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CORRELATION OF LABORATORY AND CLINICAL FINDINGS WITH THE LOCATION OF Xp21 DELETION IN DUCHENNE MUSCULAR DYSTROPHY*

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SUMMARY: Taşdemir HA, Topaloğlu H, Dinçer P, Göğüş S. Kotiloğlu E, Özdirim E, Yalaz K. (Neurology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Correlation of laboratory and clinical findings with the location of Xp21 deletion in Duchenne muscular dystrophy. Turk J Pediatr 1997; 39: 317-324.

Laboratory and clinical features of 28 Duchenne muscular dystrophy patients were evaluated. Positive family history was present in only two cases (7.1%). Dystrophin I-positive fibers were present in 33 percent of the cases with the deletion close to the 5' end of the gene. In the cases with deletion concerning the central part of the gene, all fibers were dystrophin I-negative. In five of the six cases with short stature, the deletion was close to the 5' end of the gene, and short stature was especially seen together with 8th and 13th exon deletion. Statistical analysis concerning the age at which the patient began to have difficulty in standing up and at which he could not walk, did not correlate with the clinical severity and deletion zone, location or extent. *Key words: Duchenne muscular dystrophy, Xp21 deletion, dystrophin, laboratory findings, clinical features.*

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder transmitted by female carriers to their male offspring[1-5]. On rare occasions, it occurs in females[2-4]. It is well-known that deletions on the Xp21 chromosome result differently in the quality and quantity of dystrophin, leading to muscle weakness[6-8]. However, it has been shown that the presence of dystrophin-positive (+) fibers does not correlate with clinical severity[9]. Mental retardation (MR), cardiomyopathy (CMP), respiratory disorders and obesity are seen in some but not all cases of DMD[2, 3, 10]. Some DMD patients cannot walk at the age of seven, and others at 12 years of age[3, 10, 11]. Factors contributing to these different clinical pictures are being researched by several investigators.

This study was performed to shed light on the relationship between the clinical and laboratory findings and the location of the deletions.

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Material and Methods

The study was completed between January 1990 December 1993 and included 28 patients diagnosed with DMD with Xp21 deletion found by DNA analysis. The patients' family histories were taken and a thorough physical examination performed. Short stature was identified as length below three percent of the value appropriate for age obesity was defined as weight above 20 percent of the value appropriate for length[12, 13]. The total muscle strength score (TMSS) was calculated according to the guidelines of the Medical Research Center (MRC)[14]. Intelligence tests were given to the patients, their IQ levels were determined and MR was scored[15]. The presence of cardiomyopathy (CMP) was determined according to the findings of cardiologic examination, radiography, electrocardiography (EKG) and echocardiography. Left ventricle functions were evaluated by ejection fraction (EF) and contraction fraction (CF)[16, 17]. Tests for respiratory functions were performed, and vital capacity (VC) as a representative was measured[18-20].

Histopathologic evaluation included immunohistochemical study of open biopsy samples by using dystrophin I (mid-rod domain), dystrophin II (carboxyl terminal) and dystrophin III (amino terminal) antibodies, and the ratio of dystrophin-positive [Dys(+)], partially positive (±) and negative (-) fibers were calculated.

Patient DNAs were amplified by multiplex polymerase chain reaction (PCR). Deletion analysis was performed by using the 9-exon multiplex primer PCR set (4, 8, 12, 17, 19, 44, 45, 48, 51 exons) and 5-exon multiplex PCR set (Pm, 13, 43, 50, 52 exons)[21, 22]. The exact deletion extent was able to be determined in 13 cases.

Statistical analysis was done by SPSS for Windows V5.01.

Results

In this study, the mean age was 115.6 ± 42.1 months, with a range of 33 to 220 months. Positive family history was detected in two patients (7.1%) whose uncles had DMD. Family history was negative in the majority of cases (26 out of 28, 92.9%). Nine patients (32.1%) were obese and six of them could not walk. Six patients (21.4%) had short stature. Intelligence tests were given to 24 of the 28 patients, of whom 11 (45.8%) had normal or high and 13 (54.2%) had low IQ levels. Respiratory function tests were performed in 16 cases, and five of them (31.3%) had VC with predicted values below 80 percent. Overall, 14 patients (50%) had CMP, and two (7.1%) had left ventricle dysfunction. Nineteen patients (67.9%) began to have difficulty in standing before four years of age, and nine patients (32.1%) after that age. Thirteen patients could not walk, and this symptom occurred before nine years of age in seven of them (53.9%) and after nine years of age in six of them (46.2%).

Dys I (+) fibers were present in three (10.7%), Dys I (±) in five (17.9%), Dys III (+) in four (14.3%) and Dys III (±) in two (7.1%) cases. Dys II was negative in all cases.

Deletions found in our cases were focused in two regions of the dystrophin gene: 4-19 exons zone and 45-52 exons zone (Table I). In nine cases (32.1%), deletions were close to the 5' end (4-19 exons) and in 18 cases (64.3%) close to the 3' end (45-52 exons). One case had a large deletion beginning from the 5' end and extending to the central zone (4-44 exons). The most frequently deleted exons were the 47th, 48th and 49th exons, detected in eight cases (28.6%). Deletion of the 8th exon was seen in seven cases (25%).

Table I: Deleted Zones and Extent of Deletion in 28 DMD Patients

Case No.	Deletion Detected	Exact Extent of Deletion	Case No.	Deletion Detected	Exact Extent of Deletion
1	8-13		15	45-50	45-50
2	8-13		16	48-50	
3	13-19		17	45	45
4	13-19		18	45-52	45-52
5	8		19	47	
6	8		20	46-47	46-47
7	50-52	50-52	21	48-52	
8	45-50		22	47-48	47-48
9	46-47	46-47	23	45	45
10	45-50	45-50	24	48	
11	19		25	51	51
12	51-52		26	50	50
13	48	48	27	8	
14	51	51	28	4-44	

There was no correlation between the extent of deletion and other parameters [TMSS, EF, CF, VC pred %, IQ levels and Dys I, III (+) or (±) fiber ratios] (Table II). However, when the location of the deletion was taken into account, the ratio of Dys I (+) fibers was significantly different in the two groups ($p < 0.002$). The results of other laboratory findings did not differ significantly (Table II). Another interesting observation concerning the location of the deletion was that five of the six (83.3%) short patients had a deletion close to the 5' end (4-19 exons zone), especially in the 8th and 13th exons (Table III).

Walking capability is such an important parameter that it is included in the diagnostic criteria[23]. Hence, the relationship between the location of the deletion and the age at which the patient could not walk was analyzed by a more detailed (x^2 test) analysis (Table IV), and no correlation was found.

Figure 1 depicts a graph of the relationship between age and walking capability. The aim was to show that the deleted zone has no effect on the age at which the patient could not walk, and to reveal the possible walking ability of a DMD patient at a proposed age.

Table II: Correlation Between Deleted Zones and Selected Clinical and Laboratory Findings (Covariant Analysis)

	4-19 Exons Deletion		45-52 Exons Deletion			p*
	Mean	Corrected Mean	Mean	Corrected Mean	Age	Deletion Groups
Number of cases		9		18		
Age (months)	101.2 ± 49.9	–	121.6 ± 38.3	–	–	0.25
TMSS	54.4 ± 21.8	52.2	50.7 ± 12.9	52.9	0.001	0.84
EF	66.8 ± 8.0	66.4	63.6 ± 6.8	64.0	0.23	0.44
CF	36.4 ± 6.0	36.2	34.9 ± 6.3	35.2	0.42	0.70
Dystrophin I (+) %	0.17 ± 0.25	0.18	–	–	0.06	0.002
Dystrophin I (±) %	1.17 ± 1.95	1.19	1.00 ± 4.00	0.98	0.91	0.89
Dystrophin III (+) %	–	–	0.42 ± 0.91	0.44	0.60	0.17
Dystrophin III (±) %	–	–	1.17 ± 4.71	1.28	0.54	0.40
IQ	84.6 ± 14.8	84.2	84.8 ± 20.1	85.2	0.53	0.91

* The difference is significant if $p < 0.05$
CF : Contraction fraction
EF : Ejection fraction
TMSS: Total muscle strength score

Table III: Statistical Comparison (p values) of the Frequencies of Signs and Symptoms in the Presence and Absence of Different Exon Deletions (x^2 Analysis)

	Symptoms and Findings					
Deletion Zone Deleted Exon	Difficulty in Standing Before 4 Years	Short MR	CMP	VC < 80%	Obesity	Stature
Deletion zone 4-19/45-52	0.61	0.68	0.72	0.64	0.22	0.008
Deleted 8th exon	0.78	0.23	0.46	0.56	0.69	0.02
Deleted 13th exon	–	0.23	0.55	0.36	0.45	0.05
Deleted 45th exon	0.51	0.22	0.36	0.48	0.21	0.62
Deleted 47th exon	0.29	0.58	0.62	0.38	0.59	0.43
Deleted 48th exon	0.44	0.55	0.73	0.52	0.59	0.43
Deleted 49th exon	–	0.42	0.66	0.92	0.57	0.62
Deleted 50th exon	0.44	0.62	0.39	0.73	0.59	0.43
Deleted 51st exon	–	0.12	0.42	0.32	0.43	0.62
Deleted 52nd exon	–	0.37	0.44	0.18	0.24	0.64

MR : Mental retardation
CMP : Cardiomyopathy
VC : Vital capacity

Table IV: Cox Regression Model Where Age and Deleted Exon or Exon Zone are Covariates, and Age at Which the Patient cannot Walk is Independent Variable (*)

Deleted Zone Deleted Exon	Effect of Deletion Zone (p)	Effect of Age (p)	Relative Risk	95% Confidence Limits of Relative Risk	
4-19/45-52	0.99	0.40	1.01	0.26	3.91
			45-52-4-19		
8th exon	0.91	0.46	0.92	0.24	3.49
45th exon	0.83	0.44	0.87	0.25	3.03
47th exon	0.47	0.61	0.60	0.15	2.42
48th exon	0.49	0.62	0.62	0.16	2.42
50th exon	0.52	0.48	1.49	0.44	5.07

* 2nd column shows p values representing the relation between deletion zone and age at which the patient could not walk; 3rd column shows p values representing the relation between the present age of the patient and the age he could not walk.

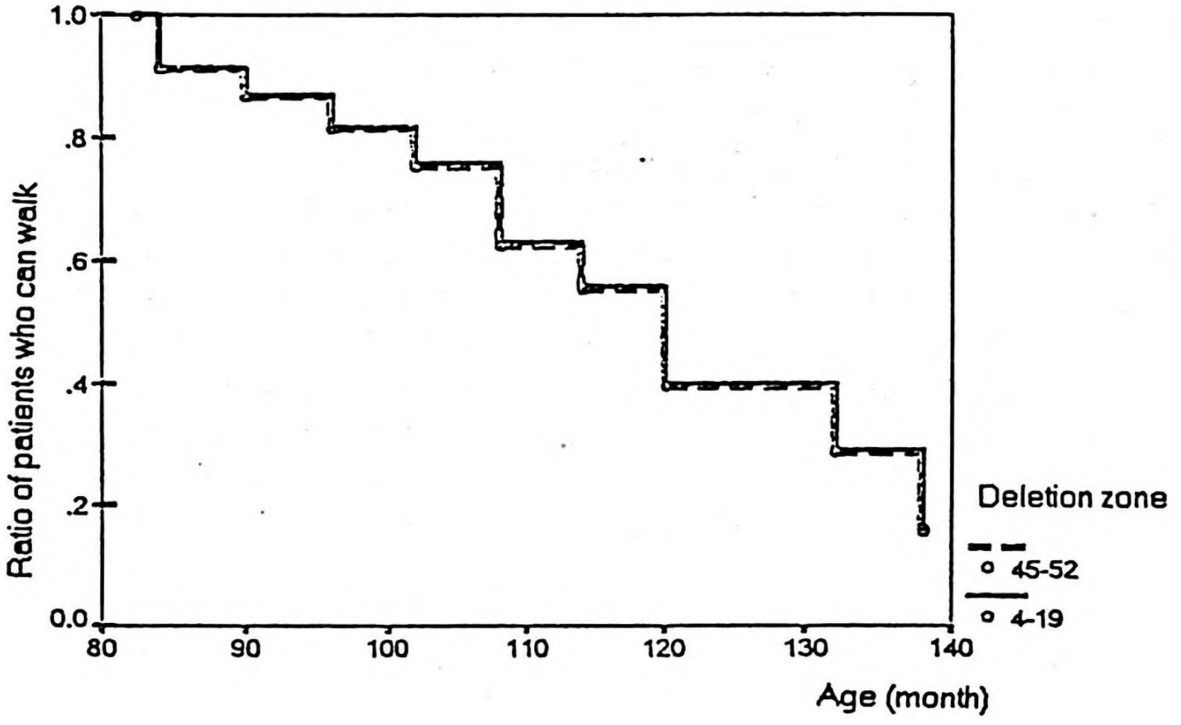

Fig. 1: Graph showing the relationship between age and walking ability in patients with deletions of 4-19 and 45-52 exons.

Discussion

In one-third of the DMD cases, there was a positive family history; another one-third were offspring of possible mutant carriers not diagnosed earlier, while the remainder were due to new mutations[2, 5, 6]. In our study group, a positive family history was present only in 7.1 percent of cases.

There was no difference in TMSS, EF, KF, IQ, Dys I (±) or Dys III (+) or (±) fiber ratios between the groups with 4-19 exons deletion and 45-52 exons deletions (Table II). On the other hand, three of nine cases (33.3%) with the deletion close to the 5' end (4-19 exons) had Dys I (+) fibers, in contrast to all cases with central exon deletions and Dys (-) fibers (Table II).

In our group, the location of the deletions or certain deleted exons did not affect the presence of MR, CMP, obesity or disturbances in VC (Table III). Hodgson[24] found that IQ levels were normal in seven of the 12 DMD patients and abnormal in the remaining five cases (41.7%). However, in the series of Rappaport et al.[25], the MR rate was much higher. Among the 27 cases in their group, 19 (70.4%) patients were mentally retarded.

In our study group, five out of six patients with short stature (83.3%) had a deletion close to the 5' end, especially deleted 8^{th} and 13^{th} exons (Table III). No correlation was found between short stature and the location of the deletion by Hodgson et al.[26].

Since 1988, studies have focused on mapping of the dystrophin gene in order to formulize the suggestion that deletions of certain regions of the gene lead to a certain clinical severity of DMD or Becker muscular dystrophy (BMD)[27, 28-30]. Medori et al.[28] reported that 8 of 11 mild DMD cases had deletions of the 5' end, and that none of the classical DMD cases had similar deletions. There have been further studies showing the regions responsible for BMD and DMD[26, 28]. Read et al.[30] tried to define specific deletions for BMD and DMD. However, there are several reports showing no correlation between the clinical severity and the location extent of the deletion[31, 32-34]. Similar results were achieved in our study with the detailed statistical analysis, using age at the onset of walking and standing disturbances as a parameter of clinical severity (Tables III-IV, Fig. 1).

REFERENCES

1. Swaiman KF. Pediatric Neurology. Missouri: CV Mosby Company; 1989: 1067-1079, 1139-1149.
2. Asbury AK, McKhan GM, McDonald WI. Diseases of the Nervous System Clinical Neurobiology. Philadelphia: WB. Saunders; 1992: 123-132.
3. Walton SJ. Disorders of Voluntary Muscle. New York: Churchill Livingstone; 1988: 32-65, 109-137, 519-540.
4. Aicardi J. Diseases of the Nervous System in Childhood. London: MacKeith Press; 1992: 1172-1178.
5. Adams JH, Duchen LW. Greenfield's Neuropathology. London: Edward Arnold; 1992: 1456-1460.
6. Hoffman EP, Schwartz L. Dystrophin and disease. Mol Aspect Med 1991; 12: 175-194.
7. Love DR, Byth BC, Tinsley JM, et al. Dystrophin and dystrophin-related proteins: a review of protein and RNA studies. Neuromuscul Disord 1993; 3: 5-21.
8. Matsumura K, Campbell KP. Deficiency of dystrophin-associated proteins: a common mechanism leading to muscle cell necrosis in severe childhood muscular dystrophies. Neuromusc Disord 1993; 3: 109-118.

9. Fanin M, Danieli GA, Vitiello L, Senter L, Angelini C. Prevalence of dystrophin-positive fibers in 85 Duchenne muscular dystrophy patients. Neuromusc Disord 1992; 2: 41-45.

10. Iannaccone ST. Current status of Duchenne muscular dystrophy. Pediatr Clin North Am 1992; 39: 879-894.

11. Dubowitz V. Muscle Disorders in Childhood (2nd ed). London: WB. Saunders; 1978: 19-49.

12. Judith J, Henry RN. Routine growth monitoring and assessment of growth disorders. J Ped Health Care; 1992; 6: 291-301.

13. Hung W. Clinical Pediatric Endocrinology. St. Louis: CV Mosby; 1992: 356.

14. Brett EM, Lake BD. Neuromuscular disorder: I. Primary muscle disease and anterior horn cell disorders. In: Brett EM (ed). Pediatric Neurology (2nd ed). London: Churchill Livingstone; 1991: 53-68.

15. American Association on Mental deficiency Adaptive Behaviour Scale. 1974 Manual. Washington, D.C.

16. Nigro G, Comi LI, Politano L, Bain RJ. The incidence and evolution of cardiomyopathy in Duchenne muscular dystrophy. Int J Card 1990; 26: 271-277.

17. D'Orsogna L, O'Shea JP, Miller G. Cardiomyopathy of Duchenne muscular dystrophy. Pediatr Cardiol 1988; 9: 205-213.

18. Burke SS, Grove NM, Houser CR, Johnson DM. Respiratory aspects of pesudohypertrophic muscular dystrophy. Am J Dis Child 1974; 121: 230-234.

19. Inkley SR, Oldenburg FC, Vignos PJ. Pulmonary function in Duchenne muscular dystrophy related to stage of disease. Am J Med 1974; 56: 297-306.

20. Hapke EJ, Meek JC, Jacops J. Pulmonary function in progressive muscular dystrophy. Chest 1972; 61: 41-47.

21. Chamberlain JS, Gibbs RA, Rainer JE, Caskey CT. Multiplex PCR for the diagnosis of DMD. In: Innis MA, Gelfand DH, Sninski J, White T (Eds.). PCR Protocols: A guide to Methods and Applications. San Diego: Academic Press; 1990: 272-281.

22. Dinçer P, Topaloğlu H, Ayter Ş, Özgüç M, Taşdemir HA, Renda Y. Molecular deletion patterns in Turkish Duchenne and Becker dystrophy patients. Brain Dev 1996; 18: 91-94.

23. Jennekens FGI, ten kate LP, de Visser M, Wintzen AR. Diagnostic criteria for Duchenne and Becker muscular dystrophy and myotonic dystrophy. Neuromuscul Disord 1991; 1: 389-391.

24. Hodgson SV, Abbs S, Clark S, et al. Correlation of clinical and deletion data in Duchenne and Becker muscular dystrophy with special reference to mental ability. Neuromuscul Disord 1992; 2: 269-276.

25. Rapaport D, Passos-Bueno MR, Brandao L, et al. Apparent association of mental retardation and specific patterns of deletions screened with probes cf56a and cf23a in Duchenne muscular dystrophy. Am J Med Genet 1991; 39: 437-441.

26. Hodgson S, Hart K, Abbs S, et al. Correlation of clinical and deletion data in Duchenne and Becker muscular dystrophy. J Med Genet 1989; 26: 682-693.

27. Beggs AH, Hoffman EP, Snyder JR, et al. Exploring the molecular basis for variability among patients with Becker muscular dystrophy: dystrophin gene and protein studies. Am J Hum Genet 1991; 49: 54-67.

28. Medori R, Brooke MH, Waterston RH. Genetic abnormalities in Duchenne and Becker dystrophies: clinical correlations. Neurology 1989: 39: 461-465.

29. Koenig M, Beggs AH, Moyer M, et al. The molecular basis for Duchenne versus Becker muscular dystrophy: correlation of severity with type of deletion. Am J Genet 1989; 45: 498-506.

30. Read AP, Mountford RC, Forrest SM, et al. Patterns of exon deletions in Duchenne and Becker muscular dystrophy. Hum Genet 1988, 80: 152-156.

31. Darras BT, Blattner P, Harper JF, et al. Intragenic deletions in 21 DMD/BMD families studied with the dystrophin cDNA: location of break points on Hind III and Bg III exon-containing fragment maps, meiotic and mitotic origin of the mutations. Am J Hum Genet 1988, 43: 620-629.

32. Baumbach LL, Chamberlain JS, Ward PA, Farwell NJ, Caskey CT. Molecular and clinical correlations of deletions leading to Duchenne and Becker muscular dystrophies. Neurology 1989; 39: 465-474.

33. Davies KE, Smith TJ, Bundey S, et al. Mild and severe muscular dystrophy associated with deletions in Xp21 of the human X chromosome. J Med Genet 1988; 25: 9-13.

34. Norman AM, Thomas NS, Kingston HM, Harper PS. Becker muscular dystrophy: correlation of deletion type with clinical severity. J Med Genet 1990; 27: 236-239.