Deletion mutations in the dystrophin gene of Saudi patients with Duchenne and Becker muscular dystrophy

Mohammed Al-Jumah, MD, Ramanath Majumdar, PhD, Saad Al-Rajeh, MD, Enrique Chaves-Carballo, MD, Mustafa M. Salih, MD, Adnan Awada, MD, Saad Al-Shahwan, MD, Shifa Al-Uthaim, BSc.

Objective: The deletion in the dystrophin gene has been reported for many ethnic groups, but until now the mutations in this gene have not been thoroughly investigated in Saudi patients with Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD). We examined the deletion pattern in the dystrophin gene of the Saudi patients applying multiplex-polymerase chain reaction (PCR). The aim of this study is to describe the outcome of our initial effort to identify mutations in the dystrophin gene in a representative group of Saudi patients with DMD and BMD.

Methods: Genomic deoxyribose nucleic acid was isolated from 41 patients with DMD and BMD (27 patients confirmed by muscle biopsy and 14 patients with clinical suspicion), 3 patients with limb girdle muscular dystrophy, 12 male relatives of the patients, and 5 healthy Saudi volunteers. A total of 25 exons around the deletion prone regions (hot spots) of the dystrophin gene were amplified. The study was carried out at the King Fahad National Guard Hospital, Riyadh, Kingdom of Saudi Arabia between 2000 and 2002.

Results: The deletion of one or more exons was found in 21 of 27 DMD and BMD patients confirmed by muscle biopsy. The deletion in the gene was detected in 5 of 14 patients with DMD diagnosis, but not confirmed by dystrophin staining of muscle biopsy. No deletion in the dystrophin gene was detected in control Saudi volunteers, the limb girdle dystrophy patients, and the relatives of patients, as expected.

Conclusion: The present study suggests that intragenic dystrophin gene deletions occur with the same frequency in Saudi patients compared with other ethnic groups. The PCR-based deletion analysis provides a reasonable first step in the diagnostic care of Saudi patients who may be afflicted with DMD and BMD.

Saudi Med J 2002; Vol. 23 (12): 1478-1482

Abstract

Duchenne muscular dystrophy (DMD) is among the most common human genetic diseases worldwide, occurring approximately one in 3500 male births. This lethal disease, which exhibits an x-linked recessive mode of inheritance, is characterized by progressive proximal weakness beginning in early childhood, wheelchair dependence by early teens and death from respiratory failure by the end of the 2nd or 3rd decade. Becker muscular dystrophy (BMD) is similar but begins later and is milder; however, most BMD patients walk well into adult life. The incidence of BMD is 10% of reported DMD cases worldwide. Both disorders arise from defects in the dystrophin gene. This gene maps to the

From the Neuromuscular Laboratory (Al-Jumah, Majumdar, Al-Uthaim), Neurology Section (Al-Jumah, Awada), King Fahad National Guard, Division of Neurology (Al-Rajeh), Department of Pediatrics (Salih), King Saud University, Department of Neurosciences (Chaves-Carballo), King Faisal Specialist Hospital & Research Center, Department of Pediatrics (Al-Shahwan) Armed Forces Hospital, Riyadh, Kingdom of Saudi Arabia.

Received 29th May 2002. Accepted for publication in final form 24th August 2002.

Address correspondence and reprint request to: Dr. Ramanath Majumdar, Department of Medicine, Mail Code 1143, King Fahad National Guard Hospital, PO Box 22490, Riyadh 11426, Kingdom of Saudi Arabia. Tel. +966 (1) 2520088. Ext. 6663. Fax. +966 (1) 2520040. E-mail: ramanathm@hotmail.com
Deletion mutations in Saudi DMD patients ... Al-Jumah et al

chromosome Xp21 region and the largest gene isolated to date, spanning 2.5 x 10^6 base pairs. It contains 79 exons and codes for a 14 kilobase (kb) messenger ribonucleic acid (mRNA). The deletions in this huge gene are non-randomly distributed. Approximately 60-70% of the DMD and BMD cases are due to intragenic deletions in the dystrophin gene, and occur primarily in the center and less frequently near the 5 prime (5') end of the gene, which have been described as "hot spots" or deletion prone regions. It has been estimated that one third of the DMD and BMD cases are the result of new duplications. Duplication has been found in approximately 5-10% of the patients. The frameshift mutations. Duplication has been found in DMD and BMD cases are the result of new

Dystrophin. The deletion of the dystrophin gene with deficiency with adhalin (α-sarcoglycan), and not dystrophin which lacks part or the entire carboxyl-terminus. The truncated proteins are presumably unstable, and little or no dystrophin is detected on Western blot analysis of muscle biopsy samples. Southern blots and multiplex polymerase chain reaction (PCR) assays have been designed to detect almost 60-65% of the intragenic deletions in DMD and BMD as well as the majority of duplications. The prevalence of DMD and BMD cases in the Kingdom of Saudi Arabia (KSA) is unknown. Another form of muscular dystrophy that is known as severe childhood autosomal recessive muscular dystrophy (SCARMD) has a clinical features overlapping with DMD and BMD. This disease is quite frequent in KSA and most of the Arab world. However, it is associated with deficiency with adhalin (α-sarcoglycan), and not dystrophin. The deletion of the dystrophin gene has been reported for many ethnic groups, but until now the mutations in the same gene of Saudi patients with DMD and BMD have not been thoroughly investigated. In this paper, we describe the outcome of our initial effort to identify mutations in the dystrophin gene in a representative group of Saudi patients with DMD and BMD.

Methods. Patient selection. Neurologists evaluated all patients. The patients were diagnosed using standard clinical diagnosis criteria for DMD and BMD in conjunction with typical myopathic electromyographic studies, elevated creatine phosphokinase (CPK) activity and typical dystrophic changes on muscle biopsy including dystrophin immunostaining. Twenty-six Saudi male patients with DMD type, one male patient with BMD type (confirmed by muscle biopsy and dystrophin staining), and 14 patients with clinical suspicion of DMD but without confirmatory biopsy were included in the study. Three patients with autosomal recessive limb girdle muscular dystrophy (LGMD) or SCARMD, resembling Duchenne type dystrophy but with no abnormality in dystrophin protein were also included. In addition, 12 relatives and 5 healthy Saudi male subjects were used as control. The local ethics committee reviewed the study, and an informed consent was obtained from the parents of the children who were included in the study. The study was carried out at the King Fahad National Guard Hospital, Riyadh, Kingdom of Saudi Arabia between 2000 and 2002.

Blood collection and deoxyribose nucleic acid extraction. For molecular studies 5-10ml blood were collected in vacutainer tubes (acid citrate-dextrose, yellow top) from patients and their unaffected relatives. Blood was generally processed within 24 hours after collection. Human genomic DNA was prepared from peripheral blood by a conventional lysis method. Briefly, anticoagulated blood was treated with hypotonic solution (155 mm ammonium chloride, 10mM ammonium bicarbonate). From the remaining lymphocytes, DNA was liberated with sodium dodecyl sulfate and proteinase K digestion and then extracted with phenol and chloroform.

Multiplex-polymerase chain reaction. Dystrophin gene deletions were detected using 3 multiplex-PCR reactions. Twenty-five exons were analyzed. Set one contained primers of promoter (Pm) (535 base pair), exons 3 (410 base pair), 43 (357 base pair), 50 (271 base pair), 13 (238 base pair), 6 (202 base pair), 47 (181 base pair), 60 (139 base pair) and 52 (113 base pair). Set 2 contained primers of exons 45 (547 base pair), 48 (506 base pair), 19 (459 base pair), 17 (439 base pair), 51 (388 base pair), 8 (360 base pair), 12 (331 base pair), 44 (268 base pair), and 4 (196 base pair). Set 3 contained exons 49 (439 base pair), one of 332 base pair), 16 (290 base pair), 41 (274 base pair), 32 (253 base pair), 42 (195 base pair) and 34 (171 base pair). Following reaction conditions were adopted: in a volume of 30µl containing 1x PCR buffer (Pharmacia), 200 mM deoxy nucleoside triphosphates (dTTP), 1.5 mM magnesium chloride, 200 ng genomic DNA and 100 ng of individual primer, the amplification was carried out with a hot predenaturation start for 5 minutes at 94°C and subsequently with 25 cycles (of denaturation for 45 seconds at 94°C, annealing and extension for 240 seconds at 64°C) followed by an extension at 72°C for 10 minutes. The amplified products were run on 2.5% agarose gel and subsequently visualized by ethidium bromide staining.

Results. The DNA samples from all patients and controls were amplified using the multiplex PCR methods as described in the methods. The deletions
Deletion mutations in Saudi DMD patients ... Al-Jumah et al

Figure 1 - Detection of deletion in the dystrophin gene by multiplex polymerase chain reaction (PCR) amplification (Set 1). Lane 1: 100 base pair deoxyribonucleic acid (DNA) ladder; the lowest band corresponds to 100 base pair. Lane 2: normal Saudi control DNA. Lane 3: deletion of exons 50 and 47. Lanes 4-7: no deletion detected. Lane 8: blank containing all PCR ingredients except genomic DNA.

Figure 2 - Detection of deletions in the dystrophin gene by multiplex polymerase chain reaction (PCR) amplification (Set 2). Lane 1: 100 base pair deoxyribonucleic acid (DNA) ladder; the lowest band corresponds to 100 base pair. Lane 2: normal Saudi control DNA. Lane 3: deletion of exons 48 and 45. Lanes 4-7: no deletion detected. Lane 8: blank containing all PCR ingredients except genomic DNA.

Figure 3 - Detection of deletions in the dystrophin gene by multiplex polymerase chain reaction (PCR) amplification (Set 3). Lane 1: 100 base pair deoxyribonucleic acid (DNA) ladder; the lowest band corresponds to 100 base pair. Lane 2: normal Saudi control DNA. Lane 3: deletion of exon 42. Lanes 4-7: no deletion detected. Lane 8: blank containing all PCR ingredients except genomic DNA.

Table 1 - Duchenne muscular dystrophy and BMD patients with or without deletion in the dystrophin gene.

<table>
<thead>
<tr>
<th>Patients</th>
<th>n</th>
<th>Deletion</th>
<th>No deletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>*DMD</td>
<td>26</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>*BMD</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>†DMD</td>
<td>14</td>
<td>5</td>
<td>9</td>
</tr>
</tbody>
</table>

* with muscle biopsy, †without muscle biopsy or muscle biopsy refused
DMD - Duchenne muscular dystrophy, BMD - Becker muscular dystrophy, n - number
were detected by multiplex sets one, 2 and 3 are shown in Figure 1-3. The pattern of deletions of the dystrophin gene in Saudi patients are depicted in Table 1. The DNA analysis showed that the dystrophin gene was interrupted in 21 out of 27 patients (78%) whose clinical diagnosis are confirmed by negative dystrophin staining. The deletions in one or more exons were detected in 5 out of 14 patients suspected to have DMD but without confirmation by muscle biopsy. The deletion in the dystrophin gene was not detected in the 3 SCARMD patients, as expected. All control Saudi volunteers and family members of the patients showed no deletion in the dystrophin gene using the method described here.

**Discussion.** While the incidence of DMD and BMD in many ethnic groups in Asia, Europe and North America, has been well documented over the past several years, little attention has been given to its molecular diagnosis in Saudi population in general and Arab population as a whole. In this study, we have extended the analysis of the dystrophin gene deletions in 41 Saudi DMD and BMD patients. Our study has shown the deletion of one or more exons of the dystrophin gene in 26 out of 41 (63%) Saudi patients with DMD and BMD. However, the percentage of deletions in the dystrophin gene increased to 78% when the molecular analysis was restricted only to 27 patients who were categorized under dystrophinopathy. The detection of deletion mutation in Saudi patients appears to be similar to a recent report on Kuwaiti and Egyptian DMD patients. Out of 14 suspected DMD cases, the deletion in the dystrophin gene was detected in 5 patients. Since genetic tests do not allow identification of all patients with dystrophinopathy, muscle biopsy is recommended in suspected cases. Six DMD patients, confirmed by muscle biopsy, did not show any deletion in the dystrophin gene using current multiplex-PCR methods that account for 25 of total 79 exons. Since the current method detects 60-65% deletions in the dystrophin gene, it is possible that the mutation has occurred in other areas of this gene in these patients. There are now several reports of small mutations (point mutations and small deletions and duplications) detected in dystrophin gene of DMD patients using single strand conformation polymorphism analysis (SSCP) and denaturing high performance liquid chromatography (dHPLC). Further studies are necessary to increase the sensitivity of the detection of mutation in the dystrophin gene of Saudi DMD and BMD patients.

In conclusion, our results are in agreement with the consensus that 65% of deletions in the dystrophin gene of the Saudi DMD and BMD patients can be detected by the multiplex-PCR method. The presented PCR-based deletion analysis provides a reasonable first step in the diagnostic care of the dystrophin patients who may be afflicted with DMD and BMD.

**Acknowledgment.** This study was supported by King Fahad National Guard Hospital and King Abdul-Aziz City for Science and Technology, Riyadh, Kingdom of Saudi Arabia (KSA) (Project: AT-18-03). We also thank Dr. Ahmed Al Jarallah (King Khalid University Hospital, Riyadh, KSA), Dr. Abdullah Al Rasheed (Security Forces Hospital, Riyadh, KSA) and Dr. Al Bux (Military Hospital, Al Khairj, KSA) for providing patients’ blood samples.

**References**

Deletion mutations in Saudi DMD patients ... Al-Jumah et al


**Related Abstract**

**Source:** Saudi MedBase

Saudi MedBase CD-ROM contains all medical literature published in all medical journals in the Kingdom of Saudi Arabia. This is an electronic format with a massive database file containing useful medical facts that can be used for reference. Saudi Medbase is a prime selection of Abstracts that are useful in clinical practice and in writing papers for publication.

**Search Word:** muscular dystrophy

**Authors:**
A. Al-Fares, Hussein Malibary

**Institute:**
King Abdul-Aziz University Hospital, Jeddah, Kingdom of Saudi Arabia

**Title:**
A patient with facioscapulohumeral muscular dystrophy (Landouzy Dejerine)

**Source:**

**Abstract**

Facioscapulohumeral muscular dystrophy is a very rare disease. We believe that this is the first case reported in Saudi Arabia.

An 18-year-old male patient presented with weakness and asymmetrical involvement of the face and shoulder girdle muscles. His intelligence was impaired and he had left eye prognosis.