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PPM-X: A New X-Linked Mental Retardation Syndrome with Psychosis, Pyramidal Signs, and Macroorchidism Maps to Xq28

S. Lindsay,[1] M. Splitt,[1] S. Edney,[1] T. P. Berney,[2] S. J. L. Knight,[3] K. E. Davies,[3] O. O'Brien,[1] M. Gale,[1] and J. Burn[1]

[1]Department of Human Genetics, Newcastle upon Tyne; [2]Prudhoe Hospital, Prudhoe, Northumberland; and [3]Institute of Molecular Medicine, Oxford

Summary

We report a three-generation family manifesting a previously undescribed X-linked mental retardation syndrome. Four of the six moderately retarded males have had episodes of manic-depressive psychosis. The phenotype also includes pyramidal signs, Parkinsonian features, and macroorchidism, but there are no characteristic dysmorphic facial features. Affected males do not show fragile sites at distal Xq on cytogenetic analysis, nor do they have expansions of the CGG repeats at the FRAXA, FRAXE, or FRAXF loci. Linkage analyses were undertaken, and a maximal LOD score of 3.311 at $\theta = .0$ was observed with the microsatellite marker DXS1123 in Xq28. A recombination was detected in one of the affected males with DXS1691 (Xq28), which gives the proximal boundary of the localization. No distal recombination has been detected at any of the loci tested.

Introduction

The genetic contribution to mental retardation is well established, and X-linked mental retardation (XLMR) is widely recognized as being one of the major forms. To date, there are 120 separate X-linked entities listed on OMIM that include mental retardation as a key feature. These have been subdivided arbitrarily into five classes: syndromes, dominant disorders, metabolic disorders, neuromuscular disorders, and nonspecific (Neri et al. 1994). We report a three-generation family that represents a previously undescribed XLMR syndrome, the most striking feature of which is a predisposition to manic depressive psychosis. This association is particularly intriguing in light of the reports by several groups of a gene for manic-depressive illness localizing to Xq27-q28 (e.g., Baron et al. 1987). More recently, with the advent of large numbers of highly polymorphic markers, support for such a localization has diminished (Baron et al. 1993), but it has not been excluded conclusively (De bruyn et al. 1994; reviewed in Pauls 1993).

The size of the family allowed us to carry out meaningful linkage analyses, initially with four markers from different regions of the X, which detected linkage to DXS1123 in Xq28. A further eight loci from Xq27 and q28 were then analyzed. In addition, the CGG triplet repeats at the FRAXA, FRAXE, and FraXF loci were studied directly.

Patients and Methods

Family Data

Figure 1 shows part of a large pedigree displaying XLMR; the founder female was born in the Shetland Islands. Clinical details and DNA were obtained from the six living affected males, three unaffected male siblings, and the four obligate carrier females. Ethical approval for the study was granted by the Newcastle and North Tyneside Health Authorities Joint Ethics Committee. The essential clinical features of the six affected males are summarized in table 1. The affected males all have moderate mental retardation. Obligate carrier females have all attended mainstream school but have below-average intelligence. Affected individuals have no characteristic dysmorphic facial features (see fig. 2). They have a degree of macroorchidism. Several have pyramidal tract signs, and one has definite Parkinsonian features. Four of the six living affected males have required psychiatric intervention for recurrent episodes of depression and hypomania. Unaffected siblings have no history of mental illness.

Case Histories

III-20 (b. 1981; see fig. 2*A*) was first recognized as being developmentally delayed at the age of 18 mo. Cytogenetic, endocrine, and metabolic studies were normal, as was electroencephalography (EEG). He had a deprived and chaotic early life and at the age of 7 years

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Address for correspondence and reprints: Dr. Susan Lindsay, Department of Human Genetics, 19/20 Claremont Place, University of Newcastle upon Tyne, Newcastle upon Tyne, NE2 4AA, United Kingdom.

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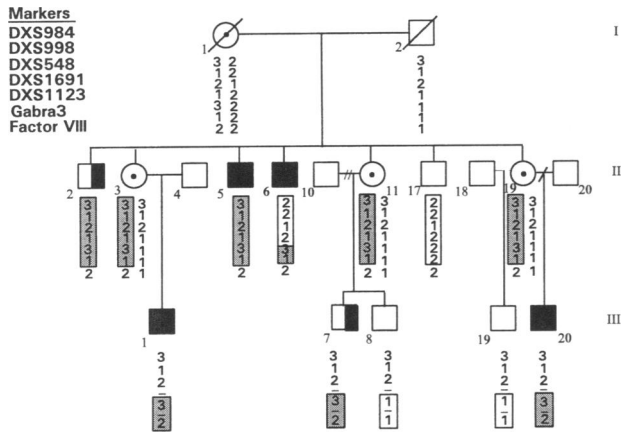

Figure 1 Pedigree of part of the family from whom samples have been taken for DNA analysis. A square with the left half unblackened and the right half blackened (◨) represents an affected male with mental retardation. A blackened square (■) represents an affected male with mental retardation and manic-depressive illness. A circle with a dot in the center (⊙) represents a carrier female. An unblackened square (□) represents an unaffected male. Symbols with diagonal lines through them represent individuals who are deceased. Shaded boxes indicate disease chromosomes. Unshaded boxes indicate unaffected chromosomes. Where no shading is given, the mother is uninformative at the locus.

was placed in the legal custody of his paternal grandmother. He was said to be cheerful and sociable. At 8 years and 6 mo he obtained an IQ score of 49 (Weschler Intelligence Test for Children). Two years later (in 1992), at age 10 years and 10 mo, he was referred to the Northern Regional Genetics Service. On examination, height and weight were in the 90th centile; head circumference (HC) was 55.5 cm (90th centile); and ear length 65 mm. He had no striking dysmorphic facial features. Average testicular volume was enlarged, at 20 ml (Prader Orchidometer) at Tanner pubic hair (PH) stage 3. On neurological examination, he was stiff, with a stooped posture, shuffling gait, and pronounced tremor at rest. He had markedly increased tone and very brisk tendon reflexes in all four limbs with bilateral up-going plantar reflexes. There was no dystonia.

At the age of 11 years and 6 mo, he developed an acute behavioral change characterized by despondency, weepiness, fearfulness, sleep disturbance, and poor appetite. An EEG was normal. A diagnosis of acute endogenous depression was made. He was treated with a tricyclic antidepressant with good initial response but remains on treatment, as he has relapsed within weeks whenever an attempt is made to stop medication.

III-1 (b. 1970; see fig. 2*B*) was of normal birth weight (3.43 kg). He was referred to the Genetics Clinic in 1985. At an early age he had been assessed as having moderate mental retardation and attended a school for children with special educational needs. At 2 years he had an epileptic seizure and was treated with carbamazepine for some years. At the age of 14 years he had an acute episode of change in mood, during which he became talkative, irritable, aggressive, overactive, and insomniac. An EEG at the time was abnormal, showing epileptic activity with sharp wave discharges from the vertex extending to the right side. This episode lasted for 6 wk until he apparently responded to treatment with carbamazepine with the return of his EEG to normal. Six months later he had a second, similar episode, again with an abnormal EEG. The diagnosis of hypomania was made; carbamazepine was ineffective; and a trial of lithium had to be discontinued after 3 wk because of marked tremor. He then began to improve, but with diurnal depressive mood swings, and was discharged

Table 1

Summary of Clinical Features in Affected Males

Subject	Age at Examination (years)	IQ or Estimate of Learning Disability	Manic-Depressive Psychosis	CNS Signs			Short Stature	Macroorchidism
				Pyr[a]	Tr[b]	Par[c]		
II-2	38	Moderate	−	NK	−	−	+	+
II-5	39	50[d]	+	+	−	−	+	NK
II-6	41	Moderate	+	+	+	−	+	+
III-1	25	Moderate	+	+	+	−	±	+
III-7	12	Moderate	−	−	−	−	−	+
III-20	11	49[e]	+	+	+	+	−	+

NOTE.—NK = not known.
[a] Pyramidal tract signs—hyperreflexia or spasticity of lower limbs.
[b] Tremor.
[c] Parkinsonian features.
[d] Stanford-Binet.
[e] Weschler Intelligence Test for Children.

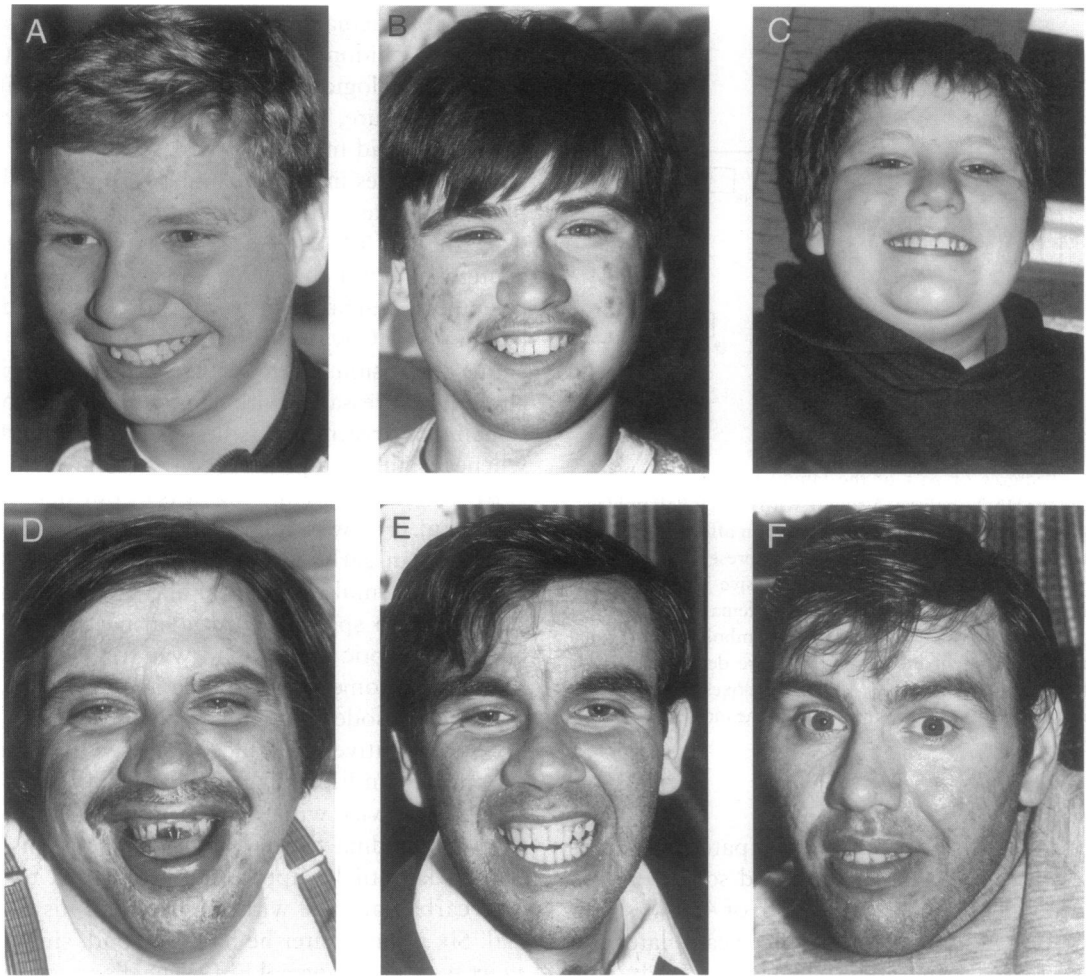

Figure 2 Photographs of the six living affected males in the family: *A*, III-20; *B*, III-1; *C*, III-7; *D*, II-2; *E*, II-5; and *F*, II-6

after 2 mo on no medication. Over the next 3 years he had three affective episodes characterized by withdrawal, weepiness, diurnal drowsiness, and nocturnal insomnia, anorexia, and agitated stereotypic behavior. He was noted to have increased tone and tremor during these episodes. EEGs done during these predominantly depressive, mixed-affective episodes were all entirely normal. The episodes responded to treatment with sodium valproate, and he remains well on this treatment.

On examination at the age of 25 years, height was 166 cm (10th centile); weight 74 kg (75th centile); HC 56.0 cm (25th centile); and ear length 64 mm (>97th centile). Average testicular volume was 45 ml. Positive signs on neurological examination were mild tremor, an unsteady, wide-based gait, and increased tone and tendon reflexes bilaterally in the lower limbs.

III-7 (b. 1983; see fig. 2*C*) was recognized to have global developmental delay in the 2d year of life and was assessed to have special educational needs. At 7 years he was fully investigated for severe behavior problems and obesity. All routine investigations, including EEG, were normal. Both his behavior and obesity improved after transfer to a residential school for children with mental retardation.

On examination at the age of 12 years he was obese; height was 157 cm (75th centile); weight was 78 kg (>97th centile); HC was 52.8 cm (25th centile); and ear length 71 mm. Testicular volume was 25 ml at Tanner PH stage 3. Neurological examination was normal.

II-2 (b. 1947; see fig. 2*D*) is moderately mentally retarded and had not presented to medical attention on the basis of mental illness or behavior problems. He developed congestive cardiac failure of unknown etiology at the age of 39 years. On examination at the age of 38 years he was obese (height 158 cm [<3d centile]; weight 95.5 kg [>97th centile]). His testicular volume was 35 ml. He was normal to a limited neurological examination.

II-5 (b. 1956; see fig. 2*E*) is moderately mentally retarded with an early IQ assessment of ~50 (Stanford-

Binet). Severe behavior problems led to his admission to a mental retardation hospital at 7 years. Throughout childhood he was said to have aggressive and destructive outbursts. Since the age of 28 years he has had multiple acute depressive episodes that have responded to tricyclic antidepressants. He has also had several episodes of agitated aggressive behavior, associated with overactivity, talkativeness, and terminal insomnia, lasting several weeks, which were treated with neuroleptics. An EEG performed during one of these episodes was normal. He was discharged to a community home at the age of 38 years and has since been treated for two further episodes of depression. Throughout his life he has been noted to have an intermittent Parkinsonian tremor and shuffling gait even when medication has been stopped.

On examination at the age of 39 years, height was 157 cm (<3d centile); weight 66 kg (50th centile); HC 53.8 cm (10th centile); and ear length 70 mm. He had facial acne. On neurological examination tone was normal, but he had abnormally brisk lower limb tendon reflexes.

II-6 (b. 1954; see fig. 2*F*) was of normal birth weight (~3.6 kg). Moderate mental retardation was noted at an early age. He spent most of his early life in a children's home. At the age of 11 years he required admission to a psychiatric ward after becoming acutely withdrawn, refusing food, with no spontaneous action. He responded to electroconvulsive therapy (ECT). Since that time he has been a long-term patient in a mental hospital. Throughout his adolescence he had many similar episodes, interspersed with episodes of overactivity, terminal insomnia, irritability, and aggression. He was treated with major tranquilizers and required ECT for depression on two further occasions. A prolonged withdrawn state responded to a selective serotonin reuptake inhibitor in 1993, and he remained on this treatment for 2 years, as attempts to withdraw medication resulted in relapse. A recent episode of hypomania has led to the start of treatment with sodium valproate.

On examination at the age of 41 years, height was 159 cm (<3d centile); weight 82.3 kg (90th centile); HC 56.2 cm (50th centile); ear length 76 mm; and testicular volume 35 ml. He had a thoracic kyphoscoliosis and a facial acneiform rash. He had a mild tremor and bilaterally increased tone and tendon reflexes in the lower limbs.

I-1 (1925–95) was of low intelligence and did not report any learning difficulties at school, but she subsequently had several convictions for child neglect. She had a cerebrovascular accident at the age of 48 years and was diagnosed as having congestive cardiac failure and dementia prior to her death.

I-2 (1917–90) was of low normal intelligence. He worked as a refuse collector but retired because of physical disability and was confined to bed in his later years. He had several convictions for child neglect.

Cytogenetic and DNA Analysis at FraXA, FRAXE, and FRAXF

Normal karyotypes, with no detectable fragile sites in distal Xq, were demonstrated in all affected males (data not shown). No amplification of the CGG triplet repeats was detected at the FRAXA, FRAXE, and FRAXF loci. The FRAXA locus was examined both by hybridization (using probe OX1.9; Hirst et al. 1991) and by PCR (Brown et al. 1993; data not shown). The triplet repeats at the FRAXE and FRAXF loci were examined by PCR, following the conditions given in Knight et al. (1993) and Ritchie et al. (1994), respectively (data not shown).

DNA Analysis

Genomic DNA was extracted from EDTA-coagulated blood by standard methods and used as the template in PCRs for each of the markers analyzed. The markers used in the study were DXS999 (Xp22.12; Donnelly et al. 1994), DXS566 (Xq13; Porteous et al. 1992), DXS178 (Xq22; Allen and Belmont 1992), DXS984 (Xq27.1; Donnelly et al. 1994), DXS998 (Xq27.3; Gyapay et al. 1994), DXS548 (Xq27.3; Riggins et al. 1992), DXS1691 (Xq28; S. J. L. Knight, unpublished observations), DXS1123 (Xq28; Donnelly et al. 1994), GABRA3 (Xq28; Hicks et al. 1991), DXS52 (Xq28; Richards et al. 1991), factor VIII (Xq28, both intron 13 and intron 22; Lalloz et al. 1994), and DXS1108 (Xq28; Freije et al. 1992). The reactions were carried out in a 10-μl/sample volume, containing 2-mM dNTPs, 1 × manufacturer's buffer (NBL Gene Sciences), 100 ng of genomic DNA, 0.5 U of *Taq* polymerase, and 1 μM forward and reverse primer. The forward primer was end-labeled using gamma-^{32}P ATP by standard methods. The primers and PCR cycle conditions are as given in the sources cited, with the exception of DXS1691, where the primers were #F322 5′-GCAATGATAATGTTGAGTTCTACC-3′ and #F010 5′-CTCAAGACCAAACTTGAAGAAACC-3′; the PCR cycle conditions were an initial cycle of 95°C for 4 min, followed by 33 cycles of 95°C for 1 min, 65°C for 1 min, and 72°C for 1 min, with a final cycle of 72°C, 4 min. All the reaction products were analyzed on 6% denaturing polyacrylamide gels.

Statistical Analysis

The results were analyzed using the LIPED program (Ott 1974). It was assumed that the mental retardation gene was fully penetrant and had a frequency of .001. Two-point linkage analysis was carried out between it and each of the markers in turn. Linkage was not tested directly between the markers and the manic-depressive illness.

Results

Initially, four markers were tested from different regions of the X chromosome: DXS999 (Xp22.12),

Table 2

Results of Two-Point Linkage Analyses Between X-linked Markers and the Disorder

Marker	Approximate Position	Location	LOD Score at θ = .000	.001	.05	.10	.20
DXS999	19.3	Xp22.12	∞	−5.993	−1.071	−.356	.140
DXS566	75.8	Xq13	∞	−9.593	−2.885	−1.775	−.775
DXS178	100.8	Xq22	∞	−14.691	−4.606	−2.917	−1.367
DXS984	140.2	Xq27.1	∞	−1.196	.371	.532	.526
DXS998	151.1	Xq27.3	∞	−1.196	.371	.532	.526
DXS548	151.4	Xq27.3	∞	−1.196	.371	.532	.526
DXS1691	152.1	Xq28	∞	−1.196	.371	.532	.526
DXS1123	152.8	Xq28	3.311	3.306	3.044	2.762	2.148
GABRA3	155.8	Xq28	1.806	1.803	1.650	1.486	1.128
Factor VIII	158.9	Xq28	1.505	1.503	1.394	1.276	1.021

Note.—Approximate position of the marker (given in Mb) from the telomere at Xp. The entire length of the X chromosome is ~160 Mb (taken from Nelson et al. 1995).

DXS566 (Xq13), DXS178 (Xq22), and DXS1123 (Xq28). As can be seen from table 2, many recombinants were detected with the first three markers, while none were seen with DXS1123 (LOD = 3.311 at θ = .0).

A further eight markers were then tested that mapped proximal and distal to DXS1123 in Xq27 and Xq28, respectively. The results for six of the markers (DXS984, DXS998, DXS548, DXS1691, GABRA3, and factor VIII) are given both in table 2 and in figure 1. The other two markers, DXS52 and DXS1108, were uninformative. The order of the markers is taken from Donnelly et al. (1994) and Nelson et al. (1995).

The results show a recombination event in individual II-6 between DXS1691 and DXS1123 and between DXS1691 and the disorder. This recombination gives the proximal boundary for the disease gene location. Both markers tested in the factor VIII locus (given as single result in table 2 and fig. 1) and at the GABRA3 locus were only informative in part of the family, and no distal recombination event was detected. DXS1691 lies distal to FRAXE (S. J. L. Knight, unpublished observations) and FRAXA (Nelson et al. 1995), and thus the position of this recombination event excludes these two loci as candidates for the disorder. This finding was confirmed by direct analysis of the CGG triplet repeats at each locus, which did not show any expansions in the affected males (data not shown). Direct analysis of the CGG triplet repeat was also carried out at the FRAXF locus, which lies distal to DXS1123 (Nelson et al. 1995), where, similarly, no expansion of the repeat was detected (data not shown).

Discussion

The phenotypic feature that focused attention on this family was the apparent predisposition to manic-depressive psychosis in affected males. The high psychiatric morbidity of individuals with learning difficulties is well known (Szymanski and Crocker 1989), and a specific association between mental retardation and atypical bipolar affective disorder is recognized (Berney et al. 1988). Nevertheless, the frequency and severity of psychosis in this family and its consistent association with mental retardation strongly suggest a syndromic basis. The four males who have had psychotic episodes all have some degree of pyramidal tract involvement. III-20 also had definite Parkinsonian features that were known to predate the onset of his first psychotic episode. Tremor and gait disturbance have been noted at various times in the other three males with psychosis. It is difficult, however, to be sure that these extrapyramidal features are not secondary to treatment with neuroleptic drugs. III-1 had EEG abnormalities with an epileptogenic focus during the early periods of mania, which subsequently resolved. These neurological abnormalities point to an underlying organic disturbance as the basis for the psychiatric disorder. The XLMR syndrome described by Laxova et al. (1985), which includes early-onset Parkinsonism, also maps to Xq27.3-qter (Gregg et al. 1991). In addition to basal ganglia signs, the propositus in the family had hyperactive and disruptive behavior. Affected individuals were not reported to have manic-depressive psychosis or pyramidal tract signs. Most affected males had frontal bossing and increased head circumference, which is not a feature in our family.

While the family reported here displayed a range of neuropsychiatric disorders, the propensity to psychosis and the frequency of pyramidal signs were felt to be cardinal and are highlighted in the acronym, PPM-X. Macroorchidism (>25 ml) was found in all the adult males examined. In addition, III-7 and III-20 had testicu-

lar volumes well above the normal range for their pubertal stage. Inclusion of "M" (for "macroorchidism") identifies this syndrome as an alternative to fragile X syndrome in retarded males with enlarged testes. The significance of the short stature in II-2, II-5, and II-6 is unclear. In II-6 it is accentuated by kyphoscoliosis. It seems more likely to be constitutional rather than part of the syndrome. Their mother was known to be small, and two of the affected boys in the next generation are well above average height for their age.

The linkage analyses indicate that the disorder in this family maps distal to DXS1691 in Xq28. This localization excludes two of the genes that map to distal Xq and that cause mental retardation, *FMR1* at FRAXA (Kremer et al. 1991; Verkerk et al. 1991) and FRAXE (Knight et al. 1993). These loci and a third fragile site in distal Xq, FRAXF (Xq28) have also been excluded by direct analysis of their CGG triplet repeats (data not shown). Gedeon et al. (1995) have reported the identification of deletions just distal to FRAXE in two unrelated patients with developmental delay. Neither patient is reported to have a history of mental illness. The deletions were detected with probes from DXS295 and DXS296. They have also shown that a probe at DXS296 detects transcripts in RNAs from several tissues, including the brain. A probe at DXS296 (VK21A) hybridizes to the same fragments in unaffected and affected males from our family, indicating that there is no detectable deletion at this locus (data not shown).

The region distal to DXS1691 that contains the causative gene(s) in this family is ~8 Mb in size (Nelson et al. 1995). No recombination events were detected by distal markers GABRA3 and FVIII, which were informative in generations I and II, respectively (fig. 1; table 2). Other distal markers were found to be uninformative in the family (DXS52 and DXS1108; data not shown). The information from GABRA3 and FVIII suggests that it is unlikely that a distal recombination event exists. In light of this information, fruitful approaches to identifying the causative gene(s) may be screening for a large-scale deletion and analysis of candidate genes as they are characterized.

One possible candidate is the neural cell adhesion molecule L1 (L1CAM). Mutations in L1CAM result in both X-linked hydrocephalus and MASA syndrome (Kenwrick et al. 1996). L1CAM plays an important role in the morphogenesis of the nervous system and continues to be expressed in the adult brain and therefore must be a good candidate for any Xq28-linked disorder with a neurological component. Lower limb spasticity, hyperreflexia, and short stature are all key features of MASA syndrome (mental retardation, aphasia, shuffling gait, and adducted thumbs), and the condition shows a high degree of inter- and intrafamilial variation. However, there are no reports of a psychiatric condition being part of the spectrum of disease seen with L1CAM mutations.

A second candidate in Xq28 is the gene for the alpha 3 subunit of the receptor for the gamma-aminobutyric acid neurotransmitter (GABRA3; Buckle et al. 1989). Two genes for nonspecific XLMR have also been mapped to this region of the X chromosome, MRX3 and MRX25 (Gedeon et al. 1991; and Nordstroem et al. 1992, respectively). In both these disorders the only feature described is mental retardation. Nevertheless, it is possible that they are deleted as part of a contiguous gene defect. As yet, neither MRX3 nor MRX25 has been cloned, so this possibility cannot be tested directly.

We have described a new XLMR phenotype, PPM-X syndrome, with prominent neuropsychiatric features. Linkage to Xq28 strengthens the suggestion of a gene in this region involved in the genesis of bipolar affective disorder. Identification of the causative gene(s) in this family may prove of particular interest both in the elucidation of manic-depressive psychosis and in its role in mental retardation.

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