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Molecular Analysis of Iranian Patients with Duchenne/Becker Muscular Dystrophies

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Abstract

Duchenne Muscular Dystrophy (DMD) and the milder allelic Becker Muscular Dystrophy (BMD) are X-linked disorders. Both DMD & BMD result from heterogenous mutation in the dystrophin gene and in about 65% of the cases one or more exons of the gene are deleted or duplicated. One third of cases arise from new mutation and the rest are familial. To analyze the prevalence of deletion in Iranian patients, a deletion screening was performed on group 18 exons of dystrophin gene. Deletions were detected in 56.8% of patients. Seventy four percent of deleted exons were located in the major hot spot region, whereas 26% were in the minor hot spot one. The most frequently deleted exons were exons 50, 48 & 47 16.2%, 16.2% & 12% respectively. No deletion was detected in exon 43. The intragenic RFLP analysis (pERT87-15/BamHI & pERT87-8/Taql) were carried out on DNA samples obtained from 22 Iranian unrelated families (196 males & females) showing DMD & BMD clinical symptoms, that 45% of them had informative patterns. The percentage of heterozygosity was 22.75% for BamHl intragenic RFLP, and 22.75% for Taql intragenic RFLP.

Keywords: Duchenne, Becker, Muscular dystrophies, DMD/BMD, Dystrophin gene, Deletion, Iran

Introduction

Duchenne and Becker Muscular Dystrophies (DMD/BMD) are allelic neuromuscular disorders caused by mutations to the dystrophin gene at Xp21 (1). Because of the extremely large size of this gene (nearly 2400 kb) the majority of mutations are intragenic deletions (~65%) or duplication (~5%) (2). In both DMD and BMD, partial deletions and duplications cluster in two recombinatiion hot spots, one proximal at the 5' end of the gene, comprising exons 2-20 (30%), and one more distal, composing exons 44-53 (70%) (3). The sever Duchenne form is caused by the absence of detectable dystrophin, which, at the DNA level, is usually a result of mutations that alter translational reading frame leading to production of a truncated and the presumably unstable dystrophin muscle. On the other hand, the milder BMD result from internal deletions or duplications that do not disrupt the reading frame, so partially functional portion can still be produced (4,5). Two multiplex polymerase chain reactions based assays encompassing 18 of 79 exons of the dystrophin gene have been developed and detect approximately 98% of deletions in DMD/BMD patients (6, 7, 8). Because of the severity of DMD and the lack of any effective treatment, it is imperative that an accurate carrier test be available to identify those female relatives, including mothers, of an affected individual who are at risk of having an affected son. Linkage analysis using marker flanking and within the dystrophin gene can predict with high accuracy whether the woman has inherited the same chromosome a common maternal relative as has an affected male.

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Materials and Methods

A total of 389 blood samples were collected from 106 Iranian DMD/BMD individual families with 207 affected males. The age of affected males ranged from 3 to 33 years. Ascertainment of clinical disease was made by the criteria described previously (9, 10), therefore 88 unrelated families with 182 patients were selected. Only one affected member per family was considered. For carrier detection with RFLP method we had 38 unrelated families with familial history that 22 of them were analyzed (they had at least one candidate for carrier detection). Genomic DNA was isolated from peripheral blood lymphocytes by the standard protocol (11). Screening for deletions was carried out essentially as described (7, 8). For Multiplex analysis 18 exons (Pm, 3, 4, 6, 8, 12,13, 17, 19, 43, 44, 45, 47, 48, 50, 51, 52, 60) were amplified in three multiplex reactioins (Set I, II, III). After amplification PCR products were electrophoresed in 3% agarose gel. The gel was stained with ethedium bromide and the bands were visualized by UV illumination (Fig. 1) demonstrates a multiplex reaction in 3 panels. Two intragenic RFLP systems (PERT 87-8/TaqI, PERT87-15/ BamHI) were used for familial cases (11). After amplification, 20µl of PCR products were digested with 5 units of either BamHI or TaqI for at least 1 h at 37ºC or 60ºC, as appropriate, electrophoresed on 3% agarose gel and visualized by ethidium bromide staining or 8% polyacrylamide gel detected by silver staining (Fig. 2).

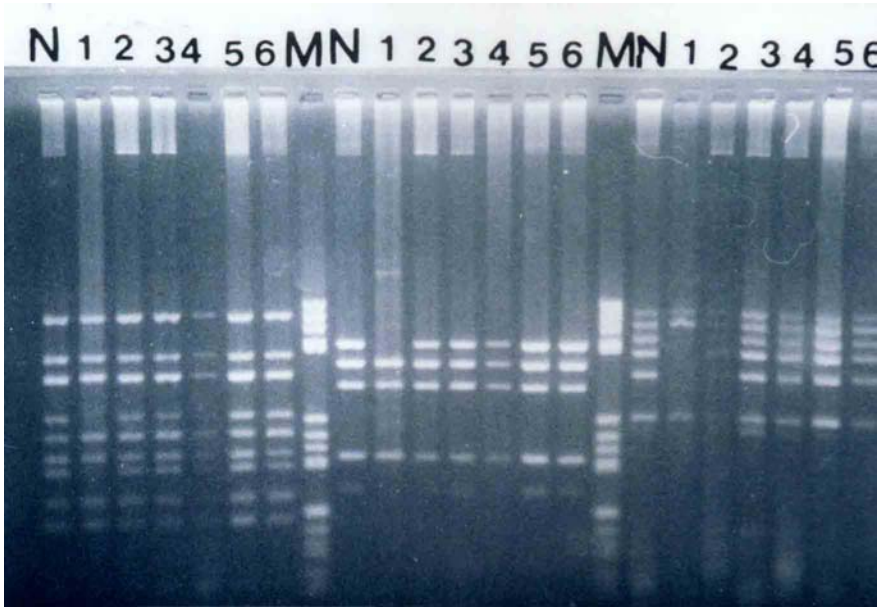

Fig. 1: DMD/BMD deletion screening for 20 exons of dystrophine gene
Set I: Pm Exons 3, 43, 50, 13, 6, 47, 60, 52 (Lane 1 exon 50 and 47 deleted)
Set II: Exons 49, 51, 12, 4, 46 (Lane 1 exon 49 and 46 deleted)
Set III: Exons 45, 48, 19, 17, 8, 44 (Lane 1 exon 19,17,8 deleted)
M: DNA size marker
Lane: 1-6 different patients
N: Normal

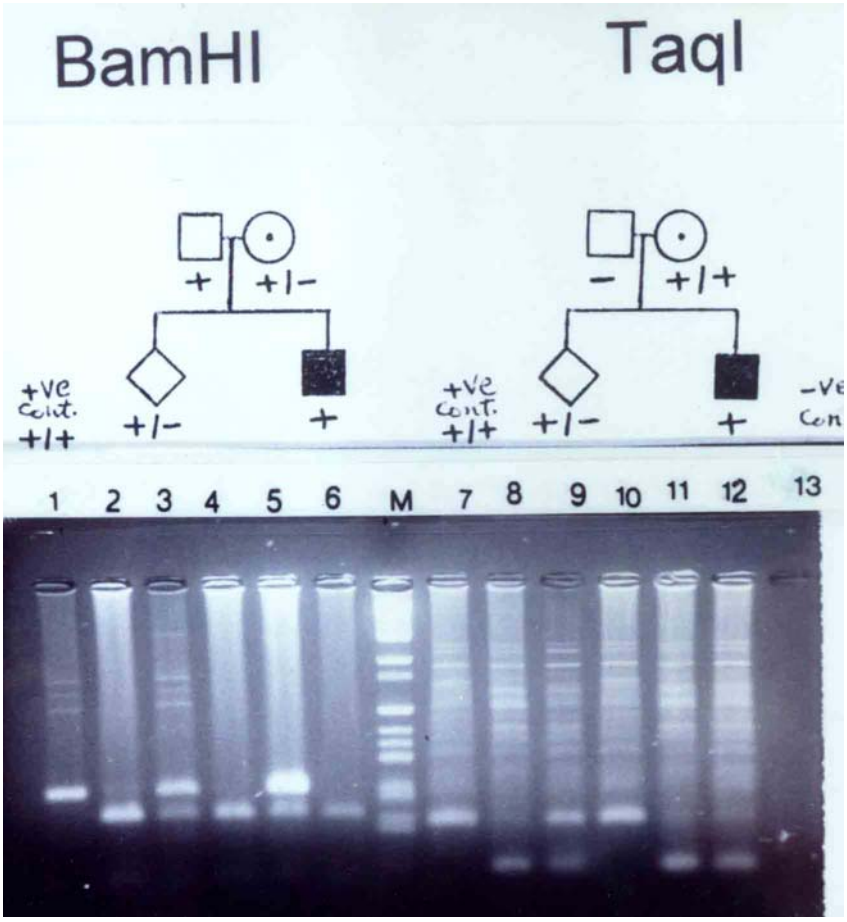

Fig. 2: BamHI and TaqI, RFLP gel electrophoresis BamHI was informative for this family

Results

Examples of deletions detected by multiplex sets I, II, III are shown in Figure 1. Deletions were detected in 56.8% of patients. Seventy four percent of deleted exons were located in the major hot spot region, whereas 26% were in the minor hot spot region. The most frequently deleted exons were exons 50, 48 & 47 (16.2%, 16.2% and 12% respectively) (Fig. 3). No deletion was detected in exon 43. 36% of patients had only one exon deletion and 64% of them had more than one exon deletion. The number of exons deleted in a given child varied from 1 to 9. The intragenic RFLP analysis (pERT87-15/BamHI & pERT87-8/TaqI) were carried out on DNA samples obtained from 22 Iranian unrelated families with familial history of DMD/BMD (196 males & females), that 45.5% of them had informative patterns. The percentage of heterozygosity was 22.75% for BamHI intragenic RFLP and same result for TaqI intragenic RFLP. Only two families were informative for both systems. Frequency of positive and negative allele in BamHI were 75%, 25% respectively and for TaqI site were 63.9% , 36.1% respectively.

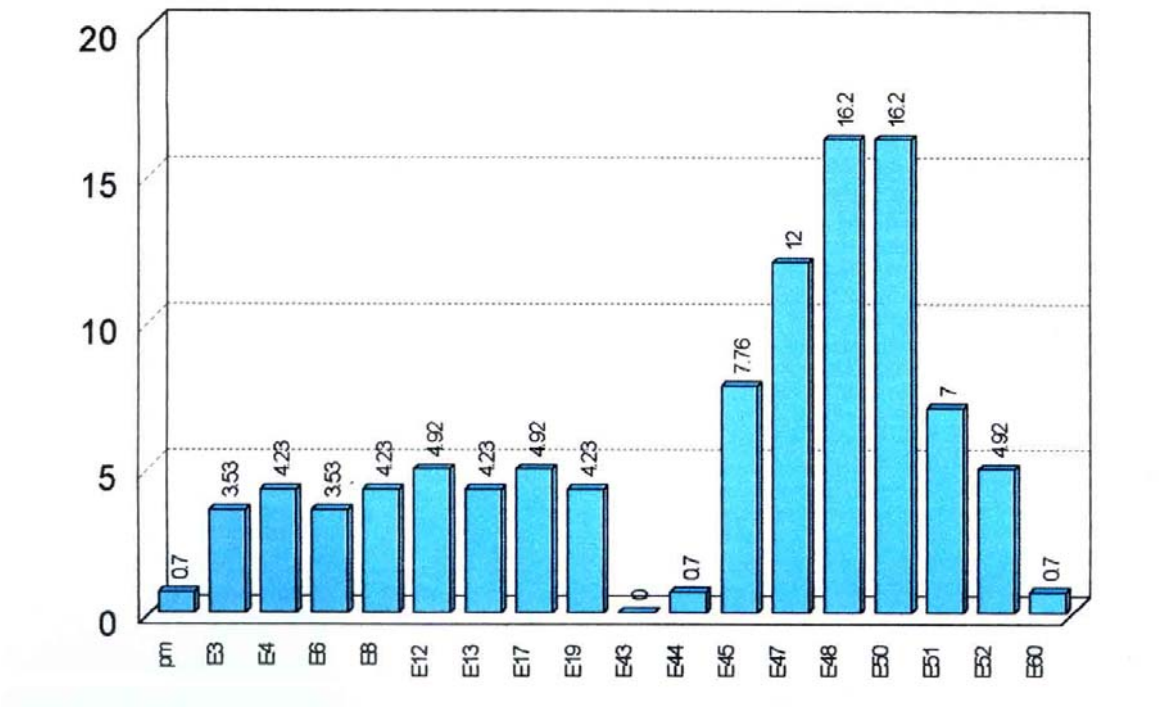

Fig. 3: Frequency of dystrophin gene deletion among 18 exons in this study

Discussion

This study showed 56.8% deletion rate in DMD/BMD patients. Fig. 4 compares the detection rates from North American & European studies varied from 55 to 70% (12,13,14), while in Asia it was lower, being 40-43% in Japan (15), 45-50% in China (16) and 55% in Thailand (17). In Turkish DMD patients, deletions were found in 52-59% (18, 19).But in Arab patients, deletions were found in 86% (20). In Greece, deletions were in 63% cases (21). The lowest rate of deletion mutations in DMD patients has been reported from the Philippines (33%) (22) and Israel (37%) (23). Amongst the Europeans, the lowest deletion rate is reported from Germany, Hungary, Czechoslovakia and Spain (39-45%) (24). In Mexico DMD patients, deletions were detected in 52.5% cases (25). In 74% cases, deleted exons were restricted to the distal hot spot (exons 44-60), in 26% cases they were found in the proximal hot spot (exons 3-19), which is compatible with other reported studies. We have used PCR for rapid analysis of intragenic RFLP to permit both carriers and prenatal diagnosis in the majority of familial cases. Over 45% of all mothers at risk of carrying DMD/BMD are expected to be heterozygous for at least one of the two intragenic RFLP, which have studied. These results are compatible with other studies (11).

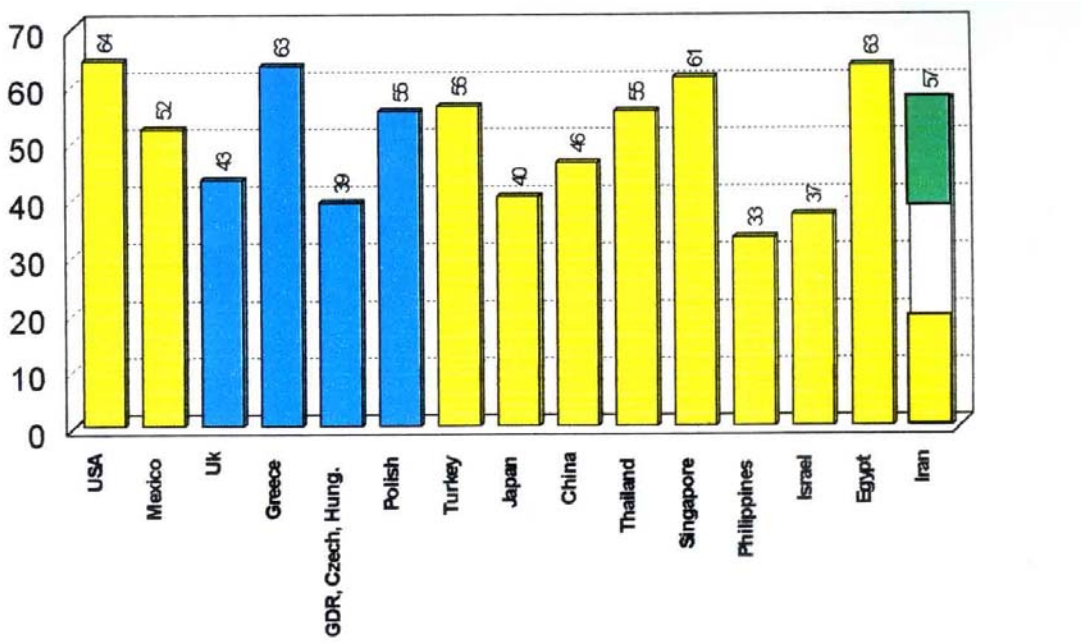

Fig. 4: Frequency of dystrophin gene deletion in the world

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