Non-specific X linked mental retardation with aphasia exhibiting genetic linkage to chromosomal region Xp11

Golder N Wilson, C Sue Richards, Kathy Katz, Gail S Brookshire

Abstract
A new type of non-specific X linked mental retardation is described in a three generation family. The three affected males had severe mental retardation (IQ 20 to 30), mutism, growth failure, frequent infections, seizures, and the following minor anomalies: brachycephaly, frontal hair whorl, square face, large mouth, thick lips, and prognathism. There was not a characteristic facies. Normal laboratory studies on the proband included a karyotype with fragile X screening, skeletal survey, blood amino acid, urine organic acid, and HGPRT levels. Linkage analysis was performed with 10 X chromosome DNA probes of which probe DXS255 at chromosomal region Xp11.22 gave a maximal two point lod score of 2.10 if phase was inferred and 1.20 if it was not. Crossovers were shown with probes mapping to regions Xp22, Xp21, and Xq28. Comparison of these patients with 80 X linked causes of mental retardation, including 41 which might be classified as 'non-specific', showed no other disorders compatible with the phenotypic and linkage data.


It is ironic that the human X chromosome, noted for its conservation in mammalian evolution, should wreak such havoc upon the mental function of hemizygous persons. Genes on the X chromosome may account for about a third of mental retardation in males and numerous surveys document a 20 to 50% excess of males over females when selected for severe retardation. While disorders such as the Lowe and Lesch-Nyhan syndromes are associated with definitive morphological or metabolic findings which aid delineation, many subjects with X linked mental retardation do not have striking phenotypes. These disorders have been grouped under the term 'non-specific' X linked mental retardation (XLMR) and have a cumulative incidence greater than 1 to 2 per 1000 male births. Over 30 types of XLMR have now been defined and many have been mapped to specific regions of the X chromosome (fig 1). Here we describe a new variety of XLMR which exhibits striking aphasia and linkage to the Xp11 region.

Case reports
Case 1 (IV.1, fig 2) was referred for evaluation at 15 months of age because of developmental delay. The height was 81 cm (75th centile), weight 11 kg (50th centile), and head circumference 46 cm (25th centile). At birth the patient weighed 3100 g with Apgar scores of 7 and 9 after a 37 week gestation to a 26 year old primigravida. There was hypotension and 'flu' in the last trimester, when antepartum amniocentesis to determine the L/S ratio showed meconium. After caesarian section, the

<table>
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<th>Enzyme</th>
<th>Alleles</th>
<th>Symbols</th>
<th>Frequency</th>
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<td>Multiple</td>
<td>X1–X4</td>
<td></td>
</tr>
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<td>PvuII</td>
<td>6-6/6</td>
<td>Aa</td>
<td>0-55/0-45</td>
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<tr>
<td>PstI</td>
<td>22/13</td>
<td>Bb</td>
<td>0-56/0-44</td>
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<td>EcoRV</td>
<td>87-5</td>
<td>Cc</td>
<td>0-16/0-85</td>
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<td>PstI</td>
<td>1-5/1-45</td>
<td>Dc</td>
<td>0-60/0-30</td>
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<tr>
<td>BamHI</td>
<td>21/5</td>
<td>Ee</td>
<td>0-21/0-79</td>
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<td>Ff</td>
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<td>M1–M4</td>
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<tr>
<td>BstXI</td>
<td>1-05/0-9</td>
<td>Gg</td>
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</tbody>
</table>

Figure 1  Idiogram of the human X chromosome showing the locations of mapped XLMR loci. The locations of informative DNA probes along with RFLP fragment sizes, allele designations, and frequencies in the general population are also shown.

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Hirschsprung disease which was reportedly negative despite continued administration of laxatives. Developmental delay was recognised at 1 year and an eventual IQ of less than 30 was measured. The patient can imitate sounds, understand his name and simple commands, knows limited sign language, has mild myopia, and normal hearing, and no speech. Care givers describe a limited attention span, cold and reddened extremities, rhythmic breathing, frequent tongue protrusion with licking and tasting of objects, an expressionless face, and a good natured demeanour. Physical examination showed an extremely withdrawn and 'autistic' adolescent with the abnormalities listed in table 1. His height was 145 cm (below the 3rd centile, 50th centile for 11½ years), weight 27 kg (below the 3rd centile, 50th centile for 9 years), and head circumference 50 cm (below the 3rd centile, 50th centile for 3 years). Photographs at ages 8 months, 3½ years, and 9 years are shown in fig 3E–G, respectively. Normal laboratory findings included karyotype, serum amino acids, CBC and electrolytes, and SMAC 20 serum screen including hepatic enzymes.

Case 3 (II.1, fig 2) is 27 years old and resides in a state school for the retarded. Birth weight was 3650 g after a long labour; hypotonia, somnolence, and feeding problems were noted in infancy. Frequent infections including otitis and pneumonia occurred in early childhood. Developmental delay was recognised by the age of 6 months, with an IQ of 40 being measured in adulthood. He has inappropriate behaviour with self-injury and resistance requiring occasional constraint. This behaviour has improved somewhat on Tegretol® , Serentil® , and Artane® therapy for a diagnosis of organic brain disorder, mixed type (DSM-IIR). The patient can dress himself and maintain personal hygiene, function in a sheltered workshop, count up to 10 objects, but cannot handle small sums of money or keep a locker key. He is completely mute, has mild myopia, excellent comprehension of instructions, a 50 word vocabulary in sign language, and normal hearing. Much of his spare time is spent listening to the radio. Physical examination disclosed an extremely curious, interactive, and friendly young man with the minor anomalies listed in table 1. His height was 163 cm (5th centile), weight 57 kg (20th centile), and head circumference 54.5 cm (25th centile). His appearance is shown at ages 6 months, 14 years, and 27 years in fig 3A–D. Laboratory studies included normal karyotype with fragile X screening, normal serum amino acids, and normal haematological and liver functions.

The carrier females II.1, II.3, and III.2 (fig 2) were all examined and found to have normal intellectual function without evidence of dysmorphology.

Methods

DNA BANKING

Heparinised blood samples from family members were collected in Leucoprep™ tubes,
Non-specific X linked mental retardation with aphasia exhibiting genetic linkage to chromosomal region Xp11

Table 1 Clinical manifestations of affected males.

<table>
<thead>
<tr>
<th></th>
<th>IV.1</th>
<th>III.3</th>
<th>III.5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>3</td>
<td>27</td>
<td>16</td>
<td>3/3</td>
</tr>
<tr>
<td>Severe MR (IQ)</td>
<td>(&lt;50)</td>
<td>(&lt;30)</td>
<td>(&lt;30)</td>
<td>(&lt;30)</td>
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<tr>
<td>Growth failure</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Brachycephaly</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Frontal whorl/upsweep</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Square jaw</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hypertelorism</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Thick lips</td>
<td>+</td>
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<td>-</td>
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</tr>
<tr>
<td>Large mouth</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tongue thrusting</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Prognathism</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inguinal hernia/hydrocele</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Seizures</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Frequent infections</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Decreased myelination</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

of DNA was obtained after chloroform extraction, RNase and Pronase treatment, and ethanol spooling. DNA was stored frozen before use.

DNA ANALYSIS AND LINKAGE STUDIES
The DNA probe DXYS14 was obtained from Lifecodes Corporation (New York),
DXS43, DXS41, and DXS270 from L Kunkel,
DXS28 from J Mandel,
DXS268 and DXS84 from G van Ommeren,
DXS255 from I Craig and Y Boyd,
POG from B Vogelstein,
and F8C from the Human Mutant Cell Repository, catalogue no 57204. Cloned DNA segments were separated from vector segments on 0.8% agarose minigels and purified using GeneClean (Bio 101 Inc, La Jolla CA). For RFLP analysis, 5 to 10 µg of DNA was restricted using the manufacturer’s protocol and excess enzyme. DNA transfer to Hybond N+ membranes (Amersham) was accomplished by capillary action after electrophoresis on 0.8% agarose gels and alkali denaturation/neutralisation. Prehybridisation, hybridisation, and washing procedures were undertaken using the conditions of Church and Gilbert with 50 ng of DNA segment labelled to a specific activity of 106 cpm/µg with 32P-dCTP by random hexanucleotide primer extension.9 Washed membranes were exposed to Kodak x-ray film for one to two days at 70°C using an intensifying screen. Of the 10 X linked probes used (fig 1), seven gave informative results as displayed in table 2. Two point lod scores (Z) were calculated using the program MLINK as described by Ott.1415

Figure 3 Affected males III.3 (A, 16 months, B, 14 years, C, D, 27 years), III.5 (E, 8 months, F, 3 years, G, 9 years), and IV.1 (H, 10 months) as designated in fig 2.
Table 2 Pair wise lod scores; putative XLMR gene versus X chromosome marker loci.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Location</th>
<th>0.001</th>
<th>0.01</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>Z at 0 of</th>
</tr>
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<tbody>
<tr>
<td>DXS14</td>
<td>Xp22.2</td>
<td>-3.9</td>
<td>-1.9</td>
<td>-0.12</td>
<td>-0.22</td>
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<td>0.3</td>
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<td>Xp21</td>
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<td>0.30</td>
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<td>0.001</td>
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<tr>
<td>DXS41</td>
<td>Xp21</td>
<td>-4.1</td>
<td>-2.2</td>
<td>-0.38</td>
<td>0.020</td>
<td>0.14</td>
<td>0.12</td>
<td>0.3</td>
</tr>
<tr>
<td>DXS28</td>
<td>Xp21</td>
<td>-9.9</td>
<td>-5.9</td>
<td>-2.0</td>
<td>-0.98</td>
<td>-0.45</td>
<td>-0.15</td>
<td>0.5</td>
</tr>
<tr>
<td>DXS255</td>
<td>Xp11.22</td>
<td>2.1</td>
<td>2.1</td>
<td>1.8</td>
<td>1.4</td>
<td>1.1</td>
<td>0.56</td>
<td>0.001</td>
</tr>
<tr>
<td>PGK</td>
<td>Xq13</td>
<td>-7.8</td>
<td>-4.8</td>
<td>-1.8</td>
<td>-0.99</td>
<td>-0.52</td>
<td>-0.21</td>
<td>0.4</td>
</tr>
<tr>
<td>F8C</td>
<td>Xq28</td>
<td>-4.1</td>
<td>-2.2</td>
<td>-0.38</td>
<td>0.020</td>
<td>0.15</td>
<td>0.08</td>
<td>0.3</td>
</tr>
</tbody>
</table>

**Results**

The family pedigree (fig 2) and clinical summary (table 1) of the affected males are consistent with an X linked form of mental retardation which could be mapped to a specific X chromosome region by RFLP linkage analysis. Representative Southern analyses of DNA from 11 family members are shown in fig 4. Fig 4A shows three recombinations between the putative XLMR gene and the smaller EcoRV allele (c) shown by the probe DXS28 (fig 1). Fig 4B shows cosegregation of the putative XLMR gene with allele M3 of the multiallelic probe DXS255 (fig 1). In fig 2, the deduced alleles from these and other restriction analyses are displayed alongside X chromosomes of family members. The marker haplotypes were deduced assuming a minimum number of crossovers and implied that recombinations were shown by the DNA probes DXS14 (alleles X1 to X4), DXS28 (alleles C, c), and F8C (alleles H, h). Table 2 shows two point lod scores (Z) as a function of recombination fraction (θ) with phase inferred from the pedigree and marker configuration. The maximal two point lod score (Z) was 2:10 at a recombination fraction of 0.01 for the DNA probe DXS255 located at Xp11.22. If phase was not inferred, then Z for DXS255 was 1:20 at a θ of 0.

**Discussion**

In 1943, Martin and Bell16 first described a family with XLMR. Two additional families with XLMR were soon reported,17 18 raising the question of whether one or many genes were involved. For a time, all types of XLMR were named 'Martin-Bell syndrome' or 'Reno- penning syndrome' after the authors of these first descriptions. This confusion began to lessen when a more specific phenotype of prominent jaw, large ears, lax connective tissue, and megalotestes was delineated for the Martin-Bell syndrome. Soon it was recognised that most patients with this phenotype had a fragile X marker which could be shown by culture of lymphocytes or fibroblasts in folate deficient medium. Improved analysis and widespread screening has now shown that the fragile X syndrome affects about 1 in 1000 males and accounts for most patients with Martin-Bell phenotype as well as 30 to 40% of families with XLMR.2

Review of the catalogue of genetic disorders updated periodically by McKusick29 shows 60 X linked conditions which cause mental retardation, many with distinctive associated features. An estimated 21 of these disorders (depending on the perspective of the reviewer) have been placed in the category of 'non-specific X linked mental retardation (XLMR)', that is, mental retardation without a striking metabolic or morphological phenotype. Recent surveys29 30 list an additional 20 conditions with XLMR defined in single families which have not been assigned a McKusick number. In many of these conditions classified as XLMR, careful assessment can establish a syndrome which allows separation from 'true' XLMR with normal appearance. Differentiating clinical features include a characteristic facies (Prieto XLMR,24 Atkin-Flaitz XLMR,25 Chudley-Lowry-How XLMR,26 Pettigrew XLMR,27 Vasquez XLMR28), palmo-plantar hyperkeratosis (Fitzsimmons XLMR29), macrotubes without fragile X (Clark-Baraitser XLMR,27 Tranebjaerg II XLMR,29 Tariverdian XLMR29), Marfanoid habitus (Lujan-Fryns XLMR29), and skeletal dysplasia (Christian XLMR29). Several forms of XLMR are associated with spastic paraplegia and ataxia,
including Allan-Herndon XLMR,18 Gareis-Mason XLMR,19 Paine-Seemanova XLMR,20 Smith-Fineman-Myers XLMR,21 Davis XLMR,22 and Schimke XLMR.23

In the present family, a characteristic facial appearance, metabolic abnormality, or chromosome aberration could not be found. However, physical examination disclosed the minor anomalies listed in table 1 which suggest that this condition is different from previously reported types of XLMR. Prominent among the minor anomalies was a frontal hair whorl or upswipe; the presence of this manifestation allowed natal recognition of the condition by the proband's mother. This anomaly is found in two other types of X linked mental retardation, but in neither is the full spectrum of malformation compatible with that in the proband and his male relatives. The Opitz-Kaveggia FG syndrome24 includes a frontal cowlick with a prominent anterior fontanelle, hypertelorism, long philtrum, abnormal ears, hypotonia, agenesis of the corpus callosum, and gastrointestinal defects. The Pallister W syndrome25 includes a frontal cowlick with pugilistic facies, hypertelorism, midline notch of the upper lip, skeletal anomalies, and seizures. Comparison with the 39 other XLMR conditions showed no comparable malformation pattern either.

Linkage analysis of this family does not suggest a match with previously reported forms of XLMR. Coordinate segregation with the DNA marker DXS255 at chromosomal region Xp11.22 provided a maximal two point lod score (Z) of 2.1 based on seven informative meioses in this family (table 2). Although the distal X short (p21pter) and long arms (q28) are ruled out by crossovers, a gene location anywhere within the Xp11-Xq11 pericentric region would be compatible with the linkage data. Additional families with this disorder must be ascertained to refine the gene location and to provide a lod score above the value of 3 considered to establish linkage. Comparison with the X chromosome regions implicated in 13 other forms of XLMR (fig 1) indicates overlap with the Prieto,21 Carpenter,22 Sutherland,23 and Sammons24 forms of XLMR with phenotypes that are quite different from the present family. The Xp11.31-q11 region is also a candidate for gene localisation in two XLMR families25-27 where affected males lack even subtle anomalies. If these and the present family involve the same locus, then allelic heterogeneity or variable expressivity must be invoked to explain the different phenotypes.

The three affected males in the family were striking for their absent or delayed speech. The oldest affected subject (III.9) was particularly remarkable for aphasia despite an interactive personality and considerable facility with sign language. Speech is mentioned as delayed or abnormal in seven of the 41 XLMR disorders so far described. In one of them (Garais-Mason XLMR23), speech was absent while in others it was dysarthric24-26 or described as verbal disability.27 The severe mutism in the present family despite normal hearing suggests a gene particularly involved with speech, that is, through control of brain asymmetry/lateralisation, rather than one which merely impacts speech along with other higher cortical functions. Such associations of X chromosome loci and particular cognitive or behavioural traits emphasize the insights these characters can provide toward neural development and mental function.49-51

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