Human Molecular Genetics, 1993, Vol. 2, No. 11 1945–1947

Genetic heterogeneity for Duchenne-like muscular dystrophy (DLMD) based on linkage and 50 DAG analysis

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Received July 26, 1993; Revised and Accepted September 7, 1993

Duchenne-like muscular dystrophy (DLMD) is an autosomal recessive (AR) muscular dystrophy which presents a clinical course indistinguishable from the Xp21 Duchenne muscular dystrophy or DMD. Recently, Othmane *et al.* (11), based on a linkage study with 13q12 markers in 3 highly inbred DLMD families from Tunisia, suggested that the gene for this myopathy lies in the pericentromeric region of chromosome 13q. It is unknown if there is genetic heterogeneity causing the DLMD phenotype. Therefore, the aim of the present report is to describe the results of linkage analysis in 4 Brazilian DLMD families with 13q12 markers (D13S115 and D13S120), which were also tested for 50DAG. It was possible to exclude the 13q gene at $\theta = 0.10$ as responsible for the DLMD phenotype in our families using both 13q12 markers, if the lod scores of each family were added up. Interestingly, 3 families were deficient for 50 DAG while one showed a positive pattern for this glycoprotein. Therefore, these results suggest: a) the DLMD phenotype is caused by more than one recessive gene; b) a gene, not located at 13q, causes deficiency of 50 DAG as a primary or secondary defect.

INTRODUCTION

The term Duchenne-like muscular dystrophy (DLMD) or severe childhood autosomal recessive muscular dystrophy (SCARMD) has been used to describe male and female patients with a form of autosomal recessive (AR) muscular dystrophy which presents a clinical course indistinguishable from the Xp21 Duchenne muscular dystrophy or DMD (1, 2). The distinction between DLMD or DMD when the proband is an isolated male patient or if he has only affected brothers, depends on DNA and dystrophin analysis (3, 4).

The frequency of DLMD seems to vary among different populations. It is relatively frequent in countries where the rate of consanguinity is high, such as Algeria and Tunisia (5, 6). In other countries, it seems to be a rare disease: in Brazil, it has been estimated that 6–8% of families with the Duchenne phenotype are due to an AR gene (2), while in Britain and North America it is apparently twenty times less common than the Xp21 form (7).

Due to the phenotypic resemblance between X-linked DMD and DLMD it has been first proposed that the dystrophin-related locus, mapped at 6q24 (8, 9), might be a candidate gene for this AR condition. However, based on linkage studies in Brazilian (10) and Tunisian families (11) and on the analysis of the expression of the dystrophin-related protein in patients with DLMD (12), the 6q24 gene was excluded as a candidate gene for this form of muscular dystrophy. In addition, the 15q gene which was found to be responsible for a mild form of Limb-girdle muscular dystrophy (LGMD), has also been excluded as the cause for the DLMD phenotype in Brazilian (13) and Tunisian families (11).

Recently, Othmane *et al.* (11), based on a linkage study of 135 microsatellite markers in 3 highly inbred DLMD families from Tunisia, reported a significant lod score with the D13S115 13q12 marker ($Z = 9.15$; $\theta = 0.03$); positive lod scores were also observed with two other markers in this region, D13S143 ($Z = 8.36$; $\theta = 0.03$) and D13S120 ($Z = 2.65$; $\theta = 0.16$), suggesting that the primary DLMD defect gene lies in the pericentromeric region of chromosome 13q.

However, it is unknown if there is one or more genes responsible for the DLMD phenotype in all the families around the world. The aim of the present report is to describe the results of linkage analysis with 13q12 markers in Brazilian DLMD families, who were also analysed for 50 DAG deficiency, in order to investigate the possibility of genetic heterogeneity.

RESULTS

The lod scores results for the two-point linkage analysis estimated for each family are described in Table 1. All families showed negative lod-scores with the two markers. If the lod scores of each family are added up, it is possible to exclude the 13q gene (at $\theta = 0.10$) as responsible for the DLMD using both 13q12 markers. With the marker D13S115 it is possible the exclusion

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Table 1. Two point lod scores for DLMD and chromosome 13q markers

	D13S115 Recombination fraction (θ)						D13SI20 Recombination fraction (θ)					
Family	0.0	0.01	0.05	0.10	0.20	0.30	0.0	0.01	0.05	0.10	0.20	0.30
a	$-\infty$	−3.32	−1.38	−0.67	−0.16	−0.01	$-\infty$	−2.64	−1.33	−0.80	−0.35	−0.14
b	$-\infty$	−2.21	−0.95	−0.52	−0.22	−0.11	$-\infty$	−2.20	−0.95	−0.52	−0.22	−0.11
c	$-\infty$	−2.80	−1.44	−0.89	−0.39	−0.15	$-\infty$	−1.06	−0.42	−0.19	−0.03	0.003
d	−339	−2.19	−1.14	−0.65	−0.22	−0.06	$-\infty$	−2.46	−1.10	−0.57	−0.16	0.0
total	∞	−10.5	−4.91	−2.73	−0.99	−0.33	$-\infty$	−8.36	−3.80	−2.08	−0.76	−0.29

at $\theta = 0.01$ for the 4 families, while with the marker D13S120 it is possible the exclusion at $\theta = 0.01$ for 3 of the 4 DLMD families.

DISCUSSION

The recent use of molecular biology techniques is revealing that genetic heterogeneity is apparently frequent for genetic human diseases. The AR forms of limb-girdle muscular dystrophies are characterized by a great variability in the clinical course, which can be due to alielic and/or loci genetic heterogeneity. The fact that there is no description of genealogies with severe (or DLMD) and mild LGMD patients in the same sibship, support this hypothesis.

Results of linkage studies in Brazilian LGMD families with markers close to the 15q gene suggest loci heterogeneity for the mild form of LGMD. In addition, the more severe and the benign forms of LGMD dystrophy are apparently caused by different non-allelic genes (13, 14).

The order of the 13q markers that showed linkage to the DLMD gene in families from Tunisia is still not established: D13S115 and D13S120 are 15−17 cM apart, and D13S143 has not been mapped in relation to any of these markers. Apparently the DLMD gene is closer to D13S115 and D13S143, with a maximum lod score at $\theta = 0.03$ (11). Although the size of our families is not sufficient to allow for a lod-score of −2.0 for each one independently at $\theta = 0.03$ with the marker D13S115, the results of linkage analysis between D13S115, D13S120 and the disease gene in our families strongly suggest that the DLMD phenotype in Brazilian genealogies is mainly caused by another gene (or genes).

Searching the biochemical defect causing the DLMD phenotype will be very important for the understanding of the mechanisms involved in its pathogenesis. Recently, Matsumara *et al.* (15), reported that the 50 kda glycoprotein, which is an integral component of the dystrophin complex, is absent in muscle from DLMD patients from Argelia. Antibodies against this glycoprotein have been tested in 10 Brazilian DLMD patients from 9 unrelated families (including the four genealogies from the present report): in 6 unrelated patients a negative reaction of 50 DAG was observed through imunocytochemistry while the others dystrophin-associated glycoproteins were present, suggesting genetic heterogeneity at the protein level for this severe condition in our population (16).

Interestingly, in the four present Brazilian DLMD families apparently unlinked to the 13q markers, 3 were deficient for 50 DAG (families a, b and d) while one was positive for this glycoprotein (family c).

Taking into account the present results of linkage as well as 50 DAG analysis, the following hypothesis are raised: a) DLMD phenotype is caused by more than one gene; b) a gene, not located at 13q, causes deficiency of 50 DAG as a primary or secondary defect. Therefore, if 50 DAG deficiency is found in patients linked to the 13q gene, it will be very important to assess if it represents the primary defect of this gene.

METHODOLOGY

Family data

Four families (including 12 patients and 33 unaffected relatives) who were ascertained in the Centro de Miopatias, Instituto de Biociências, Universidade de São Paulo, were included in this study. These families, including 2 consanguineous (a, d) and 2 non-consanguineous(b,c) genealogies, have been previously described (13). Diagnosis was based on clinical examination and course of the disease, family history, grossly elevated serum creatine-kinase levels, DNA analysis through polymerase chain reaction using 18 pairs of primers for the dystrophin gene (17, 18) and assessment of dystrophin (through IF and Western blot) in muscle biopsies. Clinical details are described elsewhere (16).

DNA analysis

DNA was extracted from whole blood, according to the method of Miller et al (19). Individuals were genotyped using the microsatellites D13SI15 and D13SI20 (20), which are about 15−17 cM apart (I 1). PCR was performed using 60 ng of genomic DNA and the PCR reaction was done as previously reported (13). Two to four μl of each reaction product were separated in 6.5% denaturing polyacrylamide gels for 3−4 hs. The gels were dried and exposed to X ray films for 1−2 days.

Linkage analysis

Two point linkage analysis between the disease gene and each of the 13q markers was performed using the computer program Linkage (21). The gene frequency for the DLMD allele was considered as 0.001. The recombination rate was assumed to be equal in males and females.

ACKNOWLEDGEMENTS

The authors are very grateful to Simone Campiotto, Antonia M.P.Cerqueira, Marta Canovas, Dr Rita C.Pavanello, Dr Ivo Panavello and Dr Ana Maria Tsanaclis; to Dr Anne Bowcock and Dr Kamel Ben Othmane for providing the sequences of the primers; also, for financial support from FAPESP, CNPq, ABDIM and MOA. KPC is an investigator of the Howard Hughes Medical Institute.

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