ORIGINAL ARTICLE

CDKL5 mutations cause infantile spasms, early onset seizures, and severe mental retardation in female patients

H L Archer*, J Evans*, S Edwards, J Colley, R Newbury-Ecob, F O'Callaghan, M Huyton, M O'Regan, J Tolmie, J Sampson, A Clarke, J Osborne

J Med Genet 2006;**43**:729–734. doi: 10.1136/jmg.2006.041467

See end of article for authors' affiliations

Correspondence to: Dr Hayley Archer, Institute of Medical Genetics, University Hospital of Wales, Cardiff CF14 4XN, UK; archerhl@cf.ac.uk

Received 7 February 2006
Revised version received 23 March 2006
Accepted for publication 26 March 2006
Published Online First 12 April 2006

*These authors made an equal contribution

Objective: To determine the frequency of mutations in *CDKL5* in both male and female patients with infantile spasms or early onset epilepsy of unknown cause, and to consider whether the breadth of the reported phenotype would be extended by studying a different patient group.
Methods: Two groups of patients were investigated for *CDKL5* mutations. Group 1 comprised 73 patients (57 female, 16 male) referred to Cardiff for *CDKL5* analysis, of whom 49 (42 female, 7 male) had epileptic seizure onset in the first six months of life. Group 2 comprised 26 patients (11 female, 15 male) with infantile spasms previously recruited to a clinical trial, the UK Infantile Spasms Study. Where a likely pathogenic mutation was identified, further clinical data were reviewed.
Results: Seven likely pathogenic mutations were found among female patients from group 1 with epileptic seizure onset in the first six months of life, accounting for seven of the 42 in this group (17%). No mutations other than the already published mutation were found in female patients from group 2, or in any male patient from either study group. All patients with mutations had early signs of developmental delay and most had made little developmental progress. Further clinical information was available for six patients: autistic features and tactile hypersensitivity were common but only one had suggestive Rett-like features. All had a severe epileptic seizure disorder, all but one of whom had myoclonic jerks. The EEG showed focal or generalised changes and in those with infantile spasms, hypsarrhythmia. Slow frequencies were seen frequently with a frontal or fronto-temporal predominance and high amplitudes.
Conclusions: The spectrum of the epileptic seizure disorder, and associated EEG changes, in those with *CDKL5* mutations is broader than previously reported. *CDKL5* mutations are a significant cause of infantile spasms and early epileptic seizures in female patients, and of a later intractable seizure disorder, irrespective of whether they have suspected Rett syndrome. Analysis should be considered in these patients in the clinical setting.

The term ''infantile spasms'' defines a severe seizure disorder that usually starts early in life and in which seizures tend to occur in clusters. They are typically, although not invariably, associated with hypsarrhythmia on the EEG.[1] Infantile spasms often occur in infants with severe underlying neurological abnormalities[1] and may be preceded by or followed by other seizure types. Infantile spasms can occur in many different conditions including tuberous sclerosis[2] and neuronal migration defects.[3] They have also been reported in association with several different chromosomal anomalies including 15q duplication[4] and subtelomeric rearrangements.[5] Mutations in three genes have been associated with X linked infantile spasms (ISSX, OMIM 308350): Aristaless related gene (*ARX,* OMIM 300382),[6] cyclin dependent kinase like 5 (*CDKL5,* OMIM 300203),[7] and the sodium channel neuronal type 1 α subunit (*SCN1A*, OMIM 182389).[8] Pathogenic *ARX* mutations have so far only been reported in males, some of whom have infantile spasms.[6] The mutation frequency of *ARX* in patients with infantile spasms has not been reported, but the combined published detection rate in singleton male cases with mental retardation is extremely low (2/1500 cases).[9]

Mutations in *CDKL5* were initially found in two female patients with infantile spasms, severe mental retardation, and X;autosome translocations that disrupted the gene.[7] Mutations were subsequently identified in further patients with suspected Rett syndrome (RTT, OMIM 312750), but only in those with seizure onset before six months of age.[10–14] All but one of the 18 patients with mutations reported so far had early seizure onset with a subsequent severe mixed seizure disorder, most of whom had normal brain magnetic resonance imaging (MRI). Very few male patients have been tested, and patients with infantile spasms or a severe early onset seizure disorder have not been investigated as a separate group.

Our aim in this study was to determine the frequency of mutations in *CDKL5* in both male and female patients with infantile spasms or early onset epilepsy of unknown cause, and to consider whether the breadth of the reported phenotype would be extended by studying a different patient group.

METHODS

Patient recruitment

Cardiff patients (group 1)

Patients were identified from those referred to Cardiff for *CDKL5* analysis (table 1). Seventy five patients had severe mental retardation and seizure onset in the first year of life, 39 of whom also had infantile spasms. Two were excluded when alternative underlying genetic aetiologies were identified. Phenotypic data were ascertained by clinical questionnaire.

Abbreviations: UKISS, United Kingdom Infantile Spasms Study

Table 1 The age of onset of seizures in the 73 patients in group 1

	Age at onset of seizures			
	0–5 months	6–12 months	<12 months (exact age not known)	Total
Male	7	4	5	16
Female	42	6	9	57
Total	49	10	14	73

UKISS patients (group 2)

Group 2 consisted of patients with infantile spasms previously recruited to a clinical trial, the United Kingdom Infantile Spasms Study (UKISS), between June 1999 and December 2002.[15] In all, 208 patients were included in UKISS, 107 of whom were recruited in to a randomised controlled trial. DNA samples were previously collected from 60 of these patients, with appropriate consent, for genetic analysis. DNA was of sufficient quality for analysis in 22 females and 33 males. The underlying aetiology was classified after assessment of history, examination, and investigations which included cranial scanning, usually by MRI. Twenty six patients with infantile spasms of unknown aetiology formed group 2, including one UKISS patient had already been reported (patient 2 in Evans *et al*, 2005[10]) in order to allow assessment of frequency of CDKL5 mutations in this cohort. Phenotypic data were ascertained by a UKISS questionnaire completed by the clinician, and in most cases a seizure diary from the family. Age at onset of infantile spasms was documented where known. Randomised trial cases had Vineland developmental assessments at the age of 14 months and some at an average of 4.2 years.

Further clinical data were reviewed for six of the seven with identified mutations. EEG reports were collected retrospectively, the number depending on availability.

Molecular methods

The open reading frame of the *CDKL5* gene was analysed in lymphocytic DNA from each of the patients, as previously described.[10] Exons 2–21 of the *CDKL5* gene were amplified by polymerase chain reaction (PCR). Mutation screening was carried out by denaturing high performance liquid chromatography (DHPLC) analysis. For males, equal amounts of DNA sample were mixed in pairs to allow formation of heteroduplexes. Any amplicons generating aberrant traces were sequenced. Exons 4 and 16 were screened by DNA sequencing because of the high polymorphism in these exons.

X chromosome inactivation status (XCI) was ascertained in those female patients in whom a mutation was identified. XCI was determined using standard methods. Briefly, aliquots of DNA were predigested with the enzymes HpaII and McrBC (New England Biolabs, Beverly, Massachusetts, USA). The triplet repeat at the HUMARA locus was then amplified by PCR using fluorescent primers and analysed using an ABI 3100 automated sequencer and Genotyper software (Applied Biosystems, Foster City, California, USA). Allele peak areas were compared for HpaII digested, McrBC digested, and undigested DNA.[16]

RESULTS

CDKL5 mutations

In all, seven likely pathogenic mutations and three further mutations of uncertain significance were identified (table 3). Only patient 1 had skewed X chromosome inactivation (80:20). All were found among those females from group one with seizure onset in the first six months of life, accounting for seven of the 42 patients in this group (17%). No further mutations other than the one already published[10] were found in female patients from group 2, or in any male patient from either study group. The frequency of mutations in females from the UKISS group presenting with infantile spasms of unknown aetiology in the first 12 months of life was 1/10 (10%).

Mutations of uncertain pathogenicity

Intronic mutation (IVS8-19C→G)

This single base change lies towards the extreme end of the exon 9 splice acceptor consensus sequence. Computer analysis predicted that this very slightly reduced the splice site score from 0.97 to 0.96 using BDGP Splice Site Predictor (http://www.fruitfly.org/seq_tools/splice.html). It was not part of the consensus sequence according to Splice Site Finder (http://www.genet.sickkids.on.ca/~ali/splicesitefinder.html). Parental samples were not available for analysis.

Missense mutation (c.2378T→C; V793A)

This missense mutation was found in a patient from group 2. The amino acid change is conservative and it is only two amino acids away from a known single nucleotide polymorphism (SNP), Q791P. The amino acid was conserved in fugu but not in mouse. There were no parental samples available for analysis.

Intronic mutation (c.IVS11-42_50del9bp)

This intronic change was outside of the consensus splice site sequence and distant from exon eleven. Parental samples were not available for analysis.

Clinical phenotype

Epilepsy

Detailed information was available for six of the seven patients with likely pathogenic mutations, all of whom had

Table 2 Characteristics of 26 patients in group 2 with infantile spasms of unknown cause

			Age at onset of spasms		
		n	0–5 m	6–11 m	Not known
Male (n = 15)	Aetiology not proven	14	8	6	0
	Data incomplete	1	0	0	1
Female (n = 11)	Aetiology not proven	10*	6	4*	0
	Data incomplete	1	1	0	0
Total		26	15	10	1

Age at onset of infantile spasms rather than age at onset of seizures was recorded.
*Case with known mutation and published previously (Evans *et al*, 2005[10]).
m, months.

Table 3 Mutations identified in the *CDKL5* gene

Type of variant	Sequence change	Comments
Splice site	IVS6-1G→T	Pathogenic, predicted to result in exon 7 skipping (patient 2)
	IVS11-2A→G	Pathogenic (patient 3)
	IVS16+1G→A	Pathogenic (patient 5)
Deletion/ insertion	c.2362_2366delAAGAA	Pathogenic (patient 1)
	c.del678_691ins683_673	Pathogenic (patient 4)
Nonsense	c.175C→T (R59X)	Pathogenic (patient 6)
Missense	c.539C→T (P180L)	Pathogenic in conserved catalytic domain (patient 7)
	c.2378T→C (V793A)	Uncertain pathogenicity (patient 11)
Intronic	IVS 11-42_50del9bp	Uncertain pathogenicity (patient 8)
	IVS8-19C→G	Uncertain pathogenicity (patients 9 and 10)

severe mental retardation, marked hypotonia, and a severe epileptic seizure disorder starting within the first two months of life; three also had infantile spasms (patients 1, 2, and 4). Two patients had infantile spasms before six months and the other at 11 months; these did not respond to numerous antiepileptic drugs. Initial seizures were often brief, lasting 10–15 seconds, but seizure frequency, intensity, and duration gradually increased. Early seizures consisted of subtle oro-facial seizures, ''breath holding''/''choking'' episodes, tonic seizures, tonic axial flexor seizures, or star shaped seizures. Once seizures had been noted they tended to occur at least on a daily basis. Four patients had a period without seizures in the second year of life, lasting between six weeks and nine months (patients 1, 2, 5, and 7). Honeymoon periods with new drugs were described in all patients but were followed by more severe seizures or by a change in seizure type. All but one had multiple seizure types and experienced one or more major seizures (such as generalised tonic-clonic or complex partial seizures) as well as numerous (up to 200) brief seizures (such as absences, drop fits, myoclonic jerks) every day. Patient 5, who was two years old, had the mildest disorder with just two generalised tonic clonic seizures a week while in the early phase of sleeping. Drug treatment was generally ineffective. In patient 1, seizures worsened during puberty. The seizure disorder improved, but remained severe, in early adulthood. At least one episode of either convulsive or non-convulsive status epilepticus was reported in two patients and was associated with loss of skills (patients 2 and 7).

Neuroimaging

None of the six patients with likely pathogenic mutations and detailed clinical information available was reported to have abnormalities on brain MRI.

EEG findings

EEG reports were available for six patients. The EEGs varied with age and seizure type and included hypsarrhythmia in one patient with infantile spasms. Three infants had normal EEGs initially for some months. No unique pattern was seen. Very slow activity was common, as were high voltage focal or multifocal spikes. Frontal or fronto-temporal activity was most frequently seen, and activity could suggest a focal pathology. At times activity was almost continuous. In one case two seizures were recorded which were accompanied by electro-decremental change; during these seizures there was a sudden jerk, lifting of the head off the pillow, stiffened of the body, and vacant staring.

Developmental progress

All patients had severe developmental delay which was apparent in the first year of life. Five presented with a severe phenotype with virtually no developmental progress (patients 2–6) and were similar to previously reported severely affected patients with mutations.[7 10 13] The previously published case (patient 2[1]) had a Vineland adaptive behaviour composite score of 72 at 14 months of age, but this had fallen to 28 by 5 years 9 months. Loss of skills was reported in two patients from study group 1. Patient 1 had much better physical skills than these severely affected patients: she could swim independently and, while hand use was limited, she was able to use normal cutlery to feed herself. She spoke in phrases and sang the words to favourite songs but her understanding was very limited. She gave poor eye contact and had repetitive behaviours including hand stereotypies

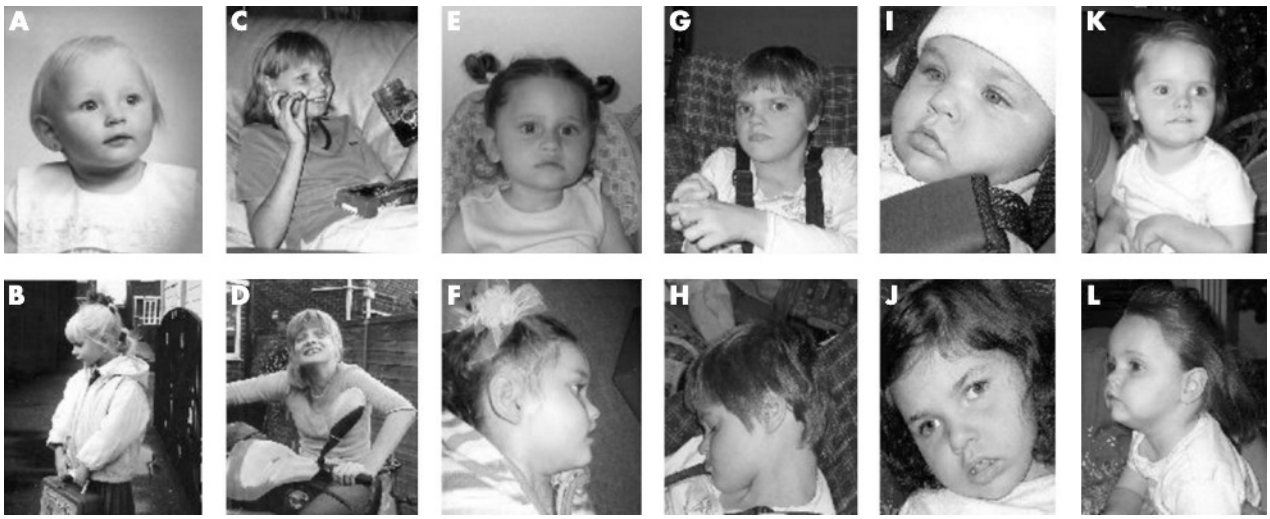

Figure 1 Clinical photographs of patients. (A–D): Patient 1, age 4 months, 5 years, 16 years, and 19 years, respectively. (E, F): Patient 2, age 2 years and 2 years 5 months, respectively. (G, H): Patient 3, age 7 years. (I, J): Patient 4 aged less than 1 year and 4 years, respectively. (K, L): Patient 5, age 2 years 5 months. Note the deep set eyes, straight eyebrows, slightly short upturned nose, relatively large ears with large earlobes, and high forehead. Written permission for publication of these photographs was obtained from the parents of the children.

Table 4 A comparison of new cases with *CDKL5* mutations identified in this study with those previously reported[7 10–14 17] and the classical presentation of Rett syndrome[18]

			Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Diagnosis	CR	CDKL5	ISSX/autism	ISSX	EE	ISSX	EE*	EE	EE
Sex	Usually F	15 F:3 M	F	F	F	F	F	F	F
Age (years)	Any	2–41 y (mean 15)	18	2	7	4	2	NK	13
Normal pre/perinatal period	+	11/18	+	+	+	+	−	NK	+
Near normal early development	+	10/18	−	−	−	−	−	NK	−
Normal OFC at birth	Usually	14/14	+	+	+	+	+	NK	+
Deceleration of OFC from birth	+	6/11	−	+	+	+	+	NK	+
Current OFC centile	Varies, mean 3rd centile	6/15 <3rd centile	9–25	0.4–2	<0.4	<0.4	<0.4	NK	9
Regression	+	5/17	−	+	−	−	−	NK	−
Hand stereotypies	+	12/14	+	+	+	+	+	NK	+
Severe intellectual disability	+	16/17	+	+	+	+	+	+	+
Motor dyspraxia	+	6/12	+	−	−	+	−	NK	+
Hypotonia	+	7/12	+	+	+	+	+	NK	+
Best gross motor skill	Varies	6/16 walk	Walks and swims	None	None	None	Sits unsupported	Limited	Crawls
Limited hand skills	+	15/16	+	+	+	+	+	NK	+
Seizure onset	After regression, extremely rare before 6 m	Birth – 5 m† (mean 2 m)	2 m	10 d	3 d	1 mh	5 w	Early	6 w
Growth retardation	+	5/10	−	−	+	−	+	NK	+
Autonomic features	+	11/16	−	−	+	−	+	NK	−
Scoliosis	+	9/14	−	−	−	−	−	NK	+
Good eye contact	+	4/15	−	−	−	−	+	NK	+
Bruxism	+	4/15	+	+	+	−	+	NK	+
Mood lability	+	3/15	+	−	+	+	−	NK	+
Sleep disturbance	+	1/14	+	−	+	+	−	NK	−
Speech in phrases	Often none	1/16	+	−	−	−	−	NK	−
Gastro-oesophageal reflux	Often	8/16	−	−	+	−	−	NK	−

*Infantile spasms status not known.
†One patient never experienced seizures.[14]
CR, classic Rett syndrome; d, days; EE, epileptic encephalopathy; F, female; ISSX, X linked West syndrome; m, months; M, male; NK, not known; OFC, occipito-frontal head circumference; w, weeks; y, years; +, feature present; −, feature absent.

and flicking through photographs. Her behaviour became difficult at puberty, with frustration and occasional aggressive outbursts. She had a more autistic presentation.

Other features

Reduced fetal movements were reported in four patients (Nos 1, 2, 4, and 5). Tactile hypersensitivity, such as dislike of hair brushing, was reported in five patients (Nos 1, 2, 3, 4, and 7). In five patients, clinical photographs were obtained and a subtle dysmorphic appearance was noted (fig 1). Only one patient had a Rett-like phenotype, although early seizure onset mitigated against the diagnosis (patient 3). Further clinical details are provided in table 4.

DISCUSSION

Our data have shown that *CDKL5* mutations are a significant cause of severe mental retardation and early seizures in female patients. *CDKL5* mutations were also a significant cause of infantile spasms but only in patients with severe mental retardation. This study has added to the evidence suggesting that while *CDKL5* mutations may be found in male patients[14 17] they are rare overall.[10] We have also extended the description of the epilepsy phenotype and the EEG findings in these patients.

Five of seven patients with pathogenic mutations presented with a phenotype comparable to the severe presentation that has already been described.[7 10 13] All presented with very early onset of a severe epileptic seizure disorder, marked hypotonia, and very limited developmental progress. While those patients who were only two years of age at assessment may have potential for further developmental progress, patient 2 had documented deterioration in development.

One further patient in our study had a severe but relatively milder presentation which may be accounted for by skewed X chromosome inactivation. Her physical and intellectual skills exceeded those of all the patients so far reported, except for one patient with autism who did not have an epileptic seizure disorder.[14] Her phenotype was more autistic, with limited eye contact and interaction with people on her own terms. She did not have any disturbance of autonomic function. Tactile hypersensitivity was present and was also a common symptom among our other patients. Poor eye contact was also found in all but one of the remaining five patients. Some autistic features have been reported previously.[11 14] A careful history may be revealing in other patients with severe mental retardation and an autistic tendency, where an early onset seizure disorder has remained difficult to control. The role of CDKL5 in similar patients without seizures needs to be explored.

One patient presented with a phenotype that might appear to overlap with Rett syndrome. However, her neonatal onset of epileptic seizures and lack of eye contact and interest in people made a diagnosis of Rett syndrome unlikely. The relative lack of patients with a Rett syndrome-like phenotype in this study may reflect our patient recruitment strategy with selection on the basis of infantile spasms or a severe early onset epileptic seizure disorder rather than having features of Rett syndrome.

The seizure disorder is the key to identifying patients likely to have *CDKL5* mutations and to differentiate them from those likely to have *MECP2* (methyl CpG binding protein 2, OMIM 300005) mutations. Only one of 345 *MECP2* mutation positive female Rett syndrome patients registered to the British Isles Rett survey had epileptic seizure onset in the first six months of life, without an underlying metabolic disturbance or other identifiable cause unrelated to Rett syndrome (Kerr A, unpublished data, personal communication). In one girl severely affected by Rett syndrome, there was difficulty in differentiating between severe autonomic episodes and early seizures, and this has been noted in other studies.[19] There is only one reported female patient with epileptic seizures in the first six months and an *MECP2* mutation, and none presenting with infantile spasms in the same time period.[20] The profile of Rett syndrome characterises the disorder and consists of many recognisable clinical features.[21] While about half of all patients with this syndrome are reported to have ongoing epilepsy, more than a quarter never have an epileptic seizure.[20] Autonomic features are an inconsistent finding in people with *CDKL5* mutations but are almost invariably present in Rett syndrome [22]. The Rett phenotype is clearly different from that of *CDKL5*, where the history usually centres around an early onset, intractable, and typically polymorphic seizure disorder in association with severe mental retardation.

The evidence so far suggests that frequent myoclonic jerks are an important component of the CDKL5 related epileptic seizures.[10] It has been suggested that the seizure disorder is an encephalopathy beginning with tonic seizures and evolving into myoclonic seizures in cases older than many of those in our study.[23] Myoclonic epilepsy also formed part of the seizure disorder in five of our six patients, and in all three of our previously reported cases.[10] Our patient without such seizures is now two years old and may develop them later. In contrast to the patients in the Buoni paper, who only experienced one or two seizures a week, five of our six patients and all our previously reported cases with an epileptic seizure disorder[10 14] were experiencing multiple seizures on a daily basis. The spectrum of the severity of the seizure disorder appears to be broad and ranges from no seizures at all to hundreds of seizures a day.

The features of the patients reported here suggest that the CDKL5 associated phenotype suggested by Buoni *et al* is too narrow.[23] A normal interictal EEG was initially seen in some of our patients in the first year of life, even though the EEG can be very abnormal in the first year. Slow frequencies are often seen, frequently with a frontal or fronto-temporal predominance and high amplitudes, which is unlike the patients reported by Buoni *et al*. Our patients often had focal EEG abnormalities, often frontal, and clinical evidence of focal features without associated MRI abnormalities. Complex partial, tonic tonic-clonic, and myoclonic seizures occur later in life. In one infant, electro-decremental episodes were seizure related. Whether some of our younger patients may show the high voltage sharp waves of 6–7 Hz or the rhythmic diffuse 15 Hz activity accompanied by tonic seizures reported by Buoni when they are older remains to be seen. Some of the differences observed in our study may be age related.

It is of interest that the seizures are largely resistant to drug treatment and that vagus nerve stimulators have been of limited benefit, if any.[10] Little is known about the role of CDKL5 and its function in the brain. There are conflicting views in two studies on whether or not there is a direct interaction between MeCP2 and CDKL5,[11 24] though both studies agree that loss of kinase activity, resulting from mutations in the gene, is likely to be responsible for the clinical phenotype and that the C-terminus is important for the function of the protein. However, the specific biological pathways and neurodevelopmental role of CDKL5 has not yet been established, and elucidating this will be important for considering appropriate targets for therapeutic interventions in patients with this severe, largely drug resistant epilepsy.

Our study has shown that *CDKL5* mutations are a significant and important aetiological factor among female patients with severe mental retardation and a seizure disorder starting in the first six months of life, which is very difficult to treat. Other investigations, including cranial MRI, are usually normal. Our study of similarly affected males

suggests that *CDKL5* mutations are a very rare cause of severe mental retardation, infantile spasms, or early onset epilepsy in males, although further work is needed to substantiate this. A detailed study following the time course of developmental progress, detailed assessment of autism, epilepsy, and associated EEG findings may be most helpful in further characterisation of this disorder.

ACKNOWLEDGEMENTS

We gratefully acknowledge clinicians who have referred patients to Cardiff for *CDKL5* analysis or to the UKISS study, in particular Dr Emma McCann, Dr Richard Appleton, Dr Helen Kingston, Dr Angela Brady, Dr Bronwyn Kerr, Dr Ana Moreiro, and Professor Joseph McMenamin. Dr Alison Kerr contributed data from the British Isles Rett survey. Vineland adaptive behaviour scales were undertaken by Dr Andrew Lux and Dr Katrina Darke. We thank Professor Mark Gardiner and Dr Robert Robinson for extracting and storing the DNA. This work was supported by the Health Care Foundation, Jeans for Genes, Rett Syndrome Association UK, and the Bath Unit for Research in Paediatrics.

Authors' affiliations

H L Archer*, J Evans*, J Sampson, A Clarke, Department of Medical Genetics, Cardiff University, University Hospital of Wales, Cardiff, UK
S Edwards, R Newbury-Ecob, J Osborne, Royal United Hospital Bath NHS Trust, Bath, UK
J Colley, Wales Gene Park, University Hospital of Wales
F O'Callaghan, The Royal Hospital for Sick Children, Bristol, UK
M Huyton, The David Lewis Centre, Alderley Edge, Cheshire, UK
M O'Regan, Fraser of Alexander Neurosciences Unit, Royal Hospital for Sick Children, Glasgow, UK
J Tolmie, Department of Medical Genetics, Yorkhill Hospital, Glasgow

*These authors made an equal contribution

Conflicts of interest: none declared.

REFERENCES

1 **Lux AL**, Osborne JP. A proposal for case definitions and outcome measures in studies of infantile spasms and West syndrome: consensus statement of the West Delphi group. *Epilepsia* 2004;**45**:1416–28.
2 **Manson JI**. Tuberose sclerosis in childhood. *Proc Aust Assoc Neurol* 1973;**9**:57–61.
3 **Palmini A**, Andermann F, Aicardi J, Dulac O, Chaves F, Ponsot G, Pinard JM, Goutieres F, Livingston J, Tampieri D. Diffuse cortical dysplasia, or the ''double cortex'' syndrome: the clinical and epileptic spectrum in 10 patients. *Neurology* 1991;**41**:1656–62.
4 **Bingham PM**, Spinner NB, Sovinsky L, Zackai EH, Chance PF. Infantile spasms associated with proximal duplication of chromosome 15q. *Pediatr Neurol* 1996;**15**:163–5.
5 **Gerard-Blanluet M**, Romana S, Munier C, Le Lorc'h M, Kanafani S, Sinico M, Touboul C, Levaillant JM, Haddad B, Lopez N, Lelong F, De Villemeur TB, Verloes A, Borghi E. Classical West ''syndrome'' phenotype with a subtelomeric 4p trisomy. *Am J Med Genet* 2004;**130A**:299–302.
6 **Stromme P**, Mangelsdorf ME, Scheffer IE, Gecz J. Infantile spasms, dystonia, and other X-linked phenotypes caused by mutations in Aristaless related homeobox gene, ARX. *Brain* Dev, 2002;**24**:266–8.
7 **Kalscheuer VM**, Tao J, Donnelly A, Hollway G, Schwinger E, Kubart S, Menzel C, Hoeltzenbein M, Tommerup N, Eyre H, Harbord M, Haan E, Sutherland GR, Ropers HH, Gecz J. Disruption of the serine/threonine kinase 9 gene causes severe X-linked infantile spasms and mental retardation. *Am J Hum Genet* 2003;**72**:1401–11.
8 **Wallace RH**, Hodgson BL, Grinton BE, Gardiner RM, Robinson R, Rodriguez-Casero V, Sadleir L, Morgan J, Harkin LA, Dibbens LM, Yamamoto T, Andermann E, Mulley JC, Berkovic SF, Scheffer IE. Sodium channel alpha1-subunit mutations in severe myoclonic epilepsy of infancy and infantile spasms. *Neurology* 2003;**61**:765–9.
9 **Raymond FL**. X-linked mental retardation: a clinical guide. *J Med Genet* 2006;**43**:193–200.
10 **Evans JC**, Archer HL, Colley JP, Ravn K, Nielsen JB, Kerr A, Williams E, Christodoulou J, Gecz J, Jardine PE, Wright MJ, Pilz DT, Lazarou L, Cooper DN, Sampson JR, Butler R, Whatley SD, Clarke AJ. Early onset seizures and Rett-like features associated with mutations in CDKL5. *Eur J Hum Genet* 2005;**13**:1113–20.
11 **Mari F**, Azimonti S, Bertani I, Bolognese F, Colombo E, Caselli R, Scala E, Longo I, Grosso S, Pescucci C, Ariani F, Hayek G, Balestri P, Bergo A, Badaracco G, Zappella M, Broccoli V, Renieri A, Kilstrup-Nielsen C, Landsberger N. CDKL5 belongs to the same molecular pathway of MeCP2 and it is responsible for the early-onset seizure variant of Rett syndrome. *Hum Mol Genet* 2005;**14**:1935–46.
12 **Scala E**, Ariani F, Mari F, Caselli R, Pescucci C, Longo I, Meloni I, Giachino D, Bruttini M, Hayek G, Zappella M, Renieri A. CDKL5/STK9 is mutated in Rett syndrome variant with infantile spasms. *J Med Genet* 2005;**42**:103–7.
13 **Tao J**, Van Esch H, Hagedorn-Greiwe M, Hoffmann K, Moser B, Raynaud M, Sperner J, Fryns JP, Schwinger E, Gecz J, Ropers HH, Kalscheuer VM. Mutations in the X-linked cyclin-dependent kinase-like 5 (CDKL5/STK9) gene are associated with severe neurodevelopmental retardation. *Am J Hum Genet* 2004;**75**:1149–54.
14 **Weaving LS**, Christodoulou J, Williamson SL, Friend KL, McKenzie OL, Archer H, Evans J, Clarke A, Pelka GJ, Tam PP, Watson C, Lahooti H, Ellaway CJ, Bennetts B, Leonard H, Gecz J. Mutations of CDKL5 cause a severe neurodevelopmental disorder with infantile spasms and mental retardation. *Am J Hum Genet* 2004;**75**:1079–93.
15 **Lux AL**, Edwards SW, Hancock E, Johnson AL, Kennedy CR, Newton RW, O'Callaghan FJ, Verity CM, Osborne JP. The United Kingdom Infantile Spasms Study comparing vigabatrin with prednisolone or tetracosactide at 14 days: a multicentre, randomised controlled trial. *Lancet* 2004;**364**:1773–8.
16 **Allen RC**, Zoghbi HY, Moseley AB, Rosenblatt HM, Belmont JW. Methylation of HpaII and HhaI sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. *Am J Hum Genet* 1992;**51**:1229–39.
17 **Huopaniemi L**, Tyynismaa H, Rantala A, Rosenberg T, Alitalo T. Characterization of two unusual RS1 gene deletions segregating in Danish retinoschisis families. *Hum Mutat* 2000;**16**:307–14.
18 **Hagberg B**, Hanefeld F, Percy A, Skjeldal O. An update on clinically applicable diagnostic criteria in Rett syndrome. Comments to Rett Syndrome Clinical Criteria Consensus Panel Satellite to European Paediatric Neurology Society Meeting, Baden Baden, Germany, 11 September 2001. *Eur J Paediatr Neurol* 2002;**6**:293–7.
19 **Glaze DG**, Schultz RJ, Frost JD. Rett syndrome: characterization of seizures versus non-seizures. *Electroencephalogr Clin Neurophysiol* 1998;**106**:79–83.
20 **Steffenburg U**, Hagberg G, Hagberg B. Epilepsy in a representative series of Rett syndrome. *Acta Paediatr* 2001;**90**:34–9.
21 **Kerr AM**, Nomura Y, Armstrong D, Anvret M, Belichenko PV, Budden S, Cass H, Christodoulou J, Clarke A, Ellaway C, D'Esposito M, Francke U, Hulten M, Julu P, Leonard H, Naidu S, Schanen C, Webb T, Engerstrom IW, Yamashita Y, Segawa M. Guidelines for reporting clinical features in cases with MECP2 mutations. *Brain Dev* 2001;**23**:208–11.
22 **Nomura Y**, Kimura K, Arai H, Segawa M. Involvement of the autonomic nervous system in the pathophysiology of Rett syndrome. *Eur Child Adolesc Psychiatry* 1997;**6**(suppl 1):42–6.
23 **Buoni S**, Zannolli R, Colamaria V, Macucci F, di Bartolo RM, Corbini L, Orsi A, Zappella M, Hayek J. Myoclonic encephalopathy in the CDKL5 gene mutation. *Clin Neurophysiol* 2006;**117**:223–7.
24 **Lin C**, Franco B, Rosner MR. CDKL5/Stk9 kinase inactivation is associated with neuronal developmental disorders. *Hum Mol Genet* 2005;**14**:3775–86.