Letter to the Editor

Increasing Evidence for a New X-Linked Mental Retardation/Epilepsy Gene Localized to Xp21.3-Xp22.1

To the Editor:

We read with interest the article, published recently in this journal by Ronce et al. [1999] describing three generations of a French family segregating with a syndromal form of X-linked mental retardation (XLMR) characterized by hypotonia, intractable seizures, and severe mental deficiency. We wish to draw attention to four phenotypically similar families that were reported previously and described as an X-linked form of infantile spasms [Feinberg and Leahy, 1977; Rugtveit, 1986; Claes et al., 1997]. The affected males of these latter families [Ronce et al., 1999] presented with severe mental deficiency associated with early-onset intractable seizures typical of infantile spasms in most but not all cases.

We also have identified a large three-generation Canadian family with infantile spasms and mental retardation seen exclusively in male offspring from asymptomatic mothers, representing the fifth such family reported [Bruyère et al., 1999]. Molecular studies using polymorphic microsatellite markers of chromosome X allowed us to determine the locus to be between marker DXS1226 and the adrenal hypoplasia locus in a 7-cM interval contained in the interval previously reported by Claes et al. [1997] and Ronce et al. [1999]. The marker DXS451, for which the two-point lod score with the *XLMR* gene was established to be 2.90 at a θ of 0.0, is contained between the two recombinations we described. Therefore, it is likely that the French family described by Ronce et al. [1999] represents a disorder allelic to, or perhaps even typical of, the disorder increasingly recognized as X-linked West syndrome.

REFERENCES

Bruyère H, Lewis MES, Wood S, MacLeod P, Langlois S. 1999. Confirmation of linkage in X-linked infantile spasms (West syndrome) and refinement of the disease locus to Xp21.3-Xp22.1. Clin Genet 55:173–181.

Claes S, Devriendt K, Lagae L, Ceulemans B, Dom L, Casaer P, Raeymaekers P, Cassiman JJ, Fryns JP. 1997. The X-linked infantile spasms syndrome (MIM 308350) maps to Xp11.4-Xpter in two pedigrees. Ann Neurol 42:360–364.

Feinberg AP, Leahy WR. 1977. Infantile spasms: case report of sex-linked inheritance. Dev Med Child Neurol 19:524–526.

Ronce N, Raynaud M, Toutain A, Moizard M-P, Colleaux L, Gendrot C, Briault S, Moraine C. 1999. Evidence for a new X-linked mental retardation gene in Xp21-Xp22: clinical and molecular data in one family. Am J Med Genet 83:132–137.

Rugtveit J. 1986. X-linked mental retardation and infantile spasms in two brothers. Dev Med Child Neurol 28:544–546.

H. Bruyère
M.E.S. Lewis*
S. Wood
P. MacLeod
S. Langlois
Department of Medical Genetics
University of British Columbia and
the Children's and Women's Health Center
Vancouver, Canada

*Correspondence to: Dr. Suzanne Lewis, Department of Medical Genetics, University of British Columbia, and the Children's and Women's Health Center, C234, 4500 Oak Street, Vancouver, BC, Canada V6H 3N1. E-mail: slewis@cmmt.ubc.ca

Received 25 March 1999; Accepted 28 March 1999

Clin Genet 1999: 55: 173–181
Printed in Ireland. All rights reserved

CLINICAL GENETICS
ISSN 0009-9163

Original Article

Confirmation of linkage in X-linked infantile spasms (West syndrome) and refinement of the disease locus to Xp21.3-Xp22.1

Bruyere H, Lewis MES, Wood S, MacLeod PJ, Langlois S. Confirmation of linkage in X-linked infantile spasms (West syndrome) and refinement of the disease locus to Xp21.3-Xp22.1.
Clin Genet 1999: 55: 173–181. © Munksgaard, 1999

The syndrome of infantile spasms, hypsarrhythmia, and mental retardation (West syndrome) is a classical form of epilepsy, occurring in early infancy, which is etiologically heterogeneous. In rare families, West syndrome is an X-linked recessive condition, mapped to Xp11.4-Xpter (MIM 308350). We have identified a multi-generation family from Western Canada with this rare syndrome of infantile spasms, seen exclusively in male offspring from asymptomatic mothers, thereby confirming segregation as an X-linked recessive trait. Using highly polymorphic microsatellite CA-repeat probes evenly distributed over the entire X chromosome, linkage to markers DXS7110, DXS989, DXS1202, and DXS7106 was confirmed, with a maximum LOD score of 3.97 at a θ of 0.0. The identification of key recombinants refined the disease-containing interval between markers DXS1226 and the adrenal hypoplasia locus (AHC). This now maps the X-linked infantile spasms gene locus to chromosome Xp21.3-Xp22.1 and refines the interval containing the candidate gene to 7.0 cM. Furthermore, this interval overlaps several loci previously linked with either syndromic or non-syndromic X-linked mental retardation (XLMR), including one recognized locus implicated in neuroaxonal processing (radixin, RDXP2). Collectively, these studies lend strong support for the presence of one or more genes intrinsic to brain development and function, occurring within the critical interval defined between Xp21.3-Xp22.1.

Helene Bruyere[1], ME Suzanne Lewis[1], Stephen Wood, Patrick J MacLeod and Sylvie Langlois

Department of Medical Genetics, Children's and Women's Health Center of British Columbia and the University of British Columbia, Vancouver, British Columbia, Canada

Key words: gene localization – infantile spasms – linkage analysis – mental retardation – West syndrome – X chromosome

Corresponding author: Dr ME Suzanne Lewis, Department of Medical Genetics, Room C234, Children's and Women's Health Center of British Columbia, 4500 Oak Street, Vancouver, British Columbia, Canada V6H 3N1. Fax: +1-604-8752376; e-mail: slewis@cmmt.ubc.ca

Received 27 November 1998, revised and accepted for publication 6 January 1999

The syndrome of infantile spasms, also known as West syndrome, is an age-limited encephalopathy of early infancy, for which the underlying neuropathogenesis remains unclear. Diagnostically, it comprises a generalized, symptomatic and progressive myoclonic epilepsy characterized by the classical triad of 1) infantile spasms; 2) electroencephalogram (EEG) pattern of hypsarrhythmia – a massively disturbed pattern characterized by multi-focal spikes and high-voltage slow waves in all cortical areas; and 3) arrest of psychomotor development stemming from the time of seizure onset (typically between 4 and 7 months of age). Clinically, infantile spasms comprise clusters of sudden involuntary flexion or extension movements of the neck, trunk, or extremities, following a period of brief neuromuscular atonia. Moderate to severe mental retardation is found in 60–70% of patients at the onset of infantile spasms. Whereas West syndrome accounts for approximately 2% of all childhood epilepsy, it is diagnosed in a striking 28–30% of infants presenting with recurrent, unprovoked seizures (1).

Several forms of epilepsy support a strong genetic predisposition. Some forms are clearly inherited as simple Mendelian traits – either autosomal dominant, autosomal recessive, or X-linked recessive. Genetic studies that have focused on these Mendelian inherited forms of epilepsy have led to the identification of chromosomal loci for 11 epilepsy genes, respectively, on chromosomes 1q, 6p, 8q, 16p, 20q, 21q, 22q (2, 3), Xp (4); two loci, respectively, on chromosomes 19p and 19q for

[1] These authors contributed equally to this manuscript.

familial febrile convulsions (5, 6); and most recently, a novel protein tyrosine phosphatase (PTP) gene on chromosome 6q24, implicated in the autosomal recessive form of progressive myoclonus epilepsy, known as Lafora's disease (LD; MIM 254780)[2] (7). Another progressive myoclonus epilepsy of Unverricht–Lundborg type (EPM1) is an autosomal recessive disorder mapped to chromosome 21q22.3 by linkage analysis (8). After refinement of the critical region to a small interval, mutations in the gene encoding cystatin B (CSTB), a cysteine protease inhibitor, were shown to account for the majority of patients with recessively inherited myoclonic epilepsy (EPM1; MIM 254800) (9).

The highest incidence of epilepsy occurs within the first year of life. Benign familial neonatal convulsions (BFNC), an autosomal dominant condition mapped by linkage analysis to chromosome 20q13.3 (EBN1; MIM 121200) (10), was recently shown to be due to mutations in a novel potassium channel gene, KCNQ2 (11, 12). However, there is evidence that BFNC is heterogeneous, with a second locus on chromosome 8q (EBN2; MIM 121201) (13, 14). Benign familial infantile convulsions (BFIC; MIM 601764) is another autosomal dominant epileptic syndrome characterized by an age of onset within the first year of life. Onset is usually between 3.5 and 12 months, and seizures are of partial type in most cases. Genetic linkage analysis performed on 5 Italian kindreds with this disorder has recently mapped the gene for this condition to chromosome 19q (15). The causative gene is not yet identified.

An X-linked form of infantile spasms has been clearly established in four families reported in the literature. Feinberg and Leahy (16) first reported 5 affected males in four sibships of a three-generation family. Rugtveit (17) described infantile spasms in 2 brothers, who also presented with mental retardation and had 5 mentally retarded male relatives. Claes et al. (4) strengthened the existence of an X-linked locus for this syndrome by reporting two new families with clear X-linked inheritance of infantile spasms. Linkage analysis in their two families mapped the disease gene between Xp11.4 and Xpter (4).

We have studied a large multi-generation family from British Columbia and Western Canada, with this unique syndrome of infantile spasms manifesting exclusively in male offspring from asymptomatic mothers of this kindred. The affected boys appear to have normal development until the onset of infantile spasms, when loss of previously gained skills is recognized with subsequent psychomotor retardation. Extensive investigations on 3 of the affected boys have excluded a cytogenetic, metabolic, syndromic, or structural neurodevelopmental cause for their infantile spasms. This kindred, therefore, appears to be identical to the previously reported families with X-linked infantile spasms.

Identification of the genetic basis of X-linked infantile spasms may ultimately provide the stepping stone for better understanding the heterogeneous causes and or variants of this devastating condition. The purpose of the present study was to identify the disease locus on the X chromosome, as a first step toward the eventual identification of the gene defect(s) responsible for this unique form of West syndrome, or infantile spasms, clearly segregating as an X-linked recessive trait.

Subjects and methods

Ascertainment of family members

A four-generation Western Canadian family segregating for X-linked infantile spasms was identified, when the proband (IV-9) presented to BC Children's Hospital (Vancouver, BC, Canada) for genetic assessment of his infantile spasms. Fig. 1 illustrates the family pedigree obtained. Consequently, 2 similarly affected brothers within the fourth generation of this kindred (IV-5, IV-8), cousins of our initial proband (IV-9), were also clinically evaluated by two of the authors (PJM, MESL). Medical records of the affected, living maternal cousin (III-16) were reviewed. Clinical information on all other affected males was obtained from immediate family members. Peripheral venous blood samples (7.5 ml) were collected from 23 family members after informed consent was obtained.

DNA-marker analysis

Genomic DNA was isolated from the nucleated peripheral blood cells by standard phenol chloroform extraction (18). Twelve commercially available (Research Genetics®, BioCan®) polymorphic (CA repeat) microsatellite markers, evenly distributed in 10–20 cM intervals along the X chromosome (Table 1), were used for initial mapping via DNA typing of 3 affected males (IV-5, IV-8, IV-9) and 2 obligate carriers (II-2, II-8). All poly-

[2] Online Mendelian Inheritance in Man, OMIM™. Center for Medical Genetics, John Hopkins University, Baltimore, MD and National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD, USA, 1997. URL: http www.ncbi.nlm.gov omim (for MIM reference numbers).

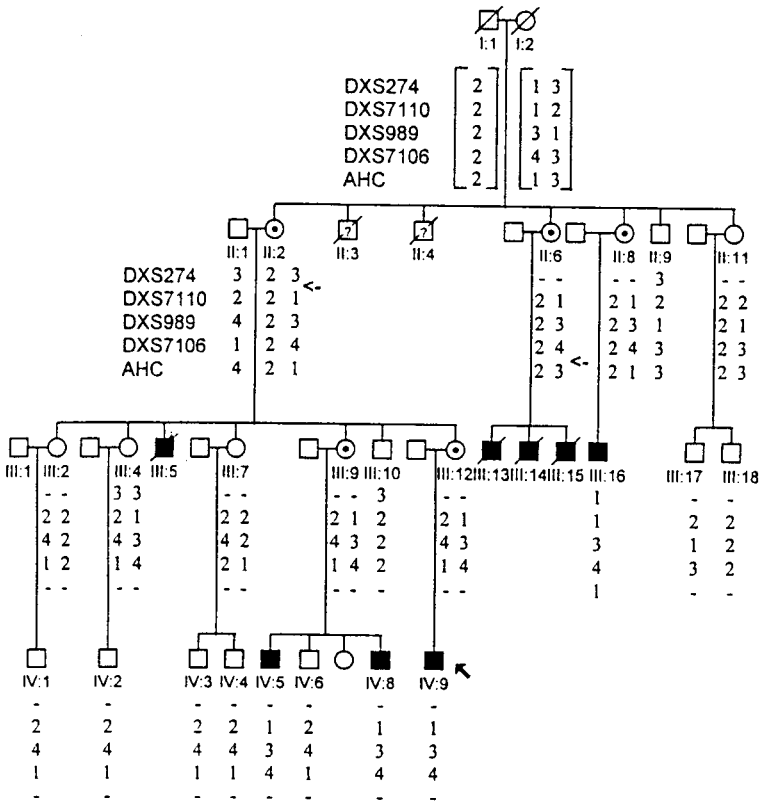

Fig. 1. Pedigree of the X-linked West syndrome family. Squares are males, circles are females, and black symbols denote clinically affected individuals. Question marks indicate possibly affected subjects. Polymorphic markers typed for the haplotype analysis are listed to the left of the first and second generations. X chromosome haplotypes are indicated below every individual. Horizontal arrows indicate recombination sites.

morphic markers were typed using the polymerase chain reaction (PCR). The microsatellite analysis was performed by PCR amplification of 100 ng of genomic DNA in a 15 µl reaction volume containing 50 mM KCL, 10 mM Tris base, 1.5–4.5 mM $MgCl_2$, pH 8.3, 0.01% gelatin, 20 pM dNTPs, 10 pM of each forward and reverse primer, 0.5 U of DNA *Taq* polymerase (Gibco BRL), and P^{32}-dCTP. Optimum $MgCl_2$ concentration was determined empirically for each pair of primers. The PCR amplification thermocycle conditions included initial denaturation for 5 min at 94°C, 35 cycles of 30 s for denaturation and 30 s for both annealing and elongation at 55°C, followed by a final 7 min for elongation at 72°C (Perkin Elmer Amp PCR 2400). The PCR products were separated on a 6% denaturing polyacrylamide sequencing gel and visualized after autoradiography. Other polymorphic markers, including the CAG polymorphism in the androgen receptor gene, the CGG repeat in the FMR1 gene, and the CA repeat in intron 49 of the dystrophin gene, were typed according to previously described techniques (19–21).

Thereafter, the second step of the mapping process involved typing of the polymorphic markers DXS7110, DXS1202, and DXS7106 in all other available family members, in an attempt to further refine the map position of the disease locus. The identification of new recombination events with the above markers, coupled with telomeric markers DXS274 and DXS1226 and centromeric markers DXS1061, DXS8039 and a CA repeat at the adrenal hypoplasia locus (AHC) in selected family members, enabled us to define a minimal region containing the X-linked infantile spasm candidate gene (Table 2). Finally, DXS7182, DXS7184, DXS1086, DXS1147, DXS1098, DXS7188 were typed in 1 affected male and 1 control to screen for a deletion within the linked interval. PCR analysis was carried out, following the protocol described above. The PCR products were electrophoresed on a 2% agarose gel and visualized under UV light after ethidium bromide staining.

Table 1. Polymorphic markers on the X chromosome

Locus	Location	Owner	Distance (cM) between each contiguous locus*
DXS1283-E	Xp22.3-Xp22.3	Ceverha, Paul	6.3
DXS1224	Xp22.3-Xp21.3	Weissenbach, Jean	5.2
DXS418	Xpter-Xp21	Minter-Morrison, Ann	2.0
DXS7099	Xp22.3-Xp21.3	Weissenbach, Jean	4.1
DXS989	Xp22-Xp22	Weissenbach, Jean	8.4
DMD-49	Xp22-Xp22	Clemens, PR	7.2
DXS1068	Xp21.1-Xp11.4	Weissenbach, Jean	6.2
DXS1367	Xp11.3-p11.23	Meindl, Alfons	16.3
AR	Xq11-Xq12	See ref. (19)	12.0
DXS441	Xq13.2-q13.3	Hudson, Jim	11.5
DXS1066	Xq21.3-Xq21.3	Weissenbach, Jean	14.4
DXS101	Xq22-Xq22	Ceverha, Paul	18.0
DXS1001	Xq24-Xq24	Weissenbach, Jean	20.5
DXS984	Xq27-Xq27	Weissenbach, Jean	9.5
FMR1	Xq27.3-Xq27.3	See ref. (20)	

* The genetic distance was determined from the chromosome X map of Genetic Location Database [URL: http: cedar.genetics.soton.ac.uk pub chrmX map (for X chromosome marker location)].

Linkage analysis

Two-point linkage analysis was performed by use of the MLINK program of the LINKAGE software package (22). Penetrances were set at 0.0 for obligate heterozygous carriers and both unaffected males and females and set at 1.0 for known affected males. Phenocopy rate was set at 0.0 and the disease gene frequency at 1 per 1 000.

Results

Clinical features

The family is of German and Russian ethnicity with no known history of consanguinity.

Table 2. Polymorphic markers on the X chromosome used to refine the area of interest

Locus	Location	Owner	Distance (cM) between each contiguous locus*
DXS7099	Xp22.3-Xp21.3	Weissenbach, Jean	3.1
DXS274	Xp22.2-Xp22.1	Hanauer, Andre	0.2
DXS1226		Weissenbach, Jean	0.4
DXS7110		Weissenbach, Jean	0.4
DXS989	Xp22-Xp22	Weissenbach, Jean	1.2
DXS1202	Xp22.1-Xp21.3	Weissenbach, Jean	1.8
DXS7106		Weissenbach, Jean	0.3
DXS1061	Xp22.1-Xp21.3	Weissenbach, Jean	2.9
AHC	Xp21.3-Xp21.3	McCabe, Edward	0.3
DXS8039		Weissenbach, Jean	1.9
DMD-49	Xp22-Xp22	Clemens, PR	

* The genetic distance was determined from the chromosome X map of Genetic Location Database [URL: http: cedar.genetics.soton.ac.uk pub chrmX map (for X chromosome marker location)].

Patient 1 (IV-9). Patient 1 (IV-9) is the proband. Pregnancy, perinatal, and neonatal histories were unremarkable. At 4.5 months of age, he developed infantile spasms, and psychomotor delay became apparent. He was examined at 5 and 10 months of age. Positive findings were again limited to neurological exam, which revealed constant roving eye movements, poor head control, truncal hypotonia, bilateral hand fisting, and typical jerking motions of the upper extremities. Detailed laboratory investigations were normal, as were cranial magnetic resonance imaging (MRI) and ophthalmologic examination. EEG demonstrated hypsarrythmia.

Patient 2 (IV-5). Patient 2 (IV-5) is the proband's maternal cousin, born at term after an uneventful pregnancy to a 20-year-old G1P0 mother. Birth weight was 3.57 kg, and no clinically recognizable abnormalities were apparent in the neonatal period. He followed normal developmental milestones until 3.5 months of age, when seizures developed. Despite ACTH therapy, uncontrolled seizures, approximating 20 infantile spasms per day, persisted. After the onset of seizures, severe psychomotor retardation ensued. At close to 8 years of age, he presented with marked developmental delay. He had poor head control and was unable to sit or ambulate independently. He had continuous roving eye movements and intermittent myoclonic jerking of both upper and lower extremities. EEG demonstrated hypsarrhythmia. Clinical and laboratory examinations excluded infectious, metabolic, and cytogenetic etiologies. Ophthalmologic and audiologic examinations were normal. Electromyography (EMG) and nerve conduction studies were normal. Cranial computed tomography (CT) scan was normal, whereas subsequent cranial MRI showed slight temporal lobe atrophy.

Patient 3 (IV-8). Patient 3 (IV-8) is the brother of patient 2. The pregnancy was uncomplicated until 6 months gestation, when decreased fetal movements were noted. Fetal monitoring was normal up until delivery at term. There were no concerns in the neonatal period. He followed normal developmental milestones until 4 months of age, when the onset of seizures occurred, and subsequent psychomotor arrest and regression followed. He responded to ACTH therapy, with seizure frequency reduced from eight to three episodes per day. Upon clinical examination at 2 years 8 months of age, noteworthy features were limited to his obvious neurodevelopmental delay, marked by poor head control and typical myoclonic flexion and extension jerking movements of the upper and lower extremities. EEG was markedly abnormal

Table 3. Pairwise LOD scores between markers and the X-linked disease locus

	Z (LOD score) at recombination frequencies							
Locus	0.0	0.001	0.01	0.05	0.1	0.15	0.2	0.25
DXS274	$-\infty$	−4.97	−2.98	−1.60	−1.02	−0.70	−0.48	−0.33
DXS7110	3.97	3.96	3.90	3.64	3.29	2.93	2.54	2.14
DXS989	3.97	3.96	3.91	3.66	3.33	2.97	2.60	2.21
DXS1202	2.77	2.76	2.72	2.52	2.27	2.01	1.73	1.45
DXS7106	3.97	3.96	3.91	3.66	3.33	2.97	2.60	2.21
AHC	$-\infty$	−1.59	−0.61	0.00	0.19	0.26	0.26	0.24

and suggestive of hypsarrhythmia. Extensive laboratory investigations, as per his older brother, failed to identify any abnormality. Ophthalmologic assessment was normal and audiologic examination showed mild hyperacusis. Both cranial CT and MRI were normal.

A detailed review of the family history also raised suspicion regarding 2 similarly affected males in the second generation (II-3, II-4). These 2 boys died in early childhood, but no information is available due to the reluctance of their mother to talk about them amongst the family.

II-6 had 3 affected sons (III-13, III-14, III-15), born in 1941, 1946, and 1950. They each had a history of epilepsy at a young age and exhibited severe psychomotor regression and typical jerking flexion extension movements, limited primarily to the upper extremities. They died at 9 years, 7 years, and 13 months of age, respectively.

II-8 has a son (III-16), who was born in 1966 after an uneventful pregnancy. His early development appeared normal until 3 months of age, when excessive irritability, apparent muscle spasms, and psychomotor delay ensued. Generalized, epileptic seizures became apparent at 15 months of age. At 5 years of age, no dysmorphic features were noted. Neurodevelopmentally, speech and language skills were absent, he was able to sit with support, head control was very poor, and he demonstrated continuous spastic jerking movements of his arms and hands. At 19 months of age, EEG suggested a diffuse encephalopathy, yet hypsarrythmia was not confirmed. EMG and ophthalmology examinations were normal.

III-5 had developmental delay. He never sat or walked. He presented with generalized seizures from 6–7 months of age. He died of pneumonia at 18 months of age. No further information was available.

DNA analysis

Initial genotyping of 3 affected males (IV-5, -8, -9) and 2 obligate carriers (III-9, -12) for the 14 polymorphic microsatellite markers spanning the X chromosome excluded most of the X chromosome (data not shown), yet indicated possible linkage between the disease locus and marker DXS989. All 3 affected males and both obligate carrier females shared one allele in common. Subsequent genotyping with marker DXS989 in all family members showed linkage, with no recombinants found with this locus, giving a maximal LOD score of 3.97 at a θ_{max} of 0.0. A significant two-point LOD score was also obtained between the disease locus and two additional markers mapping to this chromosomal interval (DXS7110 and DXS7106) (Table 3). Additional genotyping of markers mapping telomeric and centromeric to these linked loci was carried out to identify potential recombination events, in an attempt to better define the interval containing the disease gene. Individuals II-2 and III-10 were found to be recombinants for marker DXS418, which maps telomeric to the linked loci. Individual II-6 was found to be a recombinant for marker DXS992, which maps centromeric to the linked loci. Genotyping of additional markers showed that the telomeric recombination had occurred between DXS1226 and DXS7110, and the centromeric recombination had occurred between DXS7106 and AHC. Unfortunately, marker DXS1061, located between these two markers, was uninformative. Two other polymorphic markers contained in this interval (DXS8065 and DXS1065) are of low heterozygosity and were, therefore, not typed in this study. Thus, the disease locus is contained in a 7.0-cM interval between DXS1226 and AHC. Analysis of six additional markers within that interval failed to detect a deletion in the affected males.

Discussion

The syndrome of infantile spasms, known as West syndrome, appears to be a distinct syndrome complicated by heterogeneous etiology. The existence of an X-linked form of this condition (MIM 308350) has been suggested (16, 17) and confirmed

by the demonstration of linkage to Xp11.4-Xpter in two families (4). Our report confirms this X chromosomal linkage in a large four-generation family sharing a constellation of features remarkably similar to those families previously reported. The finding of key recombinants in this family has significantly refined the disease gene interval to Xp21.3-Xp22.1, between AHC proximally and DXS1226 distally, an interval of approximately 7.0 cM. As a result of further refining the gene loci for this X-linked form of infantile spasms, we have advanced the goal toward more accurate diagnosis, prognosis, and risk calculation among this group of progressive myoclonic disorders. Moreover, we have enhanced the potential for providing prenatal and presymptomatic diagnosis to members of families at risk.

Although families with clearly defined inheritance of infantile spasms may be rare, males with this condition from all causes outnumber similarly affected females 2:1 (23). There may, therefore, be a hot spot of inter-related alleles and genes on the X chromosome contributing to the high male prevalence of infantile spasms. Mutations in genes on the X chromosome are also believed to be responsible for the significant excess of males among individuals with mental retardation. The pathogenesis of infantile spasms, as well as the well-recognized concurrent development of mental retardation, remains unclear. In one study, 31 infants with West syndrome were assessed for their cognitive competence before the onset of disease and during and after the acute stage (24). The degree of transitory cognitive impairment linked to the acute epileptic stage was found to be separate and clearly less than the well-known, long-term psychomotor sequelae. Whereas the etiological role of persistent severe epileptic change leading to psychomotor deterioration can not be excluded, other, as yet undefined, neuropathologic factors may adversely influence developmental outcome in patients with X-linked infantile spasms.

In a recent update of X-linked mental retardation (XLMR) syndromes, at least 105 X-linked disorders in which mental retardation is a key, yet not exclusive, element have been identified (25). Surprisingly, the X-linked syndrome of infantile spasms (MIM 308350) was not included amongst these XLMR disorders, despite its well-characterized association with severe mental retardation.

At least 65 families with non-specific XLMR have been mapped and assigned a specific MRX number. [Human Genome Database (GDB)][3]. Nine of these MRX families [MRX2 (26), MRX13 (27), MRX21 (28), MRX29 (29), MRX32 (29), MRX33 (30), MRX34 (31), MRX36 (32), and MRX38 (33)] have been regionally mapped to an interval that contains or overlaps the interval containing the X-linked infantile spasms gene locus described in the present study (25, 34).

If one hypothesizes that these MRX families and X-linked infantile spasms families in fact represent a shared phenotype expressed from a single gene locus, this gene must map to the region shared in common by all mapped loci. Review of the linkage data from each of these ten MRX gene mapping studies identified nine MRX families and their respective flanking markers overlapping a shared common interval between DXS1202 and DXS1065 (Fig. 2) [MRX2, MRX13, MRX21, MRX29, MRX32, MRX33, MRX34, MRX36, and MRX38, the latter being the only MRX family presenting with a phenotype including both XLMR and recurrent seizures]. Interestingly, deletions have been reported in this interval: a deletion of DXS1218 was seen in all affected males of the MRX34 family and a larger deletion, extending from DXS1202 to DXS1065 (which includes DXS1218), was described by Billuart et al. in a mentally retarded male (35). This last report refines the common region to a 3.5-cM interval between DXS1202 and DXS1065 (Fig. 2). This region is within the interval defined by our study as containing the X-linked infantile spasms gene.

Our linkage data for X-linked infantile spasms also indicates that this disorder maps to an interval that overlaps with the critical X chromosome region assigned by linkage to several specific syndromic XLMR entities, including Nance–Horan syndrome [NHS: interval between DXS85 and DXS1226 (36)], Fried syndrome [interval between KAL and DXS989 (37)], Snyder–Robinson syndrome [SRS: interval between DXS443 and the 3′ end of DMD: maximum LOD score with DXS989 (38)], familial cutaneous amyloidosis [PDR: between DXS999 and DXS228: maximum LOD score with DXS989 and 5′ DYS1 within the dystrophin locus (39)] and N syndrome [NSX: defect in POLA gene, mapped between STS and DMD (40)]. Although X-linked West syndrome is unlikely to be allelic to these conditions because of clear phenotypic differences, it is reasonable to propose that a number of genes integral to neurodevelopmental structure and function map within Xp21.3-Xp22.1.

Among specific genes already mapped to Xp21.3-Xp22.1, one potential candidate gene for X-linked West syndrome is the Xp21.3 mapped radixin (RDX)-related sequence, RDXP2 (41).

[3] GDB™ Human Genome Database (database online). Johns Hopkins University, Baltimore, MD, USA, 1990. Updated daily. URL: http: www.gdb.org (for MRX, candidate gene nomenclature, marker information).

RDX is a cytoskeletal protein integral to the linking of actin to the plasma membrane and maps to chromosome 11q23. Two related RDX-like pseudogenes have also been mapped to chromosomal sites 11p (RDXP1) and Xp21.3 (RDXP2); the latter is likely to consist of a truncated copy of the 3' end of RDX (41). RDX and its related pseudogenes, RDXP1 and RDXP2, demonstrate strong homology to ezrin (MIM 123900) and moesin (MIM 309845) in forming the 'ERM' (ezrin, RDX, and moesin) complex. ERM, and specifically RDX, are queried to play a pivotal role in activation of the Rho small G protein family – Rho, Rac, and Cdc42 (42). Members of this Rho subfamily have emerged as key regulators of the actin cytoskeleton and as regulators of gene transcription in response to extracellular stimuli intrinsic to complex mechanisms of membrane-trafficking, formation of neuronal processes, and axonal guidance (43). A rhoGAP protein was recently reported to be involved in XLMR (44). The ERM proteins are also members of a family of related proteins that include at least two phosphotyrosine phosphatases (45) recently implicated in the etiology of the progressive myoclonic epilepsy, LD (7).

In summary, linkage analysis in families presenting with both syndromic and non-syndromic XLMR have demonstrated linkage to a number of different X chromosomal regions, within or including all or part of region Xp21.3-Xp22.1, which we associate with X-linked West syndrome. The localization of a gene for X-linked infantile spasms to Xp21.3-Xp22.1, between flanking markers DXS1226 and AHC, may provide the basis for testing other unmapped syndromes of infantile spasms and/or XLMR for allelism to the disorder described and mapped in the present study. The isolation of candidate genes in this interval and subsequent mutation analysis in MRX and infantile spasms families will be crucial to ultimately unraveling and better understanding their respective heterogeneous etiologies, pathogenesis, and prospects for new and successful therapies.

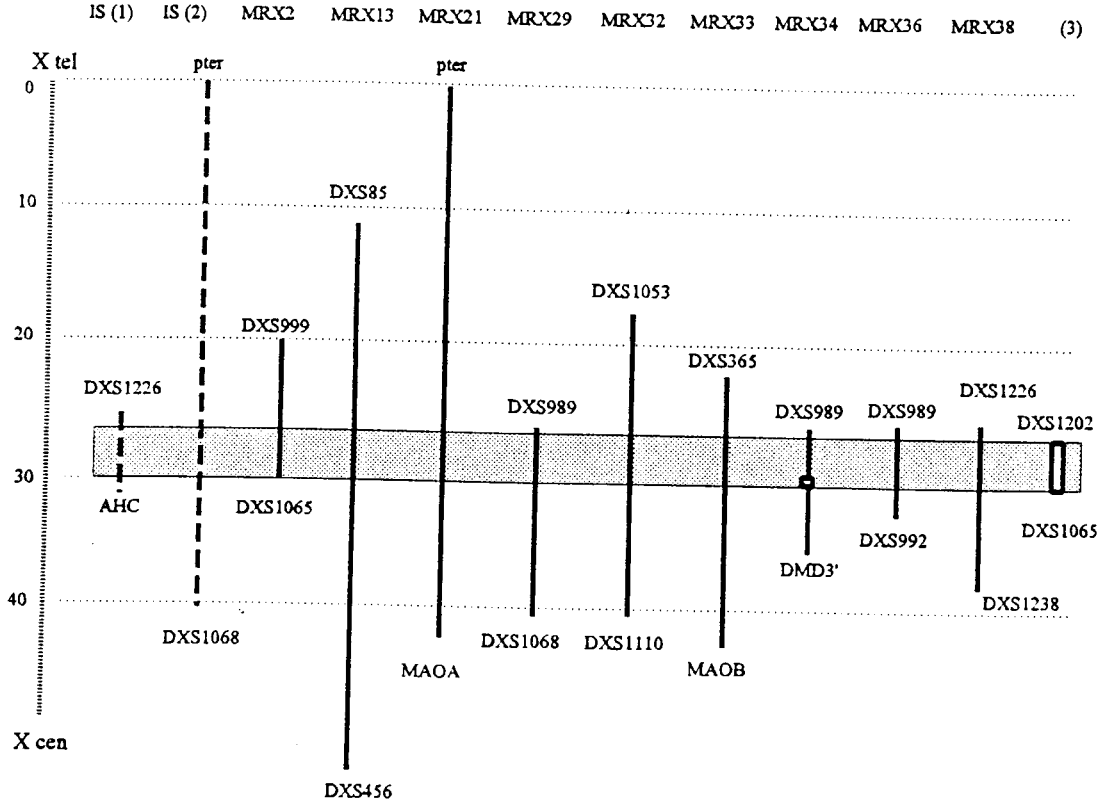

Fig. 2. Map of infantile spasms syndrome (IS) and X-linked mental retardation (XLMR) conditions assigned to Xp21.3-Xp22.1. IS(1) represents our family; IS(2) represents the family previously reported by Claes et al. (4); the numbers at the top of the figure represent the MRX numbers; (3) represents the case reported by Billuart et al. The genetic distance on the cM scale was given by the chromosome X map of Genetic Location Database [URL: http://cedar.genetics.soton.ac.uk/pub/chrmX map (for X chromosome marker location)]. The vertical lines indicate the defined interval for each described infantile spasms and MRX, with their respective flanking markers. Boxes represent deletions. The gray shaded area represents the smallest common area shared by IS and MRX.

Acknowledgements

We thank members of the family for their enthusiastic support of this study. We are grateful to Cynthia Siemens and Linda Kwong for their expert technical support. This work was supported by a grant from the British Columbia Children's Hospital Foundation, Vancouver, BC, Canada.

References

1. Hauser WA. The prevalence and incidence of convulsive disorders in children. Epilepsia 1994: 35 (suppl 2): s1–s6.
2. Delgado-Escueta AV, Serratosa JM, Liu A, Weissbecker K, Medina MT, Gee M, Treiman LJ, Sparkes RS. Progress in mapping Human Epilepsy genes. Epilepsia 1994: 35 (suppl 1): S29–S40.
3. Dichter MA, Buchhalter JR. The genetic epilepsies. In: Rosenberg RN, Prusiner SB, DiMauro S, Barchi RL, eds. The Molecular and Genetic Basis of Neurological Disease. 2nd edn. Newton, MA: Butterworth-Heinemann, 1997: 757–783.
4. Claes S, Devriendt K, Lagae L, Ceulemans B, Dom L, Casaer P, Raeymaekers P, Cassiman JJ, Fryns JP. The X-linked Infantile Spasms syndrome (MIM 308350) maps to Xp11.4-Xpter in two pedigrees. Ann Neurol 1997: 42: 360–364.
5. Johnson EW, Dubovsky J, Rich SS, O'Donovan CA, Orr HT, Anderson VE, Gil-Nigel A, Ahmann P, Dokken CG, Schneider DT, Weber JL. Evidence for a novel gene for familial febrile convulsions, FEB2, linked to chromosome 19p in an extended family from the Midwest. Hum Mol Genet 1998: 7: 63–67.
6. Wallace RH, Wang DW, Singh R, Scheffer IE, George AL Jr, Phillips HA, Saar K, Reis A, Johnson EW, Sutherland GR, Berkovic SF, Mulley JC. Febrile seizures and generalized epilepsy associated with a mutation in the Na + -channel β1 subunit gene *SCN1B*. Nat Genet 1998: 19: 366–370.
7. Minassian BA, Lee JR, Herbrick J-A, Huizenga J, Soder S, Mungall AJ, Dunham I, Gardner R, Fong C-YG, Carpenter S, Jardim L, Satishchandra P, Andermann E, Carter Snead O, Lopes-Cendes I, Tsui L-C, Delgado-Escueta AV, Rouleau GA, Scherer SW. Mutations in a gene encoding a novel protein tyrosine phosphatase cause progressive myoclonus epilepsy. Nat Genet 1998: 20 (2): 171–174.
8. Lehesjoki AE, Koskiniemi M, Sistonen P, Miao J, Hastbacka J, Norio R, de la Chapelle A. Localization of a gene for progressive myoclonus epilepsy to chromosome 21q22. Proc Natl Acad Sci USA 1991: 88: 3696–3699.
9. Pennacchio LA, Lehesjoki AE, Stone NE, Willour VL, Miao J, D'Amato E, Ramirea L, Faham M, Koskiniemi M, Warrington JA, Norio R, de la Chapelle A, Cox DR, Myers RM. Mutations in the gene encoding cystatin B in progressive myoclonus epilepsy (*EPM1*). Science 1996: 271: 1731–1734.
10. Leppert M, Anderson VE, Quattlebaum T, Stauffer D, O'Connell P, Nakamura Y, Lalouel JM, White R. Benign familial neonatal convulsions linked to genetic markers on chromosome 20. Nature 1989: 337: 647–648.
11. Biervert C, Schroeder BC, Kubisch C, Berkovic SF, Propping P, Jentsch TJ, Steinlein OK. A potassium channel mutation in neonatal human epilepsy. Science 1998: 279: 403–406.
12. Singh NA, Charlier C, Stauffer D, DuPont BR, Leach RJ, Melis R, Ronen GM, Bjerre I, Quattlebaum T, Murphy JV, McHarg ML, Gagnon D, Rosales TO, Peiffer A, Anderson VE, Leppert M. A novel potassium channel gene, *KCNQ2*, is mutated in an inherited epilepsy of newborns. Nat Genet 1998: 18: 25–29.
13. Lewis TB, Leach RJ, Ward K, O'Connell P, Ryan S. Genetic heterogeneity in benign familial neonatal convulsions: identification of a new locus on chromosome 8q. Am J Hum Genet 1993: 95: 411–415.
14. Charlier C, Singh NA, Ryan SG, Lewis TB, Reus BE, Leach RJ, Leppert M. A point mutation in a novel KQT-like potassium channel gene in an idiopathic epilepsy family. Nat Genet 1998: 18: 53–55.
15. Guipponi M, Rivier F, Vigevano F, Beck C, Crespel A, Echenne B, Lucchini P, Sebastianelli R, Baldy-Moulinier M, Malafosse A. Linkage mapping of benign familial infantile convulsions (BFIC) to chromosome 19q. Hum Mol Genet 1997: 6: 473–477.
16. Feinberg AP, Leahy WR. Infantile spasms: case report of sex-linked inheritance. Dev Med Child Neurol 1977: 19: 524–526.
17. Rugtveit J. X-linked mental retardation and infantile spasms in two brothers. Dev Med Child Neurol 1986: 28: 544–546.
18. Sambrook J, Fritsch EF, Maniatis T. In: Molecular Cloning: A Laboratory Manual, 2nd edn., Book 3, Appendix E.3. 1989.
19. La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fishbeck KH. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 1991: 352: 77–79.
20. Fu Y-H, Kuhl DP, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, Verkerk AJ, Holden JJ, Fenwick RG Jr, Warren ST, Oostra BA, Nelson DL, Caskey CT. Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. Cell 1991: 67: 1047–1048.
21. Clemens PR, Fenwick RG, Chamberlain JS, Gibbs RA, de Andrade M, Chakraborty R, Caskey CT. Carrier detection and prenatal diagnosis in Duchenne and Becker muscular dystrophy families, using dinucleotide repeat polymorphisms. Am J Med Genet 1991: 49: 951–960.
22. Lathrop GM, Lalouel JM. Fast calculations of LOD scores and genetic risks on small computers. Am J Hum Genet 1984: 36: 460–465.
23. Elmslie F, Gardiner M. The epilepsies. In: Rimoin DL, Connor JM, Pyeritz RE, eds. Principles and Practice of Medical Genetics, 3rd edn. New York, NY: Churchill Livingston, 1996: 2177–2196.
24. Guzzetta F, Crisafulli A, Crino MI. Cognitive assessment of infants with West syndrome: how useful is it for diagnosis and prognosis? Dev Med Child Neurol 1993: 35: 379–387.
25. Lubs HA, Chiurazzi P, Arena JF, Schwartz C, Tranebjaerg L, Neri G. XLMR genes: update 1996. Am J Med Genet 1996: 64: 147–157.
26. Arveiler B, Alembik Y, Hanauer A, Jacobs P, Tranebjaerg L, Mikkelsen M, Puissant H, Piet LL, Mandel JL. Linkage analysis suggests at least two loci for X-linked non-specific mental retardation. Am J Med Genet 1988: 30: 473–483.
27. Kerr B, Gedeon A, Mulley J, Turner G. Localization of non-specific X-linked mental retardation genes. Am J Med Genet 1992: 43: 392–401.
28. Kozak L, Chiurazzi P, Genuardi M, Pomponi MG, Zollino M, Neri G. Mapping of a gene for non-specific X-linked mental retardation: evidence for linkage to chromosomal region Xp21.1-Xp22.3. J Med Genet 1993: 30: 866–869.
29. Hane B, Schroer RJ, Arena JF, Lubs HA, Schwartz CE, Stevenson RE. Non-syndromic mental retardation: review and mapping of MRX29 to Xp21. Clin Genet 1996: 50: 176–183.

30. Holinski-Feder E, Golla A, Rost I, Seidel H, Rittinger O, Meindl A. Regional localization of two MRX genes to Xq28 (MRX28) and to Xp11.4-Xp22.12 (MRX33). Am J Med Genet 1996: 64: 125–130.
31. Raeymaekers P, Lin J, Gu XX, Sockarman D, Cassiman J-J, Fryns J-P, Marynen P. Non-specific mental retardation is probably caused by a microdeletion in a Belgian family. Am J Med Genet 1996: 64: 15–20.
32. Claes S, Gu XX, Legius E, Lorenzetti E, Marynen P, Fryns JP, Cassiman JJ, Raeymaekers P. Linkage analysis in three families with nonspecific X-linked mental retardation. Am J Med Genet 1996: 64: 137–146.
33. Schutz CK, Ives EJ, Chalifoux M, MacLaren L, Farrell S, Robinson PD, White BN, Holden JJA. Regional localization of an X-linked mental ratardation gene to Xp21.1-Xp22.13 (MRX38). Am J Med Genet 1996: 64: 89–96.
34. Gedeon AK, Donnelly AJ, Mulley JC, Kerr, Turner G. How many X-linked genes for non-specific mental retardation (MRX) are there? Am J Med Genet 1996: 64: 158–162.
35. Billuart P, Minet MC, de Portes V, Llense S, Richard L, Moutard ML, Recan D, Bruls T, Bienvenu T, Kahn A, Beldjord C, Chelly J. Identification by STS PCR screening of a microdeletion in Xp21.3-22.1 associated with non-specific mental retardation. Hum Mol Genet 1996: 5: 977–979.
36. Toutain A, Ronce N, Dessay B, Robb A, Francannet C, Le Merrer M, Briard M-L, Kaplan J, Moraine C, Nance–Horan syndrome: linkage analysis in 4 families refines localization in Xp22.31–p22.13 region. Hum Genet 1997: 99: 256–261.
37. Strain L, Wright AF, Bonthron DT. Fried syndrome is a distinct X-linked mental retardation syndrome mapping to Xp22. J Med Genet 1997: 34: 535–540.
38. Arena JF, Schwartz C, Ouzts L, Stevenson R, Miller M, Garza J, Nance M, Lubs H. X-linked mental retardation with thin habitus, osteoporosis, and kyphoscoliosis: linkage to Xp21.3-p22.12. Am J Med Genet 1996: 64: 50–58.
39. Gedeon AK, Mulley JC, Kozman H, Donnelly A, Partington MW. Localization of the gene for X-linked reticulate pigmentary disorder with systemic manifestation (PDR), previously known as X-linked cutaneous amyloidosis. Am J Med Genet 1994: 52: 75–78.
40. Wang T S-F, Pearson BE, Suomalainen HA, Mohandas T, Shapiro LJ, Schroder J, Korn D. Assignment of the gene for human DNA polymerase alpha to the X chromosome. Proc Nat Acad Sci 1985: 82: 5270–5274.
41. Wilgenbus KK, Milatovich A, Francke U, Furthmayr H. Molecular cloning, cDNA sequence, and chromosomal assignment of the human radixin gene and two dispersed pseudogenes. Genomics 1993: 16: 199–206.
42. Takahashi S, Sasaki T, Mammoto A, Hotta I, Takaishi K, Imamura H, Nakano K, Kodama A, Takai Y. Interaction of radixin with Rho small G protein GDP GTP exchange protein Dbl. Oncogene 1998: 16: 3279–3284.
43. Antonarakis SE, Van Aelst L. Mind the Gap, Rho, Rab and GDI. Nat Genet 1998: 19: 106–108.
44. Billuart P, Bienvenu T, Ronce N, des Portes V, Vinet MC, Zemni R, Crollius HR, Carrie A, Fauchereau F, Cherry M, Briault S, Hamel B, Fryns JP, Beldjord C, Kahn A, Moraine C, Chelly J. Oligonephrin-1 encodes a rhoGAP protein involved in X-linked mental retardation. Nature 1998: 392 (6679): 923–926.
45. Lankes WT, Schwartz-Albiez R, Furthmayr H. Cloning and sequencing of porcine moesin and radixin cDNA and identification of highly conserved domains. Biochim Biophys Acta 1993: 1216 (3): 479–482.