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Carrier Detection in Female Relatives of DMD/BMD Patients

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Abstract

Background: *Duchenne Muscular Dystrophy (DMD) is one of most common and severe neuromuscular disease in men. It is an X-linked genetic disorder of muscle, which affects about 1 in 3500 male's birth. Carrier detection is one of great importance for families with one or more sons affected with Duchenne Muscular Dystrophy or Becker Muscular Dystrophy (DMD/BMD). The aim of this study was to determine the carrier status of female relatives in south eastern of Turkey families at high risk and families having a child affected with DMD/BMD.*
Method: *The 66 female of relatives of DMD/BMD males were screened by Restriction Fragment Length Polymorphism (RFLP) using four intragenic probes.*
Results: *This study indicated that 70 % of all relatives at risk were heterozygous for at least one of these intragenic RFLP, detected by PCR-RFLP.*
Conclusion: *Marriage All these results provide useful information for DMD/BMD carrier status among female relatives of the patients for genetic counseling for South east of Turkey.*
Keywords*: Duchenne Muscular Dystrophy (DMD), Becker Muscular Dystrophy (BMD), Carrier Analysis, Restriction Fragment Length Polymorphism, Polymerase Chain Reaction (PCR).*

Introduction

Duchenne Muscular Dystrophy (DMD) is one of most common progressive X-linked muscle wasting disease, which affects about 1 in 3500 males. DMD is allelic with Becker muscular dystrophy (BMD), which is the milder and rare form of the disorder with an incidence of in 1 in 30,000 males. [1-3]These two forms of myopathy have similar symptoms but can be discriminated on the basis of their clinical progress. The DMD/BMD gene is located on Xp21.2 locus, and has been cloned and sequenced .[4]This gene is one of the largest of human gene described so far, spanning 2.4 Mb with 79 exons and coding a 14 kb mRNA. Dystrophin mRNA encodes 3685 amino acid protein of 427 kilo Daltons (kD) with overall similarity to the cytoskeletal proteins -spectrin and –actinin.[5,6]
Mutations in the DMD gene, result in a progressive muscle degeneration and early death.
Since there is no cure or effective treatment, prenatal diagnosis and carrier detection play an important role in the prevention of the disease. Carrier detection of Duchenne and Becker muscular dystrophy (DMD and BMD) by DNA methods uses Southern Blotting when a deletion is identified

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in a patient. DNA samples of women at risk in the family should be analyzed either for carrier detection by restriction fragment length polymorphism (RFLP) analysis. [7] Primer for pERT87.15/BamHI, pERT87.5/XmnI and pERT 87.15/Taq I polymorphisms within the dystrophin gene have been characterized. [8] In the present study we report the screening of carrier detection in female relatives of DMD/BMD patients, who were at risk of being carrier for pERT 87.5, /XmnI, pERT 87.15/BamHI, pERT 87-8/Taq I and 38 / Taq I polymorphisms within the dystrophin gene by PCR-RFLP analysis.

Materials and Methods

Blood samples were collected from 66 female of DMD/BMD patients attending to Neurology Department and Child Health and Disease Department of Dicle University Faculty of Medicine. Genomic DNA was extracted from peripheral leucocytes by standard procedures [9, 10] . As a routine procedure an informed consent was obtained from each participant. Polymerase chain reaction (PCR) for RFLP analyses was carried out in a final volume of 50 µl containing 10 pmoles of each primer at 25 pmol, 2 mm of each of four dNTPs, 50 mM KCl, 100 mM Tris-HCl pH 8.8, 25 mm MgCl2 and 1 unit of *Taq Polymerase* enzyme (MBI Fermentas, Germany) using DNA Thermal Cycler (Techne PHC3 thermal cycler, Cambridge UK). Thermal cycling was carried out by the following steps: pERT 87-15 /Xmn I (MBI Fermentas, Germany) ; 94 °C for 4 min. for one cycle 94 °C 40 sec, 51 °C 40 sec, 65 °C for 4 min for 37 cycle. Final elongation was at 72 °C for 7 min for one cycle. For pERT87.15 /BamHI and pERT 87.8/Taq I (MBI Fermentas, Germany) 94 °C for 4 min for 1 cycle 94 °C 48 sec, 60 °C 48 sec, 72 °C for 3 min for 35 cycle and final elongation step at 72 °C for 7 min for 1 cycle. For 138/Taq I 94 °C for 5 min for 1 cycle 94 °C 30 sec, 61,5 °C 2 min, 72 °C for 3 min for 30 cycle and final elongation step at 72 °C for 7 min for 1 cycle. PCR products were separated on 2 % agarose gel, and DNA was visualized by ethidium bromide staining. All PCR assays were performed according to Abbs et al. with slight modifications.[11] For PCR-RFLP analysis, 10 µl of the product of PCR amplification were digested with 5 units of either BamHI or XmnI for 18 hours at 37 °C and Taq I for 3 hr at 65 °C. Digested product of pERT 87-15/BamHI and pERT 87-8 /Taq I electrophoresis in a 15 % polyacrylamide gel (PAGE) and stained by silver staining and product of 87-15 XmnI and 38/TaqI electrophoresis in a 2 % Nusieve gel and visualized by ethidium bromide staining and pictures were taken under ultraviolet light with Polaroid film.

Result

The 66 female of relatives of DMD/BMD males were screened for RFLP using four intragenic probes. The four RFLP sites examined in this study were all within Xp21 and the DMD/BMD locus. The 66 females of relatives of DMD/BMD boys were screened for pERT 87-15/XMnI, pERT 87-15/BamHI, pERT 87-8/TaqI, and 38/TaqI polymorphisms within the dystrophin gene by PCR-RFLP analysis.

After PCR-RFLP of 66 of females of DMD/BMD patients relative analyzed for these polymorphisms and the frequencies of observed heterozygosity were found to be in correlation with the expected heterozygote frequencies for these polymorphisms. Our investigation indicated that 70 % of all relatives at risk were heterozygous for at least one of these intragenic RFLP, detected by PCR-RFLP. The heterozygote frequencies of four different polymorphic markers (pERT 87-15/XMnI, pERT 87-15/BamHI, pERT 87-8/TaqI, and 38/TaqI) were detected using PCR based RLFP analysis. The expected heterozygote frequency for these polymorphic markers were found to be 0,50 for pERT 87-15/ XMn I, 0,33 for 87-8/Taq I, 0,50 for pERT87.15/Bam HI and 0,49 for 138/Taq I respectively (Table I).

Table I: The expected heterozygote frequencies of RFLP markers

RFLP Markers	Total number of the chromosome	Number of (+) alleles	Frequency	Number of (-) alleles	Frequency	Expected Heterozygotes
pERT 87-15 /XmnI	132	69	0.52	63	0.48	0.50
pERT 87-8 /TaqI	132	104	0.79	28	0.21	0.33
PERT 87-15 /BamHI	132	69	0.52	63	0.48	0.50
138/TaqI	132	60	0.45	72	0.54	0.49

After screening females for the same polymorphic markers the observed heterozygote frequencies were found to be 0,77 for pERT 87-15/ XMn I, 0,36 for 87-8/Taq I, 0,77 for pERT87-15/Bam HI and 0,57 for 138/Taq I, respectively (Table II).

Table II: The observed heterozygote frequencies of RFLP markers

RFLP Markers	Number of female investigated	Number of heterozygote females	Observed heterozygote frequency
pERT 87-15/XmnI	66	51	0.77
pERT 87-8/TaqI	66	24	0.36
PERT 87-15 /BamHI	66	51	0.77
138/TaqI	66	38	0.57

Discussion

The Duchenne/Becker Muscular Dystrophy (DMD/BMD) is an X-linked recessive muscle wasting disease. There is no effective treatment for the disease. Determination of carrier status is of utmost importance for genetic counselling. The female carrier will transmit the disease gene to half of her sons and half of her daughters; half of the daughters will be carriers, while half will be normal. Half of the sons will be normal and, on average, half will have the disease. Deletion detection is a crucial step in elaborating a reliable strategy of carrier of DMD/BMD testing in families at high risk and families having a child affected with DMD/BMD. This straightforward approach is applied to all at risk families with living affected individuals referred to our department laboratory.

Advances in molecular studies have allowed the characterization of mutations responsible for DMD/BMD and accurate identifications of female carriers. In this study we used DNA probes to analyse four RFLP's in the Turkish DMD/BMD locus. RFLP data, which were shown and listed in the table we, used four markers (pERT 87-5/XMnI, pERT 87-15/BamHI, pERT87-8/TaqI, and I-38/TaqI), which have favourable allele frequencies in DMD family analysis. The study covered 66 female relatives of DMD/BMD. In all females, a 70% of female were found to be heterozygous for at least one of the four intragenic RFLP's we have studied, while 30% were non-carriers. All these results provide useful information for DMD/BMD carrier status among female relatives of the patients for genetic counseling for South east of Turkey. Further studies with higher numbers of patients and simultaneous analysis of more markers may provide better results.

References

1. Emery AEH. Differential diagnosis In: Emery AEH, ed Duchenne muscular dystrophy. Oxford: Oxford University Press: 1987;71-91

2. Monaco AP, Kunkel LM. A giant locus for the Duchenne and Becker muscular dystrophy gene. Trends Genet 1987;3:33-37

3. Muntoni F, Torelli S, Ferlini A. Dystrophin and mutations: one gene, several proteins, multiple phenotypes. Lancet Neurol. 2003;2:731–740

4. Hoffman RP Brown RH, Kunkel LM. Dystrophin the protein product of the Duchenne muscular dystrophy locus. Cell 1987;51: 917-928

5. Boyd Y, Buckle VJ. Cytogenetic heterogeneity of translocations associated with Duchenne muscular dystrophy. Clin Genet 1986;29:108

6. Koeing M, Hoffman E.P, Bertelson J, Monaco A.P, Feener C, Kunkel LM. Complete cloning of the Duchenne muscular dystrophy (DMD) cDNA and Preliminary genomic organization of the DMD gene in normal and affected individuals. Cell 1987;50:509-517

7. Cole GC, Coyne A, Hart KA, Sheridan R, Walker A, Johnson L, Hodgson S, Bobrow M. Prenatal testing for Duchenne and Becker muscular dystrophy. The Lancet February 1988;6:262-265

8. Roberts RG, Cole CG, Hart KA, Bobrow M, Bentley DR. Rapid carrier and prenatal diagnosis of Duchenne and Becker muscular dystrophy. Nucleic Acids Research 1989;17:811

9. Poncz M, Solowiejczky, D, Harpel B, Mory Y, Schwartz E, Surrey S. Construction of human gene libraries from small amount of peripheral blood. Haemoglobin 1982;6:27-36

10. Miller M, Dykes DD, Polsesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;18:1215

11. Abbs S, Bobrow M. Analysis of quantitative PCR for the diagnosis of deletion and duplication carriers in the dystrophin gene. J Med Genet 1992;29:191-196