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DELETION ANALYSIS OF DUCHENNE MUSCULAR DYSTROPHY*

*Hayat Erdem PhD***, Şükrüye Ayter PhD***, Meral Özgüç PhD****
*Meral Topçu MD*****, Haluk Topaloğlu MD*****, Yavuz Renda MD******

SUMMARY: Erdem H, Ayter Ş, Özgüç M, Topçu M, Topaloğlu H, Renda Y. (Department of Medical Biology, and the Neurology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Deletion analysis of Duchenne muscular dystrophy. Turk J Pediatr 35: 15-21, 1993.

DNA of 15 patients with Duchenne muscular dystrophy (DMD) were analyzed for deletions within the DMD gene by using recombinant DNA technology. Deletion frequency was 47 percent and six of the deletions occurred in the region of probe 7 + 8. Only one of the deletions was observed in the region of probe 9-7, and no deletions were found in the regions of probe 30-1, 30-2 and 47-4 (5b + 6). The frequency of deletions found in the Turkish DMD patients corresponds to frequencies reported for other populations. *Key words: Duchenne muscular dystrophy, DNA, cDNA probes, deletion analysis.*

Duchenne muscular dystrophy (DMD) is an X-linked recessive, neuromuscular disease characterized by progressive muscular weakness. The onset of weakness usually occurs between two and three years of age. Affected males are usually confined to a wheelchair by the age of 12, and do not survive beyond their late teens[1,2].

Until recently, as is the case with many other human genetic diseases, the biochemical basis for DMD was totally unknown. To isolate the DMD gene a "reverse genetic" approach was employed. Subsequent to the characterization of the gene on the basis of its chromosomal position, the entire cDNA for the DMD gene was isolated and found to contain 14,000 nucleotides and more than 65 exons. Dystrophin protein, the product of the gene, consists of 3685 amino acids[3-7]. Dystrophin is located in the sarcolemmic membrane of normal skeletal muscle but is absent in the muscle of DMD patients[8-12].

* From the Department of Medical Biology, and the Neurology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara.
** Research Assistant in Medical Biology, Hacettepe University Faculty of Medicine.
*** Professor of Medical Biology, Hacettepe University Faculty of Medicine.
**** Associate Professor of Medical Biology, Hacettepe University Faculty of Medicine.
***** Associate Professor of Pediatrics, Hacettepe University Faculty of Medicine.
****** Professor of Pediatrics, Hacettepe University Faculty of Medicine.

The frequency of intragenic deletions of the dystrophin gene is about 60 percent in DMD patients. Partial gene duplications have also been reported in a small number of patients (5%). In the remaining patients (30 to 35%), without detectable deletions or duplications, the nature of molecular lesions leading to the DMD phenotype remains unknown[13-15]. The high incidence of deletion mutations in patients suffering from DMD that can be identified by means of dystrophin cDNA probes has provided a direct method for genetic diagnosis that permits a high degree of diagnostic accuracy in more than 60 percent of families studied[16-22].

Material and Methods

Seventeen patients with DMD were evaluated for intragenic deletions by using 30-1, 30-2, 47-4, 44-1, 9-7 cDNA probes.

DNA extraction and digestion: 10 ml of peripheral blood was drawn in 13 nmol/L EDTA and leukocytes were separated by centrifugation and lysed in 155 mM NH_4Cl, 10 mM $KHCO_3$ and 0.1 mM EDTA in the presence of 10 mg/ml proteinase K overnight. After phenol-chloroform extractions, DNA was precipitated using ethanol 4U Hind III restriction enzyme per microgram of DNA was used for four hour digestion[23].

Southern Blotting: The resulting fragments were separated by means of electrophoresis through 0.8% agarose gels in Tris-borate, EDTA buffer overnight and at 1V/cm, denatured in 1.5 M NaCl and 0.5 M NaOH, neutralized in 0.5 M Tris-HCl (pH: 7) and 3 M NaCl and transferred overnight to nylon (Hybond) filters by Southern Blotting[23,24].

Labelling of cDNA probes and hybridization: The cDNA probes were radioactively labelled using $\propto$ ^{32}P dCTP (800 or 3000 Ci/mol) (Amersham) and commercial oligolabelling kits (Amersham). The filters were hybridized overnight with 30-1, 30-2, 47-4, 44-1, 9-7 cDNA probes that had been labelled with $\propto$ ^{32}P. Following washing at 65 °C in a 4 percent phosphate buffer, 10 ml EDTA, and 1% SDS, blots were exposed to X-ray film (X-OMAT Kodak) for 3-5 days at −70 °C[24,25].

Results

Normal Hind III restriction fragment patterns are shown in Fig. 1. The deletion of one or more of the fragments shows the deletion region within the DMD gene. There is no need to use probe 12-14, since it corresponds to the 3' untranslated sequences of the transcript of the last exon[21].

Due to low DNA concentration, lane 12 was cancelled. Lane 16 and 17 were affected siblings so they had the same results, therefore only one of them was validated in the results. Although 17 patients were studied, the data of these two were cancelled and 15 of them are being discussed.

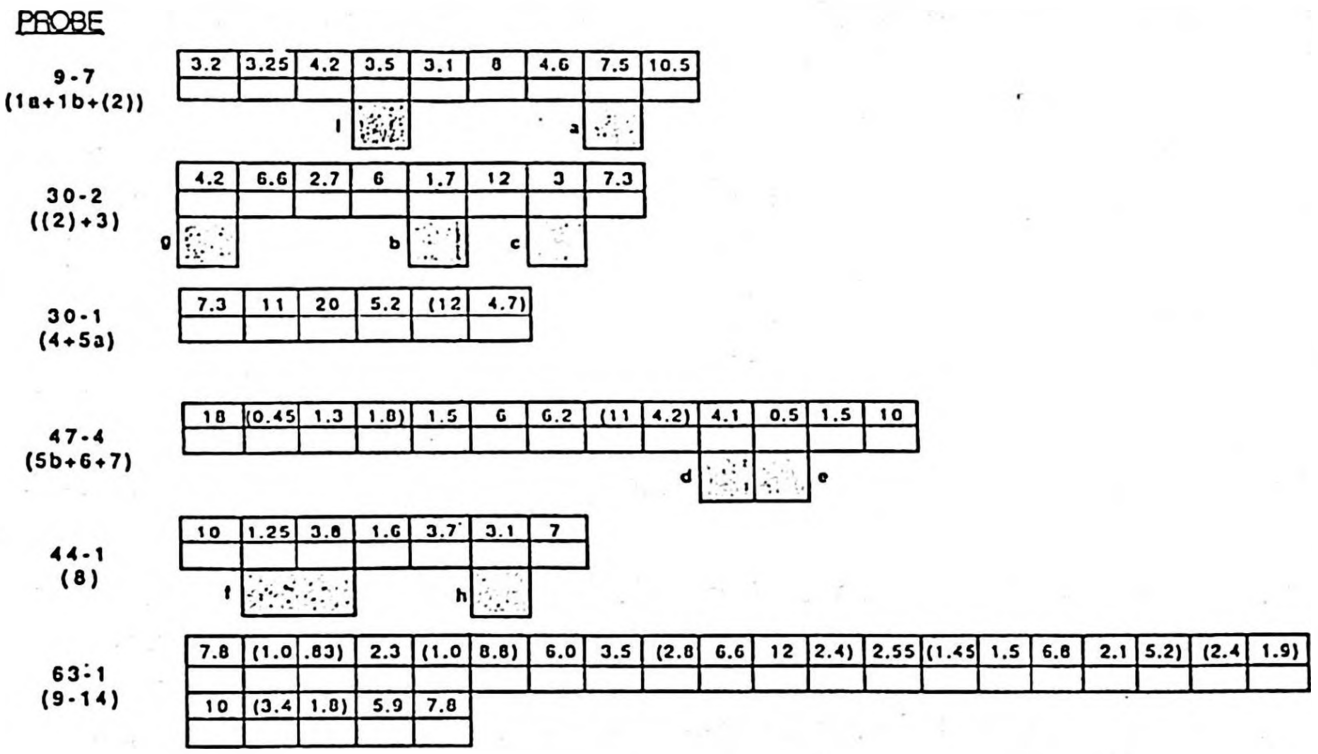

Fig. 1: Normal Hind III restriction patterns. Hind III fragments arranged in order and black bars indicate amplification sites.

20, 12+11, 7.3, 5.2, 4.7 kb fragments were analyzed with probes 30-1 and 12, 7.3, 6.0, 6.6, 4.2, 3, 2.7 1.7 kb fragments were analyzed with probe 30-2 No deletion was observed by using probes 30-1 and 30-2.

Probe 47-4 contains 5b+6+7 kb fragments digested with Hinc II restriction enzyme. The seventh region was isolated and mixed with probe 44-1 (region 8) and used together for hybridization for a better resolution.

By using probe 47-4 (5b+6) 18, 11, 6.2, 6, 4.2, 1.8, 1.5, 1.3 and 0.5 kb fragments were analyzed. As a result, none of the patients had any deletions within the probe 47-4 (5b+6) region.

Normally 8 Hind III fragments (10.5, 8.5, 8, 7.5, 4.6, 4.2, 3.25+3.20, 3.1 kb) are revealed by probe 9-7. In lane 12, results were not clear due to a low DNA concentration, therefore this patient was not included in the results. In the first 7 lanes, foreign DNA contamination was seen between 7.5-4.6 kb fragments. Except for the 3.25+3.20 fragment in lane 5, all fragments were deleted. No deletion was observed in the remaining patients (Fig. 2).

10, 7, 4.1, 3.8+3.7, 3.1, 1.6, 1.5, 1.2, 0.5 kb fragments were analysed with probe 44-1+47-4 (7+8). The 10 kb fragment in lane 8, 13, 14, 10 kb, 3.8+3.7 kb fragments in lane 4 and 6, 1.6 kb, 3.8+3.7 kb fragments in lane 7 were deleted (Fig. 3).

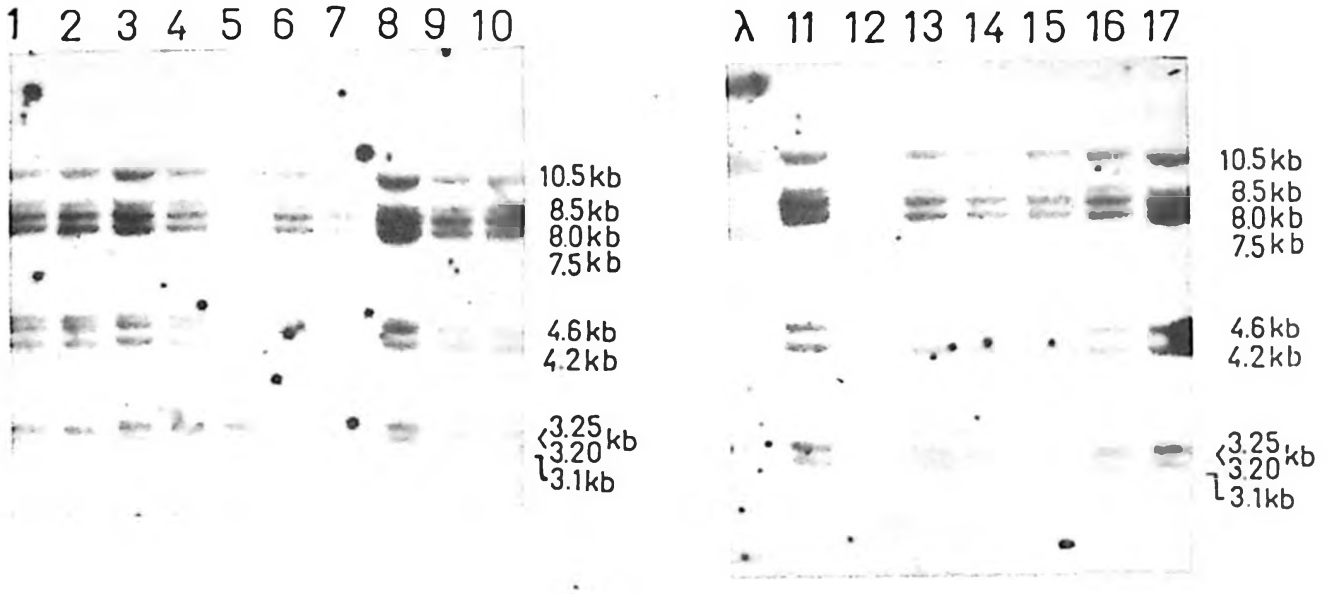

Fig. 2: Autoradiographs of two Southern Blots hybridized with probe 9-7.
λ: Lambda Hind III digested size marker.

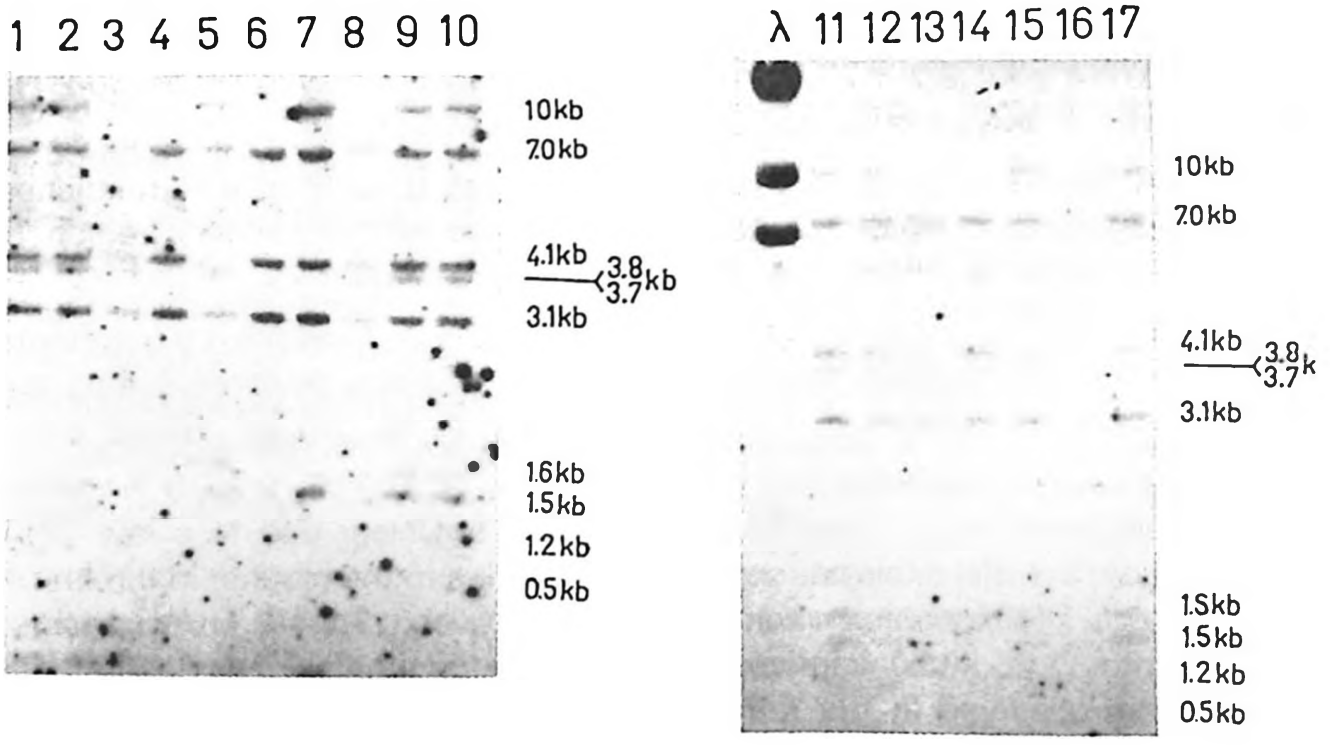

Fig. 3: Autoradiographs of two Southern Blots hybridized with probe 44 – 1 + 47 – 4 (7 + 8) λ: Lambda Hind III digested size marker.

Discussion

Since the DMD gene locus is very large and recombinations between the DMD mutations and the intragenic flanking markers are frequent, the use of linked RFLPs (restriction fragment length polymorphism) in diagnosing DMD is less accurate. The detection of deletions in the genes of DMD patients by using cDNA probes offers the highest reliability for diagnosis. With cDNA clones as probes, DMD gene deletions have been shown to be the cause of muscle dystrophy in 60 percent of DMD patients[12-14,17,22]. One alternative in detecting these deletions is the polymerase chain reaction (PCR)[26-29]. However, since 40 percent of patients have point mutations or deletions that are too small to be detected either by using the Southern Blot technique or PCR, these families should be studied with more traditional linkage analyses.

We found a 47 percent frequency of deletions in our Turkish DMD patients which is similar to other groups reported[30-32]. Although deletions arise heterogeneously they are generally large and are concentrated around the probe 7+8 and 9-7 regions of the gene. Deletions were detected in 7 out of 15 patients. The majority of deletions were shown using the probe 7+8 (lanes 4, 6, 7, 8, 13 and 14). A smaller concentration of deletions were found in the proximal region of the gene corresponding to probe 9-7 (lane 5).

The frequency of deletion mutations observed in the Turkish DMD alleles was similar to that reported in studies made of other populations. Thus the use of the above-mentioned cDNA probes can be used accurately for diagnosis and genetic counselling in our population. With the characterization of the mutations in the remaining DMD lesions, more accurate diagnoses will be available for a wider range of DMD families.

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