Deletion analysis and clinical correlations in patients with Xp21 linked muscular dystrophy

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We carried out molecular deletion analysis on 142 patients with Duchenne/Becker muscular dystrophy which covered 25 exons of the dystrophin gene. We also evaluated the results by comparing with the clinical findings and examples in the literature. A deletion ratio of 63.7% was achieved. Exon 46 was the most frequently affected region. Interestingly we also observed four cases with muscle promoter (Mp) region deletions which have been rarely reported in the literature.

Key words: dystrophinopathy, deletion analysis, clinical correlation, gene promoter mutation.

Dystrophinopathy comprises a group of hereditary muscle disorders characterized by progressive wasting and weakness of skeletal muscle, causing degeneration of muscle fibers, and can be distinguished by mode of transmission, the age at onset and proximal pattern of weakness[1-4]. The range of phenotypes associated with the region Xp21 has been expanding since identification in 1987. The mild end of the spectrum includes the phenotypes of muscle cramps with myoglobinuria and isolated quadriceps myopathy, while at the severe end, there are the progressive muscle diseases that are classified as Duchenne/Becker muscular dystrophy (D/BMD), when skeletal muscle is primarily affected, and sometimes as X-linked dilated cardiomyopathy, when the heart is primarily affected[5-11].

Dystrophin gene is the largest human gene known, spanning 2.4 Mb of DNA in Xp21, and is comprised of 79 exons. The protein product, dystrophin, has a molecular weight of 427 kDa[5,10,12-14].

Deletions of single or multiple exons within the dystrophin gene in D/BMD are responsible in about 65-70% of the cases; the remaining have point mutations (30%) or duplications (6%)[5-8, 10]. The defect causes abnormal expression of the protein product; in DMD it is missing (less than 3%) and in BMD it has 10-40% of normal dystrophin capable of synthesizing shorter or longer, less functional dystrophin protein, causing degeneration of muscle fibers. Birth prevalences of DMD and BMD are 1 in 3,500 and 18,500 live born males, respectively[3,6-8, 13-15].

The aim of this study was to evaluate the correlation of deleted regions with clinical and laboratory findings in children with D/BMD in our region and country.

Material and Methods

We have analyzed deletion patterns and their phenotypic correlation in 142 patients from 127 different families who were referred to our genetic diagnosis center from different cities of the Agean region, for suspected D/BMD based on the charasteristic clinical picture creatine phophokinase (CPK) values and electromyographic findings, and in some cases on muscle biopsies, from 1997 to 2002.

The patients were selected only if they had a strong clinical suspicion and a blood sample for DNA analysis, with or without electromyography and muscle biopsies.

The following features were recorded as appropriate: family history, age of first walk, first symptoms, first symptomatic age, age at diagnosis and the age when ability to walk independently was lost. The distinction of DMD and BMD is based on the age of wheelchair dependency and beginning of symptoms, which for DMD is less than 13 years and 3-5 years, and for BMD is greater than 16 years and 6-9 years, respectively. Among the unrelated 127 patients, 101 were considered to have Duchenne and 26 to have Becker dystrophy.

Their ability to walk, Gowers sign and pseudohypertrophy of gastrocnemius muscle, functioning of the other muscles, heart rate and murmurs, and respiratory functions were evaluated in particular by the same physicians of our department.

School performance and IQ levels were determined[16].

The peresence of cardiomyopathy and left vantricle function (EF: ejection fraction, CF: contraction fraction) were evaluated by echocardiography.

Electrophysiological (EMG: electromyography) and histopathological studies were achieved in some patients due to the different follow-up protocols applied in various centers.

Their genomic DNA was extracted from blood specimens using protein salting-out procedure[17]. Polymerase chain reaction (PCR) deletion analysis of different exons of the dystrophin gene was performed according to Chamberlain et al.[18,19]. and Gibbs et al.[20], with modifications described by Abbs et al.[21]. The amplification products were visualized by ethidium bromide after 3% NuSieve agarose (FMCBioproducts, Rockland, USA) + 1% agarose (Sigma Chemical, St. Louis, USA) gel electrophoresis. Deletions were diagnosed with presence of one of the bands in the amplified control DNA and absence of the corresponding same band in the patient's DNA. For statistics, the frequencies and distributions were compared among all groups using the SPSS for Windows VS.01.

Results

Deletion analysis by multiplex amplification of selected exons (pm+1, 3, 4, 6, 8, 12, 13, 14+15, 17, 19, 42-55, 60) showed a deletion frequency of 81/127 (63.7%) in unrelated cases.

All patients were categorized into three groups according to the localization of the deletion: group 1 comprising exons 1-19, group 2 comprising exons 42-60 and group 3 extended from proximal to central.

Deletion frequency was 65.3% (66/101) and 57.7% (15/26) in DMD and BMD patients, respectively. The majority of deletions, 62/127 (48.8%), were found within the central region of the gene (exons 42-60), 12/127 (9.4%) were mapped to the proximal hot spot (exons 3-19), and the remaining cases covered both regions 7/127 (5.5%) (Fig. 1). Patients who had deletions which covered both regions were all diagnosed as DMD.

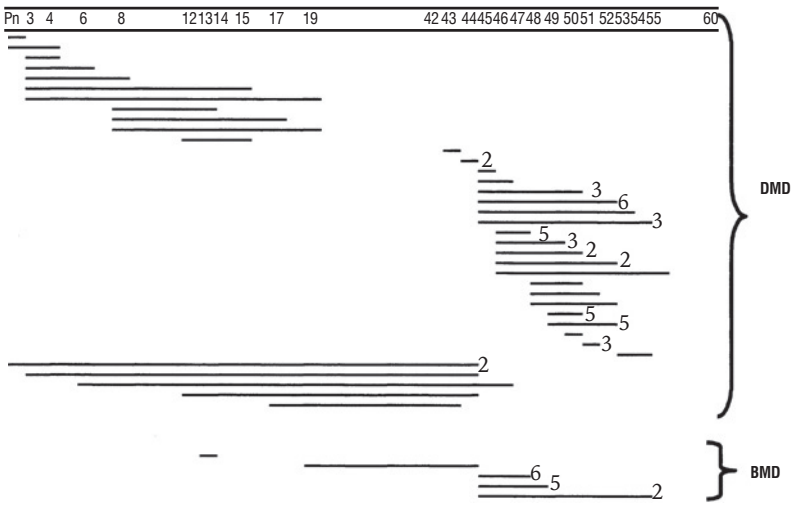

Fig. 1. Dystrophin gene deletions in patients with D/BMD. A number next to a deletion indicates the number of unrelated patients carrying the same deletion.

Exon 46 was the most frequently affected. When deletions started with exon 46, all patients were diagnosed as DMD. Table I shows genotype-phenotype correlation of patients, whose determined deletion sizes were within the central region of the gene (exons 42-60).

Four patients had muscle promoter (Mp) region deletions. The deletion pattern of all patients is summarized in Figure 1.

Single exon deletions were detected in 12 (92.3%) of the DMD patients and in 1 (7.7%) BMD, patient, respectively.

We could not find a correlation with clinical symptoms, IQ levels, EF, CPK values, or the existence of deletions.

First walking age was significantly delayed in nondeleted patients versus deleted cases (27.7 months and 16.29 months of age, respectively, p: 0.039) (Table II); however, there was no difference between DMD and BMD cases.

We could not find a correlation between phenotype and the size, location or the existence of deletion.

Discussion

Duchenne/Becker muscular dystrophies are allelic diseases caused by mutations in the middle of the short arm of the X chromosome. Both are inherited in an X linked recessive manner which is usually the problem of male genus[10]. Females, who may be affected in rare instances carrying X-autosome translocations that disrupt the dystrophin gene, may also show the same phenotype[22-28]. The patients in our study who were referred to our laboratory were all males.

The mean age of diagnosis of DMD boys without a family history was about 7 years and 10 months, with a range of 1 to 21 years, which is older than the previous reports of Zalaudek et al.[29] and Iwanczak et al.[30], who found a mean age of 4 years 10 months, with a range of 16 months to 8 years. On the other hand, the mean age of diagnosis of BMD boys without a family history was about 16 years and 6 months, with a range of 7 to 42 years[5,10,29,30].

Late diagnosis of D/BMD has implications for both the child and the family. One of our BMD families had five affected brothers. The index

Table I. Genotype-phenotype Correlation in Patients Whose Deletion Size was Determined

Exons	42	43	44	45	46	47	48	49	50	51	52	53	54	55	60
42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
43	–	DMD	–	–	–	–	–	–	–	–	–	–	–	–	–
44	–	–	DMD	–	–	–	–	–	–	–	–	–	–	–	–
45	–	–	–	DMD	BMD	BMD	BMD	–	DMD	–	DMD	BMD	B/BMD	–	–
46	–	–	–	–	–	DMD	–	DMD	DMD	–	DMD	–	–	–	–
47	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
48	–	–	–	–	–	–	–	–	DMD	BMD	DMD	–	–	–	–
49	–	–	–	–	–	–	–	–	DMD	–	DMD	–	–	–	–
50	–	–	–	–	–	–	–	–	DMD	–	–	–	–	–	–
51	–	–	–	–	–	–	–	–	–	DMD	–	–	–	–	–
52	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
53	–	–	–	–	–	–	–	–	–	–	–	–	DMD	–	–
54	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
55	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
60	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Table II. Correlations with Walking Age and Deletion Region

	Mean walking age	Ranges (months)
Nondeleted patients	27.71	12-96
Central region deletion (exons 42-60)	15.88	12-30
Proximal region deletion (exons 3-19)	18.86	12-30
Deletions extended from proximal to central	14.8	12-18

case was 21 years old, whose symptoms started at the age 11 as gait disturbance, and the family physician's first diagnosis was pes planus.

The oldest patient referred to us was a BMD case at the age of 42. He had easy fatiguability with gait disturbance, and a deletion within 45-48 exons was detected.

Our youngest patient was a six-month-old boy suffering from bronchitis when elevated activity of aminotransferases was detected. After exclusion of the hepatic and infectious causes, elevated creatine kinase value (CK: 16295 mg/dl) and electromyographic studies strongly suggested the diagnosis of D/BMD, and molecular study revealed a deletion within 8-19 exons of the gene. Ivanczak et al.[30] also reported an eight-month-old boy as an early diagnosed case.

There was an 11-year-old boy with BMD who had exon 45-48 deletion, suffering from muscle cramps after running and manifest gastrocnemius pseudohypertrophy without Gowers sign with myoglobinuria; two of his uncles (42 and 47 years old) also had similar problems and the same deleted gene regions. Neither of them had a serious difficulty in walking. Bosone et al.[31] also reported a similar case at 60 years of age.

Children with Duchenne/Becker dystrophinopathy may walk at a relatively normal age but delayed standing independently may be recognizable[28,30].

Although the walking ages of our patients with and without deletions showed significant differences, we could not find a correlation between walking age of DMD/BMDs and localization of deletions. But when deletions were extended from proximal to central, the mean walking age was surprisingly recognized as earlier, although we could not find a statistical difference. Interestingly, cases with gene duplications or point mutations may gain their standing ability later than expected (Table II).

The diagnosis of D/BMD should be based on clinical examination, biochemical tests (creatine phophokinase values), muscle biopsy, electromyography and molecular findings. As the patients in this study were referred from different medical centres, muscle biopsies of all could not be performed; however, it may not be necessary to subject them to this invasive technique if there is a strong suspicion of the disease based on clinical and biochemical aspects. The cases diagnosed without biopsies probably implicate lower deletion rates, but it is not possible to state the exact deletion rate without analysing our patients and their families for other possible deletions.

The percentage of detected deletions (63.7%) was similar to previous reports (55-65%)[13,32,33]. Some higher incidents are most probably due to the confirmed diagnosis on the basis of dystrophin-stained biopsies[15,34,35]. By the same token a high number of screened exons plays a role in catching single exon deletions and causes higher deletion ratios.

Intron 44 was found to be frequently involved in our study, which was consistent with the literature[36-40].

To date there are no reports of Turkish D/BMD cases with deletions at muscle promoter (Mp) region. We have encountered four cases of Mp deletions which hinted clinically to DMD. Clinical variety of DMD cases associated with dystrophin gene promoter mutation has been reported by Frisso et al.[41].

In both DMD and BMD, although the site and location of mutations are very heterogeneous in dystrophin gene, partial deletions and duplications are mostly seen in two hot spots: the proximal at the 5' end of the gene, comprising exons 1-19, and the more distal, comprising exons 42-60. The ratio of mutations in the proximal and central region was 1/5. In our study in approximately 76.4% of deleted cases, deletions were within the central region. The distribution of the deletions was similar to the results of Taşdemir et al.[42], Herczegfalvi et al.[43], Önengüt et al.[44], Battaloğlu et al.[45] and Dinçer et al.[46].

In general, one third of DMD cases would be expected from multigeneration families, one third from new mutations arising in the mother (producing brother pairs plus some isolated cases) and the remaining from new mutations arising in isolated males[5,13,14]. In our study, 12/110 (10.9%) of our cases were from multigeneration families, 9/110 (8.2%) from brother pairs and 89/110 (80.9%) were sporadic cases for DMD. Likewise, 6/32 (18.7%) were from multigeneration families, 6/32 (18.7%) from brother pairs and 20/32 (62.5%) were from sporadic cases for BMD.

Behavioral studies have shown that DMD boys have a cognitive impairment and a lower IQ (average 85); however, we did not find significant differences in the IQ levels of our patients (p:0.215)[10,15,47-49].

No apparent relationship has been found between the level of serum creatinine kinase activity at initial presentation to the hospital and the location of certain deleted exons.

In conclusion, alterations of the human dystrophin gene occur in muscular and cardiac disorders. It is difficult to establish genotype-phenotype correlations, particularly regarding the deletions at the 5' region of the gene, but attempts for correlations should be made based on detailed clinical findings with the type of mutations to have better follow-up protocols.

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