

Analysis of deletions and their relationship with clinical severity, family recurrence, and intelligence in Duchenne and Becker Muscular Dystrophy patients from Southern Brazil

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ABSTRACT

A total of 50 patients affected by Duchenne (DMD) or Becker (BMD) dystrophies from 41 unrelated families was investigated for deletions in the muscular promotor and in 13 exons located at two hot spots of the dystrophin gene. Twenty of the 41 probands presented deletions. None occurred at the muscular promotor, but at least two were observed in each of the exons studied. The patient with the largest identified deletion (exons 12-44) showed a mild Duchenne muscular dystrophy (DMD) clinical picture only. Three of the deletions in patients with severe DMD, and one in those with moderate DMD clearly disrupted the reading frame. No clear relationship could be inferred in the comparisons between types of deletions versus (a) disease severity, (b) sporadic and familial cases, and (c) intelligence levels.

INTRODUCTION

Despite the large amount of research already performed on Duchenne (DMD) and Becker (BMD) muscular dystrophies (reviews in Harper, 1989; Emery, 1993), a series of problems awaits solution, and three of them will be considered in this report. The first concerns the relationship between the type and extent of the deletions observed in a large number of these patients and the clinical severity of their phenotype. This question has been considered, among others, by Koenig *et al.* (1989). The second relates to deletion site

in familial as compared to isolated cases (cf. Passos-Bueno *et al.*, 1992); and the third to the relationship between mental ability and the occurrence of specific deletions in the dystrophin gene (as was done, for instance, by Rapaport *et al.*, 1991). Delineation of deletion patterns in DMD/BMD patients has been done for French Canadian (Simard *et al.*, 1992), German (Niemann-Seyde *et al.*, 1992), European in general (Danieli *et al.*, 1993), Japanese (Katayama *et al.*, 1993), and Turkish (Gökgöz *et al.*, 1993) series.

MATERIAL AND METHODS

We first contacted patients and their relatives previously studied by us but who had not been

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investigated at the molecular level. Information about them can be found in Carvalho (1987), Carvalho *et al.* (1988), and Alho (1990). Additionally, medical professionals and the media were contacted for collaboration. In the final stages of the study the Associação Gaúcha de Distrofias Musculares (AGADIM) also helped in the ascertainment of patients. As a result 67 families were contacted, but after proper screening only 41 of them were qualified for the study. They are briefly characterized in Table I. About half of them came from the interior of the State of Rio Grande do Sul, and most were Caucasoid of medium or low socioeconomic level. The total number of patients studied was 50, distributed as follows: 34 isolated cases, five sibships with two affected and two sibships with three affected.

Table I - Origin, ethnic group and socioeconomic level of the probands.

Characteristics	Sporadic		Familial DMD	Percentage
	DMD	BMD		
<i>Origin</i>				
Porto Alegre	11	1	1	32
Interior of Rio G. do Sul	11	5	8	58
Santa Catarina	2	0	2	10
<i>Ethnic group</i>				
Caucasoids	20	5	11	88
Afro-Brazilians	4	1	0	12
<i>Socioeconomic level</i>				
High	4	0	1	12
Medium	11	3	3	42
Low	9	3	7	46

DMD: Duchenne Muscular Dystrophy; BMD: Becker Muscular Dystrophy.

In a first interview of about three hours with the patients and their relatives, clinical and genealogical information was obtained and blood collected for molecular and enzymatic (creatine kinase or CK) analyses. In a second interview Raven's Progressive Matrices tests were administered to the patients, using the Portuguese version, standardized for Brazil by members of the Psychology Institute, University of São Paulo (Alves *et al.*, 1987; Campos, no date). A third and final interview was conducted after all the tests and results had been compiled, and included appropriate

genetic counseling for the potential female carriers of the gene. Since the CK results were used just for genetic counseling and clinical diagnosis, they will not be considered in detail here.

The clinical diagnosis was confirmed through CK determinations (86% of the cases), evaluation of three other enzymes (36%), electromyography (48%) and muscular biopsies (16%). Considering (a) age of the patients at the first symptoms; (b) age of ambulatory loss; (c) age at examination or death; (d) degree of muscular disability (according to Vignos scale; cf. Vignos and Archibald, 1960; Carvalho, 1987; Rapaport, 1990), and (e) in the familial cases, the course of the disease in the affected older brothers, four clinical groups were delineated: 1. *Severe Duchenne Muscular Dystrophy or DMD*: patients who stopped walking during the first nine years of life, or those with age below nine years who walked with difficulty, showing a high degree of muscular weakness; 2. *Moderate DMD*: those who had stopped walking between 9.1 and 12 years, or those with age below 12 years who still walked and showed a slower progression of muscular weakness as compared with those of group 1; 3. *Intermediate DMD*: patients who stopped walking between 12.1 and 16 years, or those with age below 16 years who could still walk but who probably would stop walking before reaching that age, and 4. *Becker Muscular Dystrophy or BMD*: cases older than 16 years who could walk, or those younger than 16 but who had motor abilities which excluded DMD (for example, jumping with two legs).

DNA extraction was performed using the technique of Debomoy and Nurnberger (1991). The primers used for the analyses were described in Beggs *et al.* (1990) and Chamberlain *et al.* (1990), and they were employed using multiplex - PCR kits acquired from Cetus, after proper permission granted by C.T. Caskey (Molecular Genetics Institute, Baylor College of Medicine, Houston, Texas, USA). The temperatures used and the times of the amplification cycles, as well as the reagent concentrations and gel conditions, followed the methods described in Chamberlain *et al.* (1990).

RESULTS

Twenty of the 41 probands had molecular deletions, described (type and position) in Table II, and classified in accordance with the clinical groups defined in the previous section. A total of 20 deletions were found. None occurred at the muscular promoter, but at least two were observed in each of the exons

Table II - Type and position of the deletions observed in the dystrophin gene among the affected individuals, classified according to the clinical group¹.

Clinical groups and patient number	Regions of the gene ²													
	Proximal							Distal						
	Mp	4	8	12	13	17	19	43	44	45	48	50	51	52
<i>DMD - Group 1</i>														
Patients no.														
5	*	*	*	*	*	*	*	*	*	*	*	*	/	*
20	*	*	*	*	*	*	*	*	*	*	/	*	*	*
32	*	*	*	*	*	*	*	*	*	*	*	/	*	*
33	*	*	/	/	/	/	*	*	*	*	*	*	*	*
41	*	*	*	*	*	*	*	/	/	*	*	*	*	*
42	*	*	*	*	*	*	*	*	*	/	*	*	*	*
48, 49, 50 ³	*	*	*	*	*	*	*	*	/	/	/	/	/	/
12	*	*	*	*	*	*	*	*	*	/	/	/	*	*
13	*	*	/	/	*	*	*	*	*	*	*	*	*	*
18	*	*	*	*	/	/	/	/	*	*	*	*	*	*
38, 39 ³	*	*	*	*	*	/	/	*	*	*	*	*	*	*
<i>DMD - Group 2</i>														
Patients no.														
16	*	*	*	*	*	*	*	*	/	*	*	*	*	*
27, 28 ³	*	*	/	*	*	*	*	*	*	*	*	*	*	*
47	*	*	*	/	/	/	/	/	/	*	*	*	*	*
2, 3 ³	*	*	*	*	*	*	*	*	*	*	/	*	*	*
6	*	*	*	*	*	*	*	*	*	/	/	*	*	*
10	*	/	/	/	/	*	*	*	*	*	*	*	*	*
<i>DMD - Group 3</i>														
Patients no.														
46	*	*	*	*	*	*	*	*	*	*	*	*	/	/
14	*	*	*	*	*	*	*	/	*	*	*	*	*	*
<i>BMD - Group 4</i>														
Patient no.														
7	*	/	*	*	*	*	*	*	*	*	*	*	*	*

¹*: without deletion; /: with deletion; ²Mp: muscular promotor; numbers refer to exon identification; ³brothers.

DMD: Duchenne Muscular Dystrophy; BMD: Becker Muscular Dystrophy.

investigated. The largest deletions occurred in patient number 47 (encompassing exons 12-44, 33 exons), and case no. 18 (exons 13-43, 31 exons). Note that while the deletions were almost identical, no. 47 showed a mild, and no. 18 a severe clinical picture. Taking into consideration the structure of the exons of the dystrophin gene reproduced in Roberts *et al.* (1993), it is possible to infer that at least three of the deletions in the severe DMD group certainly disrupted the reading frame (those observed in patients nos. 5, 12 and 18), the same being true for that found in patient no. 16, showing moderate DMD. The percentage of cases with deletions was higher in group 1 (65%) than in group 2

(43%), but the difference is statistically non-significant, possibly may be due to the small numbers involved.

Further analyses concerning our findings are presented in Table III. No clear relationships could be inferred between the severity of the phenotype or the intelligence level and the deletion site (proximal or distal), or when we compared sporadic vs familial cases. Analysis of deletion type/intelligence levels showed that although no patient achieved the very superior classification three subjects were classified as having superior intelligence (group II). Interestingly, one of them (no. 47) is the previously mentioned patient with the largest observed deletion in the present series. A

Table III - Deletion distribution according to clinical group, type of case (whether sporadic or familial) and intelligence level.

Characteristics		Without deletion	Classes of deletions			Total
			Proximal	Distal	Both	
<i>Clinical groups</i>						
DMD - Group 1	(n)	6	3	7	1	11
	(%)	35	27	64	9	65
DMD - Group 2	(n)	8	2	3	1	6
	(%)	57	33	50	17	43
DMD - Group 3	(n)	2	0	2	0	2
	(%)	50	0	100	0	50
BMD - Group 4	(n)	5	1	0	0	1
	(%)	83	100	0	0	17
<i>Type of cases</i>						
Sporadic	(n)	16	4	8	2	30
	(%)	53	29	57	14	47
Familial	(n)	5	2	4	0	6
	(%)	45	33	67	0	55
<i>Intelligence</i>						
Group II	(n)	1	1	0	1	2
	(%)	33	50	0	50	67
Group III	(n)	4	2	4	0	6
	(%)	40	33	67	0	60
Group IV	(n)	4	2	3	0	5
	(%)	44	40	60	0	56
Group V	(n)	6	1	1	1	3
	(%)	67	33	33	33	33

DMD: Duchenne Muscular Dystrophy; BMD: Becker Muscular Dystrophy.

Clinical groups: 1, Severe DMD; 2, Moderate DMD; 3, Intermediate DMD; 4, Becker Muscular Dystrophy or BMD.

Intelligence classification: I, very superior; II, superior; III, average; IV, borderline; V, mentally retarded.

previously reported association between mental retardation and deletion in exon 52 (Rapaport *et al.*, 1991) could not be confirmed here: the two patients with deletions in this exon who had their mental ability tested (nos. 49 and 46) were classified respectively as having borderline and average intelligence. Moreover, different performances in the intelligence tests were observed in affected monozygotic twins (patients nos. 2 and 3, intelligence classes III and V), in brothers who had the same deletion pattern (nos. 38 (II) and 39 (III)), and in brothers without detectable deletions in the examined exons (nos. 23 (III), 24 (V), and 25 (IV)).

DISCUSSION

How representative is the sample considered here in relation to the total number of DMD and BMD

patients living in Rio Grande do Sul, the area most thoroughly covered here? Knowing the total male population up to 20 years of age of the state, and considering the fact that persons affected by DMD have a mortality rate 2x higher than normal subjects (Emery, 1993), it is easy to verify this question. These calculations, performed elsewhere (Alho, 1994), indicated that we have studied 46% of the affected individuals living in Porto Alegre and 13% of those inhabiting the interior of the State. Therefore, at least in relation to Porto Alegre, sampling should be considered satisfactory. The ethnic prevalence exactly matches that of the total State population (in both cases, 12% Afro-Brazilians), but it is possible that families with a high socioeconomic level are over-represented (Alho, 1994).

The prevalence of deletions found in the present series (49%) is within the range (39% to 61%)

observed in eight other series studied with the PCR method in Brazil, USA, Turkey and Japan, reviewed in Alho (1994).

Among the six patients with Becker muscular dystrophy studied here only one (no. 7) showed a deletion, which included exon 4 and could have involved six other exons (1 to 7). This deletion occurred at the N-terminal region, and changes in this region are generally correlated with a more severe BMD phenotype. This is the prognosis for this patient, who had 16 years of age at the time of the study.

Our results concerning the relationships between deletion site, clinical severity, familial recurrence and intelligence level were largely inconclusive, perhaps due to the small number of patients studied. However, the percentage of mentally impaired patients in our series (29%) is quite similar to the prevalences obtained by Worden and Vignos (1962; 29%), Cohen *et al.* (1968; 22%) and Leibowitz and Dubowitz (1981; 21%). Other reports, however, presented much higher prevalences (74%, Dubowitz, 1965; 77%, Florek and Korolak, 1977). Such frequencies depend in a complex way on degrees of ascertainment and types of intelligence tests used. We verified, for instance, that the uncritical application of the Wechsler scale to Brazilian children may lead to erroneous results (Alho, 1994).

The eventual confirmation or disproof that deletions in the proximal and distal regions of the dystrophin gene are related to familial and isolated cases, respectively, (Passos-Bueno *et al.*, 1992) has more than academic interest, since if it is true it would lead to different recurrence-risk estimates.

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RESUMO

Um total de 50 pacientes afetados pelas distrofias de Duchenne (DMD) ou Becker (BMD), pertencentes a 41 famílias não relacionadas, foi investigado com relação a deleções no promotor muscular e em 13 exons localizados em duas regiões com alta variabilidade do gene da distrofina. Vinte dos 41 probandos (49%) apresentaram deleções. Nenhuma ocorreu no promotor muscular, mas pelo menos duas foram observadas em cada um dos exons estudados. O paciente com a maior deleção identificada (exons 12-44) mostrou um quadro clínico apenas moderado de distrofia muscular de Duchenne (DMD). Três das deleções nos pacientes com DMD grave e uma naqueles com DMD moderada certamente perturbaram o quadro de leitura. Nenhuma relação clara pôde ser inferida nas comparações entre os tipos de deleções versus (a) gravidade da doença; (b) casos esporádicos e familiares; e (c) níveis de inteligência.

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