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ORIGINAL ARTICLE

Deletion mutations in Duchenne muscular dystrophy (DMD) in Western Saudi children

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Abstract Duchenne and Becker muscular dystrophy (DMD and BMD) are caused, in the majority of cases, by deletions in the dystrophin gene (*DMD*). The disease is an X-linked neuromuscular diseases typically caused by disrupting (DMD) or non-disrupting (BMD) the reading frame in the dystrophin (*DMD*) gene. In the present study, amplifications of the genomic DNAs of unrelated 15 Saudi DMD males were carried out using multiplex polymerase chain reaction (PCR) for nine-hotspot regions of exons 4, 8, 12, 17, 19, 44, 45, 48 and 51. We detected six Saudi patients having deletions in a frequency of 40%. The frequency of deletions in exon 51 (20%) was the most common deletion frequently associated with our Saudi sample males. Exons 19, 45, and 48 were present in a frequency of 6.7% each. All deletions were recognized as an individual exonic deletions, while no gross deletion where detected. Finally, the molecular deletions in the Saudi males was expected to be characterized by a moderate frequency among different populations due to the geographical KSA region, which it is in the crossroad of intense migrations and admixture of people coming from continental Asia, Africa, and even Europe. In conclusion, attempts to include an extra DNA samples might reflect a valid vision of the deletions within the high frequency deletion regions (HFDR's) in the *DMD* gene mutations in KSA.

1. Introduction

Duchenne muscular dystrophy (DMD, MIM #310200) and its milder allelic variant, Becker muscular dystrophy (BMD), are both caused by mutations in the dystrophin gene (*DMD*, MIM #300377) located on Xp21. DMD represents the most common genetic neuromuscular disease of childhood. It is relatively frequent, with an incidence of 1 in 3500 male livebirth (Worton and Thompson, 1988).

Symptoms in DMD patients start to appear at the age of 3 years with progressive muscle weakness. The patient is non ambulatory by the age of 9 or 10 years and usually by 20 years old; death will occur after cardiac or respiratory complications (Sbiti et al., 2002). DMD is the more severe phenotype of muscular dystrophies (Lo et al., 2006). BMD is the allelic and the milder form of DMD, but it is less frequent in which the birth prevalence is 1 in 18,500 live born male (Zhou et al., 2006;

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Ramellia et al., 2006). It characterized by slower rate of progression in which the mean age of the onset of symptoms such as muscle weakness and poor walking, arise around 12 years of age. Loss of ambulation varies from adolescence to adulthood (Ramellia et al., 2006), while death generally occurs in the third or fourth decade (Sbiti et al., 2002).

The *DMD* gene consists of 79 exons and encodes a 14-kb mRNA (Koenig et al., 1988; Ahn and Kunkel, 1993; Freund et al., 2007). The protein product of the gene, with a molecular weight of 427 kDa, is a sarcolemma-associated protein, which binds cytoskeletal actin through its N-terminal domain, and a complex of dystrophin associated proteins (DAP) through its C-terminal domain (Ahn and Kunkel, 1993). Intragenic deletions and duplications together account for over two thirds of the mutations leading to DMD and BMD (Koenig et al., 1989; den Dunnen et al., 1989).

Intragenic deletions and duplications together account for over two thirds of mutations leading to DMD and BMD (den Dunnen et al., 1989; Koenig et al., 1989). Most can be detected by multiplex PCR (Beggs and Kunkel, 1990; Chamberlain et al., 1988) and are clustered in two high frequency deletion regions (HFDRs), one in the 5′ (centromeric) portion of the gene, the other in the 3′ half of the gene (Baumbach et al., 1989; Kim et al., 2002; Koenig et al., 1989). A small proportion ranging from 0% to 6% of the mutations within the dystrophin gene involves duplications (Hu et al., 1990; Mendell et al., 2001). More than 200 dystrophin point mutations are known (http://www.dmd.nl/). Previous study of dystrophin deletion mutations in Saudi males (Al-Jumah et al., 2002) has not dealt with the frameshifting hypothesis.

In the present paper, we presented an analysis of exceptions to the frameshift rule and their implications for dystrophin in males.

2. Subjects and methods

Dystrophin patients were selected from Saudi dystrophin families registered in the database records in neurologic out-patient clinics of governmental and military hospitals, and Handicapped Children Societies in Western Saudi regions. Informed consents were obtained from all patients' family. Clinical data sheet of a patient was registered on the database records of Molecular Genetics laboratory in the Department of Medical Genetics, Faculty of Medicine, Umm Al-Qura University. Clinical information was independent of any molecular data of *DMD* gene or its protein. For the sake of accuracy, we excluded the patients who lacked clinical evidence of the disease.

We categorized patients according to the severity of the phenotype of the disease, as 'DMD' if they were confined to a wheelchair at or before the age of 12 years and as 'BMD' if they were still ambulant at age 16 years. Patients were classified as intermediate (B/DMD) if they became wheelchair bound (WCB) between the ages of 12 and 16 years (Hodgson et al., 1989). They defined as 'ND – not determined' phenotype if the patient was not wheelchair bound or too young to be diagnosed as BMD. Our sample contained 15 unrelated proband males. The age of the DMD patients ranged from 5.0 to 19.0 years of a mean age ± standard deviation (SD) was 11.8 ± 3.4 years. Diagnosis of DMD/BMD probands included elevated serum creatine phosphokinase (CPK), age of onset, calf pseudohypertrophy, age of wheelchair confinement, presence of cardiomyopathy, and myopathic changes to EMG pattern (http://www.dmd.nl/).

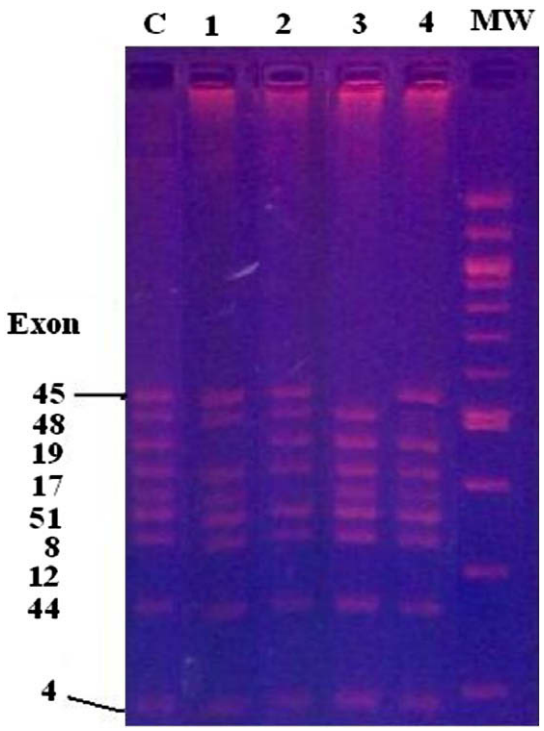

Figure 1 Multiplex PCR of the human *DMD* gene electrophoresed on a 3% NuSieve gel-ethidium dye. 'C' represented 'normal control male' with no deletions, MW is size marker (100 bp ladder). Lanes 1, 2, 3, and 4 showed missing of exon 19, 51, 45 and 48, respectively.

2.1. DNA analysis

Genomic DNA was isolated from EDTA-peripheral leukocytes using Mini Spin-Column protocol (QIAGEN, USA). Multiplex PCR of the high frequency deletion regions in the *DMD* gene was performed (Fig. 1). The amplification was carried out using multiple 18 primers flanking exons 4, 8, 12, 17, 19, 44, 45, 48, and 51 (Chamberlain et al., 1988). PCR cycling was programmed as: initial denaturing at 95 °C for 6 min (1 round), then 95 °C for 30 s; 53 °C for 30 s, 65 °C for 4 min (repeated for 23 rounds), and an initially extension 65 °C for 7 min. PCR products were separated on 3% NuSieve agarose. The gel was photographed using Gel Documentation and Analysis System (UVitec, Cambridge, UK).

Table 1 Exon deletion frequencies in 15 unrelated DMD Saudi patients.

Exon(s) deleted	No. of deletions	Deletion frequency (%)
Exon 19	1	6.7
Exon 45	1	6.7
Exon 48	1	6.7
Exon 51	3	20.0
Total deletions	6	40

Table 2 Clinical data and molecular finding of dystrophin Saudi patients within the high frequency deletion regions (HFDR's).

Fam. no.[a]	Age[b] (y)	Sex	Diagnosis[c]	WCB[d] (y)	F.H.[e]	Exon deleted[f]	Frame-shift[g]
1	15	M	DMD	4	+	19	+
5	7	M	ND	–	–	51	+
8	14	M	DMD/BMD	12	–	45	+
9	11	M	DMD	8	+	51	+
12	15	M	DMD	9	–	51	+
20	9	M	ND	–	+	48	–

[a] These numbers only refer to one affected proband.
[b] The age of initial examination.
[c] Patients were classified as intermediate (B/DMD) if they became wheelchair bound (WCB) between the ages of 12 and 16 y (Dubowitz, 1990). Patients labeled as 'not determined' "ND" were too young to permit a definitive diagnosis, and are grouped separately (Table 1).
[d] Wheelchair bound.
[e] Family history (F.H.) considered (+) if there are more than one affected individuals in the family and (–) if the affected male was a sporadic case.
[f] Deletion detection were focused to the high frequency deletion regions including exons 4, 8, 12, 17, 19, 44, 45, 48, and 51.
[g] In-frame (–) and outframeshift (+) were assigned according to Monaco et al. (1988).

3. Results and discussion

3.1. Deletion analysis

To detect *DMD* gene deletions in Chamberlain's set, genomic DNA was successfully amplified. The *DMD* deletion frequency was 40% (6 of 15 patients). The missing exons in the central region represented the majority of deletions (83%) confined to exons 51, 48 and 45, while the remaining deletions (27%) were represented only in exon 19 in the proximal region. These results might be in agreement with the Egyptian studies (Elhawary et al., 2004) and other comparative studies in Asian populations (Lu et al., 2006; Hwa et al., 2007; Hallwirth-Pillay et al., 2007; Hassan et al., 2008; Marini et al., 2008). In the present study, the deletion frequency of exon 51 was the most common deletion (20%), while exons 19, 45 and 48 showed a frequency of 6.7% each (Table 1). All deletions recognized in this study were detected as an individual exonic deletions, while no gross deletion where detected.

Again, the deletion frequency in the Saudi DMD patients (40%) is relatively lower than other Arabs or neighboring populations, but comparable with most of the Asian data (Hassan et al., 2008; Marini et al., 2008; Hwa et al., 2007). In the North countries of Africa, Egypt, for example, represented a relatively high frequency of *DMD* gene deletion frequency (51%; 78/152) (Elhawary et al., 2004), which is the case in Moroccan population (51%; 37/72) (Sbiti et al., 2002).

Our data results might lie between that of the highest frequency in Turk (60%) (Onengut et al., 2000) and the lowest deletion frequency in Philippines (33%) (Cutiongeo et al., 1995). Some Asian populations, for example, Pakistani (40.75%) (Hassan et al., 2008), Malaysian (42%) (Marini et al., 2008), Chinese (49%) (Lu et al., 2006) and Taiwanese (35.3%) (Hwa et al., 2007) have nearly the same magnitudes and patterns of deletion frequencies to this Saudi study. This might be rationalized as strong genetic linkage or genetic drifts.

3.1.1. In-frame and out-frameshifting and DMD phenotypes

A hypothesis known as the reading-frame hypothesis proposes that deletions that alter the reading frame of dystrophin mRNA produce no functional dystrophin and cause severe DMD, while in-frame deletions may produce partly-functional internally deleted dystrophin leading to the milder Becker disease (Monaco et al., 1988).

Deletion of exon 19 resulted in a disruption of the reading frame, resulting in the severe DMD phenotypes. Deletion of exon 45 resulted in the intermediate D/BMD phenotype (Table 2). On the other hand, deletion of exon 51 gave rise to severe DMD phenotypes as shown in two cases (#11 and #15) and in 'not determined phenotypes' phenotype in (#7) that might be suspected to have DMD phenotype. Deletions due to exons (4, 8, 12, 17 and 44) have never been shown deletions. Our results agreed the frameshift rule in a frequency of (66.7%) of patients. This result showed a high concordance with Monaco et al. (1988) besides other relevant studies (Elhawary et al., 2004; Lu et al., 2006; Hassan et al., 2008; Marini et al., 2008; Hwa et al., 2007). The deviation from the frameshift hypothesis was not clearly shown because of the presence of ND phenotypic cases.

In conclusion, the molecular deletions in the Saudi males should be expected to characterize a moderate frequency. This might be due to the geographical KSA region, where it is in the crossroad of intense migrations and admixture of people coming from continental Asia, Africa, and even Europe. Attempts to involve an extra DNA samples might reflect the real map of the deletions within the high frequency deletion regions (HFDR's) in the *DMD* gene mutations in KSA.

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