Original articles

Localisation of the MRX3 gene for non-specific X linked mental retardation

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Abstract

A family is described with five affected males segregating a new gene for non-specific X linked mental retardation (MRX). Linkage analysis localised the gene at Xq28-qter. The maximum lod score was 2.89 with DXS52 (St14) at $\theta=0.0$. A recombinant was observed with DXS304 (U6.2) defining the proximal limit to the localisation. No evidence for linkage was determined using markers at several points along the remainder of the X chromosome, including the regions known to contain MRX1 and MRX2. This delineates the third gene for non-specific X linked mental retardation, MRX3.

Genes responsible for non-specific X linked mental retardation (MRX)¹ can be regionally localised by linkage analysis applied to large families. At present, gene mapping is the only way in which it is possible to differentiate between families affected by this disorder. Use of the MRX nomenclature¹ provides a basis for classification. Localisation of the MRX genes in three families has established at least two MRX loci, MRX1²⁻⁴ and MRX2.²

We report the regional localisation of a third gene, MRX3, in a large pedigree where intellectual handicap is segregating. This extends the number of MRX

disorders in the genetically based classification system for these disorders.⁵

Family history

The family was identified in 1970 during a school survey to identify pairs of mentally retarded brothers.⁶ The pedigree is reproduced in fig 1. The fragile X syndrome was subsequently excluded by cytogenetic analysis. The clinical measurements of affected males are given in table 1.⁷ The obligate carriers are of normal intelligence and indistinguishable from their non-carrier sisters.

VII.4 was delivered at term after a pregnancy complicated only by maternal hypertension. The birth weight of 2800 g was commensurate with his sibs and there were no neonatal problems. He was a floppy infant, crawling at 14 months and walking at 20 months. His speech development was said to be normal. In infancy he was admitted to hospital because of oesophageal reflux and constipation, otherwise his general health has been good. He attended a special school for the moderately intellectually handicapped and now lives at home. He is independent in personal care with supervision, but has no domestic skills, although he is able to go out alone. He is given nocturnal sedation to control aggression and agitation.

On examination, aged 21 years, he has a broad forehead with a small, round, receding chin, beaked nose, and brachycephaly. His fingers are tapering; thumb position, palmar creases, and movements are normal. He has normal adult body hair distribution and no gynaecomastia. He refused venepuncture.

VII.6 was delivered vaginally at unknown gestation with a birth weight of 2200 g. There were no neonatal problems. Developmental milestones were normal, but intellectual handicap was suspected in infancy because of lack of alertness and repetitive hand and body movements. He also had oesophageal reflux in the first year. He had petit mal seizures in childhood

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Table 1 Measurements of affected males.

Patient	OFC (cm) (centile*)	Height (cm) (centile*)	Weight (kg) (centile*)	Span (cm)	LS (cm) (ratio)	Ear length (cm) (centile*)	Testicular volume (cm ³)	Hands (P/MFL†) (centile*)
V.4	60 (97)	170 (<10)	—	180	88 (0.93)	8 (>97)	10	12 (97)/8 (50)
V.17	56 (75)	161 (<3)	—	172	80 (0.99)	8.5 (>97)	—	10.5 (50)/7 (<3)
VI.13	54 (25)	170 (<10)	—	188	93 (0.82)	—	—	—
VII.4	57 (90)	173 (25)	65 (50)	182	90 (0.92)	6 (50)	25	12 (97)/8 (50)
VII.6	55 (50)	163 (<3)	49 (<3)	175	81 (1.0)	6 (50)	25	11.5 (<97)/9 (97)

*Normal standards.⁷

†Palm:mid finger length.

Figure 2 VII.6.

and has asthma as an adult. He attended a special class for the mildly intellectually disabled and is now in sheltered employment. He lives at home and is independent in his self-care and social life, requiring supervision only with money. He has none of the behavioural difficulties of his brother.

On examination, aged 19 years, he is unlike his brother in facial appearance but is brachycephalic with a triangular face (fig 2). He has fusiform swelling of the proximal interphalangeal joints of the second, third, and fourth fingers on the right hand and fourth finger on the left, with hyperextension at the proximal and flexion at the distal interphalangeal joints at rest. He has a high arched palate with crowded teeth. There is a prominent crease medial to the right tragus. Masculinisation is normal. Spinal and hand radiology and cerebral CT scan were normal.

There is no developmental history available for V.4. He lives with relatives since the death of his

parents. He has had no seizures. His general health is good, although he has hypochondriasis, leading in the past to laparotomy for functional abdominal pain. He is independent in personal care and does small tasks around the house. He is able to deliver messages and find his way around town alone.

On examination, aged 60 years, he has a round face with prominent ears and brachycephaly. He has a right convergent squint with arcus senilis, syndactyly of the second and third toes bilaterally, flexion contractures of the fifth metacarpophalangeal and proximal interphalangeal joints bilaterally, and a thoracic kyphosis. Masculinisation is normal.

V.17 lives in a hostel for the intellectually handicapped where he cooperates with household activities and attends a sheltered workshop daily. He is able to travel independently and make his own appointments. His behaviour is difficult; he has been aggressive and destructive in the past with paranoid

Figure 3 V.17.

ideation and suicidal and religious preoccupation. He is on medication to control aggression.

On examination, aged 66 years, he is brachycephalic with large ears, a high nasal bridge with deviated nasal septum, and a small mouth (fig 3). He has a mild kyphosis and bilateral fifth finger clinodactyly with ulnar deviation of the hands. He has arcus senilis and clinical evidence of mild cardiomegaly. Cerebral CT scan is normal.

VI.13 was delivered at term after an uncomplicated pregnancy with a birth weight of 3350 g. Developmental milestones were normal and his general health has been excellent. Intellectual handicap was diagnosed at school entry when he required a special class. On formal testing at the age of 8 WISC scores were: full scale 86, verbal 76, and performance 100. He has an itinerant life from which he periodically requires rescuing as he is unable to live unsupervised.

On examination, aged 34 years, he has a round face with a low anterior hairline, prominent ears and nose, and a high nasal bridge. He has hyperextensible fifth proximal interphalangeal joints bilaterally and a mild pectus excavatum.

No history is available on the dead affected males. V.11 is said to have been less severely affected than V.17; he died of a cerebral haemorrhage at the age of 42.

DNA and linkage studies

Blood was collected initially from 11 family members using EDTA as the anticoagulant. Extracted DNA samples were digested with restriction endonucleases

detecting known RFLPs, with map positions established on the X chromosome.⁸ Marker studies were carried out as previously described⁹ with exclusion of close linkage to markers spread along the remainder of the X chromosome.

Linkage analysis was carried out assuming X linked recessive inheritance using LIPED.¹⁰ Loci segregating on the X chromosome with lod scores of +2 or greater are considered very significant evidence for linkage.¹¹ Penetrance of the disease gene was assumed to be 1.0 with the MRX3 allele frequency being conservatively set at 1 in 10 000. Allele frequencies used for St14 were 0.04 for the 5.4 kb allele, 0.12 for 4.8 kb, 0.10 for 3.9 kb, and 0.74 for the grouped remaining non-segregating alleles. Results were confirmed by LINKAGE¹² version 5.03 using eight alleles (6.6, 5.4, 4.8, 4.5, 4.1, 4.0, 3.9, and 3.4 kb) with frequencies of 0.01, 0.04, 0.12, 0.36, 0.20, 0.01, 0.10, and 0.15, respectively.

Results

Two point lod scores between the MRX3 gene and markers spread along the X chromosome are given in table 2. Evidence for linkage was observed with the VNTR marker at the DXS52 locus, with known regional localisation at Xq28. The probe St14 (DXS52) was informative in all potentially informative meioses giving the maximum lod score of 2.89 at zero recombination. Since all meioses were informative, the lod score would not be increased by multipoint mapping. The 1A1 (DXS374) and DX13 (DXS15) markers were not informative in all meioses, therefore

Table 2 Pairwise lod scores: *MRX3* versus *X* chromosome marker loci.

Locus	θ						$\hat{Z}$	$\hat{\theta}$
	0.01	0.05	0.10	0.20	0.30	0.40		
<i>DXS85</i>	-1.34	-0.69	-0.45	-0.26	-0.16	-0.08	0.00	0.50
<i>DXS43</i>	-1.15	-0.50	-0.26	-0.07	0.00	0.02	0.02	0.40
<i>DXS255</i>	-0.26	0.32	0.48	0.48	0.36	0.20	0.50	0.14
<i>DXYS1</i>	-2.58	-1.23	-0.70	-0.24	-0.05	0.02	0.02	0.43
<i>DXS10</i>	-2.32	-1.00	-0.49	-0.08	0.06	0.07	0.08	0.36
<i>F9</i>	-1.38	-0.73	-0.48	-0.27	-0.16	-0.08	0.00	0.50
<i>IDS</i>	-2.15	-0.86	-0.39	-0.05	0.06	0.07	0.07	0.36
<i>DXS304</i>	-1.43	-0.75	-0.47	-0.21	-0.09	-0.02	0.00	0.50
<i>DXS374</i>	0.63	0.57	0.50	0.38	0.25	0.12	0.64	0.00
<i>DXS52</i>	2.84	2.62	2.33	1.73	1.10	0.50	2.89	0.00
<i>DXS15</i>	0.74	0.67	0.58	0.41	0.26	0.12	0.76	0.00

Table 3 RFLP genotypes (in kilobases) at informative *Xq* loci in subjects from the *MRX3* family.

Locus	<i>DXS304</i>	<i>DXS374</i>	<i>DXS52</i>	<i>DXS15</i>
Probe	U6.2	1A1	St14	DX13
Enzyme	<i>TaqI</i>	<i>TaqI</i> ¹³	<i>TaqI</i>	<i>BglII</i>
V.4	7.0	4.5	5.4	2.8
V.14	7.0/7.0	4.5/4.5	3.9/5.4	2.8
V.16	3.3/7.0	4.0/4.5	4.8/5.4	2.8/5.8
V.17	7.0	4.5	5.4	2.8
VI.4	7.0/7.0	4.5/4.5	4.8/5.4	2.8
VI.5	7.0/7.0	4.5/4.5	3.9/4.8	2.8
VI.6	7.0	4.5	3.9	2.8
VI.13	3.3	4.5	5.4	2.8
VI.14	3.3	4.0	4.8	5.8
VII.5	7.0	4.5	4.8	2.8
VII.6	7.0	4.5	5.4	2.8

their peak lod scores are lower. A recombinant with U6.2 (*DXS304*) defines the proximal limit to the localisation.

As assignment of this *MRX* gene to the *X* chromosome is accepted on the basis of pedigree information, exclusions to the rest of the chromosome may be defined in terms of negative lod scores. Those regions where *MRX1* and *MRX2* have been mapped, represented by *DXS85*, *DXS43*, and *DXYS1*, showed no evidence of linkage. Similarly, regions near *F9*, *IDS*, and *DXS304*, flanking the fragile site at Xq27.3, show no evidence of linkage. Marker genotypes showing linkage and defining the limit to the regional localisation are given in table 3.

Discussion

The DNA and linkage results indicate the localisation of the third gene responsible for *MRX*, designated *MRX3*, to Xq28 (fig 4). The recombinant distal to Xq27.3 with *DXS304* differentiates *MRX3* from the Martin-Bell or fragile X syndrome¹⁴ and from consideration as fragile X negative Martin-Bell syn-

drome.¹⁵ The distal limit for the localisation of *MRX3* is the telomere at Xqter.

The affected males in this family do not show any manifestations of the two X linked mental retardation

Figure 4 Location of the *MRX3* locus for non-specific *X* linked mental retardation in relation to the *MRX1* and *MRX2* loci. Expansion of Xq27-qter shows *MRX3* within Xq28 and distal to the fragile X. Solid lines between *IDS* and *DXS304* and between *DXS374* and *DXS52* indicate cell line breakpoints that define physical intervals in Xq28.¹³

syndromes mapped to the same area, MASA (aphasia, shuffling gait, and adducted thumbs) syndrome and X linked skeletal dysplasia (MRSD).¹⁶⁻¹⁹ Affected males are below average height with relatively large heads and limb spans. They have no common constellation of facial features. Occult skeletal abnormality could only be excluded in VII.6.

The most prominent clinical feature is the discrepancy between their behaviour and estimated levels of intellectual function. These males function in a manner consistent with upper moderate to mild intellectual disability; however, four of the five males in this family are described by their caretakers as aggressive and difficult to manage despite their self sufficiency in many areas. In 'unpublished family 1' of Lehrke,²⁰ affected males in the family were either severely retarded or had formal IQ tests in the low normal range, but their adaptive behaviour led to the description of mental retardation. This family has been established as fragile X negative.²¹

If other families with non-specific X linked mental retardation are found to map to the region defined here for MRX3, careful comparison of adaptive behaviour with IQ may be of value in defining this as a clinical entity.

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