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Severe Nonspecific X-Linked Mental Retardation Caused by a Proximally Xp Located Gene: Intragenic Heterogeneity or a New Form of X-Linked Mental Retardation?

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X-linked mental retardation (XLMR) can be subdivided into syndromic and nonsyndromic or nonspecific. Patients with nonsyndromal XLMR show no characteristic manifestations, biochemical defects, or distinct fragile sites. Nevertheless, nonspecific XLMR seems to be heterogeneous. To determine the number and location of the genes responsible for XLMR, linkage studies in large pedigrees have to be performed. Here we report the data of linkage analysis in a large Brazilian family with 7 patients affected by a severe form of XLMR, with no other associated malformations. All the obligate carriers are normal. A close linkage without recombination (lod scores 1.95 and 3.25) was found between the disease locus and polymorphic DNA loci DXS255 (Xp11.22), DXS14 (Xp11.21). These results suggest that the gene responsible for the disease in this family maps in the Xp11-cent of the X chromosome. Positive lod scores in this region have also been reported for other XLMR genealogies, but with a much milder phenotype. The possibility of intragenic or locus heterogeneity is discussed. © 1993 Wiley-Liss, Inc.

KEY WORDS: linkage analysis, genetic heterogeneity, mental retardation

INTRODUCTION

X-linked mental retardation (XLMR) represents one of the commonest genetic disorders [Sutherland and Hecht, 1985; Partington, 1986]. It has been estimated that there are at least 70 X-linked conditions in which mental retardation (MR) is the main manifestation: about 62 syndromal and 15 nonsyndromal or nonspecific, depending on the existence of a recognizable pattern or minor abnormalities and/or malformations [Neri et al., 1992].

XLMR seems to be genetically heterogeneous. In order to determine the number and location of the genes responsible for such phenotype, and if there is genetic heterogeneity, linkage studies in large pedigrees have to be performed. Here we report data from linkage analysis in a large Brazilian family in which affected patients have a severe form of XLMR, with a phenotype that apparently has not been published yet.

FAMILY AND CLINICAL DATA

The pedigree of the family, with 7 affected male patients in 2 generations and 20 normal (12 females and 8 males) individuals, is typical of X-linked recessive inheritance (Fig. 1). Except for subject II-6, already deceased, all patients were examined by us. The patients were first seen in 1978 and reevaluated recently.

The phenotype is similar in all cases (Figs. 2, 3). They are apparently normal at birth. However, they are never able to hold their head against gravity, to sit unsupported, to walk or to speak, and they all have urinary and fecal incontinence. The condition is not progressive and is apparently compatible with normal longevity since the oldest patient (II-4) is currently age 47 years.

At clinical examination a severe generalized muscle atrophy is observed in all patients, probably as a result of total physical inactivity. No malformations are identified: the head circumference and testicular volumes are normal; vision and audition are apparently normal and

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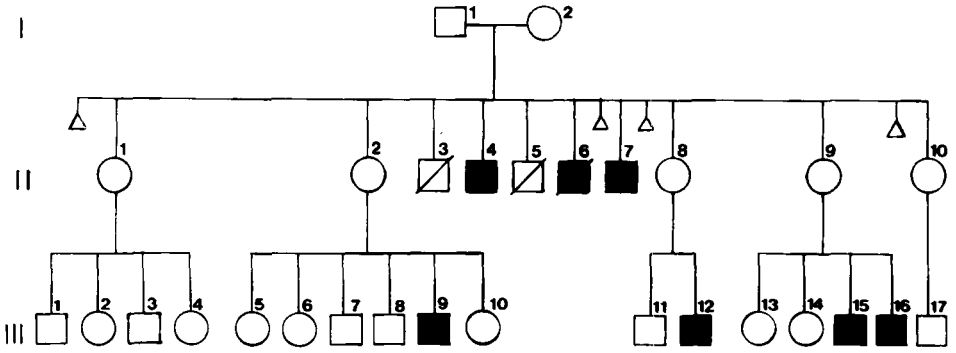

Fig. 1. Family pedigree.

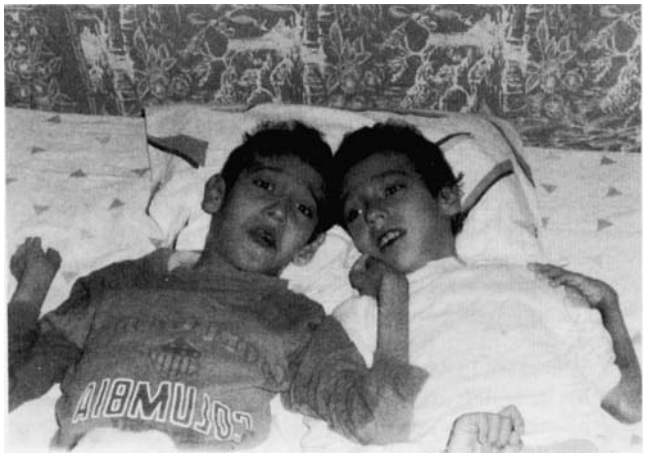

Fig. 2. Affected brothers: III-15, aged 13 years, and III-16, aged 11 years.

Fig. 3. A: Affected brothers II-4, II-6, and II-7 at childhood. B: Affected brothers II-4, II-6, and II-7 at age 42 years, 38 years, and 36 years, respectively.

they seem to be responsive to environment modifications.

Laboratory evaluation, all with normal results, included: biochemical tests for screening of inborn error of metabolism (uric acid, copper, iron, ceruloplasmin, lipids); serum creatine-kinase and pyruvate-kinase; computed tomographic (CT) scan; and nerve biopsy (performed in individual III-12).

A muscle biopsy, performed in patients III-15 and III-16, at age 10 and 11 years, respectively, showed a normal fiber structure and differentiation with no muscle degeneration, but with atrophy of type I and II fibers. There was no sign of a denervation process.

MATERIALS AND METHODS

Genomic DNA was extracted from venous blood from all living relatives by standard procedures. A total of 16 polymorphic X-chromosomal DNA markers (Table I) were tested. Except for the 5'DYSI polymorphism, all the others were analyzed through Southern blotting followed by hybridization of the probe labelled with P32 through the random primer method. The methodology used has been described previously [Passos-Bueno et al., 1990]. The polymorphism 5'DYSI was analyzed through polymerase chain reaction (PCR) according to the conditions published in Feener et al. [1991].

Two-point linkage analysis between each marker and the disease gene was performed using the computer program Linkage [Lathrop et al., 1984].

TABLE I. Polymorphic X-Chromosomal DNA Markers Used for the Linkage Analysis in This Study

Localization	Locus symbol	Probe	Enzyme
Xp21.3-p21.1	DMD	cf56a	PstI/TaqI
Xp21.3-p21.1	DMD	cf23a	PstI/TaqI
Xp21.2	DXS269	p20	EcoRV
Xp21.2	DYS	5'DYSI	PCR
Xp21.2	DXS84	754.11	BglII
Xp21.1	DSX84	L754	EcoRI
Xp11.4-11.3	DXS7	L1.28	TaqI
Xp11.22	DSX255	M27B	EcoRI
Xp11.21	DSX14	p58.1	MspI
Xq21.31	DXYS1X	pDP34	TaqI
Xq26	DXS10	36B-2	TaqI
Xq26-qter	DXS294	VK17	TaqI
Xq27.1-27.2	DXS105	cx55.7	TaqI
Xq28	F8C	ProbeC	BglI
Xq28	DXS52	St14.1	TaqI
Xq28	DXS274	p1A1.1	PstI

RESULTS

Family history suggested a new mutation in I-2.

Two-point linkage analysis (Table II) shows the results of 13 informative probes.

The region near the fragile X site (Xq26-q28) was excluded. No recombination was detected between the disease locus and the probes p58.1, M27B, and 754.11. Since I-2 is homozygous for the last 2 markers, the maximum lod scores gave lower estimates. However, with the locus DXS84 and probe L754 it was possible to identify 2 recombinants (II-1 and II-4) and a maximum lod score of 1.06 at $\hat{\theta} = 0.15$ was calculated. Recombinants were also identified with the other more distal loci at Xp21, such as 5'DYSI and DXS269. Since we also observed recombinants with the locus DXYS1X, the results suggest that the most likely location of this gene is between the locus DXS14 and the centromere.

DISCUSSION

The severe XLMR reported in the present investigation, probably resulting from a new mutation in I-2, has apparently not been described before. The most similar condition was reported in a genealogy with 24 affected males, with marked speech defects, muscle atrophy, and a nonprogressive severe form of MR [Allan et al., 1944]. This form of MR was considered congenital cerebellar hypoplasia/MR syndrome and has been classified as Allan-Herndon-Dudley syndrome (AHDS), McKusick 30960 [Opitz and Sutherland, 1984]. This family, in which 29 affected males were identified, has recently been reevaluated [Stevenson et al., 1990]. Molecular analysis showed linkage to Xq21 [Schwartz et al., 1990]. Two other families with similar clinical features to AHDS have been reported [Davis et al., 1981; Bundey et al., 1991]. Linkage analysis performed in one of them [Bialer et al., 1992] suggested that the gene responsible for the MR in this family was located at Xq12-q21, supporting that these patients also have AHDS. Linkage analysis in the family here reported, with the highest lod score at Xp11.21, suggests that it represents a different entity, which we classified as a form of nonspecific MR. This is also supported by the findings of a normal cerebellum on CT scan.

The mapping of the present gene at Xp11-cent is noteworthy since there are other reports of families with MR who have also shown positive lod scores with probes from the same region, including syndromic and nonsyndromic XLMR [Watty et al., 1991].

Suthers et al. [1988] reported a nonspecific XLMR pedigree in which a maximum lod score of 2.12 at $\hat{\theta} = 0$ was observed with the same marker locus DXS14. However, although the only major clinical symptom of the affected males was also MR, it was considered as mild. This family has been recently restudied and its gene location was more precisely defined [Kerr et al., 1992]. Arveiller et al. [1988], based on the study of 2 unrelated families in which affected males with nonspecific XLMR had different phenotypes, suggested the location of 2 separate genes for MR: one at Xq12-13 and the other at Xp22.2-p22.3. In the first family, the only significant clinical finding was a moderate MR. In the second one, male patients had short stature, high palate and bilateral macroorchidism, with variability in the expression of MR as well as slight impairment in some of the obligate carriers.

Glass et al. [1991] suggested the existence of a locus for MR at the distal part of the X chromosome, based on a study of a family with XLMR in which the main clinical sign was moderate MR but with great variability among affected individuals. Sammans et al. [1991] also identified a gene in this region (Xp11.4-q13) in a family with 5

TABLE II. Two-Point Analysis Between the Disease Gene and Each Marker

Locus	Probe	0	0.05	0.10	0.15	0.20	0.25	0.3	0.4
DXS269	p20	$-\infty$	0.37	0.54	0.57	0.55	0.50	0.43	0.20
DYS	5'DYS	$-\infty$	0.66	1.02	1.11	1.09	1.0	0.87	0.48
DXS84	L754	$-\infty$	0.55	0.94	1.06	1.06	0.99	0.87	0.51
DXS84	754.11	2.10	1.95	1.79	1.61	1.43	1.23	1.02	0.55
DXS255	M27B	1.98	1.85	1.70	1.54	1.37	1.19	0.99	0.54
DXS14	p58.1	3.66	3.37	3.05	2.73	2.38	2.01	1.61	0.77
DXYS1X	pDP34	$-\infty$	−1.58	−0.82	−0.44	−0.22	−0.09	−0.01	0.03
DXS10	36B-2	$-\infty$	−3.72	−2.54	−1.86	−1.39	−1.03	−0.74	−0.31
DXS294	VK17	$-\infty$	−2.33	−1.48	−1.02	−0.71	−0.48	−0.32	−0.10
DXS105	cx55.7	$-\infty$	−2.42	−1.56	−1.09	−0.77	−0.54	−0.37	−0.10
F8C	ProbeC	$-\infty$	−0.63	−0.16	0.04	0.14	0.18	0.17	0.08
DXS52	St14.1	$-\infty$	−0.02	0.42	0.61	0.67	0.67	0.62	0.38
DXS374	p1A1.1	$-\infty$	−0.02	0.42	0.61	0.67	0.67	0.62	0.38

affected male patients in which the main manifestations were moderate MR, hyperactivity, verbal disability, and hypotonia. In both cases, the obligate carriers were normal.

Kerr et al. [1992] have reported 4 new families with XLMR. The main manifestation was MR, which varies within families but was most often moderate. Females with unexplained retardation in 3 of these families were also observed. In all of them the most likely location is at the pericentromeric region of the X chromosome.

Linkage analysis in the above families suggests the existence of at least 3 loci responsible for nonspecific XLMR: one at Xp22.1-22.2, another at Xp11.3-q13, and the third one at Xq12-q22. With exception of the family in which the gene is probably located at Xp22, in the others MR with variable expression was the main significant abnormal finding.

In our family, although more severe, MR is also the only sign. Therefore, it could be due to a mutation in one of the above loci with intragenic heterogeneity leading to a more benign or severe phenotype, as it is known to occur for other diseases such as the Xp21 muscular dystrophies [Kunkel et al., 1986; Davies et al., 1988]. Alternatively, the MR in our family could be caused by a mutation in a different locus nearby or even contiguous to that at Xp11.3-q13. Another possibility is the existence of a small deletion (in the same or in a nearby gene) which would be responsible for the abnormal phenotype in our family.

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