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FULL-LENGTH ORIGINAL RESEARCH

Focal seizures with affective symptoms are a major feature of *PCDH19* gene–related epilepsy

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Summary

Purpose: **Mutations of the protocadherin19 gene (*PCDH19*) cause a female-related epilepsy of variable severity, with or without mental retardation and autistic features. Despite the increasing number of patients and mutations reported, the epilepsy phenotype associated with *PCDH19* mutations is still unclear. We analyzed seizure semiology through ictal video–electroencephalography (EEG) recordings in a large series of patients.**

Methods: **We studied 35 patients with *PCDH19* gene–related epilepsy and analyzed clinical history and ictal video-EEG recordings obtained in 34 of them.**

Key Findings: **Clusters of focal febrile and afebrile seizures had occurred in 34 patients, at a mean age of 10 months. The predominant and more consistent ictal sign was fearful screaming, occurring in 24 patients (70.5%); it was present since epilepsy onset in 12 and appeared later on, during the course in the remaining 12 patients. In infancy, fearful screaming mainly appeared within the context of seizures with prominent hypomotor semiology, whereas during follow-up it was associated with prominent early motor manifestations. In 16 patients, seizures were video-EEG recorded both at onset and during follow-up: in five patients (31%) seizure semiology remained identical, in 7 (44%) semiology varied and in four patients it was unclear whether ictal semiology changed with age. Three patients (9%) had both focal and generalized seizures, the latter consisting of absences and myoclonus. Ictal EEG during focal seizures showed a prominent involvement of the frontotemporal regions (22 patients). About 45% of patients had an alternating EEG pattern, with the ictal discharge migrating from one hemisphere to the contralateral during the same ictal event. Status epilepticus occurred in 30% of patients. Cognitive impairment occurred in 70%, ranging from mild (42%) to moderate (54%) and severe (4%); autistic features occurred in 28.5%. Direct sequencing detected 33 different heterozygous candidate mutations, 8 of which were novel. Mutations were missense substitutions (48.5%), premature termination (10 frameshift, 4 nonsense, and 2 splice-site mutations; 48.5%), and one in-frame deletion. Thirty candidate mutations (91%) were de novo. No specific genotype–phenotype correlation could be established, as missense and truncating mutations were associated with phenotypes of comparable severity.**

Significance: **Most patients with *PCDH19* mutations exhibit a distinctive electroclinical pattern of focal seizures with affective symptoms, suggesting an epileptogenic dysfunction involving the frontotemporal limbic system. Awareness of this distinctive phenotype will likely enhance recognition of this disorder.**

KEY WORDS: **Focal seizures, Focal epilepsy, Affective ictal semiology, Genetic epilepsy, PCDH19.**

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Mutations of the X-linked gene protocadherin19 (*PCDH19*) cause epilepsy of variable severity, with or without mental retardation and autistic features (Dibbens et al., 2008; Scheffer et al., 2008). The disorder is either sporadic or familial, in which case the *PCDH19* mutation may be transmitted through unaffected fathers or an affected mother (Marini et al., 2010). Familial cases were originally defined as ''epilepsy and mental retardation limited to females'' (EFMR) (Scheffer et al., 2008). Most patients have normal or borderline cognitive skills before seizure onset, yet cognitive impairment predating seizure onset has been reported (Scheffer et al., 2008). Some of the characteristics of epilepsy in these patients have been elucidated. In most patients, seizures begin around 12 months of age, commonly in clusters, often during a febrile illness (Marini et al., 2010), as focal or generalized, including tonic–clonic, absence, and myoclonic seizures. Affected patients at the most severe end of the phenotypic spectrum may exhibit features resembling those of Dravet syndrome (Depienne et al., 2009).

Diagnostic screening of *PCDH19* has revealed that mutations of this gene are more frequent than previously appreciated. Although formal epidemiologic studies are not available, *PCDH19* screening of large cohorts of girls with epilepsy has yielded a rate of approximately 10% of mutation-positive patients (Marini et al., 2010; Depienne et al., 2011). This percentage is likely oversized as, based on insights drawn from previous reports, the above cohorts were biased toward patients exhibiting fever-related seizure clusters and therefore more likely to carry mutations.

In previous studies, we had observed that patients carrying *PCDH19* mutations often manifest clusters of infantile-onset seizures (Marini et al., 2010; Specchio et al., 2011) whose characteristics are somewhat different from those observed in Dravet syndrome. Herein we present the results of a new study aiming at further screening of patients with epilepsy phenotypes suggestive of *PCDH19* mutations and at reevaluating seizure semiology and ictal electroencephalography (EEG) patterns of new and previously published patients. We analyzed clinical, ictal, and interictal video-EEG recordings and genetic data obtained from 34 patients in whom seizures had been recorded.

We identified in most patients a distinctive electroclinical pattern of focal seizures with prominent affective symptoms, suggesting an epileptogenic dysfunction involving the limbic system. Awareness of this distinctive phenotype will likely enhance recognition of this disorder.

Methods

We studied 35 girls and women with mutations or candidate mutations of the *PCDH19* gene. These patients represented all mutation-positive subjects who had been screened in two neurogenetics laboratories. All patients were ascertained from national neurology and epilepsy centers and had been referred for mutation screening because of epilepsy of possible genetic origin. All treating specialists participated in a meeting devoted to reviewing clinical and EEG data. Clinical data included seizure semiology at onset and throughout follow-up, with particular attention to evolution of seizure semiology through age. Ictal and interictal EEGs were obtained for 34 of the 35 patients. To properly classify seizures, we matched seizure semiology, as observed during ictal video-EEG recordings, with descriptions of seizures as initially obtained by clinical questionnaires and interviews of parents or other eyewitnesses. For all patients we also collected data on cognitive and psychomotor development, including psychiatric symptoms and autistic features, brain magnetic resonance imaging (MRI), and neurologic examination. Informed consent for genetic analysis and for video-EEG studies was obtained for all patients in each participating institution from parents or legal guardians. The study presented here is part of a clinical and genetic research project approved by the review board of the Meyer Children's Hospital. Our cohort includes 17 previously reported patients (Marini et al., 2010; Specchio et al., 2011) in whom we further defined seizure semiology by retrieving and reviewing available ictal video-EEG recordings.

Mutation analysis

All patients included in this study carried a *PCDH19* mutation. Genomic DNA was extracted from peripheral blood leukocytes using an automated DNA isolation robot (QIASymphony; Qiagen, Hilden, Germany). The six exons covering the coding regions of *PCDH19* (Entrez Gene, GeneID: 57526, Accession Number: EF676096.1) and their respective intron–exon boundaries were amplified by polymerase chain reaction (PCR), sequenced using BigDye Terminator v.1.1 (Applied Biosystems, Foster City, CA, U.S.A.) and analyzed on a 3130XL DNA sequencer (Applied Biosystems). The first exon was amplified as a fragment of 2.3 kb and cycle sequenced using internal primers. Primer sequences and PCR/sequencing conditions are available on request. The identified *PCDH19* alterations were not found in a control population of 190 ethnically matched subjects.

Bioinformatics analyses of *PCDH19* mutations

We used the Kaviar (http://db.systemsbiology.net/kaviar/cgi-pub/Kaviar.pl) Web tool and the Exome Variant Server (http://evs.gs.washington.edu/EVS/) to ascertain that the *PCDH19* variants identified in this cohort were not present in variants databases. We assessed amino acid conservation in orthologs using the Consurf Server (http://consurf.tau.ac.il/). Because the PCDH19 protein structure is not available, we used the PCDH19 amino acid sequence as input. We classified the conservation score as follow: variable (1–3), average (4–6), and conserved (7–9). For missense mutations, in order to classify an amino acid substitution as disease-associated or neutral we used MutPred (http://mutpred.mutdb.org/) and Polyphen 2

(http://genetics.bwh.harvard.edu/pph2/) as prediction tools. For Polyphen 2 analysis we chose the HumVar-trained PolyPhen 2 that is best suitable for the diagnostic of Mendelian diseases (Adzhubei et al., 2010).

Twin zygosity determination

To establish twin zygosity, we used the AmpFlSTR Identifiler kit (Life Tech, Carlsbad, CA, U.S.A.), which analyzed 15 loci plus the amelogenin for gender determination. The multiplex PCR products were run on a 3130XL genetic analyzer. The fragment analysis runs obtained for each individual of the twin pair, were compared to establish if the twins were dizygotic or monozygotic.

Results

The 35 patients had a mean age of 11 years at the time of the study (median 10, range 2–36). Clusters of focal febrile and afebrile seizures had occurred in 34 of the 35 patients, with a mean age at seizure onset of 10 months (median 8, range 1–38). In 31 of the 34 patients, a second cluster of seizures occurred at a mean age of 16 months (median 14; range 4–48). For most patients there was thus a 6-month "honeymoon" period from seizure onset to subsequent recurrent clusters of seizures. Mean follow-up was 10 years (median 9.5; range 1–35 years). Epilepsy and EEG information of the whole cohort, including electroclinical seizure recordings, is summarized in Table S1A,B. Clinical and genetic data of each patient are listed in Table 1.

Epilepsy

Seizures were recorded in 34 patients, at different ages and times with respect to epilepsy onset: at onset in 4 patients, at follow-up in 14, both at onset and during follow-up in 16. During the course of their epilepsy, 4 patients had a single seizure recorded on video-EEG, whereas in each of the remaining 30 patients several seizures were captured. Ictal semiology was analyzed according to whether seizures had been recorded at epilepsy onset or during follow-up.

Seizure semiology at onset

Clinical description of seizures was obtained by questioning the parents and other eyewitnesses (15 patients), and was corroborated by analysis of ictal video-EEG studies in 14 patients and of ictal EEG reports in 6. According to the initial ictal manifestations we identified four major seizure patterns:

1 Staring and psychomotor arrest (hypomotor seizures) observed in 16 patients (46%), followed in order of decreasing frequency by stiffening (11 patients), cyanosis (4 patients), eye and head deviation (4 patients) oral automatisms (4 patients), fearful screaming (3 patients), and secondary generalization (3 patients).

2 Bilateral, either symmetrical or asymmetrical, or focal clonic jerking, observed in 13 patients (37%), followed by diffuse stiffening (7 patients), fearful screaming (6 patients), facial flushing, eye and head deviation, cyanosis and secondary generalization, observed in one patient each.

3 Whole body stiffening observed in four patients (11%) with subsequent head deviation (two patients), fearful screaming (two patients), and cyanosis (two patients).

4 Clonic jerking or hypomotor seizures co-occurred in the same cluster in two patients (6%), with fearful screaming in one.

Overall, fearful screaming was present in 12 patients (34%) at seizure onset. Seizures occurred in clusters in 33 (94%) of the 35 patients and were triggered by fever in 23 (66%). In seven patients, the presence of an increased body temperature was uncertain and five were afebrile at seizure onset.

Seizure semiology during follow-up

Data on seizure semiology was obtained through analysis of ictal episodes captured by video-EEG in 30 patients and by questioning the parents or other eyewitnesses in the remaining five patients:

1 Twenty-one patients (60%) exhibited an early motor component with focal clonic jerking of one arm or leg, or rarely, with hemiclonic distribution, accompanied by fearful screaming (14 patients), deviation of the head and eyes (18 patients), diffuse stiffening (11 patients) or dystonic posturing (7 patients), oral automatisms (3 patients), cyanosis (3 patients), visual/olfactory illusions (one patient). Three of these patients exhibited ambulatory automatisms and psychomotor confusion, and two had postictal transitory hemiparesis.

2 Eight patients (23%) had hypomotor seizure onset, followed by fearful screaming (four patients), stiffening (four patients), eye and head deviation (four patients), oral automatisms (two patients), vomiting (two patients), cyanosis (two patients), and dystonic posturing (one patient).

3 Six patients (17%) had both hypomotor seizures and attacks with an early motor component within the same cluster, including fearful screaming in four patients.

Fearful screaming was therefore present in 22 patients (63%) at follow-up.

Seizure evolution: from onset to follow-up

Overall, the predominant and more consistent ictal sign was fearful screaming, occurring in 24 (70.5%) of the 34 patients whose seizures had been recorded. A similar semiology was also described by the parents of the only patient in whom seizure semiology was inferred through the recollection of eyewitness. Therefore, fearful screaming occurred in a total of 25 patients (71%) (Table 1); it was noticed since epilepsy onset in 13 and appeared later on during the course in 12 additional patients, at a mean of age of 6 years (median 4.5, range 2–17).

Table 1. Summary of the clinical and genetic data of the 35 patients with *PCDH19* mutations

ID	Age (years)	sz onset (months)	sz type/s	Affective semiology	Current AEDs	*PCDH19* mutation cDNA/protein	Inheritance
1	9	16	Focal sz – motor semiology	Yes	GVG, OxCZ, LTG	c.83C>A p.Ser28*	De novo
2	7.5	5	Focal sz – motor semiology	Yes	LEV, LTG	c.1464_1466del p.Ser489del	De novo
3	5.7	10.5	Focal sz – motor or hypomotor semiology	Yes	VPA, PB, LEV	c.1019A>G p.Asn340Ser	De novo
4	7.5	17	Focal sz – hypomotor semiology	No	VPA, TPM	c.1091dupC p.Tyr366Leu fs*10	De novo
5	20	8	Focal sz – hypomotor semiology	Yes	VPA, LCM	c.83C>A p.Ser28*	De novo
6	10.6	19	Focal sz – motor or hypomotor semiology	Yes	LEV	c.2903dupA p.Asp968Glufs*18	De novo
7	8	6	Focal sz – motor or hypomotor semiology + Abs + My	No	VPA, LEV	c.695A>G p.Asn232Ser	De novo
8	18	12	Focal sz – motor semiology + Abs	No	CZP, PB, ESM	c.1804C>T p.Arg602*	De novo
9	10	7	Focal sz – motor semiology	Yes	VPA	c.1211C>T p.Thr404Ile	De novo
10	11	4.5	Focal sz – motor semiology	Yes	LTG, CLB, PHT	c.1521dupC p.Ile508Hisfs*15	De novo
11[a]	12.9	14	Focal sz – motor semiology + Abs + My	Yes	VPA, TPM, CLB	c.1300_1301delCAp.Gln434Glufs*11	De novo
12[a]	12.9	24	Focal sz – hypomotor semiology	No	No AEDs	c.1300_1301delCA p.Gln434Glufs*11	De novo
13	2.2	7	Focal sz – hypomotor semiology	Yes	CBZ, CLP	c.2697dupA p.Glu900Argfs*8	De novo
14	16.2	6	Focal sz – hypomotor semiology	No	CLP, LEV	c.1019A>G p.Asn340Ser	Mother[b]
15	10.4	8	Focal sz – motor semiology	Yes	CBZ, LTG	c.1129G>C p.Asp377His	De novo
16	9.4	7	Focal sz – motor semiology	Yes	VPA, CLB	c.2676-6A>G?	De novo
17[c]	11.6	6	Focal sz – motor or hypomotor semiology	Yes	LCM, CLB	c.242T>G p.Leu81Arg	De novo
18[c]	11.6	6	Focal sz – motor or hypomotor semiology	Yes	LCM, CLB	c.242T>G p.Leu81Arg	De novo
19	12.6	7.5	Focal sz – motor or hypomotor semiology	Yes	CBZ	c.608A>C/c.617T>G p.His203Pro/p.Phe206Cys	De novo
20	11	10	Focal sz – motor semiology	Yes	CZP, VPA, LEV	c.1019A>G p.Asn340Ser	De novo
21	28	17	Focal sz – motor semiology	No	TPM, PGB, CZP	c.1786G>C p.Asp596His	Father
22	36	10	Focal sz – hypomotor semiology	Yes	VPA	c.706C>T p.Pro236Ser	De novo
23	8.6	38	Focal sz – motor semiology	Yes	CBZ, VPA	c.958dupG p.Asp320Glyfs*22	De novo
24	4	11	Focal sz – motor or hypomotor semiology	No	VPA, CBZ	c.2617-1G>A?	De novo
25	2.3	6	Focal sz – motor or hypomotor semiology	Yes	PB, CBZ	c.1091dupC p.Tyr366Leufs*10	De novo
26	1.7	13	Focal sz – hypomotor semiology	Yes	VPA, LEV, NZP	c.2341dupA p.Ile781Asnfs*3	De novo
27	16	3	Focal sz – hypomotor semiology	Yes	LEV, CBZ	c.1700C>T p.Pro567Leu	De novo
28	18.6	7	Focal sz – hypomotor semiology	Yes	LEV, PB, CLB	c.1298T>C p.Leu433Pro	De novo
29	11	8	Focal sz – motor semiology	No	VPA, LEV, NZP	c.1183C>T p.Arg395*	De novo
30	8.9	2.5	Focal sz – motor semiology	Yes	CBZ,TPM,PB,NZP	c.1091dupC p.Tyr366Leufs*10	De novo
31	19	9	Focal sz – motor semiology	Yes	TPM, CLB	c.790G>C p.Asp264His	De novo
32	9.9	5	Focal sz – hypomotor semiology	Yes	PB, LEV, DZP	c.1019A>G p.Asn340Ser	De novo
33	1.1	1	Focal sz – motor semiology	No	VPA, CLB	c.152dupTp. Ala52Argfs*37	De novo
34	8.2	12	Focal sz – motor semiology	Yes	TPM, CLB	c.1019A>G p.Asn340Ser	Mother
35	2	10	Focal sz – motor semiology	No	PB, LEV, CLB	c.1537G>C p.Gly513Arg	De novo

Abs, absences; AEDs, antiepileptic drugs; CBZ, carbamazepine; CLB, clobazam; CLP, clonazepam; ESM, ethosuximide; Focal sz, focal seizure; DZP, diazepam; GVG, vigabatrin; LCM, lacosamide; LEV, levetiracetam; LTG, lamotrigine; My, myoclonic; NZP, nitrazepam; OxCZ, oxcarbazepine; PB, phenobarbital; PHT, phenytoin; sz, seizure; TPM, topiramate; VPA, valproic acid.

[a] 1st twin pair.

[b] Affected mother.

[c] 2nd twin pair.

In 16 patients, seizures were video-EEG recorded both at onset and during follow-up. Five of them (31%) exhibited an unchanged semiology, 7 (44%) had a different semiology, and in 4 patients ictal recordings were not sufficiently clear to establish whether changes in seizure semiology had occurred with age. Three patients (9%) had both focal and generalized seizures, the latter consisting of absences and myoclonus. Accordingly, their EEG studies showed both focal and generalized paroxysmal activity, with photosensitivity in one. Clusters of seizures had occasionally evolved into convulsive status epilepticus in 10 of the 35 patients (28.5%).

Seizures had recurred in clusters in 34 of the 35 patients. At the time of the study, 25 patients (71%) were still exhibiting clusters of seizures, the frequency of which ranged from

weekly (one patient), to monthly (16 patients), and yearly (eight patients). The remaining 10 patients (28.5) had been seizure free for 1–5 years. Patients with persistent seizures had been treated with multiple antiepileptic drugs (AEDs) and no specific drug or combination of drugs appeared to have been more effective than others. At last follow up, 34 patients, including those who had been seizure free for years, were on combination therapy with a mean of two AEDs (median 2, range 1–4) (see Table 1 for specific AEDs in individual patients). Oral, rectal, or intravenous benzodiazepines, including clonazepam, lorazepam, and midazolam had been successful in arresting seizure clusters in most but not all patients.

EEG features

Ictal EEG recordings at epilepsy onset were available for 22 patients. A focal origin was apparent in 17 patients: frontotemporal in 11, temporoparietal in 2, temporoparietooccipital in 3, and in the midline frontocentral region in one (Table S1B) (Figs. 1 and 2). The ictal discharge was consistently lateralized, either right or left, in 7 patients (32%), whereas in 10 (45%) it migrated from one side to the contralateral and vice versa during the same seizure. In five patients (23%) the ictal discharge was bilateral from its onset, with topographically undetermined origin. In all patients, the interictal EEG at epilepsy onset showed normal background activity without epileptiform discharges.

During the follow-up, ictal EEG recordings were available in 30 patients. In 20 (67%) of them seizures could be lateralized. Of these, 11 had seizures arising from one frontotemporal region, 6 from one temporoparietal region, and 3 from one occipital region (Table S1B). Ictal onset was in the right hemisphere in 14 patients (70%) and in the left in 6 (30%). For the remaining 10 patients (33%), seizure

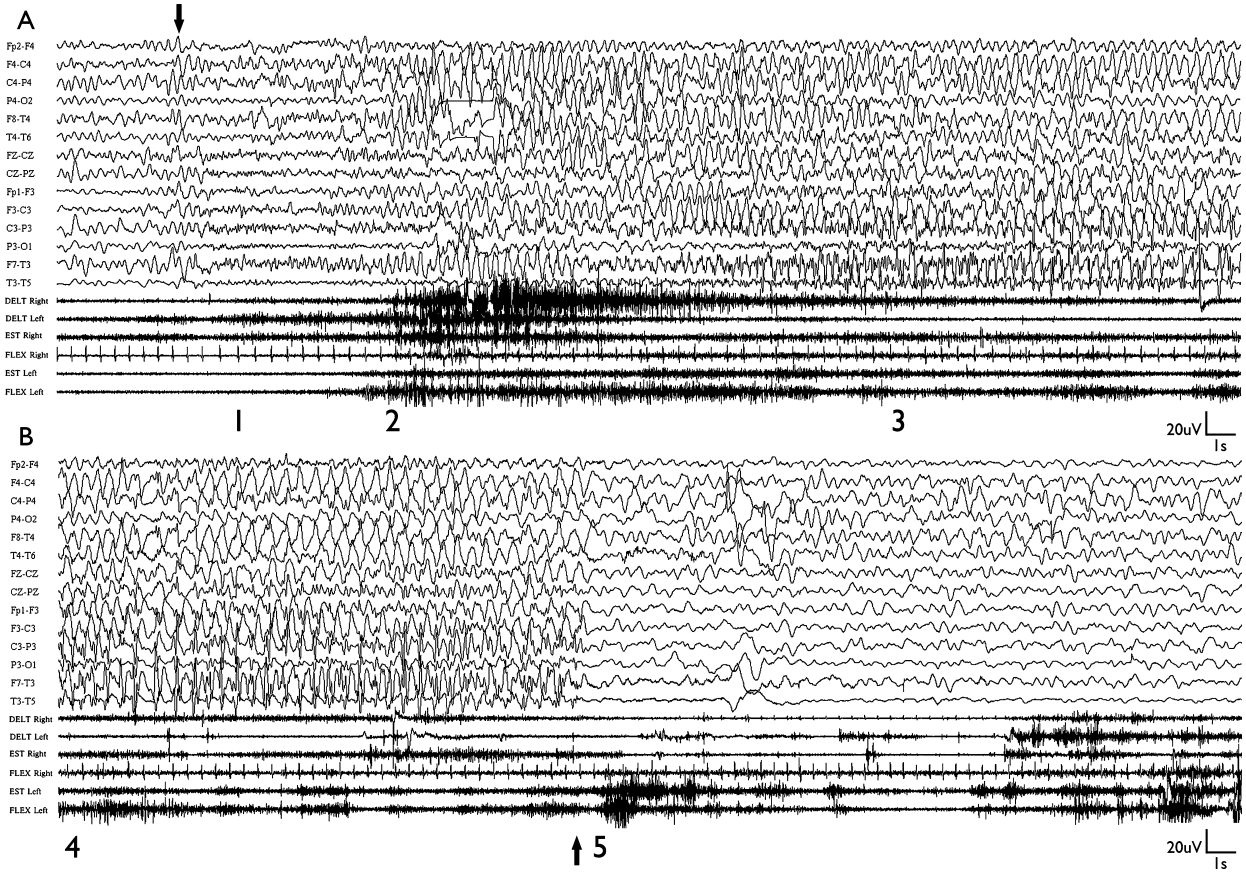

Figure 1.

Polygraphic recording including EEG and electromyography (EMG) channels of a focal seizure recorded at 1 year and 2 months of age. (**A**) Seizure onset (downward pointing arrow) with a left centroparietal and vertex ictal discharge followed, after about 10 s, by bilateral spread of slow waves, whereas rhythmic ictal discharge persists on the left temporoparietal leads. Seizure semiology and temporal correlation with the discharge is indicated by the following numbers: (1) eyes opening and deviation to the right, fearful screaming; (2) tonic vibratory phase involving the whole body; and (3) facial flushing;. (**B**) end of seizure with sudden termination of the ictal discharge (upward pointing arrow), followed by high-amplitude sharply contoured activity on the right hemisphere: (4) eyes deviation stops; (5) starts crying (DELT, deltoid; EST, wrist extensor; FLEX, wrist flexor).

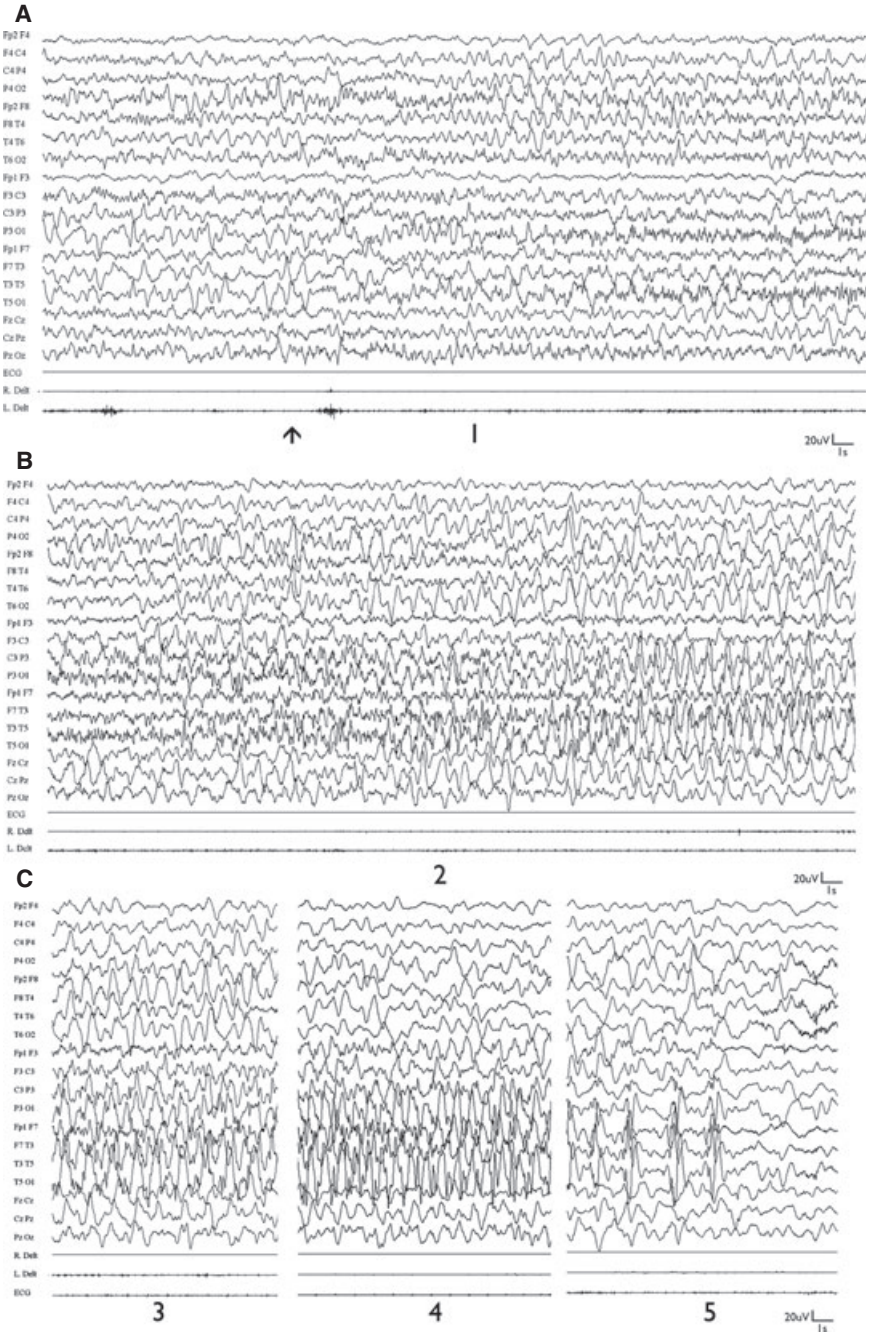

Figure 2.
Polygraphic recording including EEG and EMG channels of a focal seizure arising during sleep, recorded at age 10 months. (**A**) Seizure onset (arrow) with a bilateral ictal theta activity over both centro-parietotemporal regions, followed by (**B**) high-amplitude slow waves on the right, while a rhythmic ictal discharge persists on the left. (**C**) Twelve seconds and 23 s later (first and second panels), the ictal discharge continues on the left frontotemporal and the vertex regions, and 10 s later (third panel) there is sudden termination of the ictal discharge. Seizure semiology and temporal correlation with the discharge is indicated by the numbers: (1) eyes opening and staring; (2) psychomotor arrest; (3) minor left arm and hand movements while (4) the right side is hemiplegic; (5) starts crying and moves.
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onset could be neither localized nor lateralized. Interictal EEG studies performed during follow-up showed slow background activity in six patients and bilateral frontocentral paroxysmal activity in five. Five patients had generalized spike-wave discharges, with photosensitivity in two; multifocal paroxysmal activity was seen in five with photosensitivity in one and focal (left frontotemporal and right temporooccipital) in two. In 17 patients epileptiform EEG abnormalities were never observed.

The panel of expert epileptologists who reviewed clinical and ictal EEG data interpreted the electroclinical pattern as suggestive of an early involvement of the frontotemporal limbic structures in 74% of patients.

Cognitive and neuropsychiatric features

Before seizure onset, development was reported to be normal in 30 patients, at the borderline level in 2, and mildly delayed in 3. During follow-up, 11 patients had maintained normal cognitive abilities, whereas 24 (68.5%) were cognitively impaired: 10 (42%) exhibited mild impairment, 13 (54%) had moderate cognitive deficits, and 1 (4%) exhibited severe cognitive impairment associated with autistic

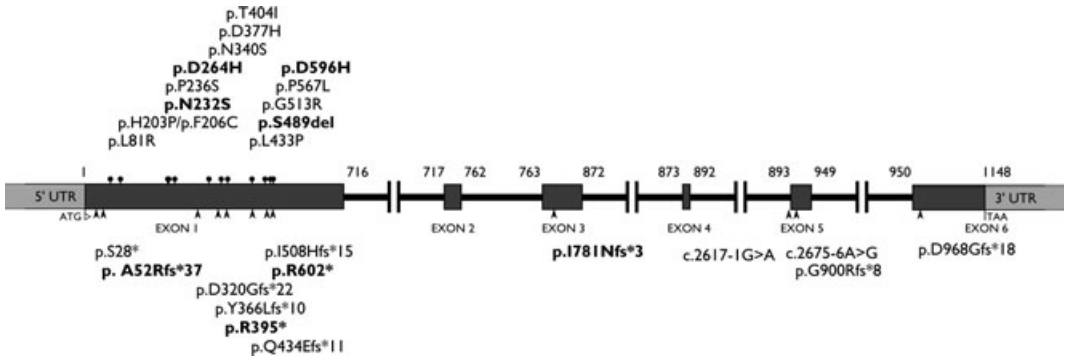

Figure 3.
Graphic representation of the PCDH19 protein showing the distribution of the 33 mutations (two were recurrent) identified in this cohort.
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features (Table S1B). In 20 patients, follow-up information was sufficiently accurate to establish that cognitive impairment had become apparent at a mean age of 2.6 years (median 2 years; range 1–7 years). Autistic features were present in 11 patients (31.5%) and other psychiatric manifestations in 1 (3%). Peculiar behavioral changes including reduced language communication, confusion, and aggressive behavior were reported between seizures of a cluster in four patients. Neurologic examination and brain MRI were normal in all patients.

PCDH19 mutations

Direct sequencing detected 33 different heterozygous mutations, 8 of which were novel (Table 1 and Fig. 3). Two mutations were identified in two twin pairs. Missense substitutions were observed in 16 patients (48.5%), and mutations leading to premature termination codon in 16 (48.5%) (10 frameshift, 4 nonsense, and 2 splice-site mutations). One additional mutation was an in-frame deletion (3%). Candidate mutations were not present in our control population of 190 ethnically matched subjects and were not reported as polymorphisms in public available databases according to the Kaviar Web tool and the Exome Variant Server. According to Consurf prediction, most missense or in-frame mutations involved a conserved amino acid of the PCDH19 protein (16 of 17; 94%) (see Table S1C). MutPred and Polyphen 2 predictions were concordant in indicating a pathogenic role for the identified missense substitutions (see Table S1C).

Missense mutations were located throughout the extracellular PCDH19 region. No missense substitutions were observed in the cytoplasmic region. Genetic analysis of the parents of all probands showed that 30 mutations (91%) were de novo. The two twin pairs were monozygotic, and the mutations arose de novo in both. Only three mutations (3 of 33; 9%) were inherited: two from the mother, one of whom had had seizures in childhood, whereas the other mother was unaffected; the third familial mutation was inherited from the unaffected father.

Thirteen patients (including a twin pair) (37%) harbored five different recurrent mutations (Table 1). These mutations were found in different unrelated patients of our cohort or were previously published by other groups (Table 1). Most recurrent mutations were de novo, indicating that they are repetitively generated through specific mechanisms.

Discussion

In most patients in this study, epilepsy history followed a recognizable pattern with occurrence of a first cluster of focal seizures precipitated by fever, around age 10 months, followed by a second cluster after a seizure-free period of about 6 months. Only a few patients had experienced isolated seizures or had later seizure onset, up to age 36 months. Clusters of focal febrile or afebrile seizures continued through age with monthly to yearly frequency, yet with an overall tendency to become less frequent over time. A few patients still had recurrent clusters of seizures evolving into status epilepticus in childhood or early adolescence, and about one third had become seizure free.

Using video-EEG monitoring, we captured seizures in 34 patients and reviewed them to detail their semiology and define their presumed area of origin. Because patients exhibiting seizures in clusters are often taken to hospital and video-EEG recorded, a large number of ictal recordings were available for the purpose of this study. Epilepsy phenotypes associated with mutations of specific genes have often been described, with limited access to direct video-EEG evidence, with unavoidable misinterpretation of seizure semiology and epilepsy types. Video-EEG monitoring, instead, provides invaluable details compared to information collected by simply interviewing eyewitness or using questionnaires, and helps to delineate whether *PCDH19*-gene–related epilepsy exhibits any specific anatomo-electroclinical pattern.

Overall, the predominant and more consistent ictal sign was fearful screaming, occurring in 24 of the 34 patients (70.5%) whose seizures had been recorded. This clinical manifestation was present since epilepsy onset in 12 patients and appeared later on, during the course, in 12 additional patients. In early infancy, fearful screaming appeared mainly within the context of seizures with prominent hypomotor semiology, whereas during follow-up it was associated with early prominent motor manifestations. Hypomotor ictal behavior is a common feature of early onset focal epilepsies and may be the consequence of the limited behavioral repertoire that is typical of infants (Hamer et al., 1999). Maturation of brain networks enriches seizure semiology so that the behavioral arrest with unresponsiveness, which may represent the whole seizure in infants, will be replaced by features that are more typical of the actual lobar origin, unless rapid spread to the motor areas occurs (Fogarasi et al., 2002). Affective symptoms manifesting as a terrified expression and fearful screaming have been associated with ictal involvement of the amygdala and hippocampus, the frontoorbital region, and the anterior cingulate gyrus (Gloor et al., 1982; Bancaud & Talairach, 1992). Panic episodes of epileptic origin, which are not easily distinguishable from the more common ictal fear, have been associated with right parietal ictal activity (Alemayehu et al., 1995) and with right amygdala activation (Zalla et al., 2000; Lanteaume et al., 2007). Overall, ictal EEG findings and clustering of symptoms in our patients were highly suggestive of a prominent involvement of frontotemporal and limbic structures during seizures with ictal fear. Rare episodes of status epilepticus had occurred in about 30% of patients.

Similar to autosomal dominant lateral temporal lobe epilepsy with *LGI1* mutations and autosomal dominant nocturnal frontal lobe epilepsy with acetylcholine receptor gene mutations, most patients with *PCDH19* mutations exhibit focal seizures that point to increased excitability within specific brain regions or system networks. Such, at present, unexplained phenomena might be related to a specific temporal or circuit-related predominant expression of the PCDH19 molecule. PCDH19 is a calcium-dependent adhesion protein involved in the neuronal circuit formation during development and in the maintenance of normal synaptic circuits in adulthood with regional and temporal expression (Hirano et al., 1999; Kim et al., 2007). A prominent expression pattern in the areas connected to the hippocampal formation, such as entorhinal cortex, lateral septum, and basolateral amygdaloid complex has been demonstrated in rats (Kim et al., 2010). Because PCDH19 is predominantly expressed in the hippocampal formation and the amygdala, mutations of this gene might well cause a dysfunction of such structures with subsequent limbic seizures.

Only a minority of patients of the whole sample (17%) had exhibited at some point more than one type of ictal semiology, with a combination of hypomotor seizures and seizures with early motor symptoms. Analysis of seizure semiology in the 16 patients whose attacks had been recorded, both at onset and at follow-up, showed that in 31% of them semiology had not changed, whereas in 44%, symptoms had evolved with an age-related enrichment of ictal behavior. Only 3 of the 35 patients (8.5%) exhibited a combination of focal and generalized seizures, including absences and myoclonic jerks, associated with generalized spike-wave discharges and photosensitivity. Therefore, only a small minority of patients had an epilepsy phenotype reminiscent of Dravet syndrome (Depienne et al., 2009).

At epilepsy onset, about 45% of patients exhibited ictal EEG activity migrating from one hemisphere to the contralateral during the same ictal event. The migrating ictal EEG pattern might make clinicians wonder whether some girls diagnosed as benign familial infantile convulsions, also presenting in clusters, might carry a *PCDH19* mutation. Likewise, series of children with the so-called ''benign psychomotor epilepsy'' (Dalla Bernardina et al., 1992), whose seizures are characterized by sudden attacks of terror with screaming, followed by oroalimentary automatisms and autonomic symptoms, might have included girls with *PCDH19* mutations and a favorable outcome.

About one third of the patients in our series had normal cognition despite the early onset and frequent clusters of seizures; the remaining 68.5% had cognitive impairment, ranging from mild (42%) to moderate (54%) and severe (4%). Autistic features were also relatively common, being reported in about one third of patients. Analysis of a subgroup of 20 patients showed that the delay had become evident around two and half years of age. Four patients had behavioral changes consisting of reduced language, confusional episodes, or aggressive behavior emerging between seizures of a cluster.

The number of reported *PCDH19* mutations is rapidly increasing, and it is likely that this gene is the second most frequently mutated epilepsy gene after *SCN1A* in girls with epilepsy (Marini et al., 2010, 2011). Mutations can be both missense or with a truncating functional effect (frameshift, nonsense, splicing), and most mutations are de novo. Compared to other epilepsy genes, *PCDH19* exhibits a higher occurrence of recurrent mutations, suggesting an underlying shared pathogenetic molecular mechanism (Table 1). For instance, the *Asn340Ser* mutation was identified in five unrelated patients of our cohort and in four previously published patients, including two unrelated individuals (Depienne et al., 2009) and two daughters and their mothers (Dibbens et al., 2011). Among the five patients in our cohort harboring the *Asn340Ser* mutation, this change occurred de novo in three girls and was inherited from their mothers in two; one mother had seizures in childhood whereas the other was unaffected. The *Asn340Ser* mutation was thus associated with variable clinical severity, ranging from normal to mild (with only a few seizures in infancy/childhood) to severe (with profound intellectual disability and frequent clusters of seizures). However, the mildly affected proband had inherited the

Asn340Ser mutation from the unaffected mother. The only additional familial mutation, the *Asp596His*, was inherited from an affected father, confirming that gender-related mechanisms prevent male carriers from being affected (Dibbens et al., 2008). No specific genotype–phenotype correlation could be established, as missense and truncating mutations were associated with phenotypes of comparable severity.

Mutations were more frequently observed within the highly conserved extracellular protein domain (amino acids 1–678). Our data confirm that missense changes are always localized in the extracellular domain, whereas nucleotide changes resulting in a truncation mutation (splicing, frameshift, and nonsense) can also be observed in the intracellular domain (Human Genome Mutation Database–HGMD professional 2011.3). This observation suggests that substitutions in the intracellular domain are either embryonic lethal or cause amino acid changes in the intracellular domain that are devoid of functional effects.

In conclusion, patients with *PCDH19* mutation–related epilepsy exhibit early onset focal seizures that are prominently hypomotor in infancy and evolve to become focal seizure with prominent affective symptoms. *PCDH19* mutations should be sought in female patients with this distinctive epilepsy phenotype.

Disclosure

The authors declare no conflicts of interest. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. (**A**–**C**) Summary of the relevant clinical characteristics of the 35 patients with *PCDH19* mutation–related epilepsy.

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