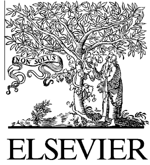
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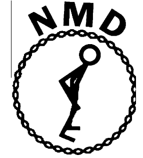

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Coexistence of two distinct intragenic dystrophin deletions in two maternal cousins with Duchenne Muscular Dystrophy

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Abstract

The identification of two independent mutations is rarely described between affected members of the same family with Duchenne Muscular Dystrophy. This study reports the presence of two distinct intragenic *dystrophin* deletions in a Turkish family. Exon 54 deletion was identified originally in the proband, whereas his maternal cousin had deletions of exons 43–50 in the *dystrophin* gene. As indicated, only the mother of the proband was identified as exon 54 deletion carrier however, the proband's cousin was detected as a sporadic case. These molecular genetic data reveal an interesting and novel mixture, in the same family, of both mutations of the same gene.
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Keywords: DMD; *DMD* gene; Deletion; Carrier detection

1. Introduction

The *dystrophin* (*DMD*) gene is one of the largest genes (approximately 2.5 million base pairs, encoding 79 exons) identified to date, and because of its size and structural characteristics, it is susceptible to a high sporadic mutation rate [1]. Consequently, for Duchenne Muscular Dystrophy (DMD) which is an X-linked lethal condition with a stable prevalence, wherein one-third of all mutated alleles reside in males and are genetic lethals, then the new mutation frequency equals one-third of the mutated allele frequency [2]. *De novo* locus specific mutation rate of DMD is 1×10^{-4}, as DMD occurs in 1/3500 male newborns [3,4]. Approximately 65% of all *DMD* mutations are caused by deletions [5]. Thus, the *de novo* deletion frequency in this large segment of the human genome is estimated at ~1:15,000 [3]. However, the identification of two independent *DMD* mutations in the same family is an uncommon event.

Here we describe the clinical and molecular features of a Turkish family with a 4 year-old male proband affected by DMD and his 11 year-old maternal cousin with similar clinical severity and report the presence of two different intragenic *DMD* deletions (del ex 54 and del ex 43–50) in the same pedigree. In addition to analyzing family specific mutation in families with multiple affected individuals, screening all exons will provide information about different *DMD* mutations as detected in our family.

2. Patients and methods

This family of Turkish origin comprises a 4 year-old male proband (Fig. 1, Case III:2) affected by DMD and his 11 year-old maternal cousin (Fig. 1, Case III:1). Briefly, our index case was originally seen at 3 years 3 months of age with slowness in gait. Growth centiles were at the 25th. The calf muscles were enlarged. Gowers sign was positive with 4½ s. The boy had a normal developmental quotient (DQ). He had a serum creatine kinase (CK) level

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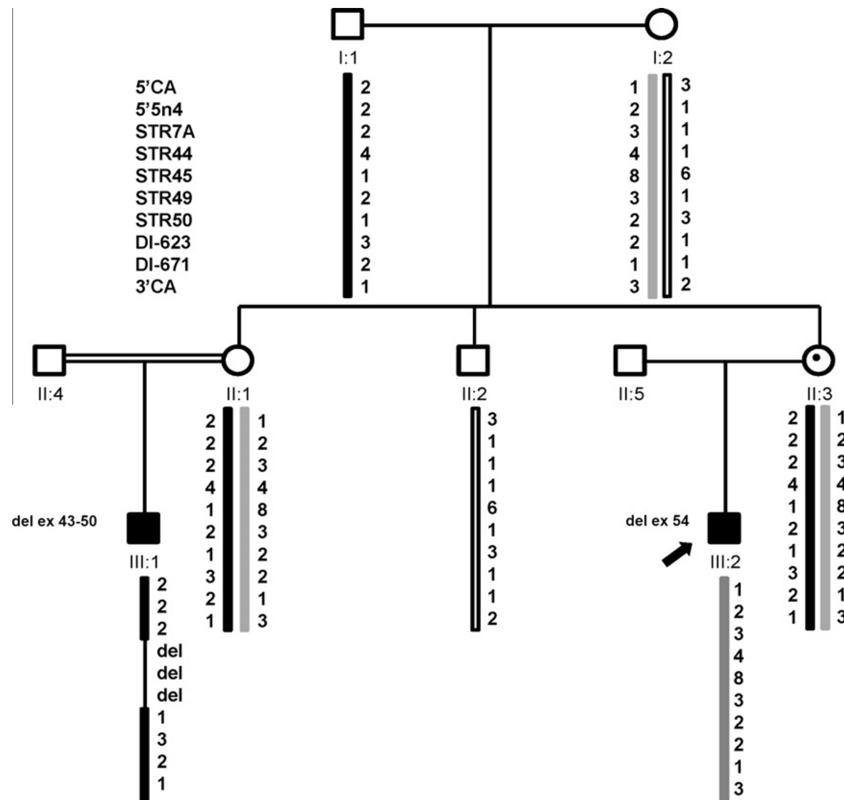

Fig. 1. Pedigree of the DMD family. Haplotype analysis by using the polymorphic microsatellite sequences is shown underneath the pedigree symbols. The proband is indicated by an arrow. Roman numerals specify pedigree position in all affected members and/or individuals.

of 12,245 U/L (<200). Currently, this boy is on low dose steroids. His cousin was evaluated first at 7½ years of age with the onset of awkward gait from age 5. This boy had a thin appearance with growth parameters being around the 10th centile. He was mentally retarded; his performance IQ was calculated as 70. Gowers sign was positive at 5 s. His CK was 9262 U/L. Low dose steroids were introduced. This patient lost ability to walk at 11 years.

Informed consent was obtained from all individuals or legal guardians of the patients included in this study. Genomic DNA was extracted from peripheral blood samples from seven available family members according to standard protocols. Analysis of the DMD patients for deletions in the *DMD* gene was performed by multiplex PCR as described previously [6,7]. To assess carrier status of del ex 43–50 within this family, the polymorphic (CA)n markers (5′CA, 5′5n4, STR7A, STR44, STR45, STR49, STR50, DI-623, DI-671, 3′CA) were used for the haplotype analysis of the family members. Carrier status of del ex 54 in the female relatives of DMD patients was determined by quantitative real-time PCR method by using specific primers for exon 54 region (F: 5′-GTTTGTCCTGAAAGGTGGGTTAC-3′, R: 5′- GAGAAGTTTCAGGGCCAAGTCATTT -3′) and dsDNA-dye SYBR green I. Quantitative analysis by real-time PCR was normalized to that of Albumin, a reference gene and conducted using comparative threshold method ($\Delta\Delta C_T$). All real-time PCR runs were performed in triplicate and each reaction mixture was prepared using the SYBR Green JumpStart Taq ReadyMix (Sigma) in a total volume of 10 μl:2 μl of PCR-grade water, 4 pmol of each primer, 5 μl of Master mix, 3 mM of $MgCl_2$ and 4 pmol of each primers and 50 ng of genomic DNA. The thermal cycling protocol was as follows: initial denaturation for 2 min at 94 °C followed by 35 cycles of 4 s at 94 °C, 5 s at 61 °C, and 15 s at 72 °C. The quantitative real-time PCR amplification was run on a Rotor-Gene 6000 instrument with software version 1.7 (Corbett Research, Sydney, Australia).

3. Results and discussion

Even it is possible to detect more than one *DMD* mutation in multiple affected members of the same family, only a few families have been reported to date [8–10]. Here we describe an extraordinary DMD family where two different intragenic deletions are present in two maternal cousins. del ex 54 deletion was identified first in the proband (Fig. 1, Case III:2). However, analysis on a related family member with DMD (Fig. 1, Case III:1) for the same mutation was negative. Further analysis of this individual led to the identification of a second mutation, del ex 43–50, in the *DMD* gene.

We then performed haplotype analysis and quantitative real-time PCR assay in order to ascertain parental origins of these two mutations. Haplotype analysis (Fig. 1) revealed that both mothers of the two affected boys

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