The clinical spectrum of female epilepsy patients with *PCDH19* mutations in a Chinese population

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Mutations in *PCDH19*, which encodes protocadherin 19, have been identified in epilepsy, mainly in affected females. We summarized the clinical spectrum of female epilepsy patients with *PCDH19* mutations in a Chinese population. We screened for *PCDH19* mutations in 75 girls diagnosed with Dravet syndrome (DS) without a *SCN1A* mutation and 29 girls with fever-sensitive and cluster seizures. We identified 11 novel and 7 reported mutations in 21 of 104 probands (20.2%), including 6 (6/75, 8%) DS girls and 15 (15/29, 51.7%) girls with fever-sensitive epilepsy. The mutations were inherited in 9 probands, de novo in 11, and undetermined in the remaining patient. Shared clinical features included early onset seizures (5–18 months), seizures sensitive to fever, focal seizures or generalized tonic–clonic seizures in clusters and brief seizures. Mental retardation was present in 17 probands. Three patients had autistic features. Two of the nine probands with inherited mutations had no family history of epilepsy, one inherited the mutation from her transmitting father and the other inherited from her asymptomatic mother. Our results confirmed that the clinical spectrum of *PCDH19* mutations includes female DS patients, epilepsy and mental retardation limited to females, epilepsy with normal development and asymptomatic female carriers.

**Conflict of interest**

All authors declare that there is no conflict of interest.
The clinical manifestations of PCDH19-related epilepsy are heterogeneous, but the cardinal features include early seizure onset, generalized or focal seizures highly sensitive to fever, and brief seizures occurring in clusters repeating over the course of several days (5). The phenotype ranges from mild epilepsy to epileptic encephalopathy (8). Brief generalized tonic–clonic seizures (GTCS) and/or focal seizures in clusters are the frequent seizure types. Other seizure types such as myoclonic seizures, atypical absences and atonic seizures are rarely observed (8–11). Cognitive development was reported to range from normal through mild to severe intellectual disability, with or without behavioral and psychiatric problems (8–10). The clinical manifestations of patients with PCDH19 mutations could overlap those of DS, which is mainly caused by SCN1A mutations (2, 3).

In the present study, we screened the PCDH19 mutations in 75 unrelated female DS patients without a SCN1A mutation and 29 female epilepsy patients with fever-sensitive and cluster seizures in the Chinese population. We aimed to assess the rate of PCDH19 mutations in these two groups of Chinese patients and further understand the clinical features of female epilepsy patients with PCDH19 mutations.

Materials and methods

Subjects

A total of 104 female probands, with either sporadic or familial fever-sensitive epilepsy were recruited to be screened for PCDH19 mutations, including 75 female DS patients without SCN1A mutations. All patients were identified in the Pediatric Clinic of Peking University First Hospital from February 2005 to December 2015. All 75 of the DS patients met the previously described diagnostic criteria for DS (12, 13). The other 29 girls had no family history of epilepsy.

Genetic analysis

Genomic DNA was extracted from peripheral blood lymphocytes by a simple salting-out procedure (15). The SCN1A mutation analysis in female DS patients was previously performed using Sanger screening and the multiplex ligation-dependent probe amplification (MLPA) method (SALSA MLPA P137) (13). The six coding exons of the PCDH19 gene and their flanking introns (reference sequence NM_001184880.1) were amplified and sequenced in both directions using an ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA). Five PCR primer pairs were used to amplify exon 1, which is longer than 2 kb. Primer sequences and PCR/sequencing conditions are available upon request. The novelty of the mutations and non-pathogenic variants found in the present study was examined using the Leiden open variation database (http://www.lovdb.nl/PCDH19), the dbSNP database and the Exome Aggregation Consortium (Exac) database. Segregation analysis was performed for all available members of a pedigree. The novel PCDH19 missense substitutions were not found in a cohort of 100 controls. Sequence alignment with PCDH19 genes in other vertebrates was performed using ClustalW (http://www.ebi.ac.uk/clustalw/). The pathogenicity of the identified variants was predicted using Mutation Taster Server (http://www.mutation.taster.org), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/).

To detect PCDH19 deletions and duplications, the MLPA method was applied using the SALSA MLPA P330-A2 probe mix in those patients determined to be PCDH19 mutation-negative by Sanger sequencing.

Results

Mutations in the PCDH19 gene

We identified 18 heterozygous mutations in 21 unrelated probands (21/104, 20.2%), including 6 (6/75, 8%) DS girls and 15 (15/29, 51.7%) girls with fever-sensitive epilepsy (Table 1, Fig. 1). Patients 1, 2, 3, 4, 17 and 18 were diagnosed with DS without SCN1A mutation. The other patients were female epilepsy patients with fever-sensitive and cluster seizures. In this study, 14 of the 21 (14/21, 66.7%) probands with PCDH19 mutations had no family history of epilepsy.

All the 18 mutations were located in exon 1, including 9 missense mutations (c.488T>G/p.V163G; c.1019A>G/p.N340S; c.790G>C/p.D264H; c.1184G>C/p.R395P; c.370G>A/p.D124N; c.1240G>A/p.E414K; c.695G>A/p.N232S; c.471C>T/p.A157E and c.964G>C/p.G322R), 4 nonsense mutations (c.2012C>G/p.S671X; c.1825G>T/p.E609X; c.1375C>T/p.Q459X and c.339C>A/p.C113X), 3 frameshift deletions (c.1017delC/p.N340MfsX28; c.1408_1417delIGGCTATACTGC/p.A470SfsX96 and c.1091delC/p.P364RfsX4), one frameshift insertion (c.1091dupC/p.Y366fsX10) and one in-frame insertion (c.1347_1348insAAC/p.N449_H450insN). Among the seven reported mutations (p.D124N; p.N340S; p.S671X; p.D264H; p.P364RfsX4; p.Y366LfsX10 and p.N232S), p.N340S and p.Y366LfsX10 were recurrent mutations identified in three (patients 3, 4 and 6) and two (patients 15 and 18) unrelated probands, respectively. Novel missense mutations (p.V163G; p.R395P; p.E414K; p.D157E and p.G322R) occurred at reasonably conserved residues (Fig. 2) and were predicted to be pathogenic by Mutation Taster, Polyphen2, and SIFT (Table 2). None of the 11 novel mutations (p.V163G; p.N449_H450insN;
| Patient ID | Seizure onset age (m): type | Seizure type(s) | Seizure sensitivity | Seizure clusters | Status epilepticus | AED(s) | Intellectual disability | Psychiatric symptoms | Age (years) and seizure frequency at last follow-up | PCDH19 mutation | Transmission | Reported/novel |
|------------|-----------------------------|-----------------|---------------------|-----------------|-------------------|--------|------------------------|---------------------|--------------------------------------------------|-----------------|-------------|---------------|
| 1 (DS)     | 8 m: GTCS                   | GTCS, focal, MS | +                   | –                |                  | PB, LTG, LEV, VPA, TPM | +                   | Aggression | 12/yearly clusters | c.548T>G p.V169G | De novo     | Novel         |
| 2 (DS)     | 9 m: GTCS                   | GTCS, focal, aTAS | +                   | –                |                  | PB, CBZ, VPA, TPM | +                   | Aggression | 19/Occasional seizures triggered by fever | c.1347_1348 insAAC p.N449_H450 | De novo     | Novel         |
| 3 (DS)     | 7 m: GTCS                   | GTCS, focal     | +                   | –                |                  | VPA, LEV, TPM | +                   | Aggression, autism | 9/3–10 months between clusters | c.1019A>G p.N340S | De novo     | Reported      |
| 4 (DS)     | 8 m: GTCS                   | GTCS, focal, MS | +                   | +                |                  | PB, VPA, LTG, CBZ, LEV, CZP | +                   | Aggression | 3/1–2 months between clusters | c.1019A>G p.N340S | Maternal inheritance | Reported      |
| 5          | 18 m: GTCS                  | GTCS            | +                   | –                |                  | TPM, VPA, LTG, LEV, PB, CZP | +                   | –                   | 7/4 months between clusters | c.2012C>G p.S671X | Familial condition | Reported      |
| 6          | 10 m: GTCS                  | GTCS            | +                   | –                |                  | VPA, CZP, TPM, OXC, LEV | –                   | –                   | 9/6–12 months between clusters | c.1017delG p.N340 M6X28 | Paternal inheritance | Novel         |
| 7          | 10 m: focal                 | Focal           | +                   | –                |                  | VPA, LEV | +                   | NA                   | 5/6 months between clusters | c.790G>C p.D264H | Paternal inheritance | Reported      |
| 8          | 15 m: focal                 | GTCS, focal     | +                   | –                |                  | LEV, VPA, TPM | +                   | Aggression, obsession | 4/2–3 months between clusters | c.1184G>C p.R369P | De novo     | Novel         |
| 9          | 18 m: GTCS                  | GTCS, focal, MS | +                   | –                |                  | PB, TPM | +                   | Aggression, obsession | 7/6–12 months between clusters | c.1408_1417 delGCCTA TCTGC p.A470 S6x96 | Paternal inheritance | Novel         |
| 10         | 8 m: GTCS                   | GTCS, focal     | +                   | –                |                  | VPA, TPM, CZP | +                   | Aggression | 4/5 months between clusters | c.1825G>T p.E609X | Unknown | Novel         |
| 11         | 8 m: GTCS                   | GTCS            | +                   | +                |                  | VPA, OXC, LEV | +                   | Aggression, obsession | 3/4 months between clusters | c.1375C>T p.Q459X | De novo     | Novel         |
| 12         | 5 m: focal                  | Focal           | +                   | –                |                  | VPA, TPM, CZP, LTG, LEV | +                   | Aggression, autism | 11/yearly clusters | c.1091delG p.P364RfsX4 | Paternal inheritance | Reported     |
| 12S        | 18 m: GTCS                  | GTCS            | +                   | +                |                  | LTG, LEV, VPA | –                   | Aggression, attention deficit | 11/7 seizures for 7 years | c.1091delG p.P364RfsX4 | Paternal inheritance | Reported     |
| 13         | 9 m: focal                  | Focal           | +                   | –                |                  | OXC, VPA, LEV, CZP | +                   | –                   | 5/4 months between clusters | c.370G>A p.D124N | Mosaic father | Reported      |
Table 1. Continued

| Patient ID | Seizure onset age (m): type | Seizure type(s) | Seizure sensitivity | Seizure clusters | Status epilepticus | AED(s) | Intellectual disability | Psychiatric symptoms | Age (years) and seizure frequency at last follow-up | PCDH19 mutation | Transmission | Reported/novel |
|------------|-----------------------------|-----------------|---------------------|------------------|-------------------|--------|-----------------------|-------------------|------------------------------------------------|-----------------|-------------|--------------|
| 13S        | 9 m: focal                  | Focal           | +                   | +                | −                 | CZP, LEV | +                    | −                 | 16/seizure free for 2 years                       | c.370G>A        | Mosaic father | Reported     |
| 14         | 14 m: GTCS                  | GTCS            | +                   | +                | −                 | TPM, LEV | +                    | Aggression | 7/seizure free for 20 months                      | c.12403>A       | Maternal inheritance | Novel        |
| 15         | 11 m: GTCS                  | Focal, GTCS     | +                   | +                | −                 | VPA, CBZ, PB, TPM, NZP | + | Attention deficit | 4/6 months between clusters | c.1019dupC, p.Y966fsX10 | De novo | Reported |
| 16         | 18 m: GTCS                  | GTCS            | +                   | +                | −                 | VPA, TPM | −                    | −                 | 7/monthly clusters                                | c.1019A>G, p.N340S | De novo | Reported |
| 17 (DS)    | 5 m: focal, GTCS, MS, AS    | +               | +                   | −                 | +                 | VPA, OXC, LEV, CZP | + | − | 2/1–2 months between clusters | c.695A>G, p.N232S | De novo | Reported |
| 18 (DS)    | 6 m: focal, GTCS            | +               | +                   | −                 | +                 | LEV, CZP, VPA | + | − | 1/5 months between clusters | c.1019dupC, p.Y966fsX10 | De novo | Reported |
| 19         | 18 m: focal, GTCS           | +               | +                   | −                 | +                 | LTG, VPA, CZP, OXC | + | Attention deficit | 15/2 months a cluster | c.339C>A, p.C113X | Familial condition | Novel |
| 20         | 8 m: focal, GTCS            | +               | +                   | −                 | +                 | VPA, LEV, TPM | − | − | 2/6 months between clusters | c.471C>A, p.D157E | De novo | Novel |
| 21         | 10 m: GTCS                  | Focal, GTCS     | +                   | +                | −                 | VPA, OXC, LTG | − | − | 2/6–8 months between clusters | c.964G>C, p.G322R | De novo | Novel |

*aCurrent medication is underlined.

12S, the dizygotic twin sister of proband 12; 13S, the elder sister of proband 13; AED, antiepileptic drugs; aTAS, atypical absence; ATS, atomic seizure; CBZ, carbamazepine; CZP, clonazepam; GTCS, generalized tonic-clonic seizure; LEV, levetiracetam; LTG, lamotrigine; MS, myoclonic seizures; NZP, nitrazepam; OXC, oxcarbazepine; PB, phenobarbital; TPM, topiramate; VPA, sodium valproate.
Fig. 1. Schematic diagram of the point mutations identified in the PCDH19 gene. EC, extracellular cadherin domain; SP, signal peptide; TM, transmembrane domain. CP, cytoplasmic domains.

Fig. 2. Alignment of the regions surrounding the five novel missense mutations in orthologous and paralogous proteins. The alignment shows the conservation of each affected amino acids in vertebrates and in the delta protocadherin paralogous genes.

p.N340MfsX28; p.R395P; p.A470FsX96; p.E609X; p.E414K; p.Q459X; p.C113X and p.D157E were detected in 100 healthy controls (Fig. 3) or the dbSNP and ExAC databases. Nevertheless, we did not detect any deletions or duplications in the PCDH19 gene in 83 PCDH19 mutation-negative patients using MLPA.

Clinical features of the patients with PCDH19 mutations

The clinical information of 21 probands together with the twin sister of patient 12 and the elder sister of patient 13 (both of whom had PCDH19 mutations) is summarized in Table 1. The mean seizure onset age of the 23 patients was 10.8 ± 4.6 months (median 9, range 5–18). GTCSs were the initial manifestation in more than half of the patients (13/23, 56.5%). Their seizure types included GTCS (18/23, 78.3%), focal seizures (18/23, 78.3%), myoclonic seizures (4/23, 17.4%), atonic seizures (1/23, 4.3%) and atypical absence seizures (2/23, 8.7%). Shared clinical features included early onset seizures (5–18 months), seizures sensitive to fever (23/23, 100%), focal seizures (18/23, 78.3%) or generalized tonic–clonic seizures (18/23, 78.3%), seizures clustering (23/23,

Table 2. Pathogenicity assessment of five novel missense mutations

| PCDH19 Mutation | Domain | Chromosome X: position | Polyphen 2     | SIFT          | Mutation taster |
|-----------------|--------|-------------------------|----------------|---------------|-----------------|
| c.488T>G p.V163G | EC2    | 99663108                | Probably damaging | Damaging       | Disease causing |
| c.1184G>C p.R395P | EC4    | 99662412                | Probably damaging | Damaging       | Disease causing |
| c.1240G>A p.E414K | EC4    | 99662356                | Probably damaging | Damaging       | Disease causing |
| c.471C>A p.D157E | EC2    | 99663125                | Probably damaging | Damaging       | Disease causing |
| c.964G>C p.G322R | EC3    | 99662632                | Probably damaging | Damaging       | Disease causing |

EC, extracellular cadherin domains.
100%) and brief seizures (23/23, 100%, duration of 5 min or less, mostly ≤1 min). Each seizure cluster with or without fever included 2–30 seizures per day, lasting 1–19 days. Two patients (patients 4 and 11) had a history of status epilepticus triggered by fever. Four of the DS patients (patients 1, 2, 4 and 17) had multiple seizure types. In addition, patient 9 also had multiple seizure types but her onset age was 18 months. Mental retardation was present in 18 (78.3%, 18/23) patients (including 17 probands and the sister of patient 13) with PCDH19 mutations, and normal intelligence was present in 5 patients (patients 6, 16, 20, 21 and the twin sister of patient 12). Three probands (DS patients 3, 4 and patient 12) had autistic features. Other psychiatric features were also observed, including aggression (11/21), obsessive-compulsive disorders (3/21), and attention deficit (3/21).

All 21 of the probands had at least one ictal or interictal EEGs. Ten patients had at least one ictal EEG recorded. Focal or multifocal seizures from the centroparieto-occipital regions or temporal region were recorded in five patients (probands 1, 6, 19, 20 and 21). Generalized seizures including tonic–clonic seizures, tonic seizures and clonic–tonic–clonic seizures were recorded in four patients (probands 5, 9, 14 and 18). Multiple seizure types were recorded in proband

Fig. 3. Sequence chromatograms of 11 novel mutations.
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17, including focal seizures, myoclonic seizures and generalized tonic–clonic seizures. Seven patients (probands 3, 5, 11, 12, 14, 18 and 20) had at least one period of normal interictal EEGs. Interictal EEG abnormalities were heterogeneous, including slow background activity in 5 patients (probands 1, 6, 16, 18 and 21), focal or multifocal epileptic discharges in the centroparieto-occipital or frontotemporal regions in 14 patients (probands 2–4, 6–11, 13, 15, 18, 19 and 20), and theta activity in 3 patients (probands 1, 14 and 22). Cranial magnetic resonance imaging (MRI) was performed in all 21 probands with $PCDH19$ mutations, and no abnormalities were detected.

Seizures were refractory to antiepileptic drugs (AEDs) at onset in all 23 patients. The AEDs used include valproate (VPA), carbamazepine (CBZ), topiramate (TPM), phenobarbital (PB), levetiracetam (LEV), oxcarbazepine (OXC), clonazepam (CZP), nitrazepam (NZP) and lamotrigine (LTG). At the most recent follow-up (the ages of patients ranged from 1 year to 19 years), 5 (patients 8, 12S, 13, 13S and 14) used monotherapy (either VPA or LEV), 11 patients used bitherapy, and 7 patients used polytherapy. The frequency of seizures decreased significantly in 13 patients, and the seizure-free interval ranged from 4 months to 7 years.

The inheritance of $PCDH19$ mutations in 9 families

The mutations were inherited in 9 probands (45%, 9/20), de novo in 11 probands, and undetermined in the remaining patient (patient 10) because the patient’s father’s DNA was unavailable. The segregation of inherited variants was performed in families as shown in Figure 4. Two of the nine probands with inherited mutations had no family history of epilepsy, one (proband 9) inherited the mutation from her transmitting father and the

Fig. 4. Pedigrees of the nine families with inherited $PCDH19$ mutations. Arrow, proband; a dot in a square, asymptomatic mutation-carrying male; a dot in a circle, asymptomatic mutation-carrying female; black circle, affected mutation-carrying female; gray-filled circle, affected female without $PCDH19$ mutation. m/+; individuals heterozygous or mosaic for the mutation; m: individuals hemizygous for the mutation; +/+ and +: individuals homozygous and hemizygous for the wild-type allele.
other (proband 14) inherited the mutation from her asymptomatic mother. For the seven familial cases, two (probands 5 and 19) were three-generation pedigrees, two (probands 4 and 6) were two-generation pedigrees and three (probands 7, 12 and 13) were single-generation pedigrees.

The clinical phenotype of the females with PCDH19 mutations showed wide inter- and intra-familial variability, even between twins. Proband 5 was from a three-generation family, and her paternal grandmother never experienced febrile seizures or epilepsy but had behavioral disturbances including mild depression symptoms, agitation and aggressive features. In the three families in which the probands inherited the PCDH19 mutations from their mother, two mothers were affected and one was asymptomatic. Variability between siblings was also evident, even in twins. In our study, the mother and aunt of proband 6 were monozygotic twins and proband 12 and her sister were dizygotic twins. The mother of proband 6 had febrile seizures with normal cognitive function starting at approximately 3 years of age. Her monozygotic twin sister (no DNA sample available) also experienced fever-sensitive seizures beginning at approximately 3 years, had cognitive impairment and died of status epilepticus at 21 years. Proband 12 experienced her first brief focal febrile seizure cluster at 5 months and later developed frequent febrile and afebrile seizures before 6 years. She had intellectual disability and distinct autistic characteristics. However, her twin sister experienced her first brief focal afebrile seizure cluster at 18 months. Seizures were controlled by VPA and LEV treatment at 3 years. She had normal cognitive function except mild attention deficit.

Discussion

In this study, we searched for PCDH19 mutations in DS patients without SCN1A mutations and girls with fever-sensitive and cluster seizures in the Chinese population. PCDH19 mutations were detected in 21/104 (20.2%) females with fever-sensitive epilepsy. The percentage of PCDH19 mutations in 6 DS girls without SCN1A mutations (8%) is much lower than that (51.7%) in 15 girls whose clinical features fulfill the diagnosis of PCDH19-related epilepsy.

Up to now, more than 150 PCDH19 mutations including missense, nonsense, small deletions and insertions, splice site mutations, intragenic deletion and whole gene deletions, have been reported, with most of the mutations (>90%) located in the largest exon, exon 1, which encodes the extracellular cadherin domain of the protein (6). In this study, we identified 18 mutations clustered in exon 1, including nine missense mutations, four nonsense mutations, three small deletions, and two small insertions. Eleven were novel mutations and 7 were reported previously (1, 2, 5, 9–11, 14, 16–19). The missense mutation p.N340S was identified in three unrelated patients (two DS and one non-DS), and the frameshift mutation p.Y366LfsX10 was identified in two unrelated patients. Both of these mutations were reported previously (1, 2, 5, 9–11, 14, 16–18). The high occurrence of recurrent mutations in our cohort and previous studies suggests that PCDH19 is an important gene in epilepsy. The majority of PCDH19 mutations in our patients are unique. Hence, our reports expand the spectrum of PCDH19 mutations associated with epilepsy in females.

Previous studies have claimed that chromosomal rearrangements may occur (2, 4, 20); thus, screening for PCDH19 rearrangements should be performed in addition to direct sequencing of the entire coding sequence. Nonetheless, our MLPA screening involving the PCDH19 gene did not identify any deletions or duplications.

Recently, the increasing number of PCDH19-related epilepsy patients have been sporadic cases, rather than familial cases (5, 11). De novo mutations accounted for more than half of the sporadic cases in our study; and the remaining mutations were inherited from asymptomatic fathers, asymptomatic mothers, or from mothers with only a history of febrile seizures (2–5, 8, 9, 11, 19, 21). In this study, 14 of the 21 probands were isolated cases. In 11 out of the 20 probands in whom inheritance could be assessed, the mutation arose de novo. Two of the nine probands with inherited mutations had no family history of epilepsy, one was inherited from her transmitting father and the other was inherited from her asymptomatic mother. The transmitting fathers were presumably asymptomatic due to the unusual X-linked inheritance of PCDH19. However, sporadic asymptomatic mother carriers have also been reported (1, 4, 20, 22). Completely skewed X-inactivation or a somatic mosaicism may explain the asymptomatic mothers with the mutations (22, 23).

Genotype–phenotype correlations were difficult to establish in this study because of distinct phenotypic variability, even in individuals with the same mutation. In our study, phenotypic variability was even evident in members of the same families including a twin sibling. The grandmother (II-4) of proband 5 carrying the PCDH19 mutation had only mild behavioral problems without seizures. The mother of patient 14 was asymptomatic, and the mother of patient 6 had only febrile seizures in her childhood. Partial or completely skewed X-inactivation may play a great role in females with a mild phenotype or asymptomatic carrier (4). Other genes or environmental factors may also be involved in the phenotypes associated with PCDH19 mutations (9).

Our six DS patients with PCDH19 mutations all fulfilled the main criteria for DS including age of seizure onset (before one-year-old), seizures triggered by fever, multiple seizure types, and cognitive impairment. As previously reported, DS patients with PCDH19 mutations had a slightly older onset age (median age of 9.5 months, with a range from 7.5 to 12 months, vs 5.3 months with a range of 2–11 months in our series of SCN1A-positive DS patients), less frequent status epilepticus (SE), fewer myoclonic seizures, fewer photosensitivity, and a better long-term outcomes than DS patients with SCN1A mutations (2, 5, 13). In our six DS patients, the seizure onset was 6.5 months, only one had SE, three had myoclonic seizures, and none had photosensitivity. Two DS patients...
also exhibited autistic features. We followed up with four of the six DS patients with PCDH19 mutations for 1–6 years. In the first few years, seizure frequency increased along with cognitive impairment. Seizures then became less frequent, and cognitive impairment was less severe than in those DS patients with SCN1A mutations. Specchio et al. reported that antiepileptic treatment could not prevent the recurrence of seizure clusters and that the seizures improved with growth and fewer episodes of febrile illnesses (9). Therefore, PCDH19 mutation screening should be performed in DS female patients without SCN1A mutations.

The other 15 patients with PCDH19 mutations were recruited from a group of female epilepsy patients with fever-sensitive and cluster seizures, including six familial EFMR cases. The clinical features of these patients were in accord with the features of reported cases with PCDH19 mutations (2–5, 8, 9, 14, 18). Seizures were resistant to treatment during infancy and childhood, but seizure frequency and intractability tended to decrease over time. Similarly, most of the patients presented a variable degree of mental retardation, though some patients may have normal cognitive function (8, 9, 18). Only one of our 15 patients had SE during the follow-up period. Female sporadic epilepsy patients with clusters of attacks and fever sensitivity should also be tested for PCDH19 mutations.

In summary, the clinical spectrum of our patients with PCDH19 mutations include female DS patients, familial or isolated epilepsy and mental retardation limited to females (EFMR), epilepsy with normal development and asymptomatic female carriers. Our data confirmed that female DS patients without SCN1A mutations and females with fever-sensitive and cluster seizures should be tested for the PCDH19 mutation. In addition to the clinical features of epilepsy described for PCDH19-related patients, females with PCDH19 mutations may present behavioral problems without seizures or may even be asymptomatic.

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