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Original article

Phenotypic manifestations between male and female children with *CDKL5* mutations

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Abstract

Background: Cyclin-dependent kinase-like 5 (*CDKL5*), which maps to chromosome Xp22.13 and contains 20 coding exons, has been recognized as the gene responsible for early-onset epileptic encephalopathy (EoEE). A retrospective study is carried out to analyze potential genotypic and phenotypic differences between male and female patients with *CDKL5* mutations.

Materials and methods: Targeted next-generation DNA sequencing was employed to search for mutations in patients with cryptogenic EE. A total of 44 patients with EoEE/infantile spasms (ISs)/West syndrome were enrolled for pathogenic mutation screening. The clinical phenotypes of patients with *CDKL5* mutations were analyzed and compared with those of 166 published cases.

Results: One novel and three recurrent mutations were found in four enrolled patients (two boys and two girls). One female patient had partial seizures during the early infantile period and epileptic spasms and tonic seizures several weeks thereafter. The other female patient had IS with hypsarrhythmia. The two male patients had IS without typical hypsarrhythmia and were bedridden. Brain MRIs of the male patients revealed brain atrophy and white matter hyperintensity. The female patients exhibited autistic features with hand stereotypies.

Conclusion: Our study highlights that both girls and boys with IS harbor *CDKL5* mutations. Male children with *CDKL5* mutations demonstrate a higher frequency of infantile spasms and brain atrophy, whereas female children often exhibit atypical Rett syndrome with EoEE. In addition, male children have a more severe phenotype than female children.

Keywords: *CDKL5*; Epileptic encephalopathy; Infantile spasms; Phenotype

1. Introduction

Cyclin-dependent kinase-like 5 (*CDKL5*) mutations have been found in patients with early-onset epileptic encephalopathy (EoEE) or the Hanefeld variant of Rett syndrome with early-onset seizures; these seizures often take the form of spasms with or without hypsarrhythmic electroencephalogram (EEG) [1,2,8,29,30]. The core features of the *CDKL5* mutation-associated phenotype are early-onset seizures, severe developmental delay with hypotonia, and impaired communication skills [4]. Seizure types are polymorphous, but infantile spasms (ISs) have often been observed [1,3,13,26]. However, few studies have focused on the differences in epileptic phenotypes and clinical features between male and female patients with *CDKL5*-related EE [6,13,14].

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Recently, advances in next-generation DNA sequencing (NGS) technology have facilitated the use of direct DNA sequencing in EE research. Consequently, more patients with *CDKL5* mutations have been identified using either targeted NGS or whole exome sequencing through genetic testing [14,34]. The present study analyzed the clinical features of male and female patients with *CDKL5* mutations, and the phenotypic characteristics of male and female patients with *CDKL5* mutations were analyzed in detail. We conducted a systematic literature review of patients with *CDKL5* mutations reported in the last 10 years. We statistically analyzed and summarized the findings genetically and phenotypically. Future challenges and perspectives in *CDKL5*-associated EE were also discussed.

2. Materials and methods

2.1. Targeted next generation sequencing

This study was approved by the Ethics Committee of Far Eastern Memorial Hospital, Taiwan (Approval Number: 105142F), and written consent was obtained from all parents of the enrolled children. A total of 44 Taiwanese children (19 boys and 25 girls) with EoEE, IS, or West syndrome (WS) were enrolled for targeted NGS or conventional DNA sequencing of the *CDKL5* gene for mutational screening. A targeted NGS panel comprising 90 genes related to EE was custom designed into a HaloPlex probe capture library for target enrichment, followed by sequencing on an Illumina Miseq platform (Illumina, Inc. San Diego, CA, USA). The obtained NGS sequencing data were verified and aligned against the Ensembl human reference genome database according to a standard analytical pipeline, and the sequence variants were determined using a Genome Analysis Toolkit, followed by further analyses using Illumina VariantStudio 3.0 software (Illumina, Inc.). All sequence variants in *CDKL5* of probands and their families, if available, were confirmed through Sanger sequencing. Their clinical features were systematically reviewed and summarized.

2.2. Statistical analysis

To further investigate the differences in clinical symptoms between male and female patients with *CDKL5* mutations, we pooled cases from 33 published reports on children with *CDKL5* mutations who were aged 0–18 years [1–21,23–30,32–35], in addition to the patients with *CDKL5* mutations identified in our study. Both genetic and clinical data were extracted and statistically analyzed using SPSS 20.0 software (IBM, Armonk, NY, USA). Categorical variables were compared using the Chi-square test or Fisher's exact test if the expected value was <5. A p value <0.05 was considered statistically significant.

3. Results

3.1. Molecular characteristics

A total of 43 cryptogenic EoEE and IS/WS patients were analyzed using targeted NGS with a panel of 90 genes related to EE, with an average read depth of 274 ± 57× (ranging from 181 to 378×). As shown in Fig. 1, identified *CDKL5* mutations included a 2-bp deletion (NM_003159.2(CDKL5_v001): c.2635_2636delCT (p.Leu879Glufs*30)), a base substitution (NM_003159.2 (CDKL5_v001): c.1670C>G (p.Ser557*)), and a 1-bp insertion (NM_003159.2(CDKL5_v001): c.849dupT (p.Asp284*)). In addition, a female patient with IS was selected for *CDKL5* mutational analysis using conventional Sanger sequencing, which revealed a base substitution leading to a nonsense mutation (NM_003159.2 (CDKL5_v001): c.1519C>T (p.Gln507*)). *CDKL5* mutations were identified in four patients (two boys and two girls), accounting for 9.1% of the examined Taiwanese cases of EoEE/IS/WS; these mutations all resulted in the premature termination of CDKL5 with or without extra noncanonical amino acid sequences. All identified mutations were *de novo* mutations, except for c.1670C>G, for which the DNA samples from family members were not available for analysis. Only the c.849dupT mutation was a novel mutation; the other mutations have been reported as pathogenic in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/). All identified mutations fulfilled the "pathogenic" variant standard and guideline proposed by the American College of Medical Genetics and Genomics [22].

3.2. Phenotypic analyses of CDKL5-associated encephalopathy

Of the 44 individuals studied, 4 nonrelated patients had pathogenic *CDKL5* mutations. Their clinical data are listed in Table 1. All patients were born at full term following an uneventful delivery. In all patients, birth head circumference was within the normal range for gestational age, and the perinatal period was uneventful.

3.3. Onset of symptoms and epilepsy

In all patients, the first epileptic seizures started within the first 3 months of life. The initial seizures were partial, and all patients had epileptic spasms. Three of the patients had hypsarrhythmia or modified hypsarrhythmia on EEG. All patients had intractable seizures, and one female patient (case #1) received a callosotomy. Her seizure frequency decreased initially but returned to

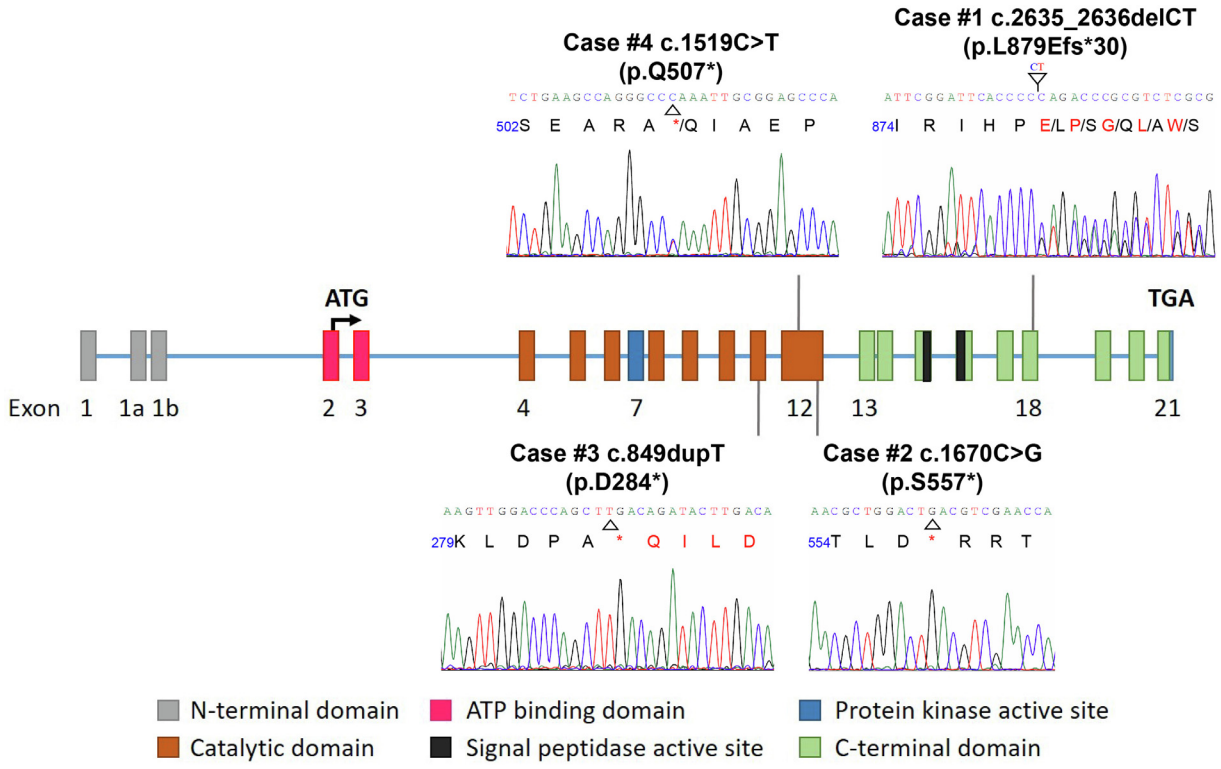

Fig. 1. A schematic representation of the *CDKL5* gene (NM_003159.2 (CDKL5_v001); protein_id: NP_003150.1) and Sanger DNA sequencing from probands. The encoding amino acids for the reference and mutant sequences are shown in black and red, respectively. DNA sequencing results from parents are provided in Fig. S1 of the supplementary material. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the presurgical state after 6 months. However, the severity of her seizures improved after the callosotomy. At the age of seizure onset, all patients presented with hypotonia.

3.4. Neurological phenotype

None of the patients showed a period of apparent normal development. The female patients showed progress, although their development remained significantly delayed, and no individual demonstrated regression after 6 months. At the final evaluation, both male patients were bedridden. By contrast, the female patients had developed head control and were able to sit without support. Hand stereotypies were evident only in the female patients with *CDKL5* mutations, but autistic features were found in all enrolled female patients and in one male patient (case #2). Brain images of the female patients were negative; however, MRI of both male patients revealed brain atrophy or ventriculomegaly with white matter hyperintensity.

3.5. Statistical analysis

In the literature, we identified a total of 170 cases from 33 studies (2003–2017), including the 4 cases with *CDKL5* mutations in the present study. A total of 139 cases involved female patients and 31 involved male patients. The average ages of the girls and boys involved were 81.0 months and 62.4 months, respectively. The mutation types showed significantly different distributions between the girls and boys. Frameshift mutations (44/139, 31.7%) were the predominant mutation type among the girls, whereas missense mutations (11/31, 35.5%) were the predominant mutation type among the boys ($p = 0.003$). No significant difference was found in the mutation localization, as the regard of either exon or protein domain distribution, between the girls and boys. In addition, no statistically significant association between the mutation localization and seizure onset or the severity of development delay was observed (data not shown). Majority of the mutations were localized to the catalytic domain followed by C-terminal domain of CDKL5. It is also noteworthy that only one (1/31; 3.2%) boy but 25 (25/139; 18.0%) girls carry splicing mutations.

The boys and girls also exhibited significant differences in their clinical symptoms. A higher proportion of the boys showed brain atrophy, as revealed by MRI, than the girls (9/17, 52.9% vs. 18/70, 25.7%; $p = 0.041$). Although EoEE was the most common diagnosis in both male and female children, atypical Rett syndrome was more common in the female children (40/132, 30.3%), whereas WS/IS was more common in

Table 1
Clinical features and identified *CDKL5* (NM_003159.2 (CDKL5_v001); protein_id: NP_003150.1) mutations in the studied cases.

Case No.	1	2	3	4
Gender	F	M	M	F
Age	5 y	4 y	10 y	5 y
Diagnosis	Han-RTT	WS	WS	WS
Mutation	**c.2635_2636delCT**	**c.1670C>G**	**c.849dupT**	**c.1519C>T**
AA change	**p.Leu879Glufs*30**	**p.Ser557***	**p.Asp284***	**p.Gln507***
Exon	18	12	11	12
De novo/Novel	Yes/No	ND/No	Yes/Yes	Yes/No
Microcephaly	–	+	–	–
Seizure onset	2m	3m	3m	2m
Initial seizures types	Partial seizure (tonic and myoclonic jerk with eye blinking)	Partial seizure	Partial seizure (tonic and myoclonic jerk with eye blinking)	Partial seizure (clonic and myoclonic seizure)
Tonic seizures	+	+	+	+
Epileptic spasms	+	+	+	+
Prognosis	Intractable seizures Can sit with aid No word	Intractable seizures with bedridden	Intractable seizures with bedridden	Intractable seizures Can stand with aid No word
EEG features				
At onset	Focal sharp waves at bilateral frontal areas	suppression burst	suppression burst	Focal spikes and sharp waves at bilateral temporo-occipital areas, Generalized spikes-waves
Hypsarrhythmia	–	+	+	+
Neurologic features				
Hypotonia	+	+	+	+
Autistic features	+	+	–	+
Hand stereotypic movement	+	–	–	+
Brain MRI	Negative	Brain atrophy (left frontal, temporal and parietal)	Ventriculomegaly with white matter hyperintensity	Negative

Abbreviations: ND – not determined; WS – West syndrome.

the male children (7/31, 22.6%; $p = 0.004$). Furthermore, the occurrence of spasms, hand stereotypies, intractable epilepsy, hypotonia, hypsarrhythmia, acquired microcephaly, and developmental delay was significantly different between the female and male children (all $p < 0.05$). Statistical analyses of clinical features and mutation types in both male and female children are summarized in Tables 2 and 3, respectively.

4. Discussion

CDKL5 mutations were first identified in two female patients with IS [12]. Mutations in the CDKL5 gene have been considered a major cause of X-linked infantile spasms (ISSX) in girls, although *CDKL5* mutations may also be associated with other diverse phenotypes [1,3–6,8,10,15,18,20,23,26,30]. Similar to methyl-CpG binding protein 2 (*MECP2*) mutation-associated Rett syndrome, the inheritance of *CDKL5* mutation-associated ISSX is considered to be X-linked dominant because of its localization on Xp22.13 and its predominance in female patients. However, the lack of CDKL5 protein may not be lethal in male patients. Liang et al. reported non-significant phenotypic differences between male and female patients with *CDKL5* mutations [13]. By contrast, Wong and Kwong revealed that a *CDKL5* variant identified in one boy was also present in his asymptomatic mother and elder sister, although the pathogenic nature of the identified variant has not been confirmed [31]. Other studies have also reported that male patients have a more severe phenotype than female patients [6,14]. In the present study, we also noted that the neurological outcome was more severe in boys with *CDKL5* mutations, though the case numbers are limited. The male patients were all bedridden with intractable seizures, whereas the female patients had motor development that allowed them to sit or stand. Brain imaging revealed that the male patients also had a higher probability of abnormalities.

Through a literature review and statistical analysis, we revealed phenotypic differences between male and female patients. In addition to the most common diagnosis of

Table 2
Comparison of clinical symptoms between female and male patients with *CDKL5* mutations.

Symptom	Subject No. (%)		*p* value
	Female	Male	
Brain MRI			
Normal	52 (74)	8 (47)	0.041
Brain atrophy	18 (26)	9 (53)	
Total	70	17	
Diagnosis			
EoEE	64 (48)	23 (74)	0.004
Aty RTT	40 (30)	1 (3)	
NSEE	2 (2)	0 (0)	
West/ISSX	26 (20)	7 (23)	
Total	132	31	
Spasms			
Yes	44 (54)	14 (88)	0.013
No	37 (46)	2 (12)	
Total	81	16	
Hand stereotypies			
Yes	97 (83)	10 (40)	<0.001
No	20 (17)	15 (60)	
Total	117	25	
Intractable epilepsy			
Yes	114 (83)	31 (100)	0.009
No	23 (17)	0 (0)	
Total	137	31	
Hypotonia			
Yes	87 (95)	21 (81)	0.041
No	5 (5)	5 (19)	
Total	92	26	
Hypsarrhythmia			
Yes	22 (23)	13 (48)	0.011
No	73 (77)	14 (52)	
Total	95	27	
Acquired microcephaly			
Yes	59 (50)	6 (24)	0.016
No	58 (50)	19 (76)	
Total	117	25	
DD			
Mild	50 (40)	28 (90)	0.011
Intermediate	67 (53)	3 (10)	
Severe	9 (7)	0 (0)	
Total	126	31	

Abbreviations: EoEE – early-onset epileptic encephalopathy; Aty RTT – atypical Rett syndrome; NSEE – nonspecific epileptic encephalopathy; West/ISSX – West syndrome/X-linked infantile spasms; DD – developmental delays.

Table 3
Types of *CDKL5* mutations in female and male patients.

	Types of Mutation						Total
	D	F	M	N	R	S	
Female	15	44	34	18	3	25	139
Male	7	3	11	9	0	1	31
Total	22	47	45	27	3	26	170

$p = 0.003$, D – deletion; F – frameshift; M – missense; N – nonsense; R – rearrangement; S – splicing.

EoEE in both male and female patients, diagnoses of WS (i.e., IS with hypsarrhythmia and mental retardation) were more frequent in male patients. By contrast, atypical Rett syndrome was more commonly diagnosed in female patients. Furthermore, hand stereotypies were more common in female patients. Consistently, similar observations were also found in our patient series. The pattern of X chromosome inactivation in affected female was the most plausible reason for gender differences in phenotypic expression of X-linked disorders. The level of somatic mosaicism may also be the contributing factor to variable severity of the disease. Further investigation of the X-inactivation pattern and level of mosaicism among patients with *CDKL5* mutation may provide direct correlation.

The core symptoms of patients with *CDKL5* mutations were early-onset seizure and severe-to-profound psychomotor retardation. These symptoms were consistent across both genders in our study. Epileptic spasms were the prominent seizure type in patients with *CDKL5* mutations, although hypsarrhythmia may not be concomitant. Only one of the two male patients in our study had hypsarrhythmia. Hypsarrhythmia has been observed in children with *CDKL5* mutations in the age group of 5–9 years, and late hypsarrhythmia has been proposed as a characteristic of *CDKL5*-related EE, with severe and adverse outcomes [11]. We did not record late hypsarrhythmia in our study population, except for in the male patient (case #2) who exhibited suppression-burst pattern during early infancy. Epilepsy in patients with *CDKL5* mutations is always intractable even under multiple anticonvulsants. One of our female patients (case #1) underwent a callosotomy. Her seizures were controlled initially but relapsed 6 months later, indicating that a callosotomy for tonic spasms or atonic seizures in patients with *CDKL5* mutations may not be effective.

A total of 31 male patients, including our identified patients, with *CDKL5* mutations have been reported [6,13,14,16,17,25,30–32,34]. The observed higher frequencies of frameshift (31.7% vs. 9.6%) and splicing (18.0% vs. 3.2%) mutations in female than in male patients may implicate potential detrimental outcomes, including lethality in males, of severely aberrant CDKL5 protein. Recently, advances in NGS technology have facilitated the use of direct DNA sequencing in the clinical diagnosis of children with EoEE. Consequently, more male patients with *CDKL5* mutations have been found [14,34]. Currently, there were only limited number of cases with the same mutation in both genders in the literature (Table S1) and the highest motor skill or epilepsy control could not compare easily due to the reported evaluation at different ages [4,14]. Nevertheless, the motor functions of female patients with premature CDKL5 termination in the same exon were more likely

not as severe as those observed in male patients [1–21, 23–30,32–35]. Compared with female patients, male patients always present with more severe phenotypes, manifested as severe-to-profound mental and motor retardation. Further investigation of the phenotypic differences between male and female patients resulting from *CDKL5* gene mutations is warranted.

5. Conclusion

In conclusion, both girls and boys with IS harbor *CDKL5* mutations. Male children with *CDKL5* mutations have a higher frequency of IS and brain atrophy, whereas female patients often present with atypical Rett syndrome besides EoEE. Hypotonia is commonly seen in both male and female children. Despite their similar manifestations, phenotypic differences do exist in male and female children with *CDKL5* mutations.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.braindev.2019.05.003.

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