Table 4
List of histopathological diagnoses of tumors and their incidence.

Types	No. of cases (%[a])		
	Infantile $n = 8$	Childhood $n = 66$	Juvenile/adult $n = 38$
Dysembryoplastic neuroepithelial tumor	0 (0.0)	17 (25.8)	10 (26.3)
Ganglioglioma	3 (37.5)	16 (24.2)	8 (21.1)
Pleomorphic xanthoastrocytoma	0 (0.0)	0 (0.0)	2 (3.0)
Astrocytoma	4 (50.0)	13 (19.7)	12 (31.6)
Other glioneuronal tumor	1 (12.5)	15 (22.7)	2 (5.3)
Other glial tumor	0 (0.0)	5 (7.6)	4 (6.1)

[a] Data are given as percentages.

III) were evident in 72 (92.3%) of the patients in the juvenile group and 49 (64.5%) of those in the adolescent/adult group. Patients considered to have FCD type IIIa [1] accounted for nearly half of both groups (Table 3).

Tumors were observed in all groups. Table 4 presents a list of the tumor histopathological diagnoses and incidences. The juvenile group included 1 papillary glioneuronal tumor and 1 rosette-forming glioneuronal tumor, which were included among other glioneuronal tumors.

Vascular lesions were also observed in all groups, but the main histological types differed among the groups. All of the infant patients suffered from Sturge–Weber syndrome. The juvenile group included 1 case of Sturge–Weber syndrome, 3 cases of arteriovenous malformation, and 4 cases of cavernous angioma (Fig. 2G), whereas the adolescent/adult group included 4 cases of arteriovenous malformation and 18 cases of cavernous angioma. A small number of infant and juvenile patients with Rusmussen encephalitis (Fig. 2H) were encountered. Destructive/scar lesions (Fig. 2I) were found in a similar proportion of patients in all three groups.

4. Discussion

The present study attempted to clarify the clinicopathological profiles of 600 consecutive patients with intractable epilepsy who underwent surgical resection of the causative lesions. A large proportion of the patients exhibited excellent seizure outcomes following the epilepsy surgeries. In infant patients, malformations of cortical development, including FCD type IIb, tuberous sclerosis, and hemimegalencephaly were predominant, whereas in juvenile and adolescent/adult patients, the frequencies of these lesions were reduced, and those of other lesions (possibly etiologically distinct lesions), including hippocampal sclerosis, tumors, and vascular lesions were increased (Table 2). In our previous study reported in 2005, we selected surgical specimens taken from 108 patients with malformations of cortical development to determine the scope of histopathological changes [2]. Since then, the number of cases we have experienced has increased. The major histopathological types appear generally consistent with those reported previously from several countries [8–12].

In the present study, categorization of the three FCD type I subtypes – types Ia, Ib and Ic [1] – was not included, because in some cases the cortical architecture was difficult to evaluate clearly, even if Neu-N immunohistochemistry was performed. Consistent with this, an evaluation survey by several neuropathologists has reported good, moderate and no agreement for diagnosis of FCD types Ia, Ib and Ic, respectively, whereas moderate to almost good agreement can be achieved if all three subtypes are combined into one category [13].

The clinicopathologic features of 5 infant and 3 juvenile patients with frontal lobe epilepsy, in whom surgical specimens showed features of FCD types IIa, IIb, and I and polymicrogyria, and involvement of the white matter and anterior striatum, have been reported elsewhere [14,15].

Recently, *de novo* somatic mutations in the PI3K-AKT3-mTOR pathway were identified in patients with hemimegalencephaly [16,17]. Variable degrees of histological abnormalities involving the cortex and subcortical white matter of the resected tissue were observed. The pathomechanisms underlying the substantial somatic mutation burden in a subset of progenitors in the developing brain, and the resulting clinicopathological phenotypes, remain to be elucidated. The present study included 9 infant patients with severe cortical dysplasia, in whom the histopathological characteristics were similar to those of hemimegalencephaly. The timing of seizure onset in the patients with severe cortical dysplasia was similar to that in patients with hemimegalencephaly, and significantly earlier in postnatal life than that of patients with FCD types IIa and IIb. These cases could be regarded as exhibiting widespread (semi-lobar, lobar, or multi-lobar), rather than focal, cortical dysplasia. Hemimegalencephaly and severe cortical dysplasia may be included in a common pathogenetic group.

Hippocampal sclerosis is the most common pathologic background for temporal lobe epilepsy. Although seizure duration was significantly longer in juvenile than in adolescent/adult patients (Table 3), it seems unlikely

that pathological grades would have advanced simply in accordance with seizure duration. Previously, we evaluated both the perikaryon and nuclear areas of residual neurons in the hippocampal end folium neurons of patients with mesial temporal lobe epilepsy (mTLE), and found that neuronal hypertrophy appeared to advance gradually as the hippocampal sclerosis becomes more severe [18]. Also, we experienced a rare example of hippocampal sclerosis with balloon cells, again a pathognomonic cellular feature of FCD type IIb, in the molecular and granule cell layer of the sclerotic hippocampus resected from a 32-year-old woman with mesial temporal lobe epilepsy and a precipitating history of non-herpetic acute limbic encephalitis [19]. The balloon cells in this case displayed immunoreactivity for CD34 in a characteristic, radiating form, similar to those in FCD type IIb (Fig. 2C). In the present study, this case was categorized as "hippocampal sclerosis" in the adolescent/adult group (Table 2). Moreover, a patient with mTLE was included under the "hippocampal sclerosis" category in the juvenile group; in this case the causative lesion was an epidermoid cyst involving the right basal cistern and inferior horn of the lateral ventricle [20].

We have previously reported the surgical pathologic features of amygdaloid melanosis in a 10-year-old girl with giant congenital pigmented nevi [21]. The lesion appeared to be hamartomatous in nature rather than a neoplasm, involving aberrant migration of melanocytes into the developing neuroepithelial tissue [21]. In the present study, this case was included in the "others" category of the juvenile group (Table 2).

In the present study, I did not include detailed clinical information on symptoms of the patients. Approximately 10% of patients in the infant group showed infantile spasms, and the surgical specimens taken from them exhibited features of FCD type II or hemimegalencephaly. Thus, as of the present patient series, only a subset of infants and children with FCD develop epileptic spasms [22]. The pathogenic mechanisms underlying epileptic spasms and significance of the histological characteristics of FCD have been unclear. I failed to identify any histological differences in FCD tissues taken from patients with/without epileptic spasms. Further studies with other strategies, including imaging techniques on human brain slices described below, for example, might provide insights on these issues. It seems exceptional that patients exhibiting Lennox-Gastaut syndrome undergo lesionectomy. In this study a female patient who exhibited the syndrome at the age of 3 years was included. The resected left fronto-parietal lesion showed tissue destructive features with prominent astrocytosis.

The term "long-term epilepsy associated tumors" (LEAT) encompasses tumorous lesions that are characteristically slow growing, benign, cortically based, often arising in younger age of onset of epilepsy, temporal lobe predominant, and exhibiting neuronal and glial differentiation [23]. A large proportion of the tumors included in the present series appeared to be consistent with the LEAT concept. LEAT cases often showed cytoarchitectural abnormalities of the cortex adjacent to the tumor lesions, leading to diagnosis of FCD type IIIb [1]. A model for future LEAT terminology has been proposed, in which ganglioglioma, DNT, mixed LEAT, and diffuse LEAT entities, as well as a LEAT NOS category for tumors that remain difficult to classify, are included [23]. Previous studies have described the clinicopathologic characteristics of astrocytomas with a better prognosis in patients with chronic epilepsy, for which a distinct subtype – isomorphic astrocytomas – has been proposed [24,25]. The present series included some astrocytomas showing histological similarities to diffuse astrocytoma, but also displaying low cellularity, a lack of mitotic activity, and a low MIB-1 labeling index (less than 1%). The affected patients showed significantly better survival without any postoperative adjuvant radiochemotherapy, being similar to that of WHO grade I. This experience seems consistent with previous reports [23–25].

Recently, we have performed flavoprotein fluorescence imaging of cortical brain slices surgically resected from patients with partial epilepsy caused by various symptomatic lesions [26]. By using this technique, we identified characteristic epileptiform propagation comprising early and late phases, suggesting that synchronized activities in the early phase may play a key role in spreading abnormal discharges [26]. This methodology has allowed us to investigate the pathophysiological and associated histological characteristics of various other lesions responsible for epilepsy. For example, we have identified highly integrated activity between periventricular heterotopic nodules and the overlying hippocampus that had been surgically removed from a patient with mTLE [27]. Further investigations of epileptogenic lesions associated with malformations of cortical development and hippocampal sclerosis may provide information on the underlying pathomechanisms involved.

The present study has shown that various histopathological entities and types, exhibiting a clear predominance depending on the age at seizure onset, are included among the cortical lesions responsible for intractable epilepsy. Such predominance may be closely associated with the pathogenesis of the lesions. The histopathological features described here appear to provide information on the pathomechanisms of the lesions as well as their clinical relevance.

Disclosure

I declare that I have no financial relationships with any pharmaceutical companies, medical equipment manufacturers, biomedical device manufacturers, or

companies with significant involvement in the field of health care. This manuscript does not report the results of a clinical trial.

Conflict of interest

I have no conflicts of interest in relation to this manuscript.

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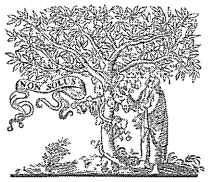

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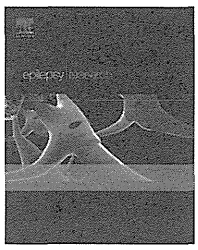

PCDH19-related female-limited epilepsy: Further details regarding early clinical features and therapeutic efficacy

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KEYWORDS
Antiepileptic drugs;
Early diagnosis;
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Seizure clusters;
Treatment

Summary Abnormalities in the protocadherin 19 (*PCDH19*) gene cause early-onset epilepsy exclusively in females. We aimed to explore the genetic and clinical characteristics of *PCDH19*-related epilepsy by focusing on its early features and treatment efficacy.

PCDH19 was analyzed in 159 Japanese female patients with early-onset epilepsy *via* direct sequencing and multiplex ligation-dependent probe amplification (MLPA) analysis.

We identified 17 patients with *PCDH19* abnormalities: point mutations were observed in 14 patients and whole *PCDH19* deletions were detected in 3 patients. One affected sister of a proband with a mild phenotype was also analyzed. The frequency of *PCDH19* deletion among all probands identified in Japan was 12.5% (3/24, including 7 probands reported previously by us). Clinical features included early onset (mean age at onset, 8.6 months), recurrent clusters of brief seizures (17/18), fever sensitivity (18/18), tonic seizures (13/18, probably including focal tonic seizures), tonic–clonic seizures (8/18), focal seizures often with subsequent generalization (17/18), intellectual disabilities (15/18), and autistic traits (13/18). Three patients exhibited delay in motor milestones before seizure onset. In 16 patients, seizures appeared in clusters from the onset of the disease. Among 6 patients for whom detailed information at onset was available, 2 onset patterns were identified: a biphasic course of short seizure clusters (each within days) in 2 patients and a prolonged course of clusters (from weeks to a month) in 4 patients. In both cases, initial seizures started during fever and transiently disappeared with the decline of fever; however, afebrile clusters recurred. In the former patients, motor development was delayed before onset, and seizures appeared in strong clusters from the onset of the disease. In the latter patients, initial development was normal and initial seizures were mild, but were followed by strong clusters lasting several weeks, even without fever. Treatment using phenytoin, potassium bromide, and clobazam showed high efficacy. Although focal seizures were the main feature in *PCDH19*-epilepsy, the efficacy of carbamazepine was poor.

This study highlighted the significance of *PCDH19* deletion, a unique pattern of initial seizure clusters, and the efficacy of antiepileptic drugs. Our data will facilitate early diagnosis and development of a treatment strategy for better clinical management of patients with *PCDH19*-related epilepsy.

Introduction

Protocadherin 19 (*PCDH19*)-related female-limited epilepsy (*PCDH19*-epilepsy) is a unique X-linked disease, in which males with *PCDH19* mutation are unaffected (Dibbens et al., 2008; Juberg and Hellman, 1971; Ryan et al., 1997; Scheffer et al., 2008). A significant number of patients with this epilepsy have been identified in the past years, and its significance has been increasing rapidly (Depienne et al., 2009, 2011; Marini et al., 2010; Specchio et al., 2011). *PCDH19* encodes protocadherin 19 and is located on chromosome Xq22.1 (Vanhalst et al., 2005). Most of the pathogenic mutations of *PCDH19* occur in exon 1, and truncation mutations have been identified exclusively in downstream exons, with the exception of exon 2 (Depienne and LeGuern, 2012). A microdeletion involving *PCDH19* has also been reported in 6 patients (Depienne et al., 2009, 2011; Vincent et al., 2012). The lack of ''cellular interference'' has been hypothesized as the mechanism underlying the male sparing observed in this disease (Depienne et al., 2009): in females, one of the X chromosomes is inactivated, which results in somatic mosaicism regarding PCDH19 expression—wild type *vs.* mutant or none (in the case of nonsense-mediated mRNA decay (Dibbens et al., 2008)). This mosaic status may be necessary for the pathogenesis of *PCDH19*-epilepsy, which is termed as ''cellular interference''.

The clinical characteristics of *PCDH19*-epilepsy have been well described (Depienne and LeGuern, 2012; Higurashi et al., 2012; Scheffer et al., 2008; Specchio et al., 2011). These include early seizure onset, fever sensitivity, seizure clusters of brief seizures—especially focal seizures, often with fearful screaming (Marini et al., 2012)—frequently associated with fever, varying degree of intellectual disability, and autistic traits. *PCDH19* mutations were initially discovered in large families (Dibbens et al., 2008) and in female patients with a Dravet syndrome (DS)-like phenotype who lacked the *SCN1A* mutation (Depienne et al., 2009; Marini et al., 2010); however, it is now well known that only a portion of patients manifest a phenotype resembling DS (Depienne and LeGuern, 2012; Higurashi et al., 2012; Marini et al., 2012). *PCDH19*-epilepsy rarely exhibits prolonged seizures, photosensitivity, or clonic, myoclonic and absence seizures. In its long-term course, seizure cessation is common after school age (Higurashi et al., 2012; Scheffer et al., 2008), and most patients achieve good motor skills and walk independently without ataxia. Accordingly, it is of critical importance to differentiate this clinical entity from DS and establish an early diagnosis of *PCDH19*-epilepsy to provide better clinical management to these patients.

For this purpose, we performed an additional genetic analysis study of *PCDH19* in Japanese female patients with early-onset epilepsy, including screening for *PCDH19* deletion *via* multiplex ligation-dependent probe amplification (MLPA) analysis, and explored the early clinical details of newly identified patients with *PCDH19*-epilepsy. Here we present the significance of *PCDH19* deletion in this disease and distinct patterns of initial seizures, which will facilitate the early diagnosis of this disease. We also summarize the efficacy of antiepileptic drugs in our patients in an attempt to identify an appropriate treatment strategy.

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Subjects and methods

Subjects and clinical information

One hundred and fifty-nine female patients with various types of epilepsy were newly enrolled in this study. These individuals were referred to our laboratory for *SCN1A* and/or *PCDH19* analyses because of their clinical features, such as early seizure onset, fever sensitivity, status epilepticus, seizure clusters, intractability of seizures, and concomitant intellectual disability, and included 53 patients with DS and 67 patients with symptomatic focal epilepsy. *SCN1A* abnormalities were screened before this study and were identified in 45 patients.

The clinical information was collected using a questionnaire and by directly contacting the physicians in charge: they were all pediatric neurology specialists and were well experienced in epilepsy care. The presence of "autistic traits" in patients was determined *via* medical interviews and observation of the patient's behavior, to determine weather they exhibited impairment in one or more of the 3 items included in DSM-IV-TR criteria for autism (American Psychiatric Association, 2000). To assess the treatment efficacy of antiepileptic drugs, the reduction of the rate of seizure frequency was determined by comparing the seizure records of periods of at least 6 months before and after the beginning of drug administration, and was classified as follows: excellent, $\geq$90% reduction; effective, $\geq$50% reduction; ineffective, <50% reduction; and exacerbation, seizures increase. Written informed consent for genetic analysis was obtained from the patients' guardians before blood sampling. This study was approved by the Human Ethics Committee of Fukuoka University (Approval No. 09-09-05).

Genetic analysis of *PCDH19*

Direct sequencing was first performed as described previously (Higurashi et al., 2012). In the present study, MLPA analysis was also performed to detect *PCDH19* deletion. Because the MLPA analysis was not performed in our previous study, the previous cohort (116 patients) was included in this analysis (Higurashi et al., 2012); however, no abnormalities were identified in that cohort. The probes used for MLPA reaction were designed to hybridize to each region of the 6 exons. The details of the MLPA probes and analytical methods, and the protocols used in the other analyses performed in this study, including fluorescence *in situ* hybridization (FISH) for *PCDH19*, array comparative genomic hybridization (aCGH), and determination of X-chromosome inactivation status at the human androgen receptor gene (*HUMARA*) (Allen et al., 1992; Mei et al., 2010), are described in the Supplementary Methods.

Results

Abnormalities in *PCDH19*

Heterozygous *PCDH19* abnormalities were identified in 17 patients: point mutations were detected in 14 patients (Table 1, Patients 1–14; GenBank accession number of the complete human *PCDH19* mRNA, EF676096.1) and whole *PCDH19* deletions were detected in 3 patients (Table 1, Patients 15–17, Supplementary Fig. S1A and B). Four affected relatives were also identified: the younger sister of Patient 4 (Patient 4s) and the mothers of Patients 6, 12, and 14 (clinical information was available for Patient 4s). Novel missense mutations (p.R198L, p.S139L, p.D90V, p.D596V, and p.D157N) occurred at highly conserved residues (Supplementary Fig. S2). Patients 10 and 14 had a nonsense mutation in exons 2 and 4, respectively; the remaining patients had a mutation in exon 1. The exon 2 mutation detected in Patient 10 was inherited from the healthy father. Although she had a typical phenotype of *PCDH19*-epilepsy (Supplementary Information), her 2 older sisters with the same mutation were completely healthy. They all exhibited a random X-inactivation pattern (paternal:maternal, 38:62 [Patient 10] and 58:43 and 28:72 [her sisters]).

PCDH19 deletion was also confirmed by FISH analysis (Supplementary Fig. S1B) In Patient 15, aCGH revealed the presence of a 4-Mbp deletion that also involved several flanking genes (Supplementary Fig. S1C), including *SPRX2*, which is responsible for rolandic epilepsy, mental retardation, and perisylvian polymicrogyria only in males (Royer et al., 2007), and other genes related to non-neuronal diseases (Bione et al., 1998; Tolppanen et al., 2010). However, as reported previously (Depienne et al., 2011; Vincent et al., 2012), no significant differences in clinical features were identified between the 3 patients with *PCDH19* deletions and patients with point mutations (the clinical details of Patients 15 and 16 are described in the Supplementary Information and those of Patient 16 are also presented in Fig. 1C). The frequency of *PCDH19* deletion among all *PCDH19*-epilepsy cases in Japan (24 probands in total, 7 reported previously (Higurashi et al., 2012)) was 12.5% (3/24).

Overview of clinical features

The clinical information of the newly identified patients (n=18, including Patient 4s) is summarized in Table 1. None of these patients had any hypoxic events during the gestational and perinatal periods. Their main features were consistent with those described previously: mean ($\pm$standard deviation) age at onset of 8.6$\pm$4.2 (4–25) months; seizure clusters (17/18; Patient 4s, who had a mild case, was the exception); fever sensitivity (18/18); brief habitual seizures (duration of a few minutes or less, often $\leq$1 min); and low incidence of prolonged seizure (defined duration: $\geq$15; 2/18, Patients 4 and 9; Patient 4 exhibited this only at onset, and Patient 9 had several prolonged complex partial seizures at the age of 6 during dose reduction of oral antiepileptics). However, some patients presented with transient severe seizure clusters that were close to status epilepticus. In Patient 10, this occurred just after the cessation of continuous administration of midazolam. Although seizure frequency was generally high during early childhood (monthly to every several months), it was highly variable among patients, and Patient 9 had no seizure between 2 years and 4 years and 4 months of age followed by recurrence.

Seizure types included tonic (13/18), tonic–clonic (8/18), and focal seizures often with subsequent

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Table 1 Clinical characteristics of the patients with *PCDH19*-related epilepsy.

Pt	*PCDH19* abnormality	Mutagenesis	Onset (m)	Development at onset	Initial sz pattern[a]	Sz type[a]	Sz cluster	FS focus[b]	Brain MRI
1	p.R198L c.593G>T	NA	7	N	Afebrile/FS, TC cluster	FS, T, TC	+	lt-O/7m, lt-CO/2y rt-P/4y	N
2	p.K120RfsX3 c.357delC	NA	10	N	Febrile/isolated TC, T → afebrile/T,FS cluster	T, TC, FS	+	rt-mTpT/10m	N
3	p.Y166* c.497_498insA	NA	4	N	Febrile → afebrile/TC, T cluster	T, TC	+	lt-aT-Fp/4y7m	N
4	p.D45GfsX43 c.134-135ACdel	Fa	8	N	Febrile/TC (SE) → (a)febrile/FS cluster	TC, FS	+	rt, lt-F, Te	Heterotopia lt-F
4s[c]	Same as above	Fa	22	N	Febrile/isolated T	T, FS	–	NA	N
5	p.N340S c.1019A>G	*De novo*	5	N	Febrile/FS, T cluster	FS, T	+	rt-C,Te/3y10m bil-F/7y11m	N
6	p.S139L c.416C>T	Mo	9	N	Febrile → afebrile/T, FS cluster	T, FS	+	NA	N
7	p.D90V c269A>T	*De novo*	11	N	Afebrile/FS, T cluster	FS, T	+	rt-C,O/4y3m	N
8	p.D596V c.1787A>T	Fa	5	N	Febrile/FS cluster	FS, T	+	rt-P,O,pT/5m rt-P/5y4m	Intensity laterality, O
9	p.Y366LfsX10 c.1091_1092ins C	NA	5	Delayed head control	Febrile → afebrile/FS cluster	FS, T	+	lt-mTaT/5m lt-aT/2y6m	Atrophy lt-PO
10	p.L719* c.2156T>G (Ex2)	Fa	13	N	Febrile/isolated TC × 2	TC, FS	+	rt-F/2y4m	N
11	p.S349* c.1048C>G	Fa	5	Delayed head control	Febrile/FS cluster	FS, T	+	rt, lt-F	N
12	p.N340S c.1019 A/G	Mo	8	N	Febrile → afebrile/TC, FS cluster	TC, FS	+	NA	N
13	p.R886* c.2656C>T (Ex4)	*De novo*	11	Delayed sitting/crawling	Febrile → afebrile/FS, TC cluster	FS, TC	+	NA	N
14	p.D157N c.469G>A	Mo	5	N	Febrile/T cluster	FS	+	lt-F	Atrophy rt-HIP
15	Whole deletion	*De novo*	9	N	Febrile/FS cluster	FS, T	+	lt, rt-F,P/2y9m	T2 high lesion lt-F WM
16	Whole deletion	*De novo*	10	N	Febrile/FS, T cluster	FS, T, TC	+	lt-O/11mo bil-C/1y4m	N
17	Whole deletion	*De novo*	8	N	Febrile/FS, T cluster	FS, T	+	NA	N

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Table 1 (*Continued*)

Pt	Acute-phase treatment[d]	AEDs effective[e]	AEDs others[f]	Present age (last sz)	ID	Motor outcome	Autistic behavior
1	MDL (buccal, iv) fPHT	CLB ZNS	PB KBr PHT TPM VPA	6y5m	DQ 80.5 2y6m	N	Autistic
2	MDL	CLB ZNS	PB KBr LEV	4y4m	DQ 56 3y6m	N	Autistic
3	MDL (DZP sup)	KBr PB	CBZ CZP TPM	7y2m	Severe 7y	N	Autistic
4	MDL	KBr VPA	(CZP) (CBZ) (PB) (PHT)	10y10m (8y)	IQ 42 9y10m	N	Autistic
4s	NA	VPA CZP		7y2m (3y)	—	N	Hyperactive
5	MDL (DZP sup)	LTG KBr CZP	PB ZNS CLB VPA NZP B6 GBP STM *CBZ PHT*	8y	DQ 44 7y4m	N	
6	(DZP sup) (PB sup)	VPA	(CBZ) (PB)	13y9m (11y9m)	IQ 62 11y9mo	N	
7	(MDL) (DZP)	VPA PHT CZP TPM	CLB PB ZNS GBP LTG	6y7m (4y11m)	Mild ~ moderate	N	Autistic
8		CLB CBZ TPM	CZP ZNS PB *VPA*	5y8m	DQ 49 4y11m	N	Autistic
9	MDL (≥4y6m)	CBZ VPA CLB	PHT TPM (CZP) (LEV)	8y4m	IQ 51 7y4m	N	Impulsive
10	MDL (PB) mPSL	LEV	CBZ CZP VPA (LTG)	3y6m	—	N	Autistic Hyperactive
11	MDL PHT	PHT	B6 CLB VPA PB KBr CZP ZNS	7y	DQ 48 3y	N	Autistic Hyperactive
12	MDL PHT	LEV	CBZ ZNS CLB CZP TPM	5y11m	DQ 38 4y7m	Truncal instability	Autistic Hyperactive
13	(MDL) TPL PB sup/iv	CLB KBr	PB CZP (CBZ)	3y4m	DQ 76 2y3m	Mildly hypotonic	Autistic Hyperactive
14		TPM	VPA CLB (CBZ) (PB)	7y	DQ 50 1y6m	Mildly ataxic	Autistic Hyperactive
15	(DZP sup)	CZP	LEV VPA OXC CLZ	2y10m	DQ 49 2y9m	Mildly unstable gait	Autistic
16	MDL TPL (PHT)	PHT	VPA PB CBZ (LEV)	2y1m	Mild	N	Hyperactive
17	MDL	TPM CLB	VPA CBZ ZNS	4y	DQ 63 2y6mo	N	Autistic Hyperactive

Pt, patient; sz, seizure; MRI, magnetic resonance imaging; NA, not analyzed; N, normal; FS, focal seizure; lt, left; O, occipital; C, central; rt, right; P, parietal; mT, mid temporal; pT, posterior temporal; aT, anterior temporal; Fp, frontopolar; Fa, father; SE, status epilepticus; F, frontal; Te, temporal; bil, bilateral; Mo, mother; HIP, hippocampus; WM, white matter. ID, intelligent disability; MDL, midazolam; fPHT, fosphenytoin; CLB, clobazam; ZNS, zonisamide; PB, phenobarbital; KBr, potassium bromide; PHT, phenytoin; TPM, topiramate; VPA, sodium valproate; DQ, developmental quotient; N, normal; LEV, levetiracetam; DZP, diazepam; sup, suppository; CBZ, carbamazepine; CZP, clonazepam; LTG, lamotrigine; NZP, nitrazepam; B6, vitamin B6; GBP, gabapentin; STM, sultiam; IQ, intelligent quotient; OXC, oxcarbazepine; CLZ, clorazepate; TPL, sodium thiopental.

[a] Tonic (T) indicates generalized, secondary generalized, or focal tonic seizures, and tonic–clonic (TC) indicates generalized or secondary generalized seizures.

[b] Determined by ictal recordings of electroencepharography.

[c] Younger sister of Patient 4.

[d] Administered intravenously otherwise specified, and drugs in parenthesis indicates "effective in some degree".

[e] Underline indicates "excellent" efficacy.

[f] Italics indicates "exacerbation", and drugs in parenthesis "undetermined" efficacy.

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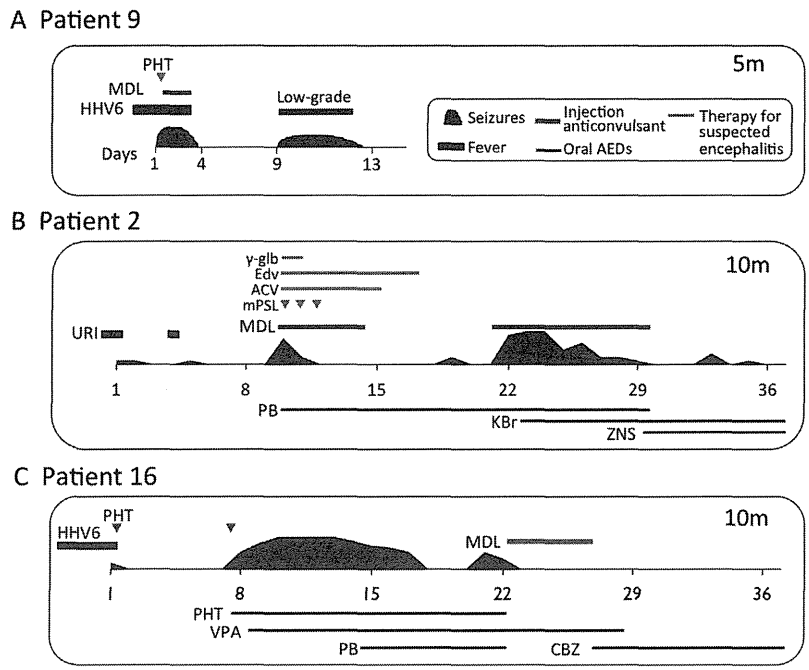

Figure 1 Examples of biphasic short seizure clusters and prolonged seizure clusters during the early phase. The early clinical courses of Patients 9 (A), 2 (B), and 16 (C) are shown. (A) Brief focal seizures consisting of motion arrest, blank eye, and facial cyanosis were repeated 33 times over 3 days during fever of exanthema subitum type and disappeared with the decline of fever. However, 5 days later, seizures accompanied by low-grade fever recurred and were repeated 26 times over 4 days. (B and C) Patients 2 and 16 had seizure onset at 10 months of age. In the initial episodes, seizures started in a mild fashion with some delay (2nd and 4th day, respectively) from the appearance of fever. Although seizures transiently disappeared with the decline of the fever, they reappeared in strong clusters without fever. (B) Brief isolated generalized convulsions associated with fever appeared on days 1, 2, and 5. Strong clusters of brief convulsions and focal seizures, often with secondary generalization, appeared on day 9; treatments for suspected acute encephalitis were administered. Although intermittently, seizure clusters were repeated over a total period of 1 month. The patient had no seizures until recurrence at the age of 2 years and 4 months. (C) The initial seizures appeared in mild clusters. ACV, acyclovir; AEDs, antiepileptic drugs; CBZ, carbamazepine; Edv, edaravone; γ-glb, gamma globulin; HHV6, human herpesvirus 6; KBr, potassium bromide; MDL, midazolam; mPSL, methylpredonisolone; PB, phenobarbital; PHT, phenytoin; URI, upper respiratory infection; VPA, sodium valproate; ZNS, zonisamide.

generalization (17/18). Focal seizures appeared from the onset of the disease in 14 patients. Here, "tonic seizure" may include focal tonic seizures. In generalized convulsions, clonic component was often less represented. The frequent symptoms of focal seizures included motion arrest, cyanosis, eye deviation, respiratory alteration, systemic jerks, mild clonic, complex movement in the face and/or extremities, and tonic symptoms. In our cases, seizures confirmed by ictal electroencephalography (EEG) included only focal seizures, and ictal activities often originated in the frontal and/or temporal regions (9/13). A posterior focus involving the occipital region was observed in 4 cases, especially during infancy. None of our patients had myoclonic and absence seizures. Patient 15 had a very brief focal seizure with momentary jerks of the trunk and limbs during sleep, independently or as the initial symptom of her longer seizure (Supplementary Video and Supplementary Fig. S3). This seizure seemingly mimicked a myoclonic seizure, which is difficult to distinguish without video-EEG recordings.

Frequent abnormalities in interictal EEG included focal (poly)spike(s)/spike(s)-and-wave discharges (14/18) and slowing of the background and basic activities (4/18). Patient 6 showed irregular spike-and-wave discharges evoked by 10–20 Hz of photic stimulation and diffuse sharp-and-wave discharges during sleep at age 11; these findings were not observed in her earlier examinations. Mild abnormalities in brain MRI were identified in 5 patients; a series of examinations revealed that those lesions were not the main cause of their epilepsy.

A mild delay in motor milestones before seizure onset was noted in 3/18 patients; their subsequent motor development was good, and all patients with the exception of Patient 12 walked independently without significant motor impairment at the time of this study. Intellectual disability [15/18 (7 mild, 7 moderate, and 1 severe)], autistic traits (13/18), and hyperactive behavior (8/18) were commonly observed. The autistic symptoms of our patients included problems in eye contact, language skills, eating, and appropriateness of behavior.

Patterns of initial seizures

Seizure clusters were highly consistent in *PCDH19*-epilepsy and appeared throughout the clinical course of patients,

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mostly from the onset of seizure (16/17, Patient 10, who had a simple febrile seizure at onset, was the exception). The detailed clinical course of the initial seizure clusters could be obtained in 6 patients, and 2 patterns of seizure occurrence were identified. The first pattern was characterized by the biphasic appearance of short seizure clusters and was observed in 2 patients (Patients 9 and 13, Fig. 1A): the earlier phase comprised strong seizure clusters during fever, which disappeared with the decline of the fever. However, seizure clusters recurred within several days without fever and remitted within days. Interestingly, both patients had motor delay since before the onset of seizure.

The second pattern was characterized by seizure clusters that intensively repeated for weeks to 1 month. This pattern was confirmed in 4 patients (Patients 2, 4, 12, and 16), who all had normal psychomotor development before the onset of seizure. The clinical course of Patients 2 and 16 are presented in Fig. 2B and C, respectively. In this pattern, several mild seizures appeared during fever. Although seizures disappeared with the decline of the fever, strong seizure clusters started without fever and continued intermittently over weeks. More specifically, Patients 2 and 4 were suspected of having some acute encephalitis/encephalopathy and their treatment included immunotherapy. However, each seizure was brief and the interictal consciousness was basically clear. After the remission of the prolonged clusters, seizures disappeared for months or longer, until the next recurrence.

Treatment of seizures

During the acute phase, the efficacy of continuous administration of midazolam in suppressing the ongoing seizure clusters was confirmed in 13 patients, often at a lower dosage ($\leq$0.2 mg/kg/h). Nevertheless, its efficacy was often insufficient to manage strong clusters during early childhood. Even when seizures were controlled by midazolam, dose reduction and discontinuation soon resulted in seizure recurrence, and sometimes even in seizure aggravation as described above (Patient 10). Intravenous administration of phenytoin/fosphenytoin or phenobarbital was also effective in 6 cases, although often transiently. Interestingly, in Patient 10, the administration of methylprednisolone (10–30 mg/kg/day for 3 days) successfully stopped ongoing clusters at several occasions at age 2. Patient 2 also exhibited transient seizure disappearance after methylprednisolone administration for suspected encephalitis at age 10 months (Fig. 1B).

The prophylactic efficacy of antiepileptic drugs, which were administered to 8 or more patients, is summarized in Fig. 2. This summary also includes information on our 8 patients who were reported previously (n=25 in total) (Higurashi et al., 2012). Phenytoin, potassium bromide, and clobazam were often beneficial for decreasing seizures. However, no definitive drug that prevented further seizures in multiple patients was identified. Carbamazepine had the poorest efficacy among the drugs recorded. Other drugs used by a small number of our patients included levetiracetam (number of patients with excellent/effective/ineffective efficacy, 0/2/2), clorazepate (1/1/1), lamotrigine (0/1/1), gabapentin (0/0/2), vitamin B6 (0/0/2), nitrazepam (0/0/1), sultiame (0/0/1), oxcarbazepine (0/0/1), and acetazolamide (0/1/0). None of our patients received oral corticosteroids, adrenocorticotropic hormone therapy, ketogenic diet, or vagal nerve stimulation.

Discussion

In this study, we found that 12.5% of Japanese probands with *PCDH19*-epilepsy carried a *PCDH19* deletion, which clearly indicates the significance of screening for this deletion in cases without point mutations. We also identified a nonsense mutation in exon 2 of *PCDH19* in a patient with a typical phenotype of *PCDH19*-epilepsy (Patient 10). Although one mutation can be associated with a significant phenotypic variation among patients (Higurashi et al., 2012), her sisters with the same mutation were completely healthy. The presence of splicing variants lacking exon 2 (Dibbens et al., 2008) may not adequately account for the differences in disease development observed among these sisters, and some triggering factors other than *PCDH19* mutation might be involved in the pathogenesis.

In our patients, 2 distinct patterns of seizure onset were identified: a biphasic course of relatively short seizure clusters (each lasting up to a few days), and a prolonged course of seizure clusters (intermittently repeated for weeks or 1 month). In both cases, initial seizures remitted with the decline of fever. In the patients with the former pattern, initial seizures appeared in strong clusters, and their motor developmental retardation started before the onset of seizure. In contrast, in the patients with the latter pattern, the development was normal at the onset of symptoms and initial seizures were mild, but were followed by strong seizure clusters lasting for weeks without fever. These findings let us to speculate that, in the former pattern, some pathological conditions had already progressed before seizure onset that induced strong clusters from seizure onset. However, in the latter pattern, such pathological conditions might gradually emerge around the onset of seizures that induce subsequent strong clusters. Considering the case in which steroid administration was strikingly effective in suppressing seizure clusters, the inflammatory process may be one possible modifier of such clinical variations. Further biological studies are necessary to address these issues.

Such prolonged seizure clusters, which are reminiscent of a type of acute encephalitis/encephalopathy, may be a key feature that suggests the presence of *PCDH19*-epilepsy, and genetic analysis should be considered in such cases. Human herpes virus 6 and the influenza virus were often the pathogen underlying the fever observed at the onset of seizures in our patients and cause acute encephalopathy, such as that presenting with biphasic seizures and late reduced diffusion (Takanashi et al., 2006). Although it exhibits a higher average age at onset, febrile infection-related epilepsy syndrome is also a peculiar form of childhood-onset epilepsy that presents with prolonged courses of seizure clusters, often evolving into status epilepticus (van Baalen et al., 2010). In our patients, status epilepticus, impairment of interictal consciousness, leukocytosis in the cerebrospinal fluid, and brain MRI

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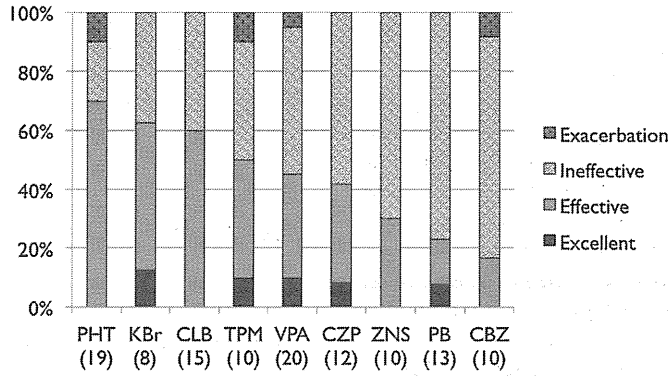

Figure 2 Prophylactic efficacy of antiepileptic drugs. Drugs administered to 8 or more patients (excluding cases of undetermined efficacy) are shown. The numbers in parenthesis indicate the number of patients assessed.

abnormalities characteristic of each type of encephalitis were rare and may be used to distinguish these diseases.

The evaluation of the prophylactic efficacy of anticonvulsants is challenging, because seizure frequency fluctuates markedly and depends significantly on the incidence of febrile illnesses and age. Such possible influences should be considered for the interpretation of the data presented in Fig. 2, and a more rigorous evaluation will be necessary in the future. However, an outline of efficacy, at least, can be speculated from our data. Although patients with *PCDH19*-epilepsy exhibited mainly focal seizures, the low efficacy of carbamazepine should be noted. The profile of drugs that showed higher efficacy was similar to that observed in DS, with the exception of phenytoin (Chiron, 2011). Unlike that which was observed in DS, phenytoin may be placed at a higher priority in cases with a high therapeutic need over the risk of gingival enlargement. Considering the unique pattern of seizure appearance in this disease, the addition of multiple antiepileptic drugs in rapid sequence should be avoided, even when seizure clusters continue.

This study highlights the significance of screening for *PCDH19* deletion, the characteristics of early seizures, and the efficacy of acute- and prophylactic treatment. Our data will contribute to the early diagnosis of the disease and the selection of more favorable treatments and may help avoid excessive treatment and provide better clinical management. In the next phase of research, the efficacy of antiepileptic drugs should be studied prospectively. In addition, it is also necessary to evaluate whether a better neurological outcome can be obtained by the improvement of seizure control.

Conflict of interest

All authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eplepsyres.2013.04.005.

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