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Localisation of a new gene for non-specific mental retardation to Xq22-q26 (MRX35)

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Abstract

Non-specific mental retardation (MR) is a condition in which MR appears to be the only consistent manifestation. The X linked form (MRX) is genetically heterogeneous. We report clinical, cytogenetic, and linkage data on a family with X linked non-specific MR. Two point and multipoint linkage analysis with 18 polymorphic markers, covering the entire chromosome, showed close linkage to DXS1001 and DXS425 with a maximal lod score of 2·41 at 0% recombination. DXS178 and the gene for hypoxanthine phosphoribosyltransferase (HPRT), located in Xq22 and Xq26 respectively, flank the mutation. All other chromosomal regions could be excluded with odds of at least 100:1. To our knowledge there is currently no other non-specific MR gene mapped to this region. Therefore, the gene causing MR in this family can be considered to be a new, independent MRX locus (MRX35).

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Key words: non-specific mental retardation; linkage; Xq22–q26.

Mental retardation (MR) is a condition which affects approximately 2 to 3% of the population in the western world.[1] X linked traits causing mental retardation have an estimated cumulated population frequency of 0·15% to 0·3%.[2–4] During recent years, considerable progress has been made in aetiological research into X linked entities in which mental retardation is a prominent manifestation and in the clinical delineation of syndromes. In their paper updating X linked MR, Neri *et al*[5] listed 127 conditions. Fifty-seven of these consist of syndromes or clinically recognisable conditions based on a characteristic pattern of physical anomalies. Six are dominant X linked disorders with lethality in males, 12 are metabolic disorders, 30 are neuromuscular disorders, that is, clinically recognisable conditions, based on a characteristic neuromuscular involvement. Finally, 22 have been described as non-specific MR or conditions where MR appears to be the only manifestation.

Non-specific X linked MR seems to be very heterogeneous. Neri *et al*[5] postulated at least three distinct loci for X linked non-specific MR, but it is expected that there are much more. Precise regional mapping by linkage analysis might be the most feasible approach to resolve the question of splitting versus lumping MR subtypes. To this end, it has been suggested analysing large families using a standardised and relatively dense map of highly informative markers.[6 7]

Here we report linkage data on a non-specific MR family with six male patients and three obligate female carriers, assigning the mutant locus to Xq22-q26 using a map of 18 highly polymorphic loci which are evenly dispersed over the X chromosome. To our knowledge the mutated gene in this family is the first non-specific MR gene assigned to this region, and thus constitutes a new non-specific MR locus on the X chromosome.

Materials and methods

CLINICAL REPORT

The present family (fig 1) came to our attention for the diagnostic evaluation of two mentally retarded brothers (III·7 and III·8). They were referred to the Department of Child Psychiatry when they were 6 and $4\frac{1}{2}$ years old respectively, because of severe hyperkinetic behaviour and moderate mental retardation (total IQ scores on WISC-R of 52 and 48 respectively). Clinical examination of the two brothers showed no gross dysmorphic stigmata except for relative macrocephaly with broad and high forehead, round facies with a flat nasal bridge, and truncal obesity: III·7, height 114 cm (50th centile), weight 24 kg (75th–97th centile), head circumference 52·5 cm (75th centile); III·8: height 97·5 cm (25th centile), weight 17 kg (75th centile), head circumference 52 cm (75th centile). Genital development was normal. Routine biochemical and metabolic screening was normal. Chromosomal analysis on peripheral blood lymphocyte cultures showed 46, XY normal male karyotypes after G banding, and fragile X (fra(X)) screening was negative in 100 cells of M199 cultures. Fra(X) was further ruled out by absence of the expansion of the $(CGG)_n$ triplet in the fra(X) mental retardation gene (FMR1). Both parents are mentally and phenotypically normal. II·5 is slightly to moderately mentally retarded and is living in a sheltered home. He dresses and cares for himself and is capable of using public transport independently, but he has no concept of money and cannot read. His head circumference is 56 cm, weight 68 kg, and height 156 cm, and testicular volume is 20 ml. Except for the relative obesity and a relatively long facies no other dysmorphic stigmata were noted. II·1 is functioning at a borderline to slightly mentally retarded level (mental developmental age was reported to be 7 years at

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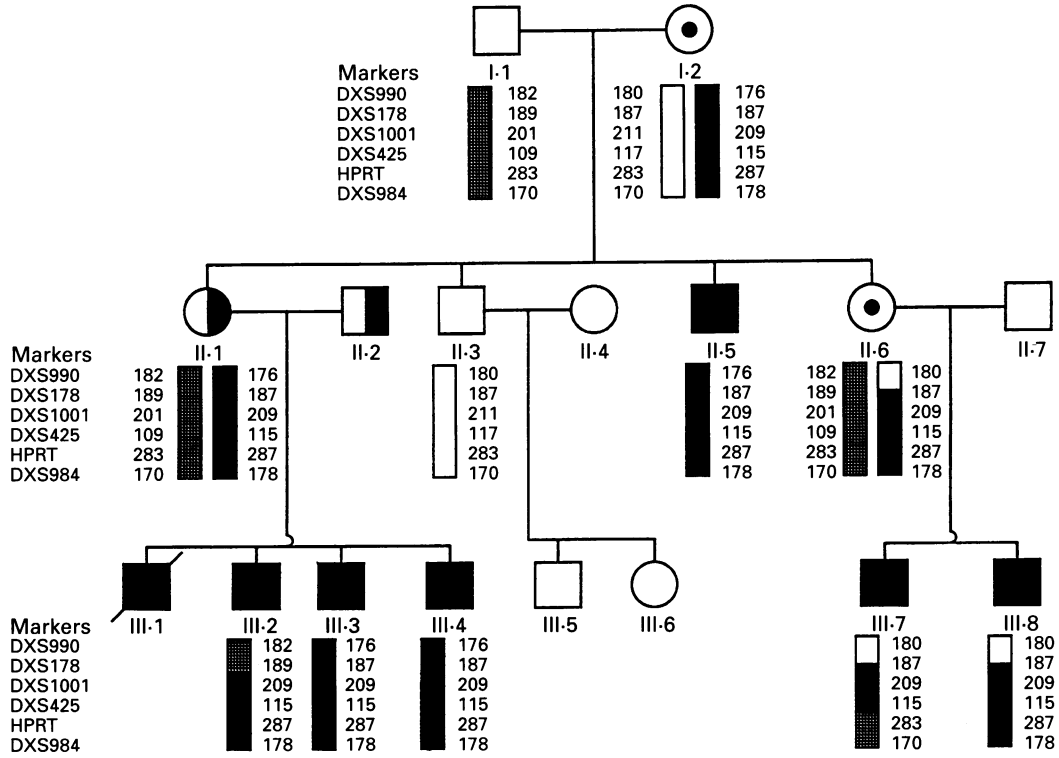

Figure 1 Pedigree showing the six sampled mentally retarded males (II·5, III·2, III·3, III·4, III·7, III·8), one borderline mentally retarded female and one male (II·1 and II·2), two normal obligate female carriers (II·6 and I·2), and two normal males (I·1 and II·3). Haplotypes are shown for the markers DXS990, DXS178, DXS425, HPRT, and DXS984.

the chronological age of 16 years) but social integration is adequate. Her husband is also reported to be borderline mentally retarded. They gave birth to four sons (III·1,2,3,4). The oldest son (III·1) died unexpectedly at the age of 9 months and was developmentally retarded (Bayley mental developmental level of 5 months at the age of 8 months). The three younger sons (III·2, III·3, III·4) are moderately mentally retarded and have been integrated in a special school for the moderately and severely mentally retarded. Physical examination at the respective ages of 19, 18, and 10 years is normal with normal testicular volumes. II·3 is physically and mentally normal and attended the regular school system. He has two children, a normal son (III·5) and a normal daughter (III·6).

DNA ANALYSIS

Genomic DNA was isolated from peripheral blood lymphocytes by a salting out method.[8] All 18 genetic markers used in this analysis are microsatellites, except DXS52 which is a variable number of tandem repeat sequence (VNTR).[9] Polymerase chain reaction (PCR) amplification of these polymorphic markers was performed using fluorescently labelled locus specific primer pairs (Genome Data Base). Marker genotypes of patients and relatives were determined by polyacrylamide gel electrophoresis of the PCR products on a Pharmacia ALF automated DNA sequencer (Pharmacia Biotech, Uppsala, Sweden). For marker DXS52, long range PCR was used and products were separated on an ethidium bromide stained 2% agarose gel.[10] Optimised PCR conditions, gel loading, and running conditions can be obtained from the authors.

LINKAGE ANALYSIS

Two and multipoint linkage analyses of the 18 markers and the disease locus were performed using the computer program package LINKAGE 5·1.[11] Initially, a model assuming X linked inheritance with a disease gene penetrance of 1·0 and 0·3 was used for males and heterozygous females respectively. The MR gene frequency was set at 0·0001. Marker order and intermarker distances were obtained from Genethon and CHLC maps.[12–15]

Results and discussion

All tested markers, except DXS292, were partially or completely informative in this family. Two point lod scores are summarised in the table. Maximal lod scores of 2·41 at 0% recombination were obtained with the markers DXS1001, located in Xq24, and DXS425, located in Xq26. All other markers showed at least one recombination, indicated by a lod score of $-\infty$ at 0% recombination. For X linked loci, lod scores exceeding +2 are considered significant indication for linkage.[16 17]

The 17 informative markers were combined in eight consecutive multipoint linkage analyses. In each of the consecutive linkage analyses, there was one completely informative marker in common. Markers contained in the multipoints are: DXS996—DXS999, DXS999—DXS989—DMD49, DMD49—DXS1068—

Two point lod scores between non-specific MR and X linked markers

Markers	Recombination frequency 0·00	0·01	0·05	0·10	0·20	0·30	0·40	Excl lim	Zmax	θmax
DXS996	−∞	−5·613	−2·906	−1·815	−0·845	−0·384	−0·133	0·09	–	–
DXS999	−∞	−7·609	−4·184	−2·769	−1·447	−0·752	−0·309	0·15	–	–
DXS989	−∞	−7·609	−4·184	−2·769	−1·447	−0·752	−0·309	0·15	–	–
DMD-49	−∞	−5·613	−2·906	−1·815	−0·845	−0·384	−0·133	0·09	–	–
DXS1068	−∞	−6·499	−3·721	−2·540	−1·387	−0·741	−0·308	0·14	–	–
DXS1003	−∞	−7·609	−4·184	−2·769	−1·447	−0·752	−0·309	0·15	–	–
DXS991	−∞	−2·507	−1·163	−0·632	−0·183	−0·005	0·043	0·02	0·04	0·40
DXS986	−∞	−3·617	−1·627	−0·860	−0·243	−0·016	0·043	0·04	0·04	0·40
DXS990	−∞	−1·622	−0·348	0·093	0·358	0·351	0·219	<0·01	0·38	0·24
DXS178	−∞	−0·512	0·115	0·322	0·418	0·362	0·219	<0·01	0·42	0·20
DXS1001	2·408	2·368	2·207	1·996	1·537	1·028	0·489	–	2·41	0·00
DXS425	2·408	2·368	2·207	1·996	1·537	1·028	0·489	–	2·41	0·00
HPRT	−∞	0·373	0·929	1·042	0·935	0·660	0·313	<0·01	1·04	0·11
DXS984	−∞	0·373	0·929	1·042	0·935	0·660	0·313	<0·01	1·04	0·11
DXS1227	−∞	0·373	0·929	1·042	0·935	0·660	0·313	<0·01	1·04	0·11
DXS1193	−∞	−0·512	0·115	0·322	0·418	0·362	0·219	<0·01	0·42	0·20
DXS52	−∞	−1·622	−0·348	0·093	0·358	0·351	0·219	<0·01	0·38	0·24

DXS1003, DXS1003—DXS991—DXS986, DXS986—DXS990, DXS990—DXS178—DXS1001, DXS1001—DXS425—HPRT—DXS984, DXS984—DXS1227—DXS1193—DXS52. From fig 2 it can be inferred that the MR mutation is excluded from the short arm of chromosome X and from regions proximal to DXS990 (lod score <-2). Distal to DXS990, the lod score becomes positive in two intervals: the region flanked by DXS178-HPRT with a maximal lod score of 2·41 at DXS1001 and DXS425, and the region between DXS1227 and DXS1193 with a maximum of 0·30. The odds of locating the gene between DXS178-HPRT versus the region DXS1227-DXS1193 or any other region distal to DXS990 are at least 125 to 1. Since all possibly informative subjects in this pedigree have been genotyped, changing the frequency of marker alleles or disease penetrance values for females did not influence the resulting lod scores (data not shown). As indicated in the table and fig 2, performing multipoint linkage analysis did not raise the peak lod score, since both the markers DXS1001 and DXS425 were completely informative in all available meioses. However, multipoint linkage analysis in other regions allows for more powerful exclusion than two point analyses. Therefore, even with a relatively small number of informative meioses, reliable positioning of the mutated locus is feasible.

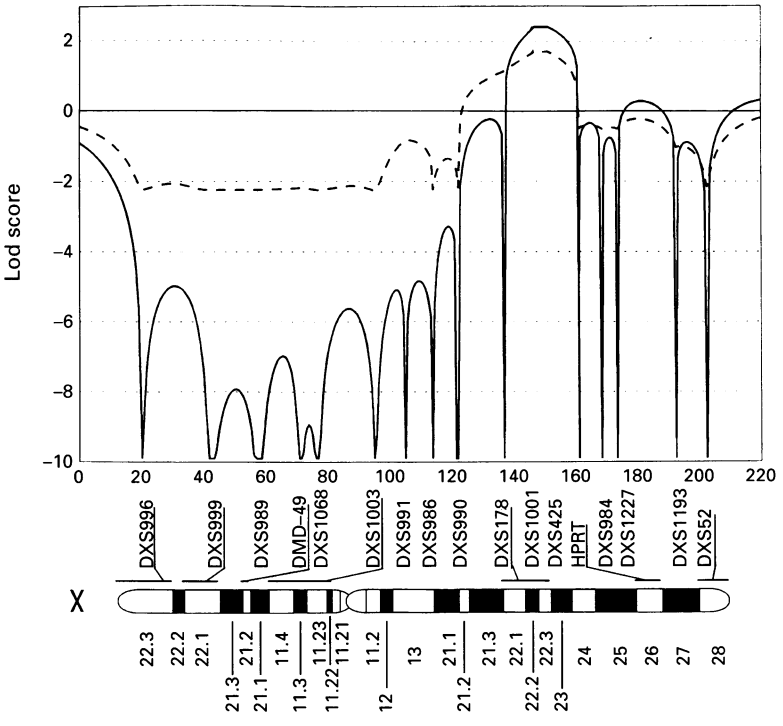

Figure 2 Multipoint lod score analysis of non-specific mental retardation versus 17 X linked markers. The continuous curve corresponds to an analysis model assuming full penetrance in males, 30% penetrance in female carriers, and 0% phenocopy frequency. The dashed curve corresponds to a similar model but allows for disease causing factors other than X linked mutations in III·2, III·3, and III·4.

A possible confounding factor in this family might be the presence of an assortative mating between II·1 and II·2. Affected sons do not inherit an X chromosome from their father. As a result, paternally inherited predisposing factors should be autosomal. In linkage analysis it would be difficult to account for the influence of autosomal factors when applying a recessive X linked model. However, by setting the phenocopy rate in the LINKAGE program to 0·5 for patients III·2, III·3, and III·4, we allow for a high probability for disease causing factors other than X linked mutations. The results of the eight multipoint analyses under this model are depicted by the dashed line in fig 2. The maximal multipoint lod score is still found in the same chromosomal region but decreases from 2·41 to 1·81. Nevertheless, the odds for locating the gene in this region versus any other region on the X chromosome remain larger than 100 to 1.

Haplotype analysis, shown in fig 1, confirms that the disease gene is located between DXS178 and HPRT. A recombination from carrier II·1 to patient III·2 occurred between markers DXS178 and DXS1001, locating the gene distal to DXS178. Furthermore, a recombination occurred between marker DXS425 and HPRT in patient III·7, mapping the MR gene proximal to HPRT.

Three non-overlapping regions for non-specific X linked mental retardation have already been established with MRX2 and MRX19 in distal Xp,[18–20] MRX3 in distal Xq,[21] and numerous MRX loci near the centromere.[5] None of these groups overlaps with the location of the gene in this family. The MRX loci closest to this location are MRX1, MRX4, MRX5, MRX7, and MRX17, all located from Xp11 to Xq21.[5] Locus MRX4 is flanked distally by

DXS178,[19] which is the proximal flanking marker in our family. MRX1, MRX5, MRX7, and MRX17 are all distally flanked by markers which are more proximal to DXS990.[22-25]

The distance between DXS178 and HPRT is estimated to be between 25 and 44 cM.[12 14 26] It can be estimated that between 500 to 1500 genes might be located in this region. Supplementary markers need to be run in order to narrow the candidate region. However, it should be kept in mind that, since neither DXS1001 nor DXS425 exhibit recombinants, the minimal candidate region will never be smaller than 5 cM, which is the distance between both markers. Two genes causing metabolic and neuromuscular disorders in which mental retardation is a consistent finding have been located to this region as well: proteolipid protein (PLP) causing Pelizaeus-Merzbacher disease[27] and oculocerebrorenal syndrome of Lowe (ORCL).[28] Mohr-Tranebjaerg and Simpson-Golabi-Behmel syndrome have also been localised to this region.[29 30] However, none of the affected members of this family shows signs typical of any of these disorders. Recently, a paracentric inversion (X)(q21.2q24) was identified and associated with MR in males.[31] Possibly, the disorder in these patients is caused by the interruption of an MR gene in Xq21.2 or in Xq24. It would be interesting to ascertain whether the distal breakpoint coincides with the linkage interval in our family.

In conclusion, we present a family with X linked mental retardation in which the causative gene is located in Xq22-q26. Our approach of using a standard panel of highly informative markers, combined with multipoint linkage and haplotype segregation analysis, is able to delineate new XMR entities even when relatively small families are used.

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