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Original article

Clinical manifestations and epilepsy treatment in Japanese patients with pathogenic *CDKL5* variants

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Abstract

Objective: Patients with pathogenic cyclin-dependent kinase-like-5 gene (*CDKL5*) variants are designated CDKL5 deficiency disorder (CDD). This study aimed to delineate the clinical characteristics of Japanese patients with CDD and elucidate possible appropriate treatments.

Methods: We recruited patients with pathogenic or likely pathogenic *CDKL5* variants from a cohort of approximately 1,100 Japanese patients with developmental and epileptic encephalopathies, who underwent genetic analysis. We retrospectively reviewed clinical, electroencephalogram, neuroimaging, and genetic information.

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Results: We identified 29 patients (21 females, eight males). All patients showed severe developmental delay, especially in males. Involuntary movements were observed in 15 patients. No antiepileptic drugs (AEDs) achieved seizure freedom by monotherapy. AEDs achieving $\geq$ 50% reduction in seizure frequency were sodium valproate in two patients, vigabatrin in one, and lamotrigine in one. Seizure aggravation was observed during the use of lamotrigine, potassium bromide, and levetiracetam. Adrenocorticotrophic hormone (ACTH) was the most effective treatment. The ketogenic diet (KD), corpus callosotomy and vagus nerve stimulation did not improve seizure frequency in most patients, but KD was remarkably effective in one. The degree of brain atrophy on magnetic resonance imaging (MRI) reflected disease severity. Compared with females, males had lower levels of attained motor development and more severe cerebral atrophy on MRI.

Conclusion: Our patients showed more severe global developmental delay than those in previous studies and had intractable epilepsy, likely because previous studies had lower numbers of males. Further studies are needed to investigate appropriate therapy for CDD, such as AED polytherapy or combination treatment involving ACTH, KD, and AEDs.

Keywords: *CDKL5*; Pathogenic variants; Developmental and epileptic encephalopathy; Involuntary movements; Antiepileptic drugs; Adrenocorticotrophic hormone; Ketogenic diet; Rational polytherapy

1. Introduction

Pathogenic variants in the cyclin-dependent kinase-like-5 gene (*CDKL5*), located on chromosome Xp22, cause early-onset developmental and epileptic encephalopathy [1], designated CDKL5 deficiency disorder (CDD, also known as early onset infantile epileptic encephalopathy-2, OMIM 300672). Because of clinical similarities with patients harboring pathogenic *MECP2* variants, CDD patients are often clinically described as having an atypical form of Rett syndrome [2]. However, CDD is now considered an independent clinical entity [3,4].

Clinical characteristics of CDKL5-related encephalopathy include infantile spasms or early-onset seizures within the first 3 months of life, and severe neurodevelopmental problems. The phenotypes associated with pathogenic *CDKL5* variants range from a mild form with controlled epilepsy and the ability to walk to a severe form with profound motor and cognitive developmental delay and refractory epilepsy [5]. Recently, the clinical spectrum of CDD has been described in several studies involving more than 100 cases [6,7]. Two case series with a small number of participants (12 and five cases, respectively) have been reported in Japan [8,9].

The new classifications for epilepsy and seizure types published in 2017 [10] emphasize the importance of understanding underlying disease mechanisms, and this etiology-based approach is already beginning to provide powerful opportunities for precision medicine in the epilepsies.

In this report, we conducted a multicenter study investigating clinical manifestations, including the type of epilepsy, treatments, neuroimaging and neurophysiological findings, and genetic information in Japanese patients with CDD. Our aim was to delineate the clinical characteristics and elucidate possible appropriate treatments for Japanese patients with CDD.

2. Methods

We performed a genetic analysis of approximately 1,100 Japanese patients with developmental and epileptic encephalopathies between 2005 and 2016 in Department of Human Genetics, Yokohama City University, or Department of Pediatrics, Yamagata University Faculty of Medicine. We identified pathogenic or likely pathogenic *CDKL5* variants in 29 patients, and enrolled all of these patients in this study. Missense variants were found in 10 patients, including one with a somatic mosaic variant and 19 with null variants including two with exonic deletions (Table 1). Genetic testing had been performed by different approaches, including high-resolution melting analysis, target capture analysis, direct sequencing, or whole exome sequencing as previously described [11].

We reviewed clinical, electroencephalogram (EEG), neuroimaging, and genetic data. All patients had undergone repeated EEG recordings and had at least one brain magnetic resonance imaging (MRI) scan. We obtained detailed clinical data for all patients through a questionnaire completed by pediatric neurologists. These questionnaires included sex, age at seizure onset, seizure types, neuropsychological status (including accompanying involuntary movements), EEG and MRI findings, the effectiveness of antiepileptic drugs (AEDs) for respective seizure types (including tonic seizure, spasms, myoclonic seizure, generalized tonic-clonic seizure, and focal seizure), additional treatments for epilepsy, and surgical intervention including corpus callosotomy and vagus nerve stimulation (VNS). Seizure types were determined on the basis of clinical history, imaging studies, and EEG findings in accordance with

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Table 1
Clinical features of the 29 patients with *CDKL5* mutations.

Patient	Sex	Age (years)	Gene variant	Epilepsy sydrome	Epilepsy onset (months)	Seizure type at onset	Seizure type in following period (age)	Honeymoon period (ages)	Interictal EEG at onset	Hypsarrhythmia	MRI findings	Regression	Seizure prognosis	Final AED	Final EEG	Final DQ	Motor skill at ascertainment	Involuntary movements	Fix and follow	Eye contact	Functional hand use	Language	Need for tube feeding or tracheostomy
1	M	11	c.533G > A, p. Arg178Gln (somatic mosaic), de novo	WS	5	GTC, tonic (daily)	spasms (6 mo); atonic, myoclonic (1 y 6 mo)	–	normal	–	normal (4 y)	–	tonic (daily)	TPM, VPA, PHT, CLB	multifocal spikes, diffuse polySWC	<20	rolling	chorea	good	poor	–	–	–
2	M	26	c.145G > A, p. Glu49Lys, de novo	OS → WS	1	tonic (daily)	spasms (1 y 7 mo)	–	hypsarrhythmia	+	mild cerebral atrophy (4 mo); mild cerebral atryophy (14 y)	NA	tonic(daily)	VPA, ZNS, CBZ, CLB	NA	<20	bedridden	HS	poor	poor	+	–	tube feeding
3	F	7	Del (Exon12)	WS	1	focal (daily)	spasms (9 mo); GTC (1 y)	6–9 mo	normal	+	normal (4 y)	–	focal, myoclonic (daily)	VPA	NA	<20	rolling	HS	–	–	–	–	–
4	F	9	c.584G > A, p. Trp195*	EOEE	2	spasms (daily)	focal (2 mo)	–	left or rigth centrotemporal dominant spikes	+	normal (3 y)	+	focal, spasms (daily)	GBP, LEV, PER, STM	nultifocal spikes, diffuse polySWC	<20	rolling	HS	poor	poor	+	–	–
5	F	5	c.404-2A > G	EOEE	0.25	focal (daily)	GTC (0.5 mo); spasms (3 y 4 mo)	3 y 1 mo-3 y 4 mo	normal	+	normal (1.5 mo)	+	GTC(daily)	VPA, CLB, ZNS, PER	diffuse polySWC, fast rhythm	<20	bedridden	–	–	–	–	–	gastrostoma
6	F	6	c.530A > G, p. Tyr177Cys	WS	3	focal (daily)	tonic (6 mo); spasms (11 mo)	–	normal	+	normal (6 mo)	+	spasms, tonic (weekly)	VPA, LEV, LTG	slow background, diffuse polySW	<20	rolling	chorea	poor	poor	–	–	NA
7	M	6	c.1648C > T, p. Arg550*, de novo	WS	0.75	focal (daily)	spasms (3 mo); tonic (6 mo)	–	bilateral temporooccipital spikes	+	chronic subdural hematoma (2 mo); normal (4 mo)	–	tonic (daily)	LEV, PB, CLB, LTG	NA	<20	bedridden	chorea	–	–	–	–	gastrostoma
8	F	37	c.2660_2669delinsCC, p.Ser887Thrfs*20, de novo	EOEE → WS	0.1	focal (daily)	spasms (3 mo); tonic	–	bilateral temporooccipital spikes	+	subdural effusion (4 mo)	+	tonic (monthly)	PHT, PB, CLB	bilateral occipital spikes	<20	bedridden	–	–	–	–	–	tube feeding, tracheotomy
9	F	5	c.65-2A > G, de novo	EOEE → WS	2	GTC (daily)	spasms (10 mo); tonic (16 mo)	3–9 mo	normal	+	normal (10 mo); mild cerebral atrophy (2 y)	+	tonic, focal, spasms (daily)	VPA, CLB, KD	diffuse polySWC, Fp1/Fp2 spikes, O1/O2 spikes	<20	sitting	chorea, HS	poor	–	–	–	–
10	F	7	c.99 + 5G > A, de novo	EOEE	0.75	GTC (daily)	focal (1 mo); spasms (2 y 3 mo)	–	normal	+	normal (3 y)	–	tonic, myoclonic (daily)	VPA, LTG, CBZ, CLB	irregular high voltage slow wave, multifocal spikes	<20	bedridden	–	poor	poor	–	–	–
11	F	20	c.1862_1877del, p. Arg621Metfs*4, de novo	WS → LGS	8	spasms (daily)	myoclonic, GTC, focal, absence (2 y); tonic (3 y)	–	hypsarrhythmia	+	normal (8 mo); mild cerebral atrophy (16 y)	–	myoclonic, tonic, atypical absence, focal, atonic (daily)	VPA, TPM, CLB, PER	diffuse polySWC	<20	rolling	HS	poor	poor	–	–	–
12	F	4	c.2046 + 1G > A, de novo	EOEE → WS	2	focal tonic (daily)	spasms (4 mo)	–	right central spikes	–	normal (23 mo)	–	focal, spasms (daily)	VPA, ZNS, TPM, PER	diffuse polySWC	<20	rolling	–	poor	poor	–	–	–
13	M	3	c.125A > G, p. Lys42Arg, de novo	WS	2	focal (daily)	spasms (4 mo)	–	left occipital sharp waves	+	normal (7 mo); severe cerebral atrophy (5 y)	–	spasms (daily)	VPA, LTG, LEV	Fp1/O1/O2 spikes	<20	bedridden	–	–	–	–	–	tube feeding
14	F	10 (Dead)	c.1591_1604del, p. Thr531Glnfs*2, de novo	WS	1	focal (daily)	spasms (4 mo); tonic (18 mo)	–	left of right occipital spikes	+	mild cerebral atrophy, crhonic subdural hematoma (3 mo); severe cerebral atrophy (10 y)	–	tonic, focal, spasms (daily)	CBZ, NZP, PB	diffuse SWC, O2 spikes	<20	bedridden	chorea, dystonia	–	–	–	–	sometimes tube feeding
15	F	6	c.587C > T, p. Ser196Leu, de novo	late infantile-onset epileptic encephalopathy	26	spasms (daily)	tonic (2 y 7 mo); atonic (3 y)	2 y 9 mo-3 y	normal	–	normal (1 y)	–	no seizure since 5y	STM, VPA	slow background, paroxysmal discharge(-)	<20	rolling	HS	poor	poor	–	–	–
16	M	3	c.528G > C, p. Trp176Cys, de novo	WS	0.75	myoclonic, GTC (daily)	spasms (3 mo)	–	multifocal spikes, polyspikes and sharp waves	+	frontal dominant cerebral atrophy, chronic subdual hematoma (5 mo); severe cerebral atrophy (5 y)	–	spasms (daily)	NA	multifocal spikes, sharp, O1/O2 spikes	<20	bedridden	–	–	–	–	–	tube feeding
17	M	16	c.587C > T, p. Ser196Leu, de novo	EOEE → WS	2	tonic (daily)	spasms (3 mo)	–	multifocal spike	+	mild cerebral atrophy, thin corpus callsum, lipoma above pineal body (9 y)	–	tonic (daily)	PRM, CLB, NZP	multifocal spikes	<20	bedridden	chorea	–	–	–	–	gastrostoma, tracheostomy
18	F	10	c.589_590del, p. Val197Glyfs*8, de novo	EOEE	1	focal (daily)	spasms (2 mo); tonic (4 mo)	–	left or right parietal polyspikes	–	normal (3 mo); mild cerebral atrophy (10 y)	–	focal, myoclonic, GTC (monthly)	LEV, CLB, CBZ, TPM	diffuse polySWC	<20	bedridden	–	poor	poor	–	–	NA

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19	F	4	c.2376_2376del, p.Val793Tyrfs*10, de novo	OS → WS	1	tonic (daily)	spasms (2 mo)	–	suppression-burst	+	normal (3 mo)	–	spasms, tonic (daily)	VPA, CZP, LCM	hypsarrhythmia, multifocal spikes, slow background	<20	bedridden	dystonia	poor	–	–	–	tube feeding, tracheotomy
20	F	4.9	c.1293delA, p.Lys432Serfs*61, de novo	EOEE	1	tonic (weekly)	clonic (2 mo)	–	multifocal spikes	–	normal (5 mo)	–	no seizure since 4 y 8 mo	VPA, LEV, LTG	δ waves in bilateral Fp	<20	sitting	–	poor	poor	+	–	tube feeding
21	M	17	c.419dupA, p.Asn140Lysfs*8, de novo	EOEE	0.75	myoclonic (daily)	spasms, tonic (3 wk)	–	multifocal spikes	–	severe cerebral atrophy (5 y)	–	spasms, tonic, focal (daily)	VPA, PB, LTG, PRM	multifocal spikes	<20	bedridden	chorea, dystonia	–	–	–	–	gastrostoma
22	F	5	c.1390C > T,p.Gln464*, de novo	EOEE	8	focal (daily)	spasms, tonic (14 mo)	–	frontal dominant slow waves, multifocal spikes and sharp waves	–	mild cerebral atrophy (3 y)	+	tonic, focal (daily)	VPA, CZP	multifocal spikes	<20	rolling	–	poor	poor	–	–	NA
23	M	2.7	c.2023_2026del, p.Phe675Ilefs*108, de novo	WS	0.9	spasms (daily)	focal (1 mo)	–	sharp waves in right hemisphere	+	hypomyelination, mild cerebral atrophy (4 mo)	–	spasms (daily)	VPA, LEV, VGB	hypsarrhythmia	<20	bedridden	–	–	–	–	–	–
24	F	16	IVS9 + 1G > A	WS	8	spasms (daily)	absence, myoclonic, tonic	–	hypsarrhythmia	+	normal (8 mo)	–	tonic, myoclonic (daily)	RUF, NZP, PER, STM	multifocal spikes, slow background	<20	walk alone	HS	poor	poor	+	babbling	–
25	F	1	c.404A > G, p.Asp135Gly	WS	2	focal (daily)	spasms (3 mo)	–	multifocal spikes	+	normal (2 mo)	NA	spasms (daily)	NA	hypsarrhythmia	NA	NA	–	NA	NA	NA	–	NA
26	F	3	c.64G > A, p.Gly22Arg, de novo	WS	2	tonic (daily)	spasms (6 mo)	–	left or right temporooccipital spikes	+	normal (2 mo); frontal dominant mild cerebral atrophy, mild delayed myelination (1 y)	–	focal (weekly)	PB, ZNS, CZP	spikes in right hemisphere	<20	bedridden	HS	poor	poor	+	–	NA
27	F	1	c.532C>,p.Arg178Trp, de novo	WS	4	focal (daily)	spasms (5 mo)	–	high voltage slow wave, Fz spikes	–	normal (1 y)	–	focal (daily)	TPM, VPA, CLB, LTG	left central dominant SWC, slow background	35–49	sitting	–	good	good	+	babbling	–
28	F	2	c.548 T > A, p.Leu183*, de novo	epilepsy with focal seizure	1	focal (daily)	spasms (3 mo)	–	occipital rhythmic slow wave, left frontal dominant SWC	–	normal (1 y)	–	focal, spasms (daily)	VPA, LTG, TPM	multifocal spikes (F7/F3/F4)	<20	bedridden	–	poor	poor	–	–	–
29	F	19	Del (exon5-21)	EOEE	8	tonic (daily)	focal (16 mo); photosensitivity	–	frontal dominat diffuse polySWC	–	mild cerebral atrophy (2 y)	+	focal (daily)	CBZ, CLB	multifocal SWC	<20	walk alone	–	good	good	+	–	–

Abbreviations: AED, antiepileptic drug; CBZ, carbamazepine; CLB, clobazam; CZP, clonazepam; EOEE, early-onset epileptic encephalopathy; F, female; GBP, gabapentin; GTC, generalized tonic-clonic; HS, hand stereotypies; KD, ketogenic diet; LCM, lacosamide; LEV, levetiracetam; LGS, Lennox-Gastaut syndrome; LTG, lamotrigine; M, male; mo, months; NA, not available; NZP, nitrazepam; OS, Ohtahara syndrome; PB, phenobarbital; PER, perampanel; PHT, phenytoin; PRM, primidone; RUF, rufinamide; STM, sulthiame; SWC, spike and wave complex; TPM, topiramate; VGB, vigabatrin; VPA, sodium valproate; wk, weeks; WS, West syndrome; y, years; ZNS, zonisamide; +, presence of the sign; -, absence of the sign

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epilepsy classifications of the International League Against Epilepsy 2017 [12]. The efficacy responder rate was determined as the extent to which the mean seizure number had reduced (seizure-freedom, ≥50% to < 100% seizure reduction, no effect, or aggravation). Very few treatments showed ≥ 50% to < 100% seizure reduction; thus, among the subjects with no effect, AEDs and additional treatments that had ≥ 25% to < 50% seizure reduction were also analyzed as having possible efficacy. Seizure-freedom was defined as the complete cessation of all types of seizures for 3 months from the time of the last onset. Patients experiencing a ≥ 50% increase in seizures compared with the seizure frequency before the initiation of drugs were considered aggravated.

All patients or parents provided written informed consent. The research protocol was approved by the Ethics Committees of Yokohama City University School of Medicine, Showa University School of Medicine, and NHO Nishiniigata Chuo Hospital.

2.1. Statistical analysis

The comparisons of clinical features and EEG and neuroimaging findings between males and females were performed using Mann–Whitney *U* test and Cochran–Armitage trend tests. Fisher's exact test was used to determine associations between categorical variables. Statistical significance was set at $p < 0.05$. IBM SPSS Statistics Ver.24.0 (IBM Corp., Armonk, New York, USA) was used for statistical calculations. Cochran–Armitage trend tests were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [13], which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander that was designed to add statistical functions frequently used in biostatistics.

3. Results

We identified 29 patients (21 females, eight males) with severe infantile-onset epileptic encephalopathy, including Ohtahara syndrome, West syndrome, or Lennox–Gastaut syndrome. Five patients had been included in previously published papers (patients 1, 9, and 14 [14]; patient 2 [15]; patient 21 [16]; and patient 29 [17]). The median age at ascertainment was 6.0 years (range, 1–37 years).

3.1. Clinical features of CDD

Clinical information, including epileptic syndrome, seizure type, and EEG and MRI findings of patients with pathogenic *CDKL5* variants, is summarized in Table 1. A comparison of key differences between females and males with CDD is shown in Table 2.

At ascertainment, all patients showed severe developmental delays and none could speak any meaningful words. The developmental level at the last evaluation is shown in Table 2. Compared with females, males had lower levels of attained motor development ($p = 0.032$). The ability to sit or walk was acquired only by female patients, and all male patients except one with a mosaic variant were bedridden. Eye fixation, eye contact, and functional hand use were poor or absent in most patients, especially in males. Involuntary movements were observed in 15 patients (52%), with hand stereotypies in eight (28%; seven females, one male), chorea in seven (24%; three females, four males), and dystonia in three (10%; two females, one male). Gastrostomy or tube feeding was required in 11 patients (five females, six males) and tracheostomy was performed in three patients (two females, one male). Patients with early epilepsy onset (under the age of 1 month) showed a more severe developmental outcome and required more gastrostomy or tube feeding than those with epilepsy onset over the age of 2 months. Patient 14 had a respiratory disturbance caused by upper airway obstruction and suddenly died at the age of 10 years.

3.2. Type of epilepsy and EEG findings

West syndrome was the most common type of epileptic syndrome in patients with pathogenic *CDKL5* variants, occurring in 19 patients (12 females, seven males). Four of 13 patients with unclassified early-onset epileptic encephalopathy (EOEE) showed the transition from EOEE to West syndrome. Two patients had Ohtahara syndrome and one had epilepsy with focal seizure or late infantile-onset epileptic encephalopathy. The median age of epilepsy onset was 2 months (range, 4 days to 26 months); in approximately two-thirds of patients ($n = 20$), seizures first appeared under 3 months of age.

Seizure types at the onset of epilepsy are shown in Table 2. These initial seizures were seen on a daily basis in all except one patient. At the onset, interictal EEG was normal in seven patients (six females, one male), and multifocal spikes were observed in six patients and occipital spikes or sharp waves were observed in five patients. Hypsarrhythmia was observed in 19 patients (Table 2), all of whom had spasms. All patients except two had spasms, which developed between 2 and 11 months of age in 21 patients and followed initial seizures including focal or tonic seizures in 19 patients. Following the onset of seizures, only four female patients had a period of more than 1 month of seizure freedom (referred to as a "honeymoon period"). Of these, the median age at onset of the honeymoon period was 19.8 months (range, 3–31 months), and the median duration was 4 months (range, 3–7 months). Regression after the onset of intractable epilepsy occurred in seven patients (26%), who were all female (Table 2).

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Table 2
Comparison of the key findings between females and males with CDKL5 deficiency disorder.

	Female (n = 21)	Male (n = 8)	p Value
Age at last evaluation, year, median (range)	6.0 (1–37)	8.5 (2–26)	0.649[a]
Age at seizure onset, months, median (range)	2.0 (0.1–26)	1.0 (0.75–5)	0.168[a]
Initial seizure type, n (%)			
focal	11/21 (52)	2/8 (25)	0.183
tonic	4/21 (19)	3/8 (38)	0.282
spasms	4/21 (19)	1/8 (13)	0.575
GTC	2/21 (10)	2/8 (25)	0.300
myoclonic	0/21 (0)	2/8 (25)	0.069
Spasms, n (%)	21/21(100)	6/8 (75)	0.069
Honeymoon period, n (%)	4/21 (19)	0/8 (0)	0.252
Seizure frequency at last evaluation, n (%)			
daily	15/21 (76)	8/8 (100)	0.123[b]
weekly	2/21 (5)	0/8 (0)	
monthly	2/21 (10)	0/8 (0)	
seizure-free	2/21 (10)	0/8 (0)	
Involuntary movement, n (%)	10/21 (48)	5/8 (63)	0.383
chorea	3/21 (14)	4/8 (50)	0.068
dystonia	2/21 (10)	1/8 (13)	0.636
handstereotypies	7/21 (38)	1/8 (13)	0.264
Development at last evaluation, n (%)			
bedridden	8/20 (40)	7/8 (88)	0.032[b]
rolling	7/20 (35)	1/8 (13)	
sitting	3/20 (15)	0/8 (0)	
walk alone	2/20 (10)	0/8 (0)	
Regression, n (%)	7/20 (35)	0/7 (0)	0.087
No fix and follow, n (%)	4/20 (8)	6/8 (75)	0.011
No eye contact, n (%)	6/20 (12)	6/8 (75)	0.040
No functional hand use, n (%)	14/20 (70)	7/8 (88)	0.327
No language, n (%)	19/21 (90)	8/8 (100)	0.517
Need for tube feeding, n (%)	5/17 (24)	6/8 (75)	0.043
Need for tracheostomy, n (%)	2/17 (12)	1/8 (13)	0.704
EEG			
Hypsarrhythmia, n (%)	13/21 (62)	6/8 (75)	0.419
Brain MRI at last evaluation, n (%)			
normal	13/21 (62)	2/8 (25)	0.017[b]
mild atrophy	6/21 (29)	3/8 (38)	
severe atrophy	1/21 (5)	3/8 (38)	

Abbreviations: GTC, generalized tonic-clonic
p Values were calculated usign Fisher's exact test; not otherwise mentioned.
[a] Mann–Whitney *U* test
[b] Cochran–Armitage trend test

Seizure types at the last evaluation were focal seizures in 13 patients, tonic seizures in 14, and spasms in 12; two-thirds of patients had two or more types of seizures. Only two female patients (7%) were seizure-free. One had late-onset epileptic seizures that first began after the age of 2 years, and another had not experienced spasms. In contrast, all of the male patients had everyday seizures (Table 2).

3.3. Epilepsy treatment

The efficacy of each AED is shown in Fig. 1. Sodium valproate (VPA) was used most often, followed by zonisamide (ZNS), carbamazepine (CBZ), clobazam (CLB), lamotorigine (LTG), and levetiracetam (LEV). No drugs were effective at achieving a seizure-free status by monotherapy. LTG achieved seizure-freedom for tonic seizure in one patient, and VPA and vigabatrin (VGB) achieved a $\geq$ 50% reduction in seizure frequency in two and one patients, respectively. VPA was effective for spasms and tonic seizure, LTG was effective for tonic seizure, and VGB was effective for spasms and focal seizure. Because very few AEDs were effective using the 50% reduction criteria, we further evaluated each AED for $\geq$ 25% to $<$ 50% seizure reduction, to identify AEDs with possible efficacy. AEDs that achieved a $\geq$ 25% to $<$ 50% reduction in seizure frequency were VPA (patient numbers with partial efficacy/total numbers of treated patients, 12/28), topiramate (TPM; 10/20), clonazepam (CZP; 9/19), sulthiame (STM; 3/4), perampanel (PER; 3/3), and lacosamide (LCM; 1/1); however, these results of $\geq$ 25% to $<$ 50% seizure reduction are likely

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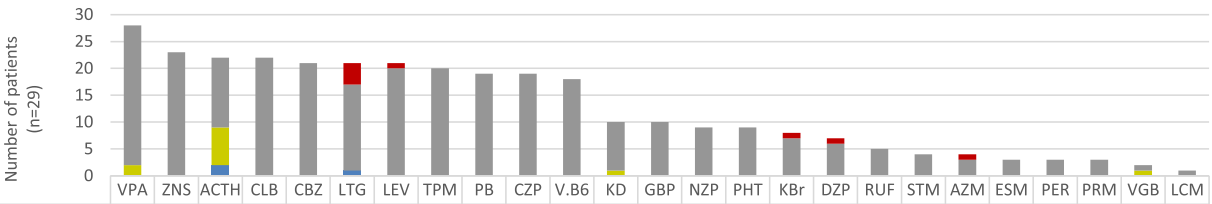

	VPA	ZNS	ACTH	CLB	CBZ	LTG	LEV	TPM	PB	CZP	V.B6	KD	GBP	NZP	PHT	KBr	DZP	RUF	STM	AZM	ESM	PER	PRM	VGB	LCM
aggravation	0	0	0	0	0	4	1	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0
no effect	26	23	13	22	21	16	20	20	19	19	18	9	10	9	9	7	6	5	4	3	3	3	3	1	1
≥50% to <100% reduction	2	0	7	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
seizure-freedom	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50% responder rate (%)	7	0	41	0	0	5	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	50	0

Fig. 1. Effectiveness of antiepileptic drugs and additional treatments for epilepsy in patients with CDKL5 deficiency disorder. Abbreviations: ACTH, Adrenocorticotrophic hormone; AZM, acetazolamide; CBZ, carbamazepine; CLB, clobazam; CZP, clonazepam; DZP, diazepam; ESM, ethosuximide; GBP, gabapentin; KBr, potassium bromide; KD, ketogenic diet; LCM, lacosamide; LEV, levetiracetam; LTG, lamotrigine; NZP, nitrazepam; PB, phenobarbital; PER, perampanel; PHT, phenytoin; PRM, primidone; RUF, rufinamide; STM, sulthiame; TPM, topiramate; V.B6, vitamin B6; VGB, vigabatrin; VPA, sodium valproate; ZNS, zonisamide

not clinically significant and their efficacy is low. In addition, in one patient with late-onset epileptic seizures, seizures were controlled by a combination of VPA and STM a long time after starting both AEDs. Conversely, CBZ (3/18), gabapentin (1/10), phenytoin (PHT; 1/9), potassium bromide (KBr; 0/8), and vitamin B6 (1/17) were less effective. LTG (4/21), acetazolamide (AZM; 1/4), diazepam (DZP; 1/7), KBr (1/8), and LEV (1/21) were reported as aggravating epileptic seizures.

Adrenocorticotrophic hormone (ACTH) therapy was performed in 22 patients and was the most effective of all treatments; however, its effects were short-lived. Ten patients received a ketogenic diet (KD), but this was ineffective in seven (four females, three males). However, KD was remarkably effective in one female patient, reducing the number of seizures by half. One patient withdrew from the KD because of adverse side effects. Total corpus callosotomy was performed in three patients; however, none of the patients achieved $\geq$ 50% reduction in seizure frequency. Two of the three patients showed possible efficacy, with a $\geq$ 25% reduction in seizure frequency, but this change was not clinically significant. In addition, three patients received VNS therapy, but this did not have a clinically significant effect on seizure frequency. The number of AEDs at the last visit was four in 12 patients, three in 10, and two in three (mean, 3.2 drugs); KD was continued for over 2 years in one patient.

3.4. Neuroimaging

Brain MRI findings shown in Table 2 revealed cerebral atrophy in 13 patients (mild in nine, severe in four). MRI was normal in 17 patients in infancy, but four patients later showed mild cerebral atrophy. Severe brain atrophy was seen in one female (5%) and three males (38%). Compared with females, males had more severe cerebral atrophy on MRI ($p = 0.017$, Table2). Other MRI findings included chronic subdural hematoma in three patients in the early infantile period.

4. Discussion

In our cohort of 29 individuals, we identified 28 different variants, and only one recurrent variant (c.587C > T) occurred in two patients. To date, numerous pathogenic *CDKL5* variants have been reported, including nonsense variants, truncating variants, and deletions [7,18]. However, the correlation between genotype and clinical phenotype is limited [19].

Pathogenic variants in *CDKL5* have been associated with X-linked disorders, and were initially reported in female patients with infantile spasms and atypical Rett syndrome [1,20]. These expanded the clinical phenotypes to include developmental and epileptic encephalopathy [4,21]. The disease course of male patients usually has a more severe phenotype than that of female patients [22,23]. In previous studies, most of the affected individuals were female and the proportion of male patients was around 10% [24]; however, 28% of our subjects were male. This difference likely reflects cohort differences. For our cohort, we recruited patients with developmental and epileptic encephalopathy, regardless of their sex. As described previously, *CDKL5* variants were initially analyzed in female patients with West syndrome or atypical Rett syndrome, so our findings might reflect a more precise *CDKL5* variant sex ratio underlying developmental and epileptic encephalopathy.

In this study, non-epileptic involuntary movements were observed in approximately half of patients. In particular, hand stereotypies, one of the main criteria for Rett syndrome [2], are a relatively common symptom in patients with CDD [4]. They were observed in 28% of our patients, which is less than the 75.3% reported in a larger study [4]. Because a smaller proportion of males than females manifested hand stereotypies [4], this

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difference in our study might reflect the different sex ratios between previous studies and the present investigation. In addition, this difference also likely reflects cohort differences as mentioned above. In our series, approximately one-third of patients also showed other involuntary movements such as chorea and dystonia. Several genes responsible for epileptic encephalopathy are associated with hyperkinetic movements [14]. However, because no large study has investigated the association of other involuntary movements with *CDKL5* deficiency, especially in male patients, further work is required to understand the comorbidity of CDD.

Patients with CDD generally show severe global developmental delay and intellectual disability, with many unable to achieve independent walking and speech [6,7]. Around 20% of CDD patients with epilepsy were reported to achieve independent walking [6], compared with only 7% (n = 2) in our study (Table 1). In our series, male patients with CDD tended to be more severely affected than females. Compared with females, males had lower levels of attained motor development. Male patients were not able to walk or sit, and eye fixation and eye contact were not observed in most. The average age at seizure onset was also slightly earlier in males than females, and the honeymoon period was only observed in females. Additionally, a few females achieved seizure-free status or had monthly seizures at their last evaluation, whereas all males were having everyday seizures. These results are mostly consistent with previous studies [4,7]. Furthermore, none of the males regressed in our study, possibly reflecting more serious developmental impairment.

Recently, precision medicine has been proposed as a therapy for genetic epileptic encephalopathy, so the identification of specific treatment for CDD is important. Although the CDD phenotype has been described, seizures are usually intractable to treatment with several AEDs and to date no disease-specific antiepileptic treatment protocol for CDD has been established. Our study also did not identify an effective therapy for CDKL5-related encephalopathy. Of the AEDs used, VPA, VGB, and LTG were effective in a small number of patients. Moseley et al. [25] found that TPM and VGB were effective in three and two out of six patients, respectively, and another study of 39 patients with pathogenic *CDKL5* variants found a 50% responder rate to at least one AED of 69% at 3 months, and 45% at 6 months, falling to 24% at 12 months [26]. Medications with the highest rates of seizure reduction at 3 months included felbamate, VGB, CLB, VPA, steroids, LTG, and ZNS [26]. In addition, the combination of VPA and STM resulted good seizure control in one patient with late-onset epileptic seizures in our study. The combination of VGB and ZNS has also been shown to elicit a response in some patients [27]. In our study, only two patients used VGB, and this was effective in one patient. Felbamate is not approved in Japan. ACTH was the most effective treatment in our study, but its effects were only temporary. These results are fairly consistent with a previous study [26]. KD was reported to reduce seizure frequency in a previous small study [25], but there was a lack of long-term efficacy [26,28]. In our study, KD did not improve seizure frequency in most patients, but it was remarkably effective in one patient, reducing seizures by half. Recently, rational combination therapy for refractory epilepsy has been proposed, but because no specific monotherapy for CDD is yet established, appropriate polytherapy with AEDs or other combination treatments such as ACTH, KD, and AEDs should be investigated.

In our study, total corpus callosotomy and VNS were performed in three patients, respectively; however, none of these patients showed seizure reduction. In a previous study regarding VNS for CDD, improvements in seizure control were reported in over two-thirds of all patients, including reductions in the frequency, duration and intensity of seizures [29]. In contrast, although the results of corpus callosotomy for CDD have not been reported, corpus callosotomy represents an important therapeutic option for patients with West syndrome without resectable MRI lesions [30]. Further studies are required to explore the therapeutic potential of surgical interventions for CDD.

Muller et al. [26] previously reported that one-third of patients with pathogenic *CDKL5* variants experienced seizure aggravation in response to at least one AED. In their study, LEV, CBZ, and LTG led to aggravation, which compares with LTG (4/21) and LEV (1/21), KBr (1/8), DZP (1/7), and AZM (1/4) in our study. Although seizure aggravation was seen in 14% of our patients, which is less frequent than in the study by Muller, several AEDs including LTG and LEV may aggravate seizures in patients with pathogenic *CDKL5* variants. Although LTG was taken by a high number of patients with aggravated seizures, one patient achieved successful seizure control with LTG. Additionally, aggravation by DZP was observed in one patient. This finding might just be a coincidence; however, it has previously been reported that benzodiazepines are correlated with the precipitation of both tonic-like microseizures in patients with West syndrome and tonic status epilepticus in patients with Lennox–Gastaut syndrome [31]. These results regarding seizure aggravation in our study are not definitive; the number of cases was small, and interactions with other AEDs or additional treatments, including ACTH, might also affect seizure aggravation. Nonetheless, we propose that ineffective overtreatment should be avoided to reduce the occurrence of adverse effects in patients with CDD [26].

A honeymoon period with seizure cessation has previously been described in children with pathogenic *CDKL5* variants [21]. In another study, almost half of

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all children with CDD had a honeymoon period with a median onset of 2 years (range, 2 months–11 years) and a median duration of 6 months [6]. In contrast, only four (14%) of our patients had a honeymoon period (median onset, 19.8 months [range, 3–31 months]; median duration, 4 months). The reason for this is unknown. No study has identified a relationship between the honeymoon period and the severity of epilepsy and developmental delay. However, our patients did experience more severe developmental outcomes than those in the other study.

Brain MRI findings in patients with CDD have been reported to be normal or cortical atrophy with frontal dominance [5,8,21]. Some studies also described T2/FLAIR high intensity in the posterior white matter [5,21]. We observed cerebral atrophy on neuroimaging in around half of our patients. All four of our patients with severe brain atrophy showed a more severe developmental outcome with involuntary movements and required gastrostomy or tube feeding; furthermore, one of them died. In addition, significantly more male patients than female patients showed mild or severe brain atrophy. Hyperintensities in the white matter were not found in our series. Neuroimaging in our patients showed nonspecific findings, and the degree of brain atrophy appeared to reflect disease severity.

In conclusion, our study describes clinical manifestations and treatments of epilepsy in Japanese patients with pathogenic *CDKL5* variants. Our patients showed more severe global developmental delay than those in a previous study and had intractable epilepsy, both likely because of the higher percentage of male patients. Because no specific therapy for CDD has been established, further studies are needed to investigate appropriate therapies, which could include AED polytherapy or specific combination treatment such as ACTH, KD, and AEDs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] Weaving LS, Christodoulou J, Williamson SL, Friend KL, McKenzie OLD, Archer H, et al. Mutations of *CDKL5* cause a severe neurodevelopmental disorder with infantile spasms and mental retardation. Am J Hum Genet 2004;75(6):1079–93. https://doi.org/10.1086/426462.

[2] Neul JL, Kaufmann WE, Glaze DG, Christodoulou J, Clarke AJ, Bahi-Buisson N, et al. Rett syndrome: revised diagnostic criteria and nomenclature. Ann Neurol 2010;68(6):944–50. https://doi.org/10.1002/ana.22124.

[3] Guerrini R, Parrini E. Epilepsy in Rett syndrome, and CDKL5- and FOXG1-gene–related encephalopathies. Epilepsia 2012;53:2067–78.

[4] Fehr S, Wilson M, Downs J, Williams S, Murgia A, Sartori S, et al. The CDKL5 disorder is an independent clinical entity associated with early-onset encephalopathy. Eur J Hum Genet 2013;21(3):266–73. https://doi.org/10.1038/ejhg.2012.156.

[5] Bahi-Buisson N, Kaminska A, Boddaert N, Rio M, Afenjar A, Gérard M, et al. The three stages of epilepsy in patients with CDKL5 mutations. Epilepsia 2008;49:1027–37.

[6] Fehr S, Wong K, Chin R, Williams S, de Klerk N, Forbes D, et al. Seizure variables and their relationship to genotype and functional abilities in the CDKL5 disorder. Neurology 2016;87(21):2206–13. https://doi.org/10.1212/WNL.0000000000003352.

[7] Fehr S, Leonard H, Ho G, Williams S, de Klerk N, Forbes D, et al. There is variability in the attainment of developmental milestones in the CDKL5 disorder. J Neurodevelop Disord 2015;7(1):2. https://doi.org/10.1186/1866-1955-7-2.

[8] Liang JS, Shimojima K, Takayama R, Natsume J, Shichiji M, Hirasawa K, et al. CDKL5 alterations lead to early epileptic encephalopathy in both genders. Epilepsia 2011;52:1835–42.

[9] Yamamoto T, Shimojima K, Kimura N, Mogami Y, Usui D, Takayama R, et al. Recurrent occurrences of *CDKL5* mutations in patients with epileptic encephalopathy. Hum Genome Var 2015;2:15042. https://doi.org/10.1038/hgv.2015.42.

[10] Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, et al. ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. Epilepsia 2017;58(4):512–21. https://doi.org/10.1111/epi.13709.

[11] Aoi H, Mizuguchi T, Ceroni JR, Kim VEH, Furquim I, Honjo RS, et al. Comprehensive genetic analysis of 57 families with clinically suspected Cornelia de Lange syndrome. J Hum Genet 2019;64(10):967–78. https://doi.org/10.1038/s10038-019-0643-z.

[12] Fisher RS, Cross JH, French JA, Higurashi N, Hirsch E, Jansen FE, et al. Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. Epilepsia 2017;58(4):522–30. https://doi.org/10.1111/epi.13670.

[13] Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant 2013;48(3):452–8. https://doi.org/10.1038/bmt.2012.244.

[14] Kobayashi Yu, Tohyama J, Kato M, Akasaka N, Magara S, Kawashima H, et al. High prevalence of genetic alterations in early-onset epileptic encephalopathies associated with infantile movement disorders. Brain Dev 2016;38(3):285–92. https://doi.org/10.1016/j.braindev.2015.09.011.

[15] Kodera H, Kato M, Nord AS, Walsh T, Lee M, Yamanaka G, et al. Targeted capture and sequencing for detection of mutations causing early onset epileptic encephalopathy. Epilepsia 2013;54(7):1262–9. https://doi.org/10.1111/epi.12203.

Please cite this article in press as: Kobayashi Y et al. Clinical manifestations and epilepsy treatment in Japanese patients with pathogenic *CDKL5* variants. Brain Dev (2021), https://doi.org/10.1016/j.braindev.2020.12.006

[16] Akamine S, Ishizaki Y, Sakai Y, Torisu H, Fukai R, Miyake N, et al. A male case with *CDKL5*-associated encephalopathy manifesting transient methylmalonic academia. Eur J Med Genet 2018;61:451–4.

[17] Saitsu H, Osaka H, Nishiyama K, Tsurusaki Y, Doi H, Miyake N, et al. A girl with early-onset epileptic encephalopathy associated with microdeletion involving *CDKL5*. Brain Dev 2012;34(5):364–7. https://doi.org/10.1016/j.braindev.2011.07.004.

[18] Hector RD, Kalscheuer VM, Hennig F, Leonard H, Downs J, Clarke A, et al. *CDKL5* variants : Improving our understanding of a rare neurologic disorder. Neurol Genet 2017;3(6):e200. https://doi.org/10.1212/NXG.0000000000000200.

[19] Olson HE, Demarest ST, Pestana-Knight EM, Swanson LC, Iqbal S, Lal D, et al. Cyclin-Dependent Kinase-Like 5 Deficiency Disorder: Clinical Review. Pediatr Neurol 2019;97:18–25. https://doi.org/10.1016/j.pediatrneurol.2019.02.015.

[20] Kalscheuer VM, Tao J, Donnelly A, Hollway G, Schwinger E, Kübart S, et al. Disruption of the serine/threonine kinase 9 gene causes severe X-linked infantile spasms and mental retardation. Am J Hum Genet 2003;72(6):1401–11. https://doi.org/10.1086/375538.

[21] Bahi-Buisson N, Nectoux J, Rosas-Vargas H, Milh M, Boddaert N, Girard B, et al. Key clinical features to identify girls with CDKL5 mutations. Brain 2008;131:2647–61.

[22] Elia M, Falco M, Ferri R, Spalletta A, Bottitta M, Calabrese G, et al. *CDKL5* mutations in boys with severe encephalopathy and early-onset intractable epilepsy. Neurology 2008;71(13):997–9. https://doi.org/10.1212/01.wnl.0000326592.37105.88.

[23] Liang JS, Huang H, Wang JS, Lu JF. Phenotypic manifestations between male and female children with *CDKL5* mutations. Brain Dev 2019;41(9):783–9. https://doi.org/10.1016/j.braindev.2019.05.003.

[24] Bahi-Buisson N, Bienvenu T. *CDKL5*-related disorders: from clinical description to molecular genetics. Mol Syndromol 2011;2:137–52.

[25] Moseley BD, Dhamija R, Wirrell EC, Nickels KC. Historic, clinical, and prognostic features of epileptic encephalopathies caused by *CDKL5* mutations. Pediatr Neurol 2012;46(2):101–5. https://doi.org/10.1016/j.pediatrneurol.2011.11.007.

[26] Müller A, Helbig I, Jansen C, Bast T, Guerrini R, Jähn J, et al. Retrospective evaluation of low long-term efficacy of antiepileptic drugs and ketogenic diet in 39 patients with *CDKL5*-related epilepsy. Eur J Paediatr Neurol 2016;20(1):147–51. https://doi.org/10.1016/j.ejpn.2015.09.001.

[27] Melikishvili G, Epitashvili N, Tabatadze N, Chikvinidze G, Dulac O, Bienvenu T, et al. New insights in phenomenology and treatment of epilepsy in CDKL5 encephalopathy. Epilepsy Behav 2019;94:308–11. https://doi.org/10.1016/j.yebeh.2019.02.013.

[28] Lim Z, Wong K, Olson HE, Bergin AM, Downs J, Leonard H. Use of the ketogenic diet to manage refractory epilepsy in CDKL5 disorder: Experience of >100 patients. Epilepsia 2017;58:1415–22.

[29] Lim Z, Wong K, Downs J, Bebbington K, Demarest S, Leonard H. Vagus nerve stimulation for the treatment of refractory epilepsy in the CDKL5 deficiency disorder. Epilepsy Res 2018;146:36–40. https://doi.org/10.1016/j.eplepsyres.2018.07.013.

[30] Baba H, Toda K, Ono T, Honda R, Baba S. Surgical and developmental outcomes of corpus callosotomy for West syndrome in patients without MRI lesions. Epilepsia 2018;59(12):2231–9. https://doi.org/10.1111/epi.14594.

[31] Guerrini R, Belmonte A, Genton P. Antiepileptic drug-induced worsening of seizures in children. Epilepsia 1998;39(s3):S2–S10. https://doi.org/10.1111/j.1528-1157.1998.tb05118.x.

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