

Molecular Genetic Diagnosis for a Family with Type I Spinal Muscular Atrophy (SMA) via Analysis of the Survival Motor Neuron (SMN) Gene

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Abstract

Autosomal recessive spinal muscular atrophy (SMA) is, after cystic fibrosis, the second most common fatal monogenic disorder. Depending on the clinical type, SMA causes early death or increasing disability in childhood. Here, we report a three month old Saudi girl presented with a history of coughing and respiratory distress who had previous admissions due to pneumonia. There was a positive family history of sibling death, at age 2 years, due to SMA. Genetically, we found homozygous deletions of the Survival Motor Neuron (*SMN1*) gene exons 7 and 8 using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. Thus, confirming this case clinical diagnosis with presumed SMA type I. DNA testing of patients, in whom SMA is suspected, is a highly reliable, fast, and noninvasive method. The ability to detect homozygous gene deletions in a high percentage of typical SMA patients will much improve genetic counseling and prenatal diagnosis in affected families.

Keywords : Spinal muscular atrophy (SMA); Survival Motor Neuron (*SMN1*) gene; Gene deletion of exon 7 and exon 8 of the *SMN1*

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Introduction

Spinal muscular atrophy (SMA, MIM #253300) is an autosomal recessive neuromuscular disease characterized by degeneration of the alpha motor neuron from anterior horn cells of the spinal cord, resulting in progressive proximal and symmetrical weakness and atrophy of limbs and trunk. The motor neurons of cranial nerves could be involved, but sensations are spared due to originates from the posterior horn cells of the spinal cord [1-3]. It is the most common autosomal recessive neurodegenerative disorder and the second most common fatal autosomal recessive disorder after cystic fibrosis. It affects 1 in 5,000 to 1 in 10,000 live births overall worldwide, due to consanguineous marriage [4-6].

The disease was first described by Werdnig and Hoffmann in the 1890s. In 1995, the genetic defect was localized to 5q11.2-q13.3 with the identification of the survival motor neuron gene (*SMN*) as the disease-causing gene [7,8]. The *SMN* gene consists of nine exons (exons 1, 2a, 2b, and 3-8) and occurs in two copies of *SMN1* (600354) or telomeric copy and *SMN2* (601627) or

centromeric copy [9, 10]. Both *SMN1* and *SMN2* sequences are homologous with only five nucleotide differences, one in intron 6, one in exon 7, two in intron 7, and one in exon 8 [11]. They encode the survival motor neuron protein and are important during RNA processing [12]. Nucleotide (C) in *SMN1* and (T) in *SMN2* is the main difference used to differentiate, which does not alter the amino acid sequence but has been shown to be important in splicing [13]. Exon 7 is included in the final mature mRNA product. When (C) is changed to (T) in *SMN2*, this leads to the splicing enhancer being disrupted [12]. Therefore, 90% of the mature mRNA transcript from *SMN2* will be without exon 7. The final protein product will be unstable and degrade rapidly due to lack of exon 7 [12]. Also in exon 8 the nucleotide difference is (G) for *SMN1* and (A) for *SMN2* [11]. A homozygous deletion of exons 7 and 8 of the *SMN1* gene occur in 93% of SMA patients and a homozygous deletion of exon 7 account for 5.6% [14]. In 90-98% of SMA patients, the *SMN1* gene is deleted or interrupted but *SMN2* is not associated with the disease [10]. The *SMN2* copy number shows an inverse relationship with disease severity. As all patients retain at least one copy of *SMN2*, and as SMN protein levels increase the severity of the disease will be reduced. Patients, such as type III, have less disease severity, but a higher *SMN2* copy number than type I patients [15].

On the basis of age of onset and motor function achieved, SMA is clinically classified into four phenotypes [16]. SMA type 1 (Werdnig-Hoffmann disease) is the common type found in 50% of SMA patients and the most severe [16]. These patients present by hypotonia, symmetrical flaccid paralysis, no head control and poor suck and swallow, with increasing swallowing and feeding difficulty over time due to involvement of bulbar motoneurons. They present before 6 months of age. According to the severity of clinical signs, SMA type I is classified into 3 clinical subgroups. SMA type II onsets between 7 and 18 months of age. Patients have the ability to sit unsupported and standing, but have the inability to walk independently. They have fine tremors of the upper extremities and absence of deep tendon reflexes. In severe cases, joint contractures and kyphoscoliosis are very common. They live beyond 4 years of age and until adolescence or later, depending on the degree of respiratory muscle involvement [16]. SMA type III (Kugelberg-Welander disease) patients are able to walk independently; with some abilities to continue to walk and live productive adult lives while others might need a wheelchair [2]. SMA type IV onsets at adulthood (years 18+). They are able to walk in adulthood and are without respiratory and nutritional problems [16].

For any patient suspected of having SMA, first there should be the search for *SMN1* gene homozygous deletion by using a PCR-RFLP assay [10, 17, 18]. The diagnosis of SMA is confirmed by the absence of *SMN1* exon 7 (with or without deletion of exon 8) [17]. This test has 95% sensitivity and nearly 100% specificity [17]. If the first level assay tests are negative other laboratory examinations, including creatine kinases dosage and electrophysiological tests, such as electromyography (EMG), and nerve conduction study,

should be performed [17]. In this case study we aim to confirm this clinical diagnosis by SMA type I with the method of molecular genetics.

Case Report

A three month old Saudi girl was presented to the emergency department of the Maternity and Children's Hospital (MCH), in Makah, KSA, with a history of coughing and respiratory distress. There is a family history of a two year old female known to have SMA, who died due to pneumonia. There is no family history of other diseases and no consanguinity between her parents. The three months old is the product of a full term pregnancy and delivered by caesarean section. At age 22 days she was admitted to the NICU of MCH due to pneumonia and stayed in the NICU for 15 days (13 days on mechanical ventilation) during that time all investigations including CBC, blood chemistry, ammonia, lactate and brain CT scan were normal. At this time she looked alert, having severe hypotonia, frog like position, seesaw breathing, with decreased breath sounds on the right side of the chest. The case was admitted to PICU and connected to mechanical ventilation. CBC, urea and electrolytes, chest x-ray, blood culture, and gene study for SMA were analysed. The investigation results were: WBC: 13.9; HB:11; PLT: 445; creatinine: 21; Na:142; K: 3.5, blood culture was staphylococcus epidermidis, and the chest x-ray showed right upper lobe collapse, then left lung collapse and both resolved. She was on cefuroxime, then changed to tazocin and cloxacillin after the blood culture results. It had been planned for a tracheostomy due to failed extubation wicet. Our genetic analysis identified *SMN1* gene deletion due to the presence of zero copies of exon 7 as well as zero copies of exon 8. Also up to two copies of *SMN2* gene were identified. According to this result she is most likely to be affected with SMA. The presence of two copies of *SMN2* gene classifies her disease severity as SMA type I.

Materials and Methods

This study was approved by the Institutional Review Board and the Research Ethics Committees (REC) in Umm al-Qura University Medical School, Makkah, KSA. Blood samples were obtained from the subjects after informed written consent was given by each subject. The Genomic DNA was isolated from EDTA treated whole blood using a Gene JET Whole Blood Genomic DNA Purification Kit (Thermo Scientific Co. Ltd.). Genotyping for *SMN1* exons 7 and 8 were performed by polymerase chain reaction (PCR) and restriction enzyme digestion. The primers for exon 7 were as follows:

Forward: 5'- CTA TCA ACT TAA TTT CTG ATC A -3', and Reverse: 5'- CCT TCC TTC TTT TTG ATT TTG TTT -3'. The primers for exon 8 were as follows: Forward: 5'- GTA ATA ACC AAA TGC AAT GTG AAA -3', and Reverse: 5'- CTA CAA CAC CCT TCT CAC AG -3'. The PCR amplification was performed in a Veriti thermal cycler (Life Technologies). DNA was amplified using GoTaq Colorless Master Mix (Promega Co. Ltd) under the following conditions: initial denaturation at 95°C for 5 mins, followed by 37 cycles of 95°C for 30s, 45°C for 30s, and 72°C for 1 min, and a final extension step at 72°C for 10 mins for both exons. These primers yielded a PCR fragment of 188 bp and 187 bp for exons 7 and 8 respectively.

Subsequently, 5U of enzymes DraI and DdeI (Promega Co. Ltd.) were added to 20 μ l PCR products for exons 7 and 8, respectively, in a total volume of 25 μ l and incubated at 37°C overnight. After restriction enzymes digestion, the products were separated on a 3% agarose gel and visualized using ethidium bromide and ultraviolet light.

Results and Discussion

Genotyping for *SMN1* exon 7 and exon 8 was performed by PCR-RFLP analysis. We found fragments of 188 bp, 165 bp and 23 bp for exon 7 and fragments of 187 bp, 123 bp and 64 bp for exon 8 (Fig. 1). This indicated there is a deletion for both exons in *SMN1* gene. Our molecular analysis has been proven to be a specific tool for the diagnosis of suspected SMA patients in our society. Saudi Arabia has one of the highest rates of consanguinity in the world [4], consequently genetic disorders, such as SMA, are prevalent in this society and for this reason. The incidence of rare diseases and congenital ones, and as in our case homozygosity for SMA alleles, are also high. In the case study, there is no consanguinity between the parents, but this does not exclude that only consanguineous partners can have the disease. For example, as in other research there were 10 consanguineous marriages among the parents of 389 index cases in the International Collaborative Study of the SMAs [19].

The diagnosis of SMA depends on clinical symptoms and investigation and the most common symptom of SMA is weakness. In the case study, the family had 5 children, 3 boys and 2 girls. The first girl was diagnosed with SMA and died. She was diagnosed according to clinical symptoms only. The second daughter had the same clinical problem and the boys were unknown. Since Daniels et al. [20] performed the first prenatal diagnosis of SMA1 in 1992, *SMN1* gene deletions have been found in more than 98% of patients with SMA, and point mutations have also been reported. The pathogenesis of SMA involves *SMN1* gene

dysfunction, and the severity of SMA is related to *SMN2* gene dysfunction. Molecular genetic research of SMA patients from the Netherlands revealed homozygosity for *SMN1* deletion in 96 (93%) of 103 subjects. In addition, neuronal apoptosis inhibitory protein deletions were found in only 38 (37%) of those subjects and occurred most frequently in SMA type I [17].

In our case genomic DNA was extracted from peripheral blood samples using a Blood DNA isolation kit. Genotyping for *SMN1* exon 7 and exon 8 was performed by polymerase chain reaction (PCR) and restriction-enzyme digestion test. We found that there is deletion for both exons to confirm SMA for this patient. Furthermore, it confirms the need to establish this diagnostic test for the SMA condition, in our society. In most cases, carrier testing is requested by siblings of patients or of parents of SMA children and is aimed at gaining information that may help with reproductive planning (prenatal diagnosis or pre-implantation diagnosis). In these cases, we suggest to test at risk individuals first and, in case of testing positive, to analyze also the partner. Thus in the case of a request for carrier testing on siblings of an affected SMA infant, a detailed neurological examination should be done and consideration given to doing the direct test to exclude SMA [4]. Currently, only individuals with a family history of SMA are routinely being offered carrier testing. However, more broad-based population carrier screening is currently recommended for a number of other genetic disorders with similar carrier frequencies [21].

SMA has no effective treatment, therefore appropriate genetic counseling should be provided with genetic testing to allow families to consider the future of the fetus. It is important to be aware that the carrier test may not identify all carriers, and that there is a possibility of false negative results. Furthermore the risk in the next pregnancy, when the two parents are carriers, is 25%, with the exception of de novo rearrangements.

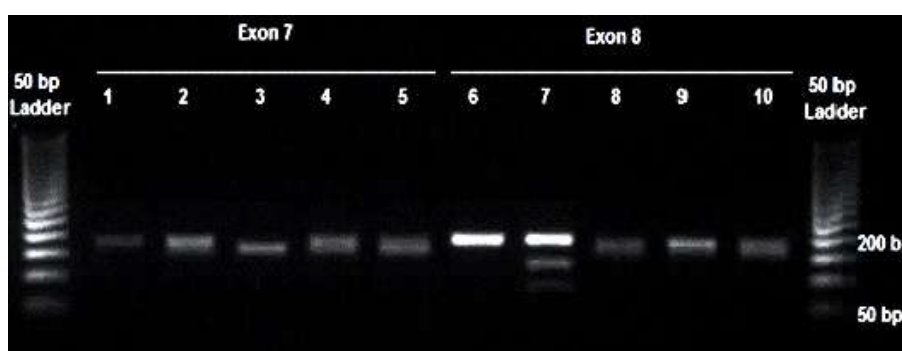


Figure 1 RFLP analysis result for exon 7 and 8 of *SMN1* gene
Lane 1: exon 7 PCR product with 188 bp. Lane 2: exon 7 PCR product from normal control incubated with DraI restriction enzyme and shows no cut. Lane 3: patient exon 7 PCR product incubated with DraI enzyme and shows homozygous cut for both alleles; 165 bp and 23 bp (lost). Lanes 4 and 5: patient father and mother respectively, show heterozygous cut with 188 bp, 165 bp and 23 bp (lost). Lane 6: exon 8 PCR product with 187 bp. Lane 7: exon 8 PCR product from normal control incubated with DdeI restriction enzyme and shows heterozygous cut with 187 bp, 123 bp and 64 bp. Lane 8-10: patient and her parents show single band with 187 bp.

In a research study prenatal diagnoses were carried out in 15 pregnancies by amniocentesis. Four fetuses were normal and carried none of the mutations. Three fetuses were affected and carried both the mutations. Five fetuses were carriers and carried one of the mutations. Two fetuses were healthy or carriers and only one fetus was a carrier or affected [4].

We hope, in the future, to prevent further diagnosis of SMA and other rare genetic diseases by early detection and counseling. From a practical point of view, as in this study, demonstration of a homozygous *SMN* deletion in a clinically presumed SMA patient should be considered as confirmation of the diagnosis. This test

can readily be used instead of diagnostic methods making use of invasive procedures such as muscle biopsy, to verify whether a patient has SMA or a different disorder that has phenotypical similarities.

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