

FREQUENCY AND PATTERN OF DNA DELETIONS IN DUCHENNE (DMD) AND BECKER (BMD) PATIENTS: COMPARISON OF DIFFERENT RACIAL BACKGROUND AND ISOLATED VERSUS INHERITED CASES

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ABSTRACT

A total of 164 unrelated Duchenne (DMD) and 21 Becker (BMD) patients from different racial backgrounds were screened for DNA deletions in the central region (probes cf23a and cf56a) of the dystrophin gene in order to determine if the frequencies of deletions differ in isolated as compared with inherited cases or in patients from different racial backgrounds (caucasoids and negroids). Among DMD patients 51 had affected relatives in their families, 107 were isolated cases and 6 were adopted children. DNA deletions were found in 42.7% of the patients (70/164) with no significant difference in frequency between caucasoids and negroids. However, a significantly greater ($P < 0.02$) proportion of deletions (51.4%) was observed in isolated as compared with inherited cases (29.4%). Eight (38%) deletions were detected in BMD cases, all starting in cf23a region.

INTRODUCTION

Duchenne (DMD) and Becker (BMD) muscular dystrophies are allelic X-linked conditions caused by the absence (DMD) or an abnormality in quantity or quality (BMD) of the protein dystrophin (Kunkel *et al.*, 1986; Hoffman *et al.*, 1988). DMD has an incidence of 1 in 3000-4000 male births while BMD is approximately 10 times less

frequent (Moser, 1984). At least 2/3 of the cases are inherited through carrier mothers (Zatz, 1986).

About 40 to 70% DMD/BMD patients have a cDNA deletion at the dystrophin gene (Koenig *et al.*, 1987; Forrest *et al.*, 1987, 1988; Koenig *et al.*, 1989; Lindlöf *et al.*, 1989; Passos-Bueno *et al.*, 1990), while 6% have a DNA duplication (Hu *et al.*, 1990).

In a preliminary study by our group (Passos-Bueno *et al.*, 1990), 34 unrelated DMD and 12 BMD patients were screened for DNA deletions with probes cf23a and cf56a, which detect 70% of DNA deletions (Forrest *et al.*, 1988). An interesting finding, apparently not reported by others, was that the frequency of these deletions was significantly greater in isolated than in inherited cases. Such observation, if confirmed in a large sample, would suggest different mechanisms of mutation through maternal and paternal lines and would have important implications for estimation of heterozygosity risks for mothers of isolated cases.

The purposes of the present investigation were: a) to assess, in a large sample of DMD patients from different racial backgrounds (caucasoids and negroids), the frequency and pattern of DNA deletions, with cDNA probes cf23a and cf56a; b) to extend our previous study on the proportion of deletions in isolated as compared with inherited cases of DMD.

PATIENTS AND METHODS

A total of 164 unrelated DMD patients (including 34 from our preliminary study) and 21 BMD patients (12 from our preliminary study) were screened for DNA deletions with probes cf56a and cf23a. Patients were clinically classified as BMD when able to walk unassisted beyond age 16. In the group of DMD patients, 51 had affected relatives, 107 were isolated cases and 6 were adopted children. Among 158 who could be classified according to racial background, 108 were caucasoids, 45 negroids and 5 mongoloids.

In the group of Becker patients, 18 had a positive familial history of BMD and 3 were isolated cases; 11 were caucasoids, 6 negroids, 2 mongoloids and 2 could not be classified as to racial background.

All patients were diagnosed in the Muscular Dystrophy Center of São Paulo, University of São Paulo, Brazil. The diagnosis was established based on clinical examination and the course of the disease, including Vignos scale (Vignos and Archibald, 1960) and motor functional ability (Scott *et al.*, 1982), family history, grossly elevated serum creatine-kinase (CK) and pyruvate-kinase (PK) activities, typical electromyography and/or muscle biopsy. The majority of the patients had a muscle biopsy which showed histopathological changes typical of a myopathic process. All patients submitted to muscle biopsy since 1989 were assessed for dystrophin by immunohistochemistry or western blot to confirm the diagnosis of DMD or BMD.

Since isolated cases diagnosed as BMD might represent the autosomal recessive limb-girdle muscular dystrophy (LGMD), the 3 isolated Becker cases were included because: a) in two of them the mothers had an increased serum CK and PK levels; b) in the third patient, dystrophin studies showed a positive reaction through immunohistochemistry but a protein of abnormal molecular weight through western blot analysis, characteristic of Becker dystrophy (unpublished observation).

DNA was extracted from whole blood according to Kunkel *et al.* (1977) and digested with PstI, TaqI and/or HindIII. Restricted DNA was size fractionated by electrophoresis through 0.8% agarose gels transferred to hybrid membrane (Amersham N), according to Southern (1975). Probes were labelled with P³² by hexanucleotide primer (Feinberg and Vogelstein, 1983). Membranes were washed with 3XSSC and 0.1% SDS and exposed for 1 to 10 days at -70°C. DNA from all patients was hybridized with cDNA probes cf23a and cf56a (Forrest *et al.*, 1988) which correspond to probes 5b-7 and 8, respectively (Koenig *et al.*, 1987). In addition, in order to determine the extent of the deletions, the distal and proximal dystrophin cDNAs, cf77a (Forrest *et al.*, 1988) and Ca 1A (Cross *et al.*, 1987) were hybridized with the DNA of selected DMD boys who had deletions of exons 52 (N = 28) or 37 (N = 5), respectively.

RESULTS

Frequency and patterns of deletions

a) Duchenne patients

A total of 70 (42.7%) independent deletions was detected: 46 (66%) in the cf56a region, 10 (14%) in the cf23a region and 14 (20%) involving both regions, with a cluster of endpoints (24% or 17/70) in exon 47 (Figure 1). Three junction fragments were detected: two with probe cf56a and one with probe cf77a.

No statistically significant differences ($X^2 = 1.20$; $P > 0.05$) were found in the pattern or frequencies of DNA deletions among caucasoids (49/108 = 45%) and negroids (16/45 = 36%). Two among 5 mongoloids had DNA deletions, but this sample is not large enough to allow statistical analysis.

In 10 among the 28 patients who had deletions in the region encompassed by exon 52, the deletion extended toward region cf772; in at least 3 among the 5 cases who had deletions involving exon 40 (cf23a) these extended toward the 5' region.

The frequency of deletions among inherited and isolated cases was 29.4% (15/51) and 51.4% (55/107), respectively. This difference is statistically significant ($X^2 = 5.91$; $P < 0.02$).

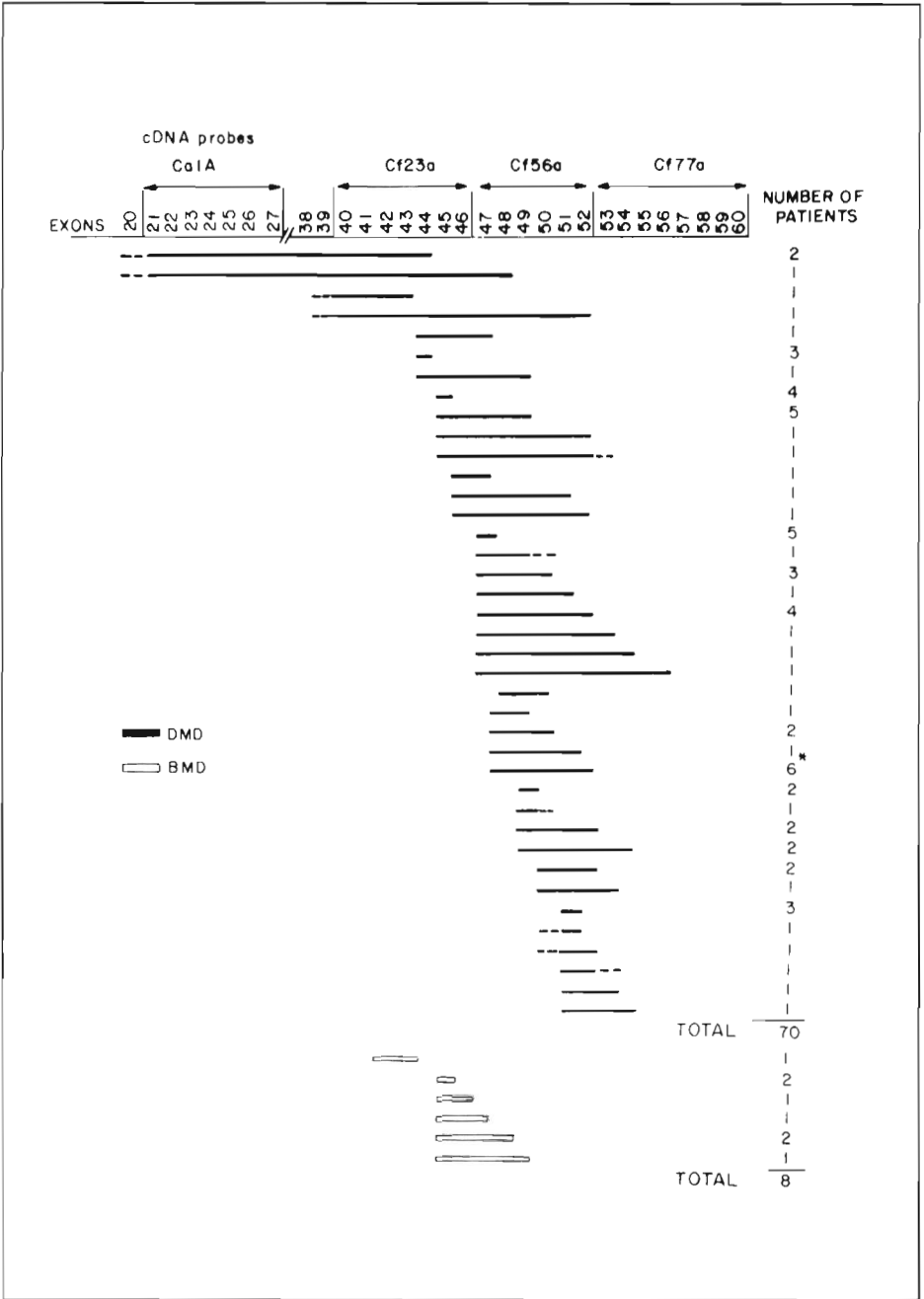


Figure 1 - Patterns of DNA deletions in DMD and BMD cases.

b) Becker patients

Among 21 BMD patients, 8 (38%) showed a DNA deletions all starting in the cf23a region; 4 extended to the region encompassed by cf56a, but none was detected exclusively in the cf56a region. No junction fragment was detected in any patient.

The frequency of DNA deletions (8/20 or 40%) in the group of BMD inherited cases (18 familial and 2 isolated cases with high serum enzyme levels in their mothers) did not differ significantly ($X^2 = 0.10$; $P > 0.05$) from that of DMD inherited cases (34.2%).

No deletion was found in two mongoloids. The frequency of deletions in the negroid patients (83% = 5/6) was higher than in caucasoids (27% = 3/11), but the sample is too small for statistical analysis.

DISCUSSION

The percentage of deletions in our DMD cases detected with probes cf23a and cf56a, the majority (86%) with probe cf56a, was similar to the one reported in previous studies with probes from the same regions (Koenig *et al.*, 1987; Forrest *et al.*, 1988; Read *et al.*, 1988; Speer *et al.*, 1989; Passos-Bueno *et al.*, 1990).

All deletions in our BMD patients began in the cf23a region, confirming previous observations (Forrest *et al.*, 1988), that BMD mutations are particularly concentrated in this region. On the other hand, among DMD boys, only 34% (24/70) of the detected deletions started in cf23a region. This difference was statistically significant ($Z = 5.64$; $P < 0.001$), in accordance with data from Gilgenkrantz *et al.* (1989).

An interesting observation, apparently not reported by others, was the difference in the proportion of DNA deletions in DMD inherited as compared with isolated cases. In the first study of Kunkel and co-authors (Kunkel *et al.*, 1986) the incidence of deletions detected with probe PERT87, although higher among DMD males with a clear family history of the disease (8.3%), was not statistically different ($X^2 = 2.8$; $P = 0.09$) from those with no family history (5.8%).

Lindlöf *et al.* (1989) did not find differences between the proportions of deletions in their study of isolated and familial cases. However, their sample included DMD and BMD patients.

In our previous report with a smaller sample, a significantly higher proportion of deletions was found among isolated DMD patients as compared with inherited DMD or BMD cases. This difference was confirmed in the present investigation in a total of 164 DMD cases, as well as the similar frequency of deletions among inherited DMD and BMD cases.

We are currently investigating if the same difference in frequency of DNA deletions in isolated as compared with inherited cases, observed in the present study, is found also for cDNA probes which detect deletions in other sites of the dystrophin gene. If confirmed, it might be the result of different mechanisms of mutation through maternal and paternal lines. If deletions (or duplications) arise more frequently in ova than in spermatozoa through different mechanisms such as unequal crossing-over restricted to female meiosis, it would explain their higher frequency in sporadic DMD patients than in familial cases. In fact, Grimm *et al.* (1988) had estimated, theoretically, that a deletion in the X-chromosome, generated by an intrachromosomal rearrangement would recur with a probability which is double in female than in male gametes. This finding was recently confirmed by Liechti-Gallati *et al.* (1990).

Such differences in frequency of DNA deletions may have important implications in estimating heterozygosity risks for mothers of isolated cases in which no DNA deletions are detected with these probes, since it would increase the recurrence risks for further pregnancies. For instance as illustrated in Zatz *et al.* (1991), a mother of an isolated boy, diagnosed as DMD, in which no DNA deletion is detected (in the central region of the gene) has greater risk of having another affected child than if a DNA deletion is found in her DMD son in that region. In addition, parents of patients in which no deletion is detected with any probe should be aware that they still have considerable risk for further pregnancies, in particular for the autosomal recessive "Duchenne-Like" forms (Vainzof *et al.*, 1991). In families with no detectable deletion in affected patients, the strategy for genetic counselling should be based on polymorphic DNA markers.

It would be interesting also to verify if the same difference in the proportion of DNA deletions is also found among sporadic as compared with inherited cases of BMD. In order to exclude a possible diagnosis of limb-girdle muscular dystrophy (LGMD) and polymyositis among isolated cases, X-linked inheritance should be confirmed (through serum enzyme determinations in all females at risk in each family) and affected patients should have their muscle dystrophin assessed through western blot analysis.

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RESUMO

Foram estudados 164 pacientes afetados por Distrofia Muscular tipo Duchenne (DMD) e 21 pacientes afetados por Distrofia Muscular tipo Becker (DMB), todos pertencentes a genealogias diferentes e a grupos raciais variados. A triagem de deleções de DNA foi feita na região central do gene (sondas Cf23a e Cf56a) da distrofina. Os pacientes foram classificados em dois grupos raciais (caucasóides e negróides) e a frequência de deleções entre os dois grupos foi comparada.

Dentre as genealogias com pacientes afetados por DMD, 51 tinham dois ou mais afetados, em 107 o probando era caso isolado e 6 eram filhos adotivos. A frequência de deleções encontrada foi de 42,7% (70/164), sem diferença significativa entre caucasóides e negróides. Porém, uma diferença significativa ($P < 0,02$) foi encontrada na frequência de deleções entre os casos isolados (51,4%) e os familiares (29,4%). Nos afetados por DMB a frequência de deleções foi de 38%, todas elas com início na região correspondente a sonda Cf23a.

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