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A Gene for Nonspecific X-Linked Mental Retardation (MRX41) Is Located in the Distal Segment of Xq28

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We report on a family in which nonsyndromal mild to moderate mental retardation segregates as an X-linked trait (MRX41). Two point linkage analysis demonstrated linkage between the disorder and marker DXS3 in Xq21.33 with a lod score of 2.56 at θ = 0.0 and marker DXS1108 in Xq28 with a lod score of 3.82 at θ = 0.0. Multipoint linkage analysis showed that the odds for a location of the gene in Xq28 vs Xq21.33 are 100:1. This is the fourth family with nonspecific X-linked mental retardation with Xq28-qter as the most likely gene localization.

KEY WORDS: nonspecific X-linked mental retardation, linkage analysis, Xq28, MRX41

INTRODUCTION

X-linked mental retardation (XLMR) occurs with a frequency of about 1/500 males, whereas 2.5/1,000 women are carrier of a mutation for XLMR [Turner and Turner, 1974; Herbst and Miller, 1980]. Neri et al. [1994] listed 127 different XLMR conditions in five categories. The category "Nonspecific XLMR" contained 19 genes, of which 18 have been mapped and 1 has been cloned (FRAXE). Several other families with nonspecific XLMR have been reported since [Gendrot et al., 1994; Baraitser et al., 1995; Lazzarini et al., 1995; Martinez et al., 1995]. Here we report the results of linkage analysis of a large family with nonspecific XLMR, in which the gene maps to the tip of the long arm (MRX41).

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MATERIALS AND METHODS

Clinical Report

In a three generation family (Fig. 1) eight males are mentally retarded. Pregnancy and delivery were uneventful in all. The retardation became apparent in the first years of life and was non-progressive. The mental retardation ranged from mild to moderate, but it could not be quantified by IQ-testing (see below). None of the affected males is institutionalized, but all attended special schools for children with (severe) learning problems. They all live and work in a sheltered environment. There was no consistent clinical phenotype other than the mental retardation. Obvious neurological symptoms and signs were not present. Height, weight, and OFC were all within normal limits. Their behaviour is unremarkable. Unfortunately, further investigations were refused by caretakers. All obligate and possible carriers were of normal intelligence. Cytogenetic analysis of several affected males and molecular study of the FMR-1 gene gave normal results.

Genetic Analysis

From all patients and relevant relatives venous blood was sampled and DNA was isolated according to the procedure of Miller [1988]. Markers were analysed by the amplification of 50 ng of genomic DNA with the appropriate primers (GDB; Isogen Bioscience BV, The Netherlands). Amplification involved 35 cycles of 1 min at 94°C, 2 min at 55°C, and 3 min 72°C, which was carried out in a 15 μl reaction mixture containing 0.06 U Supertaq in 1× Supertaq buffer (HT Biotechnology Ltd, England) and in the presence of ^{32}P-dCTP. Subsequently, labeled fragments were separated on 6.6% denaturing polyacrylamide gels. After electrophoresis, gels were exposed overnight to Kodak X-omat S film to visualize the allelic bands.

Linkage data were evaluated with the program LINKAGE [Lathrop et al., 1985, version 5.1] using the Mlink and Linkmap options. Calculations were based on complete penetrance and a disease allele frequency of 0.0001. Consecutive five-point linkage analysis was

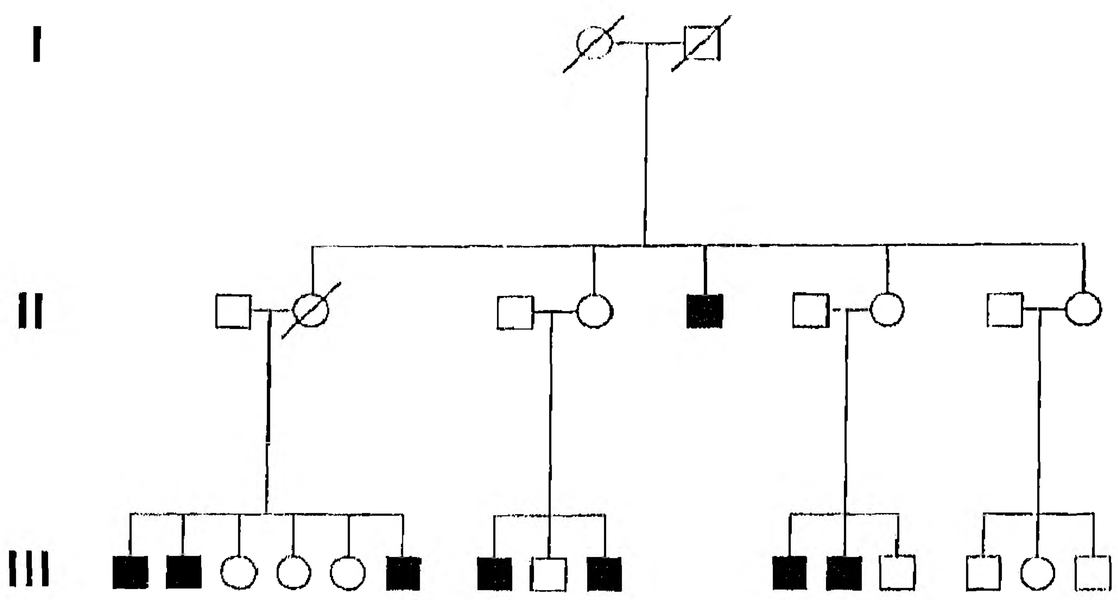

Fig. 1. Pedigree.

performed to construct the multipoint map depicted in Figure 2. Map locations, genetic distances, and allele frequencies of the marker loci were obtained from the Genome Database and from the report by Willard et al. [1994]. The total length of the X chromosome was estimated at 220 cM.

RESULTS

From the pedigree and clinical data it can be concluded that we are dealing with a nonspecific X-linked mental retardation.

To determine the location of the gene for nonspecific XLMR in the present family, linkage analysis was performed with more than 30 highly polymorphic markers distributed along the entire X chromosome. Of 24 informative markers, only three gave a positive lod score at $\theta = 0.0$ (Table I). A maximum lod score of $Z = 3.82$ was obtained with marker DXS1108 and a lower but still significant lod score of $Z = 2.56$ with DXS3. Additional information about the exact location of the gene was then pursued by the construction of a multipoint linkage map encompassing the entire X chromosome (Fig. 2). This showed that the odds for a location of the responsible gene in Xq28 vs Xq21.33 are 100:1. Marker DXS1108 was completely informative in our family, but for marker DXS3 the grandmother I.1 appeared to be homozygous. A detailed haplotype analysis with two closely flanking markers for DXS3, i.e., CHM and DXS990, further indicates genetic recombination between this region and the disorder (data not shown). Therefore, we conclude that the gene which is responsible for the nonspecific XLMR in the present family is located distal to DXS52 in Xq28-qter, a region spanning about 3 Mb.

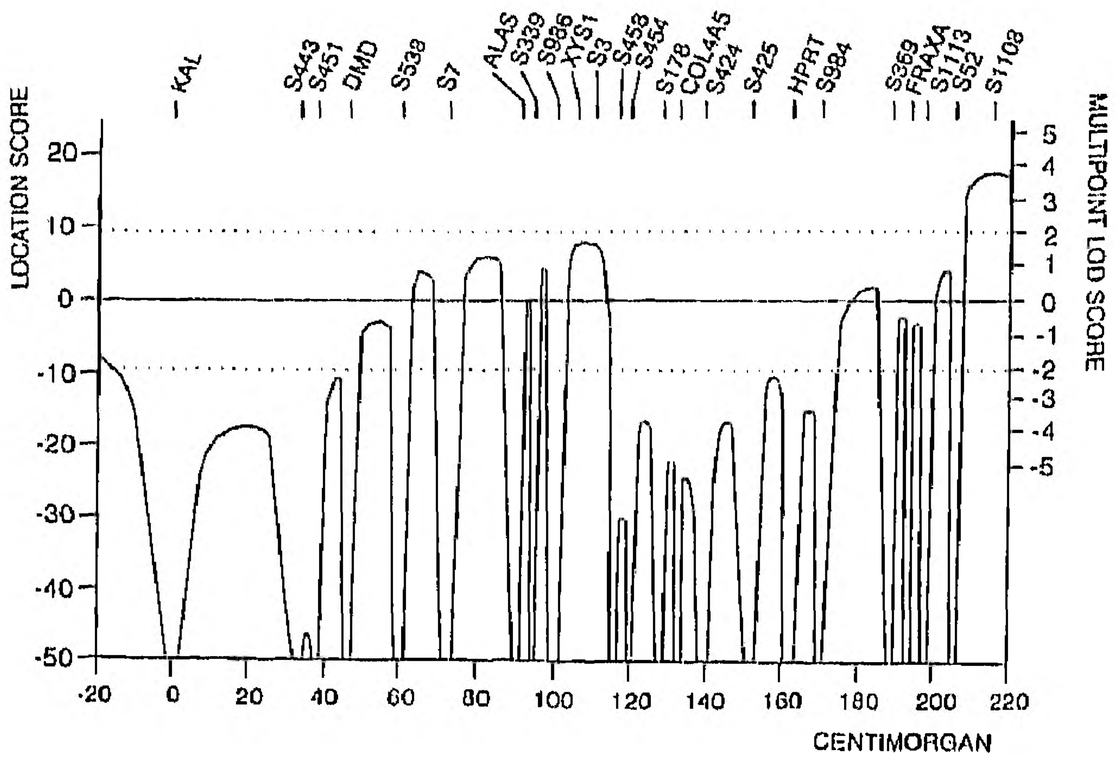

Fig. 2. Multipoint linkage analysis with microsatellite markers along the entire X chromosome.

DISCUSSION

So far, genes in three families with nonspecific X-linked mental retardation have been assigned to the region Xq28-qter. The MRX 3 gene [Gedeon et al., 1991] was localised by linkage analysis to this region with a maximum lod score of 2.89 with DXS52 at $\theta = 0.0$. Affected males had upper moderate to mild intellectual disability and the most prominent clinical trait was their aggressive and difficult-to-manage behaviour. Female carriers were normal. In the family described by Nordström et al. [1992] the gene was also assigned to the region Xq28-qter with a maximum lod score of 2.52 with DXS52 at $\theta = 0.0$. The three affected males showed all profound and the three affected females moderate mental retardation. Behaviour characteristics were not reported. In the third family [Holinski-Feder et al., 1995] no recombinants were found with the markers DXS52 and STR9120/9121, locating the gene within a 3.5 Mb interval at Xq28 ($Z = 2.8$). No alterations were detected in expressed sequences of two GABA-receptor subunits adjacent to DXS52. Clinical data were not included in the abstract. In our family, recombination with DXS52 defines the proximal limit of the mapping interval. Affected males showed mild to moderate mental retardation and inconspicuous behaviour. Clinical findings in the Gedeon et al. [1991] family, in the Nordström et al. [1992] family, and the family here reported renders pooling of the linkage data hazardous, though the respective genetic defects are in the same region. Several XLMR syndromes have been localised to the Xq28 region, such as the HSAS/MASA syndrome due to L1CAM gene mutations [Fransen et al., 1994], Waisman syndrome [Gregg et al., 1991] and the Simpson-Golabi-Behmel syndrome [Xuan et al., 1993]. These findings point to the existence in the Xq28 region of a cluster of genes that play a role in mental development. It remains to be seen whether these syndromic forms of XLMR are allelic to the nonspecific forms of XLMR that we and others assigned to the same region. In conclusion, we report on a 4th family with nonspecific XLMR in which the gene is localised in the Xq28-qter region.

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TABLE I. Results of Two-Point Linkage Analysis

		LOD-scores θ				
Marker	Locus	0.0	0.1	0.2	0.3	0.4
KAL	p22.32	−∞	−0.63	0.08	0.23	0.14
DXS443	p22.13	−∞	−1.92	−1.13	−0.69	−0.31
DXS451	p22.13-p11.12	−∞	−1.59	−0.55	−0.17	−0.07
DMD	p21.2	−∞	0.76	0.87	0.68	0.35
DXS538	p21.1-p11.21	−∞	1.30	1.32	1.01	0.52
DXS7	p11.4-p11.3	−∞	1.31	1.34	1.05	0.59
ALAS2	p11.22-p11.21	−∞	0.01	0.18	0.20	0.14
DXS339	q12	−∞	1.45	1.26	0.92	0.49
DXS986	q21.1	−∞	−1.32	−0.40	−0.02	0.08
DXYS1	q21.31	2.26	1.94	1.51	1.01	0.42
DXS3	q21.33	2.56	2.23	1.80	1.29	0.69
DXS458	q21.33	−∞	−0.64	0.06	0.23	0.17
DXS454	q21.1-q22.1	−∞	−0.26	0.04	0.09	0.05
DXS178	q22.1	−∞	−0.26	0.04	0.09	0.05
COL4A5	q22.3	−∞	−0.26	0.04	0.09	0.05
DXS424	q23-q24	−∞	−0.64	0.06	0.23	0.17
DXS425	q25	−∞	−0.46	0.06	0.18	0.13
HPRT	q26.1	−∞	−0.93	−0.26	−0.00	0.06
DXS984	q26.3-q27.1	−∞	0.06	0.46	0.45	0.27
DXS369	q27	−∞	1.00	0.92	0.65	0.28
FRAXAc2	q27.3	−∞	1.28	1.20	0.92	0.51
DXS1113	q28	−∞	1.28	1.29	1.00	0.55
DXS52	q28	−∞	2.00	1.72	1.23	0.61
DXS1108	q28	3.82	3.21	2.53	1.74	0.87

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