 500 ml EDTA mM as anticoagulant and freeze until the day of extracting genomic DNA. Falcon tubes containing samples were taken out of the freezer and melted at room temperature. DNA isolation was carried out using standard "salting out" method as described by miller et al.(20). Extracted DNA samples were assessed with bio photometer (Eppendorf AG) and the quality of DNA was confirmed. The ratio for pure DNA is between 1.7 to 2. PCR reaction was carried out for each reaction after optimization in 0.2 mL Eppendorf tubes in a final volume of 25 microliters containing dNTPs at a concentration of 200 micro molar, 0.2 ml Taq polymerase enzyme, 0.75 ml MgCl<sub>2</sub> at a concentration of 50 mM, DNA about 100 nanograms, 0.5 microliter of each primer at a concentration of 10 pico moles and finally 10X buffer at a rate of 2.5 micro liters (CinnaGen PCR Master Kit, SinaClon Co., Iran). Amplification area, sequencing primers, and amplified fragment length, PCR program and cutting enzymes have been compiled in Table 1.

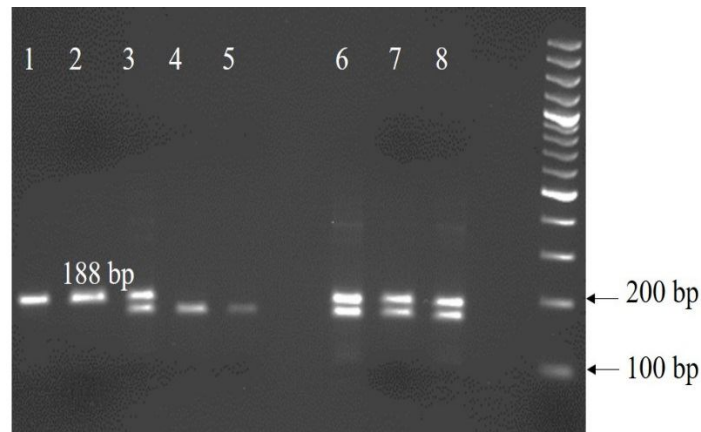
Restriction enzyme was used to detect the gene omissions in enzymatic digestion. The enzyme DraI (Thermo Scientific) was used in exon 7 and the enzyme DdeI (Thermo Scientific) was used in exon 8 to detect deletion. For digestion in 20 microliters volume, 10 ml PCR product, restriction enzyme and a buffer enzyme associated with the concentration (1X) were used. Enzymatic digestion is done at 37 ° C for according to manufacturer's brochure. To view the PCR products and enzymatic digestion, 2.5 percent agarose gel (SinaClon Co., Iran) stained with safe stain color (SinaClon Co., Iran) was used. Then the gels were analyzed under UV light and the images have been recorded using gel documentation devices.

## Results

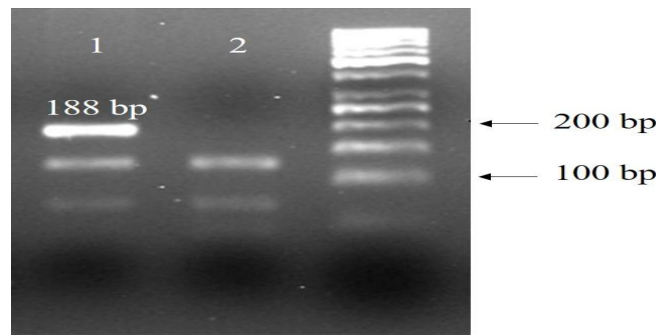
Patients participating in the study were diagnosed with SMA Type 1 and 49 (98%) of patients had the homozygous deletion of SMN1 exons 7 and 8. SMN1 gene sequencing indicates that there is a point mutation in exon 5 (c.549 del C)(p.Lys184ser fs 29) as a homozygous mutation. Gel Electrophoresis pictures of the samples are shown in Figures 1 and 2.

**Table 1.** Area amplification, sequencing primers (TAG Copenhagen A / S), and PCR amplified fragment length and restriction enzyme to that used in this study (21).

Area	Primer sequences	fragment length	Denaturation temperature / time	Hybridization temperature / time	Elongation temperature / time	The number of cycles	Cutter enzyme
Exon 7	ctatcaacttaattctgatca ccttcctctcttttgattttgtt*t	base 188	94 ° C 2 minutes	55 ° C 1 minutes	72 ° C 1 minutes	35	DraI (Thermo Scientific)
Exon 8	gtaataaccaaatgcaatgtgaa ctacaacaccttctcacag	base 188	94 ° C 1 minutes	60 ° C 1 minutes	72 ° C 1 minutes	35	DdeI (Thermo Scientific)



**Figure 1.** Evaluation of exon 7 of SMN1 gene in 8 samples using the restriction enzyme *DraI* by agarose gel electrophoresis. Wells 1 and 2 samples without cutting enzyme, wells 3, 6,7,8 are control samples, Wells 4 and 5 are studied samples (These samples are considered positive given the deletion of 188-bp band in exon 7 of SMN1 gene)



**Figure 2.** Evaluation of exon 8 of SMN1 gene in two samples using the restriction enzyme *DdeI* by agarose gel electrophoresis. Well 2 relates to the studied sample (the sample is considered positive due to the deletion of 188-bp band in exon 8 of SMN1 gene); Well 1 is related to the control sample.

## Discussion

Telomeric gene mutation or SMN1 is necessary for the SMA onset (22). There are two hypotheses for pathogenic description of SMA disease: 1-SMN protein is involved in the biogenesis of small nuclear ribonucleic proteins and pre-mRNA splicing. Thereby reducing amount of the SMN could be decisive (23). 2-SMN has a dedicated motor neuron function that is independent from the nuclear small ribonucleic proteins such as mRNA transport along the axons (24). Both hypotheses are supported by several studies: However, low levels of SMN protein are crucial for motor neurons both in terms of the exon and due to their proprietary contrast to skeletal muscle. Spinal Muscular Atrophy international consortium in 1992 categorized patients

into three groups based on the age of onset and age at death. But in 1998, this classification was revised again, and the patients were divided into four groups (25). In 1995, it was reported that SMN gene in 98.6 percent of patients with SMA type 1 has had deletions. The majority of patients with homozygous deletion of exon 7 or 8 were identified. The study of Lefebvre et al. showed that the frequency of the deletion of exon 7 and 8 in patients with SMA is about 93 percent (3). Many studies have been performed in different populations after 1995 regarding the deletion in SMN1 gene in SMA patients according to which the frequency of removal in countries such as Kuwait, Korea and India was reported as 100%, Japan 87.5% and 94% in Saudi Arabia (26-29). Rates of consanguineous marriage in Iran is very high

(especially in the area of Azerbaijan). In fact, this is one of the important genetic causes for infant and children mortality in Iran and is considered as the most common neurological-muscular diseases after Duchenne muscular dystrophy. In this regard, several studies have been conducted in Iran. According to a study in 2007 by Derakhshande and colleagues on 75 Iranian SMA Patients, the homozygous deletion of the SMN1 gene in exon 7 and 8 was equivalent to 97 percent (30). In 2009, a study was carried out by Omrani and colleagues on 75 patients with SMA (54 patients with type I, 8 patients with type II and 13 patients with type III). Homozygous deletion of exon 7 and 8 of SMN1 gene was found in 68 patients and the frequency was estimated as 90%. In the study, the deletion of SMN1 gene in exon 7 and 8 was the leading cause of SMA disease (31). On the other hand, another study was done by Hasan Zadeh and colleagues at the same year on 243 Iranian families and the deletion of exon 7 in SMN1 gene was estimated as 94 percent (32). Then, another study by Sadeghi and colleagues in 2014 was done on 71 Iranian patients. Homozygous deletion of exons 7 and 8 on 51 patients, and the frequency of removal was 71.8 percent (33). In this study with the aim to determine the SMN1 gene mutations in patients with SMA in Western Azerbaijan province's population, the frequency of the deletion of exons 7 and 8 in SMN1 gene was found in 98% of tested cases. As the molecular sensitivity of the tests was 93-95 percent, it is essential for all relative couples intending to get married to receive counseling and also the prenatal diagnoses for couples who have a child with SMA disease is essential. Prenatal diagnosis is proved when SMA disease screening test of both parents is necessary. The purpose of screening tests is to identify embryos with the disease before the appearance of symptoms. Improved public health screening programs are leading to improved quality of life and the process leads to the identification of individuals at risk of disease.

## Conclusion

Assessment of the presence or absence of exons 7 and 8 of SMN1 gene and also checking the presence of point mutations in exons 7 and 8 in patients suspected of SMA is needed to support the clinical diagnosis and genetic counseling. This is the first study conducted in the province of West Azerbaijan and shows that due to a high prevalence of the disease in the province, it is essential to develop risk screening programs to identify the people and those at risk.

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**Conflicts of Interest:** The present study has no conflict of interest.

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