A new X linked recessive syndrome of mental retardation and mild dysmorphism maps to Xq28

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Abstract
Efforts to understand the genetic basis of mental retardation are greatly assisted by the identification of families with multiple relatives with mental retardation that clinical geneticists encounter in the routine practice of their profession. Here we describe a linkage study of a four generation family in which X linked recessive mental retardation (XLMR) is associated with minor dysmorphism and premature death of the affected males. Microsatellite based polymorphic loci evenly spaced over the entire X chromosome were used initially to detect linkage to Xq28. Further analysis identified a haplotype of Xq28 markers bounded proximally by locus DXS1113 and distally by DXS1108 that cosegregated with XLMR in this family. Two point lod scores >3.0 provided strong evidence that the gene locus responsible for XLMR in this family is within this 7 Mb region of Xq28. The minor anomalies noted in some affected males were not distinctive enough to suggest a unique syndrome. None of our patients had features of the Waismann-Laxova syndrome or the PPM-X syndrome. The possibility of allelism with any of the five other non-specific XLMR syndromes (MRX3, MRX16, MRX25, MRX28, and MRX41) mapped to Xq28 could not be excluded. While the recognition of a gene responsible for this disorder needs much additional work, multiple female relatives at risk in this family benefit immediately from knowing their genotype and heterozygotes will have the opportunity to undergo prenatal diagnosis.

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X linked mental retardation (XLMR) accounts for much of the male excess noted among people with mental retardation.1 Current efforts to understand the biological basis of XLMR focus on clinical studies defining the X linked syndromes associated with mental retardation in conjunction with the mapping, cloning, sequencing, and mutation analysis of candidate genes. Spectacular advances have been made with the cloning of such important genes as FMR1 and L1CAM leading to paradigm shifts in our thinking about phenotype-genotype correlations.2 The pace of discovery has been so rapid as to require regular biannual meetings of researchers focusing on XLMR to disseminate information and exchange ideas for new directions in research. At the seventh and most recent such workshop, 146 XLMR conditions including 42 entities described as non-specific X linked mental retardation (MRX) were listed.3 Most of the MRX entities have been identified on the basis of linkage mapping while a few have been mapped by studies of structural abnormalities of the X chromosome. However, no gene for MRX has yet been cloned with the exception of the FRAXE gene.4 Since there is considerable overlap among regions containing the various MRX loci and the clinical phenotypes are not clearly distinguishable from one another, there is much speculation about the exact number of loci that may be involved in XLMR.5 6 Most researchers, however, recognise the value of identifying and studying in depth all available families with XLMR. Careful analysis of their clinical, neuropsychological, or other distinguishing features may facilitate identification of candidate genes, better phenotype-genotype correlation, and ultimately a more meaningful function based reclassification of MRX and other XLMR syndromes. The following effort is in keeping with this spirit and describes a family in which five males with severe mental retardation had variable minor facial or other anomalies, seizures, and repeated life threatening respiratory infections.

Clinical background
CASE 1
Fig 1 shows the partial pedigree of family K8300. Our proband, V.1, was seen at 10 weeks of age because of a family history of X linked recessive mental retardation identified through his maternal second cousin, V.3. He weighed 2492 g, was 45 cm long, and had a head circumference of 32 cm at birth after 37 weeks
of uncomplicated gestation and spontaneous vaginal delivery. His Apgar scores were 8 and 9 at one and five minutes, respectively. He had surgical repair of a hydrocele and right inguinal hernia at the age of 3 months. Developmental delay became obvious by the end of the first year primarily owing to delayed onset of speech and language. Standardised tests estimated a developmental quotient (DQ) of 45 at 19 months. Follow up assessments at ages 3 and 4½ years showed DQs of 38 and 50, respectively. His health has been good. His height and weight have improved from the 3rd centile at birth to the 75th centile at the age of 6 years. Head circumference, which was below the 3rd centile at birth, has consistently been on the 50th centile since the age of 5 months. Mild facial dysmorphism is apparent in the form of shallow orbits, high nasal bridge, and a small mouth (fig 2A).

Vision and hearing tests have been normal. He has developed slight hypertonia in his lower limbs with brisk deep tendon reflexes at the knees but no other evidence of an upper motor neurone lesion. At his most recent evaluation a high stepping, broad based gait was observed. There was no ataxia, tremors, or cogwheel rigidity, neurological signs, or history of regression. He remains free of seizures. His cognitive function is quite poor with severe delay in the areas of speech and language and relatively better social and adaptive skills.

The following diagnostic tests have been normal: prometaphase chromosome analysis, fragile site and FMR-1 gene analysis, urine metabolic and organic acid screens, serum levels of thyroxine, thyroid stimulating hormone, creatine kinase, aldolase, lactate, pyruvate, very long chain fatty acids, immunoglobulins, total protein, urea nitrogen, and liver enzymes. In addition, MRI scans of the brain and lumbar-sacral spine, EEG, and echocardiogram have been normal.

CASE 2
V.3 was born in 1976 after an uneventful prenatal course. He was the first born child of an 18 year old mother and 20 year old father. His birth weight of 3238 g, length of 52 cm, and head circumference of 34 cm were on the 75th, 50th, and 25th centiles, respectively. His medical history was significant for multiple episodes of pneumonia and ear infections during the first two years of life. Immunological studies at the age of 2½ years showed normal T and B cell numbers and function, and no evidence of gastro-oesophageal reflux or other predispositions to recurrent pneumonia. He
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Figure 2. Clinical photographs of (A) case 1 and (B) case 2 showing small mouth, mild proptosis (case 1), minimal epicanthic folds, tapering fingers, and normal genitalia (case 2). See text for details.

was diagnosed as having global developmental delay of unknown aetiology. An abnormal but non-specific EEG was noted. Chromosome analysis and cytogenetic tests for fragile X syndrome were undertaken at 6 years of age when he was referred for a genetic evaluation. Physical examination at that time was remarkable for several minor anomalies (fig 2B) consisting of minimal epicanthic folds, prominent ears of normal length (5 cm), a small mouth, and tapering fingers. Height, weight, and head circumference, which were below the 3rd centile at 2½ years of age had increased to the 75th centile for age. He was functioning in the severe range of mental retardation. Non-specific X linked mental retardation was diagnosed based on the pedigree (fig 1) and negative cytogenetic studies.

His subsequent course was complicated by the seizure disorder that became increasingly refractory to treatment over time. His EEGs were described as diffusely abnormal. Legg-Perthe's disease was diagnosed at the age of 9 years and treated with lower body casting. At the age of 10 years, he required admission to hospital because of repeated seizures and pneumonia that proved fatal.

CASE 3

V.2 was the 2640 g product of a term gestation of an 18 year old primipara. His birth length was 44.5 cm and head circumference was 31.75 cm (<2nd centile). His prenatal course was complicated by maternal gastrointestinal infection in the seventh month of gestation. Owing to persistent vomiting in the first two days of life he was transferred to a tertiary care centre where diagnostic studies were negative. He was discharged following antibiotic therapy for suspected sepsis. At 17 months of age he had a febrile seizure during an attack of croup. A paediatric neurologist diagnosed moderate global developmental delay of unknown aetiology at the age of 21 months. Investigation at that time showed normal karyotype, urine and plasma amino acid levels, and screening tests for urinary ketones, reducing substances, and mucopolysaccharides. Serum electrolytes, plasma thyroxine and thyroid stimulating hormone levels, and serum antibody titres against toxoplasmosis, cytomegalovirus, herpes simplex, and syphilis were within normal limits. CT scan of the brain, EEG, and cerebrospinal fluid analysis were also normal. His height of 76 cm was below the 3rd centile whereas the weight (10.2 kg) and head circumference (48.5 cm) were on the 5th and 50th centiles, respectively. At 6 years of age he had inguinal hernia repair and orchidopexy for a partially descended right testis. His clinical course deteriorated over the next few years and ended in death just before his 10th birthday. Altogether he had at least seven documented episodes of pneumonia requiring hospital
based care. No anatomical abnormalities were detected by chest radiographs or bronchoscopy. Immunoglobulins and peripheral blood counts were normal and no immunodeficiency syndrome was identified. Review of photographs provided by the family showed only the vague facial dysmorphism often noticeable in children with mental retardation. There was no coarsening of facial features over time or distinctive craniofacial or limb anomalies.

CASE 4

IV.5, born in 1963, lived for only two years. He was born at home and weighed about 4082 g according to his mother. She was treated for pelvic inflammation during the first few weeks of gestation, but continued to have a brownish vaginal discharge throughout the pregnancy. The baby apparently “never acted normal” and had his first seizure aged 8½ months in conjunction with bronchopneumonia. During his third hospital admission for pneumonia at the age of 14 months developmental delay was noted. He was unable to sit or roll over from stomach to back. Delayed bone age of 6 to 9 months at a chronological age of 14 months was recorded. He had four more episodes of pneumonia at ages 18, 19, 21, and 24 months, the last of which was the terminal event. Chest radiographs and bronchoscopy ruled out anatomical defects of the major airways and immunological studies were not done. His growth and physical appearance were considered normal and an etiology was not established for his developmental delay.

CASE 5

IV.10 was born in 1957. At the age of 8 years he was placed in an institution with the diagnosis of spastic quadriplegia, severe mental retardation, and epilepsy. His intelligible speech was limited to a few words such as mama, dada, and bye. He was described as somewhat small for his age and sickly. During his teens he learned to walk with leg braces. He died at the age of 21 years from bronchopneumonia. No further details are available. Photographs provided by the family showed a slightly built young adult of below average height and no distinctive craniofacial anomalies. Subject III.3 is also believed to be affected, but there is no documentary evidence. Female relatives of affected males in this family, including the obligate heterozygotes identified by pedigree evidence (fig 1) or test results discussed below, have no clinical diagnosis of mental retardation, epilepsy, learning disabilities, or psychiatric illness. However, we have not conducted detailed neuropsychological testing or formal assessment of psychoeducational achievement of this rather large kindred.

Methods

After informed consent, blood samples were obtained from 41 members of family K8300 including the proband, unaffected male relatives and their mothers, and the obligate heterozygotes in generations II, III, and IV who could provide information for identification of the X chromosome bearing the mutant allele.

PREPARATION OF DNA

Genomic DNA was isolated from peripheral blood using a high salt precipitation method.7 Purified DNA was diluted to a concentration of 105 µg/ml and stored at 4°C in TE buffer (10 mmol/l Tris-chloride, pH 7.6, 1 mmol/l EDTA).

MICROSATELLITE ANALYSIS

The conditions used to generate the specific di-, tri-, or tetranucleotide polymorphisms were as given in Nelson et al. The forward primers for PCR products analysed by the Automated Laser Fluorescent Sequencer (ALF, Pharmacia, Uppsala, Sweden) were synthesised with fluorescein amidite (FluoroPrime, Pharmacia). Primers for the ALF were desalted through Sephadex G-25 (NAP-10 columns, Pharmacia). Detection of the polymorphisms was done by ALF with Fragment Manager software (Pharmacia).

LINKAGE ANALYSIS

Two point disease to marker linkage analysis was conducted with the program MLINK of the LINKAGE package.7 The mutation rate and gene frequency were set at 3 x 10^-6, and 0.0001, respectively.

Results

The maximum expected lod score for this family was estimated to be Z=3.88 at θ=0.00. Initial two point lod scores for the disease locus and 14 markers spanning the X chromosome were computed using MLINK. Suggestive linkage to Xq28 was noted for DXS1108 (Z=1.85 at θ=0.00). Once suggestive linkage was established to this region, additional markers were used and significant linkage (Z=>3.0 at θ=0) was observed at DXS1684 (Z=3.65), DXS8103 (Z=3.56), DXS8061 (Z=2.35), p39 (Z=3.88), and F8C (Z=3.57), all in region Xq28 (table 1).

Haplotype analysis indicated that recombination occurred between DXS1113 and DXS1684 proximally during transmission of an X chromosome from III.2 to IV.2 (fig 1). No recombination was observed between the disease locus and our most distal marker, DXS1108. Thus, two point analysis indicated a region of linkage extending from distal to DXS1113 to at least DXS1108 in Xq28.

Discussion

In the family we report here, X linked recessive inheritance of severe mental retardation is associated with minor facial and other anomalies in some affected subjects. These include relatively non-specific findings, such as a small mouth, epicanthic folds, tapering fingers (fig 2), hydrocele, undescended testis, and inguinal hernias. None of these features is consistently present in all five affected males whose physical findings, medical records, and photographs have been personally scrutinised by one of us (GSP). Since a clinically recognisable multiple anomaly syndrome is not present in this family,
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non-specific X linked mental retardation (MRX) is the most appropriate designation for this entity at this time. Seizures occurred in four of the boys (cases 2, 3, 4, and 5) and were initially associated with febrile episodes resulting from a respiratory infection in two (cases 2 and 3). A diffusely abnormal but non-specific EEG was found in cases 2, 3, and 4 while CT or MRI scans of the brain were normal in two (cases 1 and 3) who were studied. Most remarkable was the multiple episodes of lower respiratory infections variously described as bronchopneumonia, lobar pneumonia, and diffuse pneumonitis on the basis of chest radiographs in at least three of the affected boys (cases 3, 4, and 5) and by report in another (case 2). It is noteworthy that these children, though mentally retarded, were living at home with loving parents and were well-nourished as judged by their normal growth. Thus, their infections were not nosocomial hazards often faced by institutionalised or debilitated people. Two of the boys had been immunised by live virus vaccines and had normal immunoglobulins and blood counts, thus ruling out severe immunodeficiency syndromes.

At the Seventh International Workshop on Fragile X Syndrome and X Linked Mental Retardation held in 1995, 41 non-specific XLMR loci were recognised and designated MRX1 to MRX41. Unlike the syndromic, neuromuscular, metabolic, or dominant lethal X linked mental retardation syndromes, MRX families have distinguishing clinical or laboratory features. MRX families cannot be distinguished from one another except by their location on the physical and genetic map of the X chromosome. Currently, there appear to be at least eight to 10 non-overlapping regions where the 41 MRX families cluster along the length of the X chromosome.5,6 Xq28 is one of these regions. Within Xq28, there are two syndromic (Waisman-Laxova syndrome, MIM 311510, and PPP-X syndrome, MIM 300055) and five non-syndromic forms of XLMR (MRX3, MRX16, MRX25, MRX28, and MRX41), two candidate loci (DXS295 and DXS296) identified through microdeletions found in two unrelated males, and two cloned genes (LICAM and GABRA3) that could be candidates for the phenotype observed in our family.7 The loci (DXS295 and DXS296) deleted in the patients of Mulley10 can be excluded because they are centromeric to the proximal boundary of the region of linkage in our family. Although our patients show early developmental delay, seizures, and abnormal EEG similar to the patients described by Laxova et al11 and Gregg et al,12 none of them has frontal bossing, macrocephaly, or extrapyramidal signs which are the defining characteristics of the syndrome that is now designated the Waisman-Laxova syndrome (MIM 311510). However, the possibility that they could have developed Parkinsonism at an older age had they lived long enough cannot be ignored altogether. The PPP-X syndrome3 seems unlikely because in this family there is more severe mental retardation and early onset of developmental delay and there is no evidence of manic depressive episodes, aggressive behaviour, or macro-orchidism in either of the two males (cases 2 and 5) who had lived for 10 and 21 years, respectively. Our proband is only 6 years old at present and has no macro-orchidism. However, he needs to be watched closely for signs of the PPP-X syndrome since it is not possible formally to exclude this diagnosis given the limited knowledge we have of its complete clinical spectrum. The non-syndromic XLMR syndromes MRX3, MRX16, MRX25, MRX28, and MRX41 have all been assigned to Xq28 on the basis of lod scores of greater than +2.0 and haplotype analysis to define boundaries flanking the region of linkage when possible.13-18 While these assignments follow the recommendations of the nomenclature committee of the Eleventh International Gene Mapping Workshop,19 potential pitfalls of this approach have been pointed out and the assignments are considered tentative.20 Also, there is no way of knowing whether the various families studied represent a single locus with multiple alleles or multiple loci clustered in Xq28 that are involved in the determination of normal

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cognitive and behavioural functions. The region of interest covers an estimated 7 Mb of DNA that is already known to have numerous loci responsible for syndromic disorders such as adrenoleucodystrophy, X linked myotubular myopathy, Barth syndrome, and the L1CAM associated syndromes with neuromuscular and cognitive impairment. The clinical manifestations of L1CAM mutations have been so varied within and among families that at least three clinically defined syndromes (MASA syndrome, MIM 303350, X linked hydrocephalus, MIM 307000, and X linked spastic paraplegia, MIM 312900) may have to be reconsidered in light of the new findings. In addition, the recent update of L1CAM mutations and their associated spectrum of clinical manifestations further suggests that L1CAM remains a candidate gene for the disorder in this family.

In summary we have found strong linkage to multiple loci distal to DXS1113 in Xq28 in a family segregating a non-syndromic form of severe XLMR associated with early death in affected males and no evident heterozygous manifestation in females.

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