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ORIGINAL ARTICLE

Clinical and genetic aspects of *PCDH19*-related epilepsy syndromes and the possible role of *PCDH19* mutations in males with autism spectrum disorders

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Abstract Epilepsy and mental retardation limited to females (EFMR), caused by *PCDH19* mutations, has a variable clinical expression that needs further exploration. Onset of epilepsy may be provoked by fever and can resemble Dravet syndrome. Furthermore, transmitting males have no seizures, but are reported to have rigid personalities

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suggesting possible autism spectrum disorders (ASD). Therefore, this study aimed to determine the phenotypic spectrum associated with *PCDH19* mutations in Dravet-like and EFMR female patients and in males with ASD. We screened 120 females suffering from Dravet-like epilepsy, 136 females with EFMR features and 20 males with ASD. Phenotypes and genotypes of the *PCDH19* mutation carriers were compared with those of 125 females with EFMR reported in the literature. We report 15 additional patients with a *PCDH19* mutation. Review of clinical data of all reported patients showed that the clinical picture of EFMR is heterogeneous, but epilepsy onset in infancy, fever sensitivity and occurrence of seizures in clusters are key features. Seizures remit in the majority of patients during teenage years. Intellectual disability and behavioural disturbances are common. Fifty percent of all mutations are missense mutations, located in the extracellular domains only. Truncating mutations have been identified in all protein domains. One ASD proband carried one missense mutation predicted to have a deleterious effect, suggesting that ASD in males can be associated with PCDH19 mutations.

Keywords PCDH19 · Epilepsy · X-linked · Genetics · Autism spectrum disorder

Introduction

Early infantile epileptic encephalopathy-9 (MIM 300088), also known as epilepsy and mental retardation limited to females (EFMR), is caused by mutations in the X-linked *PCDH19* gene. It is characterized by seizures with an onset in infancy and cognitive impairment. Seizures often occur in clusters and are often provoked by fever. Nevertheless, the phenotypic spectrum is heterogeneous and can fluctuate between severe generalized or multifocal epilepsy, resembling Dravet syndrome and more benign focal epilepsies with normal intelligence [1–21]. So far over 100 females and one mosaic male with a *PCDH19* mutation have been reported [6]. The X-linked inheritance pattern is remarkable: Heterozygous females are affected while transmitting hemizygous males do not show epilepsy or mental retardation. However, transmitting males in familial EFMR were reported to have controlling, rigid personalities [4]. We screened a large cohort of female patients with EFMR or a Dravet-like phenotype and describe 15 new cases with a *PCDH19* mutation. Clinical characteristics and genetic findings were compared with patients reported in literature. We also hypothesized that *PCDH19* mutations may play a role in normal intelligent males with autism spectrum disorders (ASD) and analysed the *PCDH19* gene in 20 high-functioning males with ASD.

Methods

Selection of female patients for *PCDH19* screening

Analysis of the *PCDH19* gene was performed in two patient cohorts: a cohort of 120 females with features of Dravet syndrome originally referred for analysis of the *SCN1A* gene but without mutation and a cohort of 136 patients referred for analysis of the *PCDH19* gene because of features of EFMR. Clinical data of the patients with a *PCDH19* mutation were collected from the medical records and through a questionnaire completed by the treating physicians. We collected data on age at seizure onset, seizures types, seizure frequency, seizure offset and results of EEG and magnetic resonance imaging (MRI). We also collected data on psychomotor development, behavioural features and the level of intellectual disability, assessed clinically.

Selection of males with ASD

At the department of Child and Adolescent Psychiatry of the UMC Utrecht, a repository of genomic DNA and phenotypic data on patients with ASD has been collected over the past years for research purposes. The parents or caretakers have given their informed consent for these data storage and DNA banking. We selected a total of 20 sporadic male patients with ASD and an intelligent quotient above 75. Nine of these patients had Asperger syndrome, pervasive developmental disorder—not otherwise specified was diagnosed in six, four patients had a diagnosis of multiple complex developmental disorder and autistic disorder was diagnosed in one patient.

PCDH19 mutation analysis

Genomic DNA was extracted from whole blood cells using standard procedures. PCR amplification of DNA was done using specific primer pairs for the six exons and adjacent intron–exon boundaries of the *PCDH19* gene (reference sequence EF676096.1). Primer sequences and PCR/sequencing conditions are available on request. Sequence products were run on an ABI 3730 automated DNA sequencer. Mutation analysis of the coding exons and intron /exon boundaries was undertaken using suitable mutation analysis software. The analysis of larger structural variants (deletions/duplications) was performed using MLPA (MRC Holland probe kit nr. P330-A1) or Multiplex Amplicon Quantification (http://www.multiplicom.be).

Females with a *PCDH19* mutation reported in literature

To find reports in literature on females with a *PCDH19* mutation, we performed a Medline search from 2008

onwards using the key words *EFMR*, *PCDH19*, *epilepsy female mental retardation* and *protocadherin 19*.

Results

Screening results and clinical characteristics

A PCDH19 mutation was detected in seven out of 136 (5.1 %) EFMR suspected females, eight out of 120 (6.7 %) Dravet-like females and one out of 20 (5 %) ASD males. Only three mutations were inherited, and the remaining 12 were confirmed de novo mutations. Table 1 summarizes the clinical characteristics and *PCDH19* gene mutations. Patients 2, 3, 4, 5, 10, 11, 12 and 15 were originally referred for analysis of the *SCN1A* gene. The other patients were referred for analysis of *PCDH19* based on a clinical phenotype thought to be compatible with EFMR.

Table 2 summarizes the clinical characteristics of all 125 patients with *PCDH19* mutations reported in the literature thus far [4, 6–12, 14–16, 18, 19, 21], including the 15 patients identified in our study. One study was excluded because part of all patients in this study had been reported earlier and because the focus of this study was on seizure types and EEG characteristics. The overall clinical characteristics were only summarized for the whole group of patients and not for each individual patient [20].

Epilepsy course

In all reported patients, seizure onset occurred at the end of the first year of life. The most common seizure type at onset was febrile or afebrile generalized tonic–clonic seizures. In 34 % of patients, the first seizures occurred in a cluster. Different seizure types followed, but a high prevalence of fever sensitivity (in 54 % of patients), generalized tonic–clonic seizures (in 80 %) and occurrence of seizures in clusters (in 62 %) remained. In a recent study on seizure semiology in 35 patients, "fearful screaming" in the context of focal seizures was reported as a predominant ictal sign, occurring in 70.5 % of patients [20].

In our patients, the frequency of febrile seizures (100 %), tonic seizures (60 %), myoclonic seizures (40 %), complex partial seizures (67 %) and seizures occurring in clusters (100 %) was high compared with the frequency of these seizures in previously reported patients. Clusters of seizures lasted several days or weeks. In ten of our 15 patients (67 %), the seizure-free interval in between clusters ranged from 2 to 10 months during the first 4 years of life. For many of the previously published patients, data on seizure frequency and seizure free intervals are not available. Data on seizure offset were available for patients from four studies [4, 7, 12, 14] and for our own patients. Long seizure-free intervals may hamper a reliable assessment of the percentage of patients with seizure offset. Therefore, we decided to include only patients who were seizure free for at least 2 years. Two of 20 patients (10 %) aged under 10 years, three of eight patients (38 %) aged 10 to 15 years and 24 of 30 patients (80 %) older than 15 years were seizure free for at least 2 years. The mean age of seizure offset was 12 years (range 1, 8 to 30 years).

Intellectual disability and psychiatric disorders

Thirteen of our 15 patients (87 %) showed developmental delay or intellectual disability, which is comparable with the proportion in reported patients (78 %). Half of the reported patients had mild ID while the other half had moderate to severe ID. Most patients had normal development prior to seizure onset. After seizure onset, development remained normal, showed slowing, stagnation or regression. In four patients from one family, development was slow form birth [4]. Behavioural disturbances, including autistic features, aggression and hyperactivity, were present in 73 % of our patients versus 46 % of reported patients.

EEG

Ictal or peri-ictal EEGs of our patients showed focal, multifocal or bilateral synchronous discharges, and background activity was either normal or showed slowing. For 12 patients, interictal EEGs were available and were reported to be normal.

The results of EEGs of patients reported in literature are also heterogeneous [4, 5, 7–16, 20]. Many patients had normal interictal EEGs, but a variety of EEG abnormalities have been described. Unfortunately, reports did not always specify whether these abnormal EEGs were recorded within a cluster of seizures. Background activity has been reported to be normal or showed diffuse or focal slowing. Focal, multifocal and generalized epileptic discharges have all been described. A photoparoxysmal response was seen in only two patients [7, 12], and in one patient, electrical status epilepticus in slow wave sleep was present [11]. In a series of 35 patients, the electroclinical pattern of ictal EEG data was interpreted as suggestive of an early involvement of the frontotemporal limbic structures in 74 % of patients [20].

The mutation spectrum

Table 3 gives an overview of the mutations identified in our patients and of all mutations reported in literature. Nine of the mutations identified in our patients (60 %) and 56 of the reported mutations in the literature (49 %) were missense mutations. All missense mutations were located in the

Table 1 Clinical characteristics and PCDH19 mutations in 15 females

Patient ID	Age at study (years)	Age at onset (months)	Seizure type at onset	Following seizure types	Seizure frequency onset-age 4 years	Seizure offset (years)	Behaviour	Development/ ID	MRI	PCDH19 mutation inheritance
1	4	12	FS, T, cluster	FS, T, H, clusters	Once in 10 months a cluster	Ongoing	Aggression, hyperactive	Delayed, mild ID	Normal	c.1863dupT p.Gly622fs de novo
2[a]	12	8	FS, T, cluster	FS, TC, T, M, PS, CP/abs, SE clusters	Once/3–4 months a cluster	Ongoing	Aggression	Delayed, moderate ID	Normal	c.823T > A p.Tyr275Asn maternal
3[a]	7	12	FS, TC Cluster	FS, TC, clusters	Once/3–4 months a cluster	Ongoing	–	Delayed, mild ID	Normal	c.2656C > T p.Arg886X de novo
4[a]	4	6	FS, TC, cluster	FS, TC, M, H, CP/abs, clusters	Abs, M: weekly TC: once/year	Ongoing	–	Delayed	Normal	c.91G > A p.Glu31Lys de novo
5[a]	14	8	FS, TC, cluster	FS, TC, CP/abs, SE, clusters	Once/1–2 months a cluster	Ongoing	Hyperactive	Mild ID	Normal	c.1123G > T p.Asp375Tyr de novo
6	16	12	FS, T	FS, TC, T, M, CP/abs, clusters	Once/3–4 months a cluster	Ongoing	Autistic features	Moderate ID	Normal	c.1091delC p.Pro364fs de novo
7	14	12	T, cluster	FS, TC, T, M, CP/abs, H, clusters	Once/3–4 months a cluster	Ongoing	Aggression, autistic features	Moderate ID	Normal	c.1031C > T p.Pro344Leu de novo
8	9	4	T, cluster	FS, TC, T, CP/abs, SE, clusters	Once/0.5 months a cluster	Ongoing	Autistic features	Moderate ID	Normal	c.718G > T p.Glu240X de novo
9	13	17	TC, cluster	FS, TC, clusters	Once/2–6 months a cluster	12	Autistic features	Delayed, moderate ID	Slight asymmetry frontal lobes	c.1091dupC p.Tyr366fs de novo
10[a]	6	15	FS, H,	FS, TC, PS, T, H, SE, clusters	Once/0.5–5 months a cluster	Ongoing	Autistic features, hyperactive	Moderate ID	Normal	c.1019A > Gp.Asn340Ser unknown
11[a]	5	6	H, Cluster	FS, TC, T, CP/abs, H, clusters	Once/4–6 months a cluster	Ongoing	Attention deficit	Delayed, Mild ID	Normal	c.1022A > G p.Asp341Gly de novo
12[a]	2.8	13	FS, TC, Cluster	FS, TC, T, CP/abs, clusters	Clusters with each episode of fever	Ongoing	–	Normal	Normal	c.824A > C p.Tyr275Ser maternal
13	7, 5	11	FS, H	FS, TC,PS, M, CP/abs, clusters	One cluster, 3 FS	Ongoing	Hyperactive	Mild ID	Normal	c.416C > A p.Ser139Stop de novo
14	16	10	T, cluster	FS, PS, T, M, CP/abs, clusters	Once/2 months a cluster Abs: daily	Ongoing	Aggression, autistic features	Delayed, moderate ID	Normal	c.1780G > C p.Asp594Hisde novo
15[a]	3	12	TC	FS, TC, SE, cluster	Once/2–7 months a cluster	Ongoing	–	Normal	Normal	c.1802G > A p.Gly601Asp unknown

CP/abs complex partial/(atypical) absences, *FS* febrile seizures, *H* hemiclonic, *ID* intellectual disability, *M* myoclonic, *PS* partial seizures, *SE* status epilepticus, *T* tonic, *TC* tonic–clonic seizures

[a] Originally referred for analysis of *SCN1A*

Table 2 Summary of the clinical characteristics of all 125 female patients with *PCDH19* mutations

Study	Present study	Scheffer 2008	Depienne 2009	Hynes 2010	Marini 2010	Jamal 2010	Depienne 2011	Dibbens 2011	Specchio 2011	Higurashi 2012	Vincent 2011	Camacho 2012	Dimova 2012	Kwong 2012	Terracciano 2012	All studies
Inclusion	120 females DS and 136 females with EFMR	4 EFMR families	45 females with DS, 41 probands	86 females with epilepsy with(out) ID	117 females with FS and spectrum of epilepsy	3 case reports	113 females with FS and afebrile seizures	2 EFMR families	75 females with epilepsy with(out) ID	116 females, 97 with DS[c] and 19 with other epilepsies	2 females with epilepsy and ID with deletion PCDH19	2 females	1 case report	16 SCN1A-negative DS or DS-like females	1 case report	(Marini 2012 excluded)
No. (%) of females with PCDH19 mutation	15 (6)	27, 4 probands	12, 11 (27) probands	3, 2 (2) probands	14, 13 (11) probands	3	25 18 (16) probands	4, 2 probands	6 (8)	8, 7 (6) probands	2	2	1	2 (13)	1	125
Mean age at onset in months (range)	10.5 (4–17)	*N*=25[a] 14 (6–36)	*N*=11[a] 9.3 (7.5–11)	18 (12–24)	8.5 (4.5–19)	10 (6–15)	*N*=24[a] 16 (7–60)	11 (7–17)	15.5 (9–38)	11.5 (5–25)	11 (8–14)	9 (8–10)	5	11 (7–15)	11	*N*=121[a] 12.4 (4–60)
Mean age at study in years (range)	8.9 (3–16)	31.4 (2.5–79)	7.7 (2.5–18)	18.3 (7–25)	11.5 (1–44)	9.2 (3–19)	14.2 (1–54)	11 (3–20)	13.3 (2–35)	10.8 (3.8–25)	14 (8–20)	17 (12–22)	13	22.5 (13–32)	8.3	16.1 (1–79)
Seizure type at onset, *N* (%)		NA					*N*=23[a]							NA		*N*=94[a]
FS	9 (60)		6 (50)	1 (33)	8 (57)		14 (61)	1 (25)	5 (83)			2 (100)	1 (100)		1 (100)	48 (51)
TC	6 (40)		4 (33)	2 (67)	1 (7)	2 (67)	18 (78)	3 (75)	3 (50)	6 (75)	2 (100)	1 (50)				48 (52)
T	6 (40)						1 (4)	1 (25)	2 (33)				1 (100)			11 (12)
H	3 (20)		6 (50)	1 (33)			1 (4)								1 (100)	10 (11)
M																
AS				1 (33)												1 (1)
CP/abs						1 (33)	1 (4)									2 (2)
PS			2 (17)		4 (29)				4 (67)	2 (25)						12 (13)
SE					1 (7)		2 (9)	1 (25)[b]	1 (17)			1 (50)				6 (6)
Cluster	11 (73)		2 (17)	1 (33)	1 (7)		2 (9)	3 (75)	6 (100)		1 (50)				1 (100)	32 (34)
Following seizure types, *N* (%)																
FS	15 (100)	17 (63)	12 (100)	1 (33)	2 (14)	2 (67)	5 (20)		2 (33)	7 (9)		2 (100)	1 (100)	1 (50)	1 (100)	68 (54)
TC	13 (87)	25 (93)	12 (100)		11 (79)	2 (67)	21 (84)	3 (75)	1 (17)	4 (50)	2 (100)	2 (100)	1 (100)	2 (100)		99 (80)
T	9 (60)	3 (11)					1 (4)	1 (25)		3 (38)			1 (100)			18 (15)
H	5 (33)	5 (19)	10 (83)	2 (67)	1 (7)		1 (4)									24 (19)
M	6 (40)	4 (15)	1 (8)		2 (14)		1 (4)			1 (12.5)						15 (12)
CP/abs	10 (67)	10 (37)	3 (25)		4 (29)	1 (67)	10 (40)		1 (17)							39 (31)
PS	4 (27)	3 (11)	11 (92)		10 (71)		8 (32)		5 (83)	5 (63)				2 (100)		48 (39)
SE	5 (33)	5 (27)	5 (42)	1 (33)	6 (43)		8 (33)	2 (50)		1 (13)		2 (100)		2 (100)		37 (30)
Cluster	15 (100)	NA	11 (92)	1 (33)	8 (57)	NA	23 (92)	3 (75)	4 (67)	7 (88)		2 (100)	1 (100)	1 (50)	1 (100)	77 (62)
Autistic features/ behavioural disturbances, *N* (%)	11 (73)	6 (22)	7 (58)	2 (67)	6 (43)	3 (100)	7 (28)	4 (100)	3 (50)	3 (38)	2 (100)	2 (100)	1 (100)	1 (50)	0	58 (46)
ID	13 (87)	18 (67)	12 (100)	2 (67)	12 (86)	3 (100)	*N*=24[a] 18 (75)	4 (100)	3 (50)	5 (63) [d]	2 (100)	2 (100)	1 (100)	2 (100)	0	*N*=124[a] 97 (78)

Table 2 (continued)

Study	Present study	Scheffer 2008	Depienne 2009	Hynes 2010	Marini 2010	Jamal 2010	Depienne 2011	Dibbens 2011	Specchio 2011	Higurashi 2012	Vincent 2011	Camacho 2012	Dimova 2012	Kwong 2012	Terracciano 2012	All studies
Mild	6 (40)	12 (44)	6 (50)	1 (33)	8 (57)	2 (67)	6 (25)	–	1 (17)	1 (13)	1 (50)	2 (100)		2 (100)		48 (39)
Moderate	7 (47)	2 (7)	4 (33)	1 (33)	1 (7)	1 (33)	11 (46)	2 (50)	2 (33)	–			1 (100)			32 (26)
Severe		4 (15)	2 (17)	–	3 (21)	–	1 (4)	2 (50)	–	3 (38)	1 (50)					16 (13)

AS atonic seizure, *CP/abs* complex partial/(atypical) absences, *DS* Dravet syndrome, *FS* febrile seizures, *H* hemiclonic, *ID* intellectual disability, *M* myoclonic, *NA* not available, *PS* partial seizures, *SE* status epilepticus, *T* tonic, *TC* tonic–clonic seizures

[a] Data on the variable were not available for all patients

[b] Electrical status epilepticus in slow wave sleep

[c] In 52 of the patients with Dravet syndrome, a SCN1A gene mutation had already been identified

[d] Degree of ID is unknown in one patient

extracellular domains and were never present in the cytoplasmic domain. Nonsense, frameshift and splicing mutations have been identified in all protein domains.

In our patient series, we identified three novel variants which were classified as rare polymorphisms based on the results of in silico analyses and segregation analyses: c.2598C > T (p.Asn866Asn), c.1294A > G (p.Met432Val) and c.769 G > C (p.Val257Leu).

Inheritance of mutations

For two of our patients, the inheritance of the mutation is unknown. In 11 of the other 13 patients (85 %), the mutation arose de novo. For 27 of all 130 mutations reported in Table 3, the inheritance of the mutation is unknown. For the other 103 mutations, 68 were de novo (66 %), 12 maternally inherited (12 %), 13 paternally inherited (13 %) and 10 (10 %) were familial (indicating extended families with multiple affected females). Mosaicism was demonstrated in three transmitting mothers, one affected and two asymptomatic [11, 21]. In sporadic patients, 68 out of 93 mutations (73 %) arose de novo.

Recurrent mutations and genotype–phenotype correlation

Two recurrent mutations are relatively frequent. The p.Asn340Ser mutation has been identified in 16 independent probands, including one of our patients. They showed variable phenotypes, ranging from generalized tonic–clonic convulsions only with normal development to an explosive onset of epileptic encephalopathy with developmental regression and electrical status epilepticus in slow wave sleep on EEG. Seven probands had the c.1091dupC mutation and one the c.1091delC mutation. Both the insertion and the deletion result in a frameshift. Detailed clinical data were available for three of these probands who all had fever sensitive epilepsy, behavioural disturbances and moderate to severe ID.

Intrafamilial variability and penetrance

The clinical phenotype in affected females from three of the four extended families described by Scheffer et al. [4] showed wide intrafamilial variability. Dibbens et al. reported on the identification of *PCDH19* mutations in the same four families and in three additional extended families [5]. Penetrance in these seven families was high: 66 out of 68 carrier females (97 %) were classified as affected.

Our patient 12 comes from a three generation family of African ancestry with a phenotype compatible with EFMR (Fig. 1). All female mutation carriers presented seizures in childhood, and although detailed information is lacking for the first generation, most of them had febrile and afebrile

Table 3 *PCDH19* gene mutations reported in females

Mutation	Protein level	Mutation type	Exon	Inheritance	Ref	Protein
c.74T > C	p.Leu25Pro	Missense	1	Maternal[a]	[11]	EC
c.78delG	p.Lys26AsnfsX4	Frameshift	1	De novo	[9]	
c.83C > A	p.Ser28X	Nonsense	1	De novo	[8, 20]	
				De novo	[20]	
c.91G > A	p.Glu31Lys	Missense	1	De novo	This study	
c.142G > T	p.Glu48X	Nonsense	1	Paternal	[6]	
c.152dupT	p.Ala52ArgfsX37	Frameshift	1	De novo	[20]	
c.215T > G	p.Val72Gly	Missense	1	Unknown	[14]	
c.241dupC	p.Leu81ProfsX8	Frameshift	1	De novo	[17]	
c.242T > G	p.Leu81Arg	Missense	1	Unknown	[10]	
				De novo	[20]	
c.253C > T	p.Gln85X	Nonsense	1	Familial	[4, 5]	
				Familial	[17]	
c.352G > T	p.Glu118X	Nonsense	1	De novo	[6]	
				De novo	[17]	
c.357delC	p.Ile119fsX122	Frameshift	1	Familial	[5]	
c.361G > A	p.Asp121Asn	Missense	1	Paternal	[6]	
c.415_423dup	p.Ser139_Ala141dup	In frame dup	1	Maternal	[10]	
c.416C > A	p.Ser139X	Nonsense	1	De novo	This study	
c.424delG	p.Ala142ProfsX70	Frameshift	1	Unknown	[10]	
c.437C > G	p.Thr146Arg	Missense	1	Paternal	[10]	
c.457G > A	p.Ala153Thr	Missense	1	Paternal	[17]	
c.462C > A	p.Tyr154X	Nonsense	1	Unknown	[10]	
c.506delC	p.Thr169SerfsX43	Frameshift	1	De novo	[6]	
c.514dupG	p.Glu172GlyfsX54	Frameshift	1	Unknown	[10]	
c.569T > G	p.Leu190Arg	Missense	1	Unknown	[17]	
c.571G > C	p.Val191Leu	Missense	1	Unknown	[14]	
c.595G > C	p.Glu199Gln	Missense	1	Unknown	[6]	
c.[608A > C;617T>]	p.[His203Pro;Phe206Cys]	Missense	1	De novo	[8, 20]	
c.617T > A	p.Phe206Tyr	Missense	1	Unknown	[10]	
c.695A > G	p.Asn232Ser	Missense	1	Unknown	[17]	
				De novo	[20]	
c.697_700delinsTAAC	p.Asp233X	Frameshift	1	Paternal	[10]	
c.701A > G	p.Asn234Ser	Missense	1	De novo	[17]	
c.706C > T	p.Pro236Ser	Missense	1	De novo	[12, 20]	
c.718G > T	p.Glu240X	Nonsense	1	De novo	This study	
c.729C > A	p.Tyr243X	Nonsense	1	De novo	[9]	
c.730dupG	p.Ala244GlyfsX76	Frameshift	1	De novo	[17]	
c.746A > G	p.Glu249Gly	Missense	1	De novo	[16]	
c.747A > T	p.Glu249Asp	Missense	1	Maternal	[10]	
c.772_773delAT	p.Ile258ProfsX61	Frameshift	1	Unknown	[14]	
c.785C > A	p.Ala262Asp	Missense	1	Unknown	[17]	
c.790G > C	p.Asp264His	Missense	1	De novo	[20]	
c.823T > A	p.Tyr275Asn	Missense	1	Maternal	This study	
c.824A > C	p.Tyr275Ser	Missense	1	Maternal	This study	
c.826T > C	p.Ser276Pro	Missense	1	De novo	[7]	
c.840C > G	p.Tyr280X	Missense	1	De novo	[14]	
c.859G > T	p.Glu287X	Nonsense	1	Paternal	[6]	
				De novo	[6]	

Table 3 (continued)

Mutation	Protein level	Mutation type	Exon	Inheritance	Ref	Protein
c.949C > T	p.Gln317X	Nonsense	1	Unknown	[14]	
c.958dupG	p.Asp320GlyfsX22	Frameshift	1	De novo	[12, 20]	
c.1019A > G	p.Asn340Ser	Missense	1	Unknown	This study	
				De novo	[6]	
				De novo	[6]	
				Maternal	[8, 20]	
				De novo	[8, 20]	
				Maternal[a]	[11]	
				De novo	[12]	
				De novo	[14]	
				De novo	[17]	
				De novo	[17]	
				Unknown	[17]	
				Unknown	[19]	
				Maternal	[20]	
				De novo	[20]	
				De novo	[20]	
				Maternal[a]	[21]	
c.1022A > G	p.Asp341Gly	Missense	1	De novo	This study	
c.1023C > G	p. Asp341Glu	Missense	1	De novo	[10]	
c.1026_1027delinsAA	p.Asn342_Pro343delinsLysThr	Frameshift	1	Unknown	[17]	
c.1031C > G	p.Pro344Arg	Missense	1	Unknown	[17]	
c.1031C > T	p.Pro344Leu	Missense	1	De novo	This study	
c.1036_1040dup	p.Asn347LysfsX23	Frameshift	1	Familial	[6]	
c.1091dupC	p.Tyr366LeufsX10	Frameshift	1	De novo	This study	
				Familial	[5]	
				Paternal	[14]	
				De novo	[17]	
				De novo	[20]	
				De novo	[20]	
				De novo	[20]	
c.1091delC	p.Pro364fs	Frameshift	1	De novo	This study	
c.1123G > T	p.Asp375Tyr	Missense	1	De novo	This study	
c.1129G > A	p.Asp377Asn	Missense	1	De novo	[19]	
c.1129G > C	p.Asp377His	Missense	1	De novo	[8, 20]	
c.1131C > A	p.Asp377Glu	Missense	1	De novo	[17]	
c.1143dupT	p.Gly382TrpfsX19	Frameshift	1	Unknown	[17]	
c.1183C > T	p.Arg395X	Nonsense	1	De novo	[20]	
c.1192G > T	p.Glu398X	Nonsense	1	Paternal	[8]	
c.1211C > T	p.Thr404Ile	Missense	1	De novo	[8, 20]	
c.1240G > C	p.Glu414Gln	Missense	1	Paternal	[8]	
c.1298T > C	p.Leu433Pro	Missense	1	De novo	[12, 20]	
c.1300_1301delCA	p.Gln434GlufsXII	Frameshift	1	De novo	[12, 20]	
c.1322T > A	p.Val441Glu	Missense	1	Familial	[4, 5]	
c.1464_1466del	p.Ser489del	Frameshift	1	De novo	[20]	
c.1521dupC	p.Ile508Hisfs15	Frameshift	1	De novo	[8, 20]	
c.1537G > C	p.Gly513Arg	Missense	1	De novo	[12, 20]	
c.1628T > C	p.Leu543Pro	Missense	1	Paternal	[6]	
c.1671C > G	p.Asn557Lys	Missense	1	Familial	[5, 7]	

Table 3 (continued)

Mutation	Protein level	Mutation type	Exon	Inheritance	Ref	Protein
c.1682C > G	p.Pro561Arg	Missense	1	Paternal	[10]	
c.1700C > T	p.Pro567Leu	Missense	1	Maternal	[10]	
				De novo	[20]	
c.1780G > C	p.Asp594His	Missense	1	De novo	This study	
c.1786G > C	p.Asp596His	Missense	1	Paternal	[20]	
c.1802G > A	p.Gly601Asp	Missense	1	Unknown	This study	
c.1804C > T	p.Arg602X	Nonsense	1	De novo	17	
				De novo	[20]	
c.1852G > A	p.Asp618Asn	Missense	1	Maternal	[10]	
c.1863dupT	p.Gly662fs	Frameshift	1	De novo	This study	
c.1924G > A	p.Val642Met	Missense	1	Unknown	[17]	
c.1955T > C	p.Leu652Pro	Missense	1	Paternal	[16]	
c.1956_1959delCTCT	p.Ser653ProfsX6	Frameshift	1	Unknown	[17]	
c.2012C > G	p.Ser671X	Nonsense	1	Familial	[4, 5]	
c.2019delC	p.Ser674LeufsX2	Frameshift	1	De novo	[10]	
c.2030_2031insT	p.Leu677fsX717	Frameshift	1	Familial	[4, 5]	
c.2341dupA	p.Ile781AsnfsX3	Frameshift	4	De novo	[20]	CP
c.2617-1G > A	p.?	Splicing	4	De novo	[8]	
c.2631_2634delTTTT	?	Frameshift	4	De novo	[9]	
c.2656C > T	p.Arg886X	Nonsense	4	Unknown	This study	
				Familial	[10]	
				Unknown	[17]	
c.2675+1G > C	p.?	Splicing	Intron 4	Unknown	[17]	
c.2675-6A > G	p.?	Splicing	intron 4	De novo	[8]	
c.2697dupA	p.Glu900Argfs8	Frameshift	5	De novo	[8, 20]	
c.2705dupA	p.Asp902Lysfs6	Frameshift	5	Maternal	[18]	
c.2903dupA	p.Asp968Glufs18	Frameshift	6	De novo	[8]	
Deletion of exons 1 to 3		Deletion		De novo	[10]	
Whole gene deletion		Deletion		Unknown	[10]	
				De novo	[10]	
				De novo	[15]	
				Unknown	[15]	

EC extracellular cadherin domains, *CP* cytoplasmic domain

[a] Mosaicism for the mutation demonstrated in the mother

seizures from childhood, often occurring in clusters. Cognitive impairment was very variable within the family and ranged from moderate ID to borderline intelligence.

PCDH19 mutations in males

Epilepsy has not been reported in male carriers of a *PCDH19* mutation, except for one male patient with clinical features of Dravet syndrome who proved to be mosaic for a *PCDH19* gene deletion [6]. One father who carried the mutation identified in his affected daughter had moderate ID without epilepsy. His mother and sister also had ID without epilepsy. They were not tested for the presence of the mutation [6]. Scheffer et al. observed that five obligate male carriers from four unrelated families with EFMR had obsessive, controlling, rigid, inflexible personalities [4].

Males with an autism spectrum disorder

Sequence analysis of *PCDH19* in the 20 males with ASD revealed one missense mutation: c.2359C > T in exon 3 in a 14-year-old boy diagnosed with Asperger syndrome at the age of 8.5 years. His family members were not affected by autism or epilepsy. This mutation results in the replacement of an arginine amino acid by a cysteine (p.Arg787Cys) in the cytoplasmic domain of the protein. The mutation affects a highly conserved amino acid with a Grantham score of the amino acid change of 180. A score higher than 100 indicates

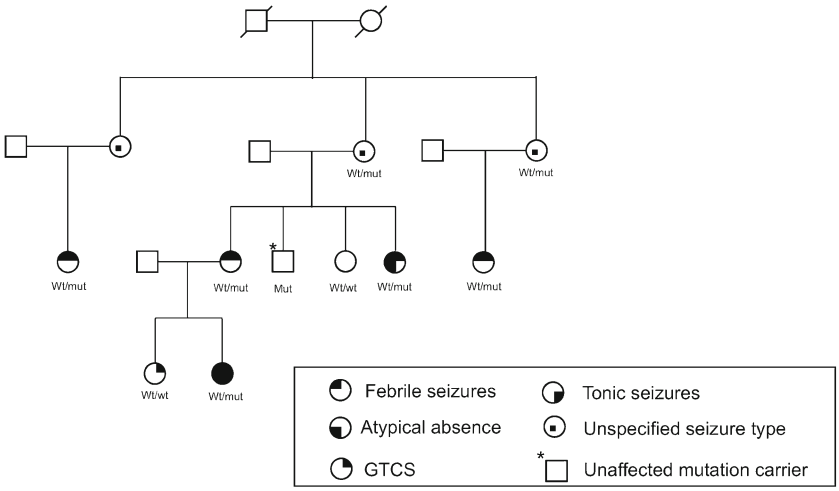

Fig. 1 Phenotypes and genotypes in a three generation family with EFMR

radical amino acid changes [22]. Two bioinformatic prediction programs show that the p.Arg787Cys is probably damaging (Polyphen2) and not tolerated (SIFT). The inheritance of the mutation is unknown.

Discussion

Clinical characteristics

We present an overview of phenotypes and the mutation spectrum in females with EFMR caused by *PCDH19* mutations. For this purpose, we included 13 case series [5, 7–10, 12–16, 18–21], two papers on families with a *PCDH19* mutation [4, 11], and we report 15 new cases with a *PCDH19* mutation. The criteria for inclusion of patients for analysis of the *PCDH19* gene varied: Some studies only included patients with *SCN1A*-negative Dravet syndrome [6, 14, 19], whereas others included females with various epilepsy syndromes including patients without ID [7, 12]. Our own patients contributed to both categories. The clinical picture associated with *PCDH19* mutations emerging from the individual case series reflects to some extent the inclusion criteria. In studies including mainly Dravet syndrome patients, onset was relatively early and seizure types other than generalized tonic–clonic seizures occurred more frequently compared with studies including patients without ID or familial cases. The combined data from these studies give an overall picture of the highly variable clinical characteristics associated with *PCDH19* gene mutations (Table 2). Most patients have febrile and afebrile, tonic–clonic seizures with onset in infancy. Focal seizures with or without impaired consciousness or status epilepticus occur in about one third of all patients. A very characteristic and discriminating feature is the occurrence of (febrile) seizures in clusters with seizure-free intervals that may last for months. Seizures remit in the majority of patients during teenage years. Interictal EEG is usually normal, and (peri-)ictal EEG shows variable abnormalities.

A subgroup of patients with a *PCDH19* mutation has a phenotype resembling Dravet syndrome. Most patients with Dravet syndrome carry a de novo mutation in the *SCN1A* gene [23] and have refractory seizures, (severe) ID and poor outcome [24–27]. *PCDH19*-positive patients differ from the classical *SCN1A*-positive Dravet patients though, first of all because prognosis seems more favourable. Seizure onset is somewhat later, seizures occur more in clusters and status epilepticus and myoclonic seizures occur less frequently. Moreover, in *SCN1A*-related Dravet syndrome, treatment with carbamazepine and lamotrigine is contraindicated as it may aggravate seizures [28, 29]. It remains to be seen if this also holds true for patients with Dravet syndrome features and a *PCDH19* mutation.

Mutation spectrum

Most mutations are found in the large exon 1, corresponding to the extracellular cadherin domains, which are pivotal for normal function. In exons 3 to 6, encoding the cytoplasmic domain, a relatively small number of only truncating mutations are found. Amino acid substitutions in the cytoplasmic domain appear to have less or no deleterious effects on protein function. The truncating mutations in the cytoplasmic domain may result in nonsense mediated decay of the mRNA and thereby cause loss of function of the whole protein. The extracellular domain being involved in all cases supports the view that adverse interaction between genetically different cell populations, expressing one or the other allelic protein, is the pivotal mechanism of disease [30].

Clinical genetic aspects

Both truncating and missense mutations are associated with variable phenotypes. Among non-related patients with the

same mutation and among affected relatives, there is variable expression, which may be explained by different patterns of X-inactivation in brain, the influence of genetic modifiers or mosaicism for the mutation in the brain of patients with de novo mutations. This variable expression makes it difficult to predict the clinical course for unborn or young children. From extended families, the penetrance in females appears to be high [4].

About 35 % of probands have an inherited mutation, with an inherent high recurrence risk for the probands' sisters and possibly also for other female relatives. Because of the unusual X-linked mode of inheritance with asymptomatic transmitting males, it is highly important to recognize the clinical picture of EFMR in probands and to screen the parents once a *PCDH19* mutation is found, also when they are asymptomatic. When providing genetic counselling, the possibility of gonadal mosaicism of the *PCDH19* mutation in one of the parents should be taken into account [11].

Males with a PCDH19 mutation

Males carrying *PCDH19* mutations are generally unaffected, although they seem to have a more rigid personality. Nevertheless, *PCDH19* mutations in the mosaic state may cause EFMR. In males with clinical characteristics highly suggestive of EFMR, analysis of *PCDH19* in different tissue samples to detect such a mosaicism might be considered.

In one out of 20 high-functioning males with ASD, we identified a missense variation in exon 3. The pathogenicity of this variation is unclear. On the one hand, the affected amino acid is highly conserved and has a high Grantham score. On the other hand, it affects the cytoplasmic domain of the protein, in which no pathogenic missense mutations have been found in females with EFMR. Previously, *PCDH19* mutations have been reported in a male with ASD [31], in a male with schizophrenia [31] and in two males with intellectual disability [32]. Larger cohorts of males with ASD, but also of males with a broader spectrum of psychiatric and neurological phenotypes, need to be tested to further assess the significance of *PCDH19* gene mutations in these disorders.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. Juberg RC, Hellman CD (1971) A new familial form of convulsive disorder and mental retardation limited to females. J Pediatr 79:726–732
2. Fabisiak K, Erickson RP (1990) A familial form of convulsive disorder with or without mental retardation limited to females: extension of a pedigree limits possible genetic mechanisms. Clin Genet 38:353–358
3. Ryan SG, Chance PF, Zou CH, Spinner NB, Golden JA, Smietana S (1997) Epilepsy and mental retardation limited to females: an X-linked dominant disorder with male sparing. Nat Genet 17:92–95
4. Scheffer IE, Turner SJ, Dibbens LM, Bayly MA, Friend K, Hodgson B, Burrows L, Shaw M, Wei C, Ullmann R, Ropers HH, Szepetowski P, Haan E, Mazarib A, Afawi Z, Neufeld MY, Andrews PI, Wallace G, Kivity S, Lev D, Lerman-Sagie T, Derry CP, Korczyn AD, Gecz J, Mulley JC, Berkovic SF (2008) Epilepsy and mental retardation limited to females: an under-recognized disorder. Brain 131:918–927
5. Dibbens LM, Tarpey PS, Hynes K, Bayly MA, Scheffer IE, Smith R, Bomar J, Sutton E, Vandeleur L, Shoubridge C, Edkins S, Turner SJ, Stevens C, O'Meara S, Tofts C, Barthorpe S, Buck G, Cole J, Halliday K, Jones D, Lee R, Madison M, Mironenko T, Varian J, West S, Widaa S, Wray P, Teague J, Dicks E, Butler A, Menzies A, Jenkinson A, Shepherd R, Gusella JF, Afawi Z, Mazarib A, Neufeld MY, Kivity S, Lev D, Lerman-Sagie T, Korczyn AD, Derry CP, Sutherland GR, Friend K, Shaw M, Corbett M, Kim HG, Geschwind DH, Thomas P, Haan E, Ryan S, McKee S, Berkovic SF, Futreal PA, Stratton MR, Mulley JC, Gécz J (2008) X-linked protocadherin 19 mutations cause female-limited epilepsy and cognitive impairment. Nat Genet 40:776–781
6. Depienne C, Bouteiller D, Keren B, Cheuret E, Poirier K, Trouillard O, Benyahia B, Quelin C, Carpentier W, Julia S, Afenjar A, Gautier A, Rivier F, Meyer S, Berquin P, Hélias M, Py I, Rivera S, Bahi-Buisson N, Gourfinkel-An I, Cazeneuve C, Ruberg M, Brice A, Nabbout R, Leguern E (2009) Sporadic infantile epileptic encephalopathy caused by mutations in PCDH19 resembles Dravet syndrome but mainly affects females. PLoS Genet 2:e1000381
7. Hynes K, Tarpey P, Dibbens LM, Bayly MA, Berkovic SF, Smith R, Raisi ZA, Turner SJ, Brown NJ, Desai TD, Haan E, Turner G, Christodoulou J, Leonard H, Gill D, Stratton MR, Gecz J, Scheffer IE (2010) Epilepsy and mental retardation limited to females with PCDH19 mutations can present de novo or in single generation families. J Med Genet 47:211–216
8. Marini C, Mei D, Parmeggiani L, Norci V, Calado E, Ferrari A, Moreira A, Pisano T, Specchio N, Vigevano F, Battaglia D, Guerrini R (2010) Protocadherin 19 mutations in girls with infantile-onset epilepsy. Neurology 75:646–653
9. Jamal SM, Basran RK, Newton S, Wang Z, Milunsky JM (2010) Novel de novo PCDH19 mutations in three unrelated females with epilepsy female restricted mental retardation syndrome. Am J Med Genet A 152A:2475–2481
10. Depienne C, Trouillard O, Bouteiller D, Gourfinkel-An I, Poirier K, Rivier F, Berquin P, Nabbout R, Chaigne D, Steschenko D, Gautier A, Hoffman-Zacharska D, Lannuzel A, Lackmy-Port-Lis M, Maurey H, Dusser A, Bru M, Gilbert-Dussardier B, Roubertie A, Kaminska A, Whalen S, Mignot C, Baulac S, Lesca G, Arzimanoglou A, LeGuern E (2011) Mutations and deletions in PCDH19 account for various familial or isolated epilepsies in females. Hum Mutat 32:E1959–E1975
11. Dibbens LM, Kneen R, Bayly MA, Heron SE, Arsov T, Damiano JA, Desai T, Gibbs J, McKenzie F, Mulley JC, Ronan A, Scheffer

IE (2011) Recurrence risk of epilepsy and mental retardation in females due to parental mosaicism of PCDH19 mutations. Neurology 76:1514–1519
12. Specchio N, Marini C, Terracciano A, Mei D, Trivisano M, Sicca F, Fusco L, Cusmai R, Darra F, Bernardina BD, Bertini E, Guerrini R, Vigevano F (2011) Spectrum of phenotypes in female patients with epilepsy due to protocadherin 19 mutations. Epilepsia 52:1251–1257
13. Specchio N, Fusco L, Vigevano F (2011) Acute-onset epilepsy triggered by fever mimicking FIRES (febrile infection-related epilepsy syndrome): the role of protocadherin 19 (PCDH19) gene mutation. Epilepsia 52:e172–e175
14. Higurashi N, Shi X, Yasumoto S, Oguni H, Sakauchi M, Itomi K, Miyamoto A, Shiraishi H, Kato T, Makita Y, Hirose S (2012) PCDH19 mutation in Japanese females with epilepsy. Epilepsy Res 99:28–37
15. Vincent AK, Noor A, Janson A, Minassian BA, Ayub M, Vincent JB, Morel CF (2011) Identification of genomic deletions spanning the PCDH19 gene in two unrelated girls with intellectual disability and seizures. Clin Genet 82:540–545
16. Camacho A, Simón R, Sanz R, Viñuela A, Martínez-Salio A, Mateos F (2012) Cognitive and behavioral profile in females with epilepsy with PCDH19 mutation: two novel mutations and review of the literature. Epilepsy Behav 24:134–137
17. Depienne C, Leguern E (2012) PCDH19-related infantile epileptic encephalopathy: an unusual X-linked inheritance disorder. Hum Mutat 33:627–634
18. Dimova PS, Kirov A, Todorova A, Todorov T, Mitev V (2012) A novel PCDH19 mutation inherited from an unaffected mother. Pediatr Neurol 46:397–400
19. Kwong AK, Fung CW, Chan SY, Wong VC (2012) Identification of SCN1A and PCDH19 mutations in Chinese children with Dravet syndrome. PloS One 7:e41802. doi:10.1371/journal.pone.0041802
20. Marini C, Darra F, Specchio N, Mei D, Terracciano A, Parmeggiani L, Ferrari A, Sicca F, Mastrangelo M, Spaccini L, Canopoli ML, Cesaroni E, Zamponi N, Caffi L, Ricciardelli P, Grosso S, Pisano T, Canevini MP, Granata T, Accorsi P, Battaglia D, Cusmai R, Vigevano F, Bernardina BD, Guerrini R (2012) Focal seizures with affective symptoms are a major feature of PCDH10 gene-related epilepsy. Epilepsia 53:2111–2119. doi:10.1111/j.1528-1167.2012.03649.x
21. Terracciano A, Specchio N, Darra F, Sferra A, Bernardina BD, Vigevano F, Bertini E (2012) Somatic mosaicism of PCDH10 mutation in a family with low-penetrance EFMR. Neurogenetics 13:341–345
22. Grantham R (1974) Amino acid difference formula to help explain protein evolution. Science 185:862–864
23. Claes L, Ceulemans B, Audenaert D, Smets K, Lofgren A, Del-Favero J, Ala-Mello S, Basel-Vanagaite L, Plecko B, Raskin S, Thiry P, Wolf NI, Van Broeckhoven C, De Jonghe P (2003) De novo SCN1A mutations are a major cause of severe myoclonic epilepsy of infancy. Hum Mutat 21:615–621
24. Wolff M, Casse-Perrot C, Dravet C (2006) Severe myoclonic epilepsy of infants (Dravet syndrome): natural history and neuropsychological findings. Epilepsia 47(Suppl 2):45–48
25. Genton P, Velizarova R, Dravet C (2011) Dravet syndrome: the long-term outcome. Epilepsia 52(Suppl 2):44–49
26. Catarino CB, Liu JY, Liagkouras I, Gibbons VS, Labrum RW, Ellis R, Woodward C, Davis MB, Smith SJ, Cross JH, Appleton RE, Yendle SC, McMahon JM, Bellows ST, Jacques TS, Zuberi SM, Koepp MJ, Martinian L, Scheffer IE, Thom M, Sisodiya SM (2011) Dravet syndrome as epileptic encephalopathy: evidence from long-term course and neuropathology. Brain 134:2982–3010
27. Petrelli C, Passamonti C, Cesaroni E, Mei D, Guerrini R, Zamponi N, Provinciali L (2012) Early clinical features in Dravet syndrome patients with and without SCN1A mutations. Epilepsy Res 99:21–27
28. Wakai S, Ito N, Sueoka H, Kawamoto Y, Hayasaka H, Chiba S (1996) Severe myoclonic epilepsy in infancy and carbamazepine. Eur J Pediatr 155:724
29. Guerrini R, Dravet C, Genton P, Belmonte A, Kaminska A, Dulac O (1998) Lamotrigine and seizure aggravation in severe myoclonic epilepsy. Epilepsia 39:508–512
30. Lindhout D (2008) Somatic mosaicism as a basic epileptogenic mechanism? Brain 131:900–901
31. Piton A, Gauthier J, Hamdan FF, Lafrenière RG, Yang Y, Henrion E, Laurent S, Noreau A, Thibodeau P, Karemera L, Spiegelman D, Kuku F, Duguay J, Destroismaisons L, Jolivet P, Côté M, Lachapelle K, Diallo O, Raymond A, Marineau C, Champagne N, Xiong L, Gaspar C, Rivière JB, Tarabeux J, Cossette P, Krebs MO, Rapoport JL, Addington A, Delisi LE, Mottron L, Joober R, Fombonne E, Drapeau P, Rouleau GA (2011) Systematic resequencing of X-chromosome synaptic genes in autism spectrum disorder and schizophrenia. Mol Psychiatry 16:867–880
32. Tarpey PS, Smith R, Pleasance E, Whibley A, Edkins S, Hardy C, O'Meara S, Latimer C, Dicks E, Menzies A, Stephens P, Blow M, Greenman C, Xue Y, Tyler-Smith C, Thompson D, Gray K, Andrews J, Barthorpe S, Buck G, Cole J, Dunmore R, Jones D, Maddison M, Mironenko T, Turner R, Turrell K, Varian J, West S, Widaa S, Wray P, Teague J, Butler A, Jenkinson A, Jia M, Richardson D, Shepherd R, Wooster R, Tejada MI, Martinez F, Carvill G, Goliath R, de Brouwer AP, van Bokhoven H, Van Esch H, Chelly J, Raynaud M, Ropers HH, Abidi FE, Srivastava AK, Cox J, Luo Y, Mallya U, Moon J, Parnau J, Mohammed S, Tolmie JL, Shoubridge C, Corbett M, Gardner A, Haan E, Rujirabanjerd S, Shaw M, Vandeleur L, Fullston T, Easton DF, Boyle J, Partington M, Hackett A, Field M, Skinner C, Stevenson RE, Bobrow M, Turner G, Schwartz CE, Gecz J, Raymond FL, Futreal PA, Stratton MR (2009) A systematic, large-scale resequencing screen of X-chromosome coding exons in mental retardation. Nat Genet 41:535–543