

# Epilepsy and mental retardation limited to females: an under-recognized disorder

Ingrid E. Scheffer,<sup>1,2</sup> Samantha J. Turner,<sup>1</sup> Leanne M. Dibbens,<sup>3,4</sup> Marta A. Bayly,<sup>3</sup> Kathryn Friend,<sup>3</sup> Bree Hodgson,<sup>3</sup> Linda Burrows,<sup>3</sup> Marie Shaw,<sup>3</sup> Chen Wei,<sup>5</sup> Reinhard Ullmann,<sup>5</sup> Hans-Hilger Ropers,<sup>5</sup> Pierre Szepietowski,<sup>6</sup> Eric Haan,<sup>3</sup> Aziz Mazarib,<sup>7</sup> Zaid Afawi,<sup>7</sup> Miriam Y. Neufeld,<sup>7</sup> P. Ian Andrews,<sup>8</sup> Geoffrey Wallace,<sup>9</sup> Sara Kivity,<sup>10</sup> Dorit Lev,<sup>11</sup> Tally Lerman-Sagie,<sup>11</sup> Christopher P. Derry,<sup>1</sup> Amos D. Korczyn,<sup>7</sup> Jozef Gecz,<sup>3,4,12</sup> John C. Mulley<sup>3,4,12</sup> and Samuel F. Berkovic<sup>1</sup>

<sup>1</sup>Epilepsy Research Centre and Department of Medicine, University of Melbourne, Austin Health, Heidelberg, Victoria,

<sup>2</sup>Department of Paediatrics, University of Melbourne, Royal Children's Hospital, Melbourne, <sup>3</sup>Department of Genetic Medicine, Women's and Children's Hospital, North Adelaide, South Australia, <sup>4</sup>School of Paediatrics and Reproductive Health, South Australia, Australia, <sup>5</sup>Department of Human Molecular Genetics, Max Planck Institute for Molecular Genetics, Berlin-Dahlem, Germany, <sup>6</sup>INSERM UMR 491, 'Genetics of Human Epilepsies' Group, Faculté de Médecine de la Timone, Université de la Méditerranée, Marseille, France, <sup>7</sup>Department of Neurology, Tel Aviv Sourasky Medical Centre and Sackler Faculty of Medicine, Tel Aviv University, Israel, <sup>8</sup>Sydney Children's Hospital, Randwick, New South Wales, <sup>9</sup>Mater Medical Centre, South Brisbane, Queensland, Australia, <sup>10</sup>Department of Neurology, Schneider Children's Medical Centre, Petaq Tikvah, Israel, <sup>11</sup>Metabolic Neurogenetic Clinic, Wolfson Medical Centre, Holon, Israel and <sup>12</sup>School of Molecular and Biomedical Sciences, University of Adelaide, South Australia, Australia

Correspondence to: Professor Ingrid E Scheffer, Epilepsy Research Centre, Department of Medicine, University of Melbourne, Austin Health, Neurosciences Building, Level I, Heidelberg Repatriation Hospital, Banksia Street, Heidelberg VIC 3081, Australia

E-mail: [scheffer@unimelb.edu.au](mailto:scheffer@unimelb.edu.au)

**Epilepsy and Mental Retardation limited to Females (EFMR) which links to Xq22 has been reported in only one family. We aimed to determine if there was a distinctive phenotype that would enhance recognition of this disorder. We ascertained four unrelated families (two Australian, two Israeli) where seizures in females were transmitted through carrier males. Detailed clinical assessment was performed on 58 individuals, using a validated seizure questionnaire, neurological examination and review of EEG and imaging studies. Gene localization was examined using Xq22 microsatellite markers. Twenty-seven affected females had a mean seizure onset of 14 months (range 6–36) typically presenting with convulsions. All had convulsive attacks at some stage, associated with fever in 17 out of 27 (63%). Multiple seizure types occurred including tonic-clonic (26), tonic (4), partial (11), absence (5), atonic (3) and myoclonic (4). Seizures ceased at mean 12 years. Developmental progress varied from normal (7), to always delayed (4) to normal followed by regression (12). Intellect ranged from normal to severe intellectual disability (ID), with 67% of females having ID or being of borderline intellect. Autistic (6), obsessive (9) and aggressive (7) features were prominent. EEGs showed generalized and focal epileptiform abnormalities. Five obligate male carriers had obsessional tendencies. Linkage to Xq22 was confirmed (maximum lod 3.5 at  $\theta = 0$ ). We conclude that EFMR is a distinctive, under-recognized familial syndrome where girls present with convulsions in infancy, often associated with intellectual impairment and autistic features. The unique inheritance pattern with transmission by males is perplexing. Clinical recognition is straightforward in multiplex families due to the unique inheritance pattern; however, this disorder should be considered in smaller families where females alone have seizures beginning in infancy, particularly in the setting of developmental delay. In single cases, diagnosis will depend on identification of the molecular basis.**

**Keywords:** epilepsy; intellectual disability; females; X-linked inheritance; autistic features

**Abbreviations:** BAC = bacterial artificial chromosome; CFNS = craniofrontonasal syndrome; EFMR = epilepsy and mental retardation limited to females; ID = intellectual disability.

Received September 24, 2007. Revised December 6, 2007. Accepted December 18, 2007. Advance Access publication January 29, 2008

## Introduction

Epilepsy and Mental Retardation limited to Females (EFMR) was described in a single North American family in three sequential reports (Juberg and Hellman, 1971; Fabisiak and Erickson, 1990; Ryan *et al.*, 1997). This family was characterized by onset of convulsions in infancy in previously normal girls who subsequently showed developmental regression, with mild to profound intellectual disability (ID). The disorder has an extraordinary pattern of inheritance, regarded as X-linked dominant with male sparing, where females are affected and males transmit the disorder; males have been regarded as phenotypically normal. The mode of inheritance is a key diagnostic feature but hard to recognize without several generations of family members showing only affected females. It is easier to appreciate when transmission through unaffected carrier males is observed. The locus for the disorder in the one reported family was mapped to Xq22 (Ryan *et al.*, 1997).

Here we describe four new EFMR families with detailed phenotyping on all affected and carrier family members. The chromosomal localization was explored in an attempt to reduce the candidate gene region.

## Methods

### Ascertainment and genealogical documentation

We ascertained four families (Fig. 1) whose pedigree and history were suggestive of EFMR; two Australian and two Israeli. Field trips were conducted to Israel, England and to three Australian states. Detailed pedigrees with specific attention to consanguinity and foetal loss were constructed. Extensive genealogical research was performed to determine if the Australian families were related.

### Clinical evaluation

Detailed electro-clinical assessment was performed on 58 individuals from the four families. Seizure histories were sought on affected and unaffected family members, including obligate carrier males, using a validated seizure questionnaire (Reutens *et al.*, 1992). Neurological examination was performed on family members where possible. The clinical description of seizures was corroborated by questioning parents, eyewitnesses and older relatives when available. Strenuous attempts were made to review all available previous medical records including electro-clinical data and neuroimaging studies. In some instances, the EEG studies were performed many years ago and were not available for re-analysis so interpretation was limited to the EEG report. Seizure types were classified according to the international classification (1981; 1985; 1989; Engel, 2001).

Intellectual assessment was based on psychological evaluation where available. If no formal assessment had been performed, the gross cognitive presentation at a clinical level, together with educational and vocational background, were used to assess intellect. This study was approved by the Human Research Ethics Committee of Austin Health and Tel Aviv Medical Centre IRB. Informed consent was obtained from all subjects, or in the case of minors and adults unable to give informed consent due to ID, parents and/or guardians.

## Molecular genetic analysis

Venous blood for molecular genetic studies was taken from all consenting affected and unaffected family members, apart from one severely affected female where cheek cells were taken. Two affected women refused to participate in molecular studies. DNA was extracted and polymerase chain reaction based microsatellite markers from the Xq22 region (*DXS1225*, *DXS995*, *DXS1222*, *DXS990*, *DXS458*, *DXS8088*, *DXS6804* and *DXS1220*) were genotyped by standard <sup>32</sup>P methods. Haplotypes were constructed from the observed segregation of each marker tested. Each family was of insufficient size to independently confirm linkage to Xq22; however, any observed recombination events have the potential to reduce the previously published regional localization, thus narrowing down the number of candidate genes. Lod scores were determined using penetrance in males set at zero and conservatively set in females at 0.9 as a single unaffected obligate carrier has been reported (Ryan *et al.*, 1997). The bacterial artificial chromosome (BAC) array used to investigate submicroscopic chromosomal copy number variations within Xq22 consisted of 36 000 overlapping BAC clones covering the entire genome. Resolution was ~70–80 kb, but by increasing specificity to consider only displacement effects involving more than one adjacent clone the resolution for this BAC set is conservatively estimated at 150 kb. Primer sequences for amplifying exons of the candidate genes *DIAPH2*, *PCDH11X*, *NAP1L3* and *SRPX2* are available on request. X-inactivation analysis was carried out using fluorescently labelled amplicons run on a 3100-Avant Genetic Analyzer (Applied Biosystems) as described by Crawford *et al.* (2006).

## Case histories illustrating clinical spectrum of EFMR

### Severely affected female in Family A

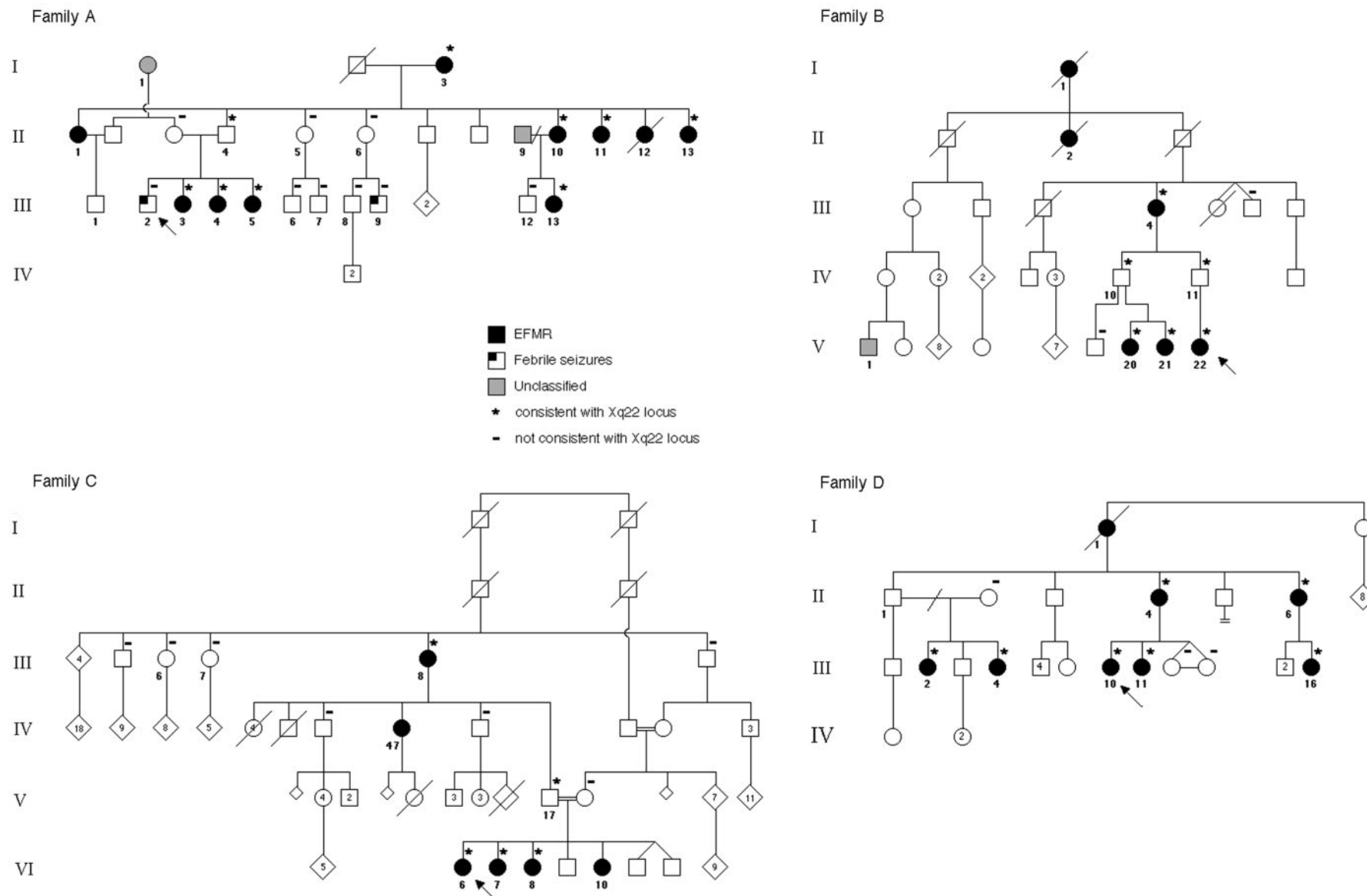
A-II-13 is a 42-year-old woman who was born following a normal pregnancy and delivery. She developed convulsive seizures at 7 months of age lasting 3–4 min, some associated with fever. Clusters of attacks occurred every 3–4 weeks. By 42 years, brief staring spells occurred 1–3 times per month, sometimes with arm jerking, and tonic-clonic seizures every few months. Tonic seizures with arm flexion and vocalization up to 30 s duration also occurred. She was treated with sodium valproate and lamotrigine.

She had severe global developmental delay from birth, and regression was not apparent with seizure onset. She walked at 2 years, never talked and remained incontinent. She was institutionalized at 4 years and never attended school. At 42 years, she walked independently, followed simple commands with physical prompting and fed herself, but had no words or signs. She was uncooperative to examination and exhibited aggressive behaviour such as spitting, slapping and biting and episodes of hyperventilation. No focal signs or dysmorphic features were evident.

EEG at 11 months showed right central high voltage slowing and sharp waves, and at 2.5 years, was unremarkable. Chromosomes, Fragile X, subtelomere analysis and urine metabolic screen were normal.

### Mildly affected female in Family A

A-II-1 is a 38-year-old woman who developed brief 5-min generalized tonic-clonic seizures at 10 months of age. Her seizures were not preceded by an aura and were sometimes associated



with fever. Seizures occurred in clusters, approximately monthly in early childhood, decreasing to every 2 months by 10 years. She was treated with phenytoin and phenobarbitone. Her last seizure was at 14 years, and medication was stopped at 18 years.

She was born following a normal pregnancy and delivery. Developmental milestones were normal and there was no regression. She passed her second last year of secondary school and worked in cleaning and catering at the local hospital. She had no dysmorphic or neurocutaneous stigmata. No abnormalities were found on examination.

Her EEG at 2 years was reported as showing frequent left central epileptiform activity. At 4 years, the EEG was more abnormal with the background predominantly comprising bilateral delta rhythms most prominent over the right central region. At 5 years 3 months, rare sharp wave discharges were seen bilaterally and synchronously in the parietal regions. At 7 years, prominent bilateral parietal and central discharges were noted. At 15, 17 and 37 years, the EEG was normal.

## Results

### Affected females

The four families (Fig. 1) had 30 affected females, four of whom (A-II-12, B-I-1, B-II-2, D-I-1) were deceased. Information was available on 27 females (Table 1), with limited histories available for four Israeli women (C-III-8, C-IV-47, D-II-4, D-II-6). The Australian families were of Anglo-Saxon origin and were unrelated. Of the Israeli families, Family C was Arab-Israeli and Family D was Jewish Moroccan. One branch of Family D lived in the UK.

### Seizure history

The mean age of seizure onset was 14 months (median 11 months, range 6–36 months). Mean seizure onset age was 9 months in the two Australian families with an identical range of 7–12 months (median 10 months) which was earlier than that of the two Israeli families (mean onset 18 months, range 6–36 months, median 17 months).

All 27 women had convulsive seizures: 26 had tonic-clonic seizures and four had tonic seizures. Seizures were associated with fever in 17 out of 27 (63%). Other seizure types included absence seizures in five, myoclonic seizures in four, atonic seizures in three and partial seizures in 11 including hemiclonic attacks in five. Staring spells of uncertain significance were noted in A-II-13 and C-VI-6. Convulsive status epilepticus occurred in five women (A-III-4, A-III-13, C-VI-6, D-III-4, D-III-11). In addition to seizures in infancy, the matriarch of Family A (A-I-3) had probable complex partial seizures of temporal lobe origin from 60 years of age.

Seizure offset occurred at a mean of 12 years (range 2.5–24 years, 7 ongoing). Several affected women had died: A-II-12 at 16 years due to a lung abscess; B-II-2 died following a seizure at 6 years, B-I-1 and D-I-1 had died of unknown causes and the twin sister of B-III-6 was stillborn. Information regarding medication was available for 24 females; 17 required two or more anti-epileptic

medications, four had not been treated and for three it was unclear if they required more than one anti-epileptic agent.

Seizures were reported in five other family members and were considered unrelated to the EFMR phenotype: A-III-2 and A-III-9 had febrile seizures, A-I-1 and A-II-9 had unclassified seizures. One distantly related male (B-V-1) had seizures, ID and cerebral palsy due to presumed other cause.

### EEG studies

EEG studies showed heterogeneous features (Table 1). In infancy, the EEG background was normal (1), showed diffuse (3) or focal slowing that was temporal (1) and in the left hemisphere (1), and showed focal (central, temporal) discharges or generalized spike wave and polyspike wave activity. After infancy, the EEG background was normal (5), showed diffuse slowing (10) or focal slowing that was bilateral fronto-temporal (2) and left temporal (1). EEG recordings showed a variety of changes: generalized spike wave and polyspike wave activity, focal discharges, focal or diffuse slowing or were normal. Photosensitivity was not seen. EEG studies were not performed at a uniform age or frequency and thus no age- or seizure-related pattern of evolution could be demonstrated.

### Imaging

Structural imaging of the brain was available from 14 cases, including nine MRI and five CT studies. Apart from an incidental retro-cerebellar arachnoid cyst (A-III-13), no abnormalities were detected.

### Developmental course

Information regarding early development was collected for 23 affected women, with early histories not available for four older women (A-I-3, C-III-8, D-II-4, D-II-6). Developmental regression occurred in half of the cases. Regression with onset of seizures was described in eight females, whereas regression occurred 7–38 months after seizure onset in four. The cause of later regression was not clear for three females (A-III-5, D-III-10, D-III-11), and was associated with seizure recurrence following withdrawal of medication in one (B-V-21). Four women from Family A had slow development from birth without regression. Seven affected females (two Australian, five Israeli) had normal development and no history of regression. Neurological examination was unremarkable in the affected individuals with the exception of dyspraxia in D-III-10, poor fine motor skills in D-III-4 and intention tremor in A-III-4.

### Intellectual function

Intellect varied markedly in affected women. Eight of the affected females from Australian families underwent formal psychological evaluation using a range of standardized assessment tools dating back to 1984. Formal testing was not available in the remaining affected women.

**Table 1** Clinical data on affected females from four families

Pedigree ref.	Age	Seizure onset	Seizure offset	Seizure types	Regression	ID	Neurological + Psychiatric features	EEG/VEM	Neuroimaging
A-I-3	76 yrs, 10 mths	Infancy 60 years	7 yrs Continuing	TCS, FS CPS	NK	Borderline	Depression	62–69 yrs: L temporal slowing and epileptiform activity	–
A-II-1	38 yrs, 6 mths	10 mths	14 yrs	GTCS, FS	Nil	Normal	–	2 yrs: L central epileptiform 4 yrs: diffuse slowing, more marked over R central region 5 yrs: bilateral parietal epileptiform 7 yrs: bilateral central and parietal epileptiform	–
A-II-10	50 yrs	8 mths	12 yrs	PSSG	Always delayed	Normal	Depression	2 yrs: normal	MRI-normal
A-II-11	46 yrs, 5 mths	8 mths	24 yrs	H, TCS, PSSG	8 mths	Severe	Obsessive, Autistic features, Depression, Self Injury	1 yr: diffuse slowing	–
A-II-12	Died 16 yrs	10 mths	6 yrs	T, abs	Always delayed	Moderate	Aggression	13 mths: 3–4Hz generalized spike wave, 5 yrs: diffuse slowing	–
A-II-13	42 yrs, 4 mths	7 mths	Continuing	TCS, T, H, staring spells, FS	Always delayed	Severe	Aggression, Self injury	11 mths: R central discharges 2.5 yrs: normal	–
A-III-3	27 yrs	12 mths	8 yrs	GTCS	Always delayed	Mild	Depression, Panic attacks, Schizophreniform Psychosis	13–18 yrs: normal	CT-normal
A-III-4	25 yrs, 9 mths	11 mths	18 yrs	GTCS, A, CPS, H, status, FS	11 mths	Mild	Panic attacks, tremor	1–11 yrs: generalized and bilateral independent discharges from both anterior and posterior regions, diffuse slowing	CT-normal
A-III-5	21 yrs, 5 mths	11 mths	16 yrs, on AED	GTCS, MJ, FS	4 yrs	Mild	–	7–21 mths: normal	–
A-III-13	20 yrs	7 mths	Continuing	focal R tonic, PSSG, TCS, A, abs, MJ, status	7 mths	Severe	Obsessive, Autistic features, Aggression	7–18 mths: L hemisphere slowing	MRI—retrocerbellar arachnoid cyst
B-III-4	64 yrs, 7 mths	12 mths	21 yrs	GTCS, FS	Nil	Borderline	Aggression, Hysteria, Schizophreniform Psychosis	32 yrs: diffuse slowing	–
B-V-20	17 yrs, 4 mths	11 mths	Continuing	GTCS, CPS, abs, FS	11 mths	Severe	Obsessive, Autism, Aggression	1 yr: focal temporal slowing and epileptiform activity 17 yrs: diffuse slowing	CT-normal



B-V-21	14 yrs, 4 mths	8 mths	12 yrs	GTCS, H, FS	3 yrs	Mild	Obsessive, Autistic features	5 yrs: L frontal discharges, diffuse slowing	CT-normal
B-V-22	6 yrs, 11 mths	7 mths	Continuing	GTCS, abs, MJ, A, FS	7 mths	Mild	Autistic features	1 yr: diffuse slowing 8 yrs (VEM) – diffuse slowing, no clinical events	MRI-normal
C-III-8	79 yrs	Childhood	11 yrs	TCS	NK	Normal <sup>a</sup>	Obsessive	–	–
C-IV-47	48 yrs	3 yrs	7 yrs	TCS	Nil	Normal <sup>a</sup>	–	–	–
C-VI-6	17.5 yrs	16 mths	10 yrs	GTCS, staring spells, status, FS	16 mths	Mild	–	16 yrs: mild generalized slowing	MRI-normal
C-VI-7	16 yrs	9 mths	10 yrs	GTCS, abs, MJ, FS	9 mths	Mild	–	6 yrs: generalized slowing 15 yrs: generalized polyspike wave	MRI-normal
C-VI-8	12 yrs	6 mths	2.5 yrs	GTCS, FS	Nil	Borderline	–	–	MRI-normal
C-VI-10	2.5 yrs	18 mths	Continuing	TCS, FS	Nil	Normal*	–	3 yrs: normal	MRI-normal
D-II-4	53 yrs	24 mths	8 yrs	TCS	NK	Normal*	–	–	–
D-II-6	40 yrs	24 mths	14 yrs	GTCS	NK	Normal*	–	–	–
D-III-2	33 yrs	30 mths	Continuing	GTCS, CPS, FS	Nil	Normal	ASD, OCD	31 yrs: L frontotemporal epilep- tiform activity, irregular gener- alized spike wave	MRI-normal
D-III-4	24 yrs	12 mths	14 yrs	GTCS, status	12 mths	Mild	Obsessive, Poor fine motor skills	Normal	CT-normal
D-III-10	21 yrs	10 mths	22 yrs	GTCS, H, CPS, FS	4 yrs	Moderate	Obsessive, Dyspraxia, Aggression	2.5 yrs: R temporal discharges 12–14 yrs: generalized polyspike wave, ictal recording no focality, bilateral fronto-temporal slowing	–
D-III-11	19 yrs	11 mths	17 yrs	TCS, CPS, status, FS	18 mths	Mild	Obsessive	7–14 yrs: independent bilateral frontotemporal discharges, bilat- eral polyspike wave, bilateral frontotemporal slowing	MRI-normal
D-III-16	18 yrs	21 mths	12 yrs	GTCS, T, FS	Nil	Normal	Aggression	–	–

<sup>a</sup>Intellect thought to be within the normal range based on activities of daily living and education, but formal assessment not possible due to language and cultural constraints. GTCS = generalized tonic-clonic seizures; TCS = tonic-clonic seizures; T = tonic; A = atonic; H = hemiclonic; abs = absence; MJ = myoclonic jerks; PSSG = partial seizures, secondarily generalized; CPS = complex partial seizures; FS = febrile seizure; NK = not known; ASD = autism spectrum disorder; OCD = obsessive compulsive disorder; AED = anti-epileptic drug; R = right, L = left; mths = months, yrs = years.

The Australian families presented a more severe picture with two women of normal intellect, two borderline and 10 with ID varying from mild (5), moderate (1) to severe (4). In contrast, the Israeli families comprised seven women of apparently normal intellect, one with borderline intellect, four with mild ID and one with moderate ID. Assessment was difficult in the Israeli women due to language and cultural constraints. All women with developmental regression were intellectually disabled; only one woman with developmental delay from birth was of normal intellect.

The most severely affected females walked independently, fed themselves and followed simple commands, but had no words or signs. The females with mild intellectual disability attended special school or required support to live independently in adult life. There was no correlation between intellect and skewing of X-inactivation in affected females, and no difference in skewing between normal and affected female family members.

### Psychiatric features

A range of psychiatric features was prominent in the families. Autism spectrum disorder and/or autistic features were identified in six females, and were particularly striking in Family B. Obsessive features were evident in nine women. One woman (D-III-2), who was diagnosed with obsessive-compulsive disorder in childhood, was concerned with contamination and obsessed with cleanliness and handwashing. Aggressive behaviour occurred in seven women whose intellect varied from normal to severe. Two individuals (B-III-4, A-III-3) had episodes of schizophreniform psychosis in their early to mid-20s that required hospitalization. Individual B-III-4 also suffered from hysteria.

Other psychiatric features were also noted in Family A. Four women (A-I-3, A-II-10, A-II-11, A-III-3) had been treated for depression, and two women (A-III-3, A-III-4) had panic attacks. Self-injury was seen in the two most severely affected women in this family (A-II-11, A-II-13).

There was limited information available regarding psychiatric features in individuals from Israel, thus the frequency of psychiatric symptoms may be underestimated.

### Transmitting males

None of the five obligate male carriers, with affected female offspring, had seizures or intellectual difficulties. A striking clinical feature of these five men was the presence of obsessive traits and interests (A-II-4). This took the form of obsessively recounting and repeating details in conversation (B-IV-10, B-IV-11, C-V-17, D-II-1); the men had controlling, rigid personalities and were inflexible.

### Genetic linkage analysis

Although each family was of insufficient size to independently demonstrate linkage, the absence of recombination events with any marker within the Xq22 region was

consistent with these families mapping to the same region identified by Ryan *et al.* (1997). The maximum two-point lod score pooled over the four families was 3.5 at  $\theta=0$  for DXS990. Unfortunately the absence of recombination events for any of the markers in any of the affected or obligate carrier family members meant that we were unable to narrow the interval containing the EFMR locus and reduce the number of candidate genes.

X chromosome inactivation potentially modulates the phenotype of females carrying mutations in X-linked genes. To determine whether altered X inactivation contributed to the inheritance mechanism in EFMR, we analysed methylation patterns within the *FMR1* gene and the androgen receptor gene *AR*, but found no evidence of skewing of X inactivation in affected females, consistent with the findings from the original pedigree (Ryan *et al.*, 1997).

### Candidate gene analysis

The EFMR gene localization remained within ~25 cM or 34 Mb of DNA containing ~246 genes, of which 150 are expressed in brain. The localization included a 4-Mb region of X/Y homology which might somehow be involved in the facilitation of a unique genetic mechanism consistent with the unusual mode of inheritance. The task of sequencing this number of candidate genes was formidable, so we first examined the genomic imbalance hypothesis and excluded submicroscopic deletion or duplication by array-CGH (comparative genomic hybridization) particularly involving the region of X/Y homology (data not shown). Then we attempted to prioritize candidate genes using biologically based hypotheses. *PCDH11X*, which has a paralogue *PCDH11Y* on the Y chromosome, was sequenced to test the X/Y homology hypothesis. Multiple isoforms are known, some of which have sex-specific expression patterns, but no pathogenic mutations were detected. The supplementary table lists other candidate genes that were negative for mutations.

### Discussion

EFMR is a syndrome hitherto described in a single North American family (Juberg and Hellman, 1971; Fabisiak and Erickson, 1990; Ryan *et al.*, 1997). We describe four new families and extend the clinical phenotype of EFMR demonstrating the heterogeneity of seizure, cognitive and psychiatric phenotypes in affected females. Our families showed an identical pattern of X-linked inheritance, with affected females and transmitting males. Linkage analysis of our families was consistent with the known chromosomal localization to Xq22 (Ryan *et al.*, 1997).

### Phenotype of females with EFMR

Our families extend the EFMR phenotype. The mean age of seizure onset was 14 months (range 6–36 months); the onset previously reported was between 4 and 18 months

(Juberg and Hellman, 1971; Fabisiak and Erickson, 1990; Ryan *et al.*, 1997). Only partial and generalized tonic-clonic seizures were reported in the American family. In contrast, generalized seizure types predominated in our families including tonic-clonic, absence, myoclonic, tonic and atonic seizures. Our cases also had both complex partial seizures and focal motor seizures. One feature reported in 1971 when the family was first published, but not mentioned in the two subsequent papers, was that seizures were often associated with febrile illnesses when young (Juberg and Hellman, 1971). This was also prominent in our families, with 17 females having at least one seizure precipitated by fever.

The developmental trajectory in EFMR is more varied than originally reported. Normal early development is the most common scenario with regression that may be coincident with seizure onset (8/12 our cases), or may occur later in the second to the fourth year of life (4/12 our cases). Regression from the second year may occur in the setting of seizure exacerbation (Juberg and Hellman, 1971; Fabisiak and Erickson, 1990). Our families showed that some females (4/23) were never normal with delayed early milestones and no regression.

The intellectual outcome for females in EFMR is generally poor; 2 out of 20 affected women in the American family were of 'grossly normal intellectual and emotional function' (Ryan *et al.*, 1997). Our families varied with regard to the proportion of affected women of normal intellect. Four of 14 women from the Australian families and 8 of 13 affected women from the Israeli families were regarded as not intellectually disabled; however, intellectual and behavioural assessment was imprecise due to language and cultural restraints.

Psychiatric features are a characteristic part of the EFMR female phenotype and have been extended here to include autism spectrum disorders and obsessive features. In the American family, disabling psychiatric symptoms were reported in three women with mild cognitive impairment, however, the nature of the psychiatric phenotype was not described (Ryan *et al.*, 1997). Autism Spectrum Disorder and/or autistic features were noted in six females from three of our families. Obsessive features were also prominent in our families. Sometimes they were part of the autistic presentation, such as the girl (B-V-20) who picked fluff from her clothing throughout the consultation and her sister (B-V-21) who always tidied the shelves when shopping in the supermarket. Obsessive features were also noteworthy in women without autistic features, such as one woman from Family C (C-III-8) who was obsessive about personal hygiene, and would check and re-check that doors were locked and the gas was turned off. One woman (D-III-2) had a formal diagnosis of both Autism Spectrum Disorder and Obsessive Compulsive Disorder. Depression, panic attacks and self-injury were also observed in some affected women (Table 1). Seven women, ranging in intellect from normal to severe intellectual disability,

had aggressive behaviour including biting and spitting. Two women, one with borderline intellect (B-III-4) and one with mild ID (A-III-3), were hospitalized following episodes of schizophreniform psychosis.

The differential diagnosis of EFMR includes classical Rett syndrome, the Hanefeld variant of Rett syndrome, Angelman syndrome and certain sodium channelopathies; the first two disorders predominantly affect girls. Rett syndrome begins with developmental regression at a similar age in infancy; however, seizures are usually of later onset, mild and easily controlled (Williamson and Christodoulou, 2006). *MECP2* mutations are found in most girls with classical Rett syndrome, but are also associated with more diverse phenotypes, which could be confused with EFMR. The Hanefeld variant is associated with intractable seizures including infantile spasms from the first few months of life and can be caused by mutations in *CDKL5*. The outcome is poorer than that seen in EFMR with profound retardation (Grosso *et al.*, 2007). Both disorders are associated with manual stereotypies which are not a feature of EFMR. Angelman syndrome has characteristic dysmorphic features associated with a happy disposition, ataxia and later seizure onset and can be easily differentiated from EFMR (Williams *et al.*, 2006). *SCN1A* mutations can be associated with infantile onset of febrile seizures and an epileptic encephalopathy with intellectual decline and could resemble an individual presenting with EFMR (Harkin *et al.*, 2007). Despite some clinical overlap with these disorders, the distinctive inheritance pattern of EFMR with women affected over multiple generations is not seen in these conditions.

### Is there a male phenotype for carriers of EFMR?

Previously males have been considered unaffected carriers of EFMR, but we suggest that subtle features may exist. Obligate male carriers have no seizures and are of normal intellect. We observed obsessive, rigid and inflexible personality traits in the five obligate carrier men in our families, however, these features were not formally assessed. This observation, together with the prominent obsessive behaviour and autistic features in affected female offspring, led us to hypothesize that these personality traits may be a subtle marker of carrier status. It is of course possible that obsessiveness was just a common unrelated feature in these families, however, it is more likely to be related. Studies of family members of patients with classical autistic disorder show that parents and siblings often have the broader autism phenotype with obsessive, rigid and inflexible features (Piven *et al.*, 1997; Pickles *et al.*, 2000; Lainhart *et al.*, 2002).

### Inheritance pattern of EFMR

The most remarkable feature of this disorder is its mode of inheritance. X-linked inheritance where females are affected



and males are normal cannot be easily explained with our current understanding of X-linked disorders. In classical X-linked modes of inheritance, males are more severely affected than females, regardless of whether the disorder follows X-linked recessive or X-linked dominant inheritance. In EFMR, carrier males have no definite clinical manifestations (obsessive features are suspected but yet to be proven as a trait of this disorder) and the females are very likely to have seizures and intellectual difficulties. Penetrance of EFMR is high in females, with non-penetrant carrier females only rarely seen (Ryan *et al.*, 1997).

The EFMR inheritance pattern is distinctive, but may only be apparent over three or more generations. The key is a pedigree where only females are affected and unaffected males or affected females transmit the disorder. Another clue is that all (or most) female offspring of a carrier male will be affected. From a counselling perspective, all female offspring of a carrier male will be likely to be affected, and 50% of the female offspring of an affected female. Half of the male offspring of a carrier female will carry the genetic mutation. Male to male inheritance does not occur. It is also important to note that females pass on this disorder to half of the daughters so inheritance only through women may be observed.

### Molecular analysis and mechanism of inheritance

Our molecular data supported EFMR gene localization to Xq22, with no evidence of locus heterogeneity, but did not succeed in narrowing the previously reported region (Ryan *et al.*, 1997). Part of the 25-Mb region includes the X/Y homology region, where a few deletions are already known to be associated with various syndromes or contiguous gene syndromes such as choroideraemia. In the case of these EFMR families, we ruled out chromosome imbalance within the X/Y homology region.

A similar, but not identical, pattern of inheritance is seen in the rare X-linked disorder craniofrontonasal syndrome (CFNS). Females are severely affected with midline and skeletal defects, whereas, male carriers typically show mild manifestations although severely affected males also occur. Mutations in *EFNB1*, encoding the ligand ephrin-B1, occur in most CFNS cases (Twigg *et al.*, 2006). Ephrin ligands bind with ephrin receptors (Eph) to regulate embryonic tissue morphogenesis. It has been postulated that alternate ephrins may bind to the receptor compensating for much of the ephrin-B1 function, explaining the generally mild features in hemizygous males. In females, because of random X-inactivation, some cells express ephrin-B1 and others do not. This is postulated to result in 'cellular interference' where the two classes of cell have aberrant interactions resulting in a more severe phenotype in women (Wieacker and Wieland, 2005). A similar explanation could underlie EFMR.

Our observations suggest that EFMR is an under recognized cause of seizures and intellectual disability in girls. The identification of families with affected girls and transmitting males is currently the key to diagnosis. As such, we found three to four generation pedigrees with affected women that have a distinctive phenotype and an inheritance pattern specific to EFMR. It is likely that smaller kindreds will be readily diagnosed as the phenotype becomes more widely recognized. Sporadic cases can be suspected based on the clinical phenotype, but will only be definitively diagnosable once the gene is identified.

### Supplementary material

Supplementary material is available at *Brain* online.

### Acknowledgements

We are very grateful to the families for their participation in the study. This study was supported by research grants from the National Health and Medical Research Council of Australia.

### References

- Commission of Classification and Terminology of the International League Against Epilepsy: Proposal for revised clinical and electroencephalographic classification of epileptic seizures. *Epilepsia* 1981; 22: 489–501.
- Commission on Classification and Terminology of the International League Against Epilepsy: Proposal for classification of epilepsies and epileptic syndromes. *Epilepsia* 1985; 26: 268–278.
- Commission on Classification and Terminology of the International League Against Epilepsy: Proposal for revised classification of epilepsies and epileptic syndromes. *Epilepsia* 1989; 30: 389–99.
- Crawford J, Lower KM, Hennekam RC, Van Esch H, Megarbane A, Lynch SA, et al. Mutation screening in Borjeson-Forssman-Lehmann syndrome: identification of a novel de novo PHF6 mutation in a female patient. *J Med Genet* 2006; 43: 238–43.
- Engel J Jr. A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: Report of the ILAE Task Force on Classification and Terminology. *Epilepsia* 2001; 42: 796–803.
- Fabisiak K, Erickson RP. A familial form of convulsive disorder with or without mental retardation limited to females: extension of a pedigree limits possible genetic mechanisms. *Clin Genet* 1990; 38: 353–8.
- Grosso S, Brogna A, Bazzotti S, Renieri A, Morgese G, Balestri P. Seizures and electroencephalographic findings in CDKL5 mutations: case report and review. *Brain Dev* 2007; 29: 239–42.
- Harkin LA, McMahon JM, Iona X, Dibbens L, Pelekanos JT, Zuberi SM, et al. The spectrum of SCN1A-related infantile epileptic encephalopathies. *Brain* 2007; 130: 843–52.
- Juergens RC, Hellman CD. A new familial form of convulsive disorder and mental retardation limited to females. *J Pediatr* 1971; 79: 726–32.
- Lainhart JE, Ozonoff S, Coon H, Krasny L, Dinh E, Nice J, et al. Autism, regression, and the broader autism phenotype. *Am J Med Genet* 2002; 113: 231–7.
- Pickles A, Starr E, Kazak S, Bolton P, Papanikolaou K, Bailey A, et al. Variable expression of the autism broader phenotype: findings from extended pedigrees. *J Child Psychol Psychiatry* 2000; 41: 491–502.
- Piven J, Palmer P, Jacobi D, Childress D, Arndt S. Broader autism phenotype: evidence from a family history study of multiple-incidence autism families. *Am J Psychiatry* 1997; 154: 185–90.
- Reutens DC, Howell RA, Gebert KE, Berkovic SF. Validation of a questionnaire of clinical seizure diagnosis. *Epilepsia* 1992; 33: 1065–71.

- Ryan SG, Chance PF, Zou CH, Spinner NB, Golden JA, Smietana S. Epilepsy and mental retardation limited to females: an X-linked dominant disorder with male sparing. *Nat Genet* 1997; 17: 92–5.
- Twigg SR, Matsumoto K, Kidd AM, Goriely A, Taylor IB, Fisher RB, et al. The origin of EFNB1 mutations in craniofrontonasal syndrome: frequent somatic mosaicism and explanation of the paucity of carrier males. *Am J Hum Genet* 2006; 78: 999–1010.
- Wieacker P, Wieland I. Clinical and genetic aspects of craniofrontonasal syndrome: towards resolving a genetic paradox. *Mol Genet Metab* 2005; 86: 110–6.
- Williams CA, Beaudet AL, Clayton-Smith J, Knoll JH, Kyllerman M, Laan LA, et al. Angelman syndrome 2005: updated consensus for diagnostic criteria. *Am J Med Genet A* 2006; 140: 413–8.
- Williamson SL, Christodoulou J. Rett syndrome: new clinical and molecular insights. *Eur J Hum Genet* 2006; 14: 896–903.