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RESEARCH REPORT

A novel variant of *CDK19* causes a severe neurodevelopmental disorder with infantile spasms

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Abstract Infantile spasms are a potentially catastrophic form of epilepsy syndrome that are usually associated with substantial developmental delay and commonly occur in children younger than 1 yr. Recent reports on four cases revealed that variants harbored in a novel gene *CDK19* were causative for the syndrome. We report a fifth affected individual, a 10-mo-old male patient who presented with a neurodevelopmental syndrome characterized by infantile spasms. We identified a novel de novo missense variant c.92C > A (p.Thr31Asn) in *CDK19* that was classified as a likely pathogenic disease-causing variant. The characterized clinical phenotypes of the proband were similar to the previously reported four patients, but he had few variable features including earlier seizure onset age and earlier occurring developmental abnormality. Protein structure modeling analysis revealed that *CDK19* variants may disable its kinase activity, which would further impede the transcriptional regulation, thus leading to detrimental pathologies. Our report expanded *CDK19* genotype spectrum and further demonstrated that a *CDK19* missense variant was causative of neurodevelopmental disorder clinically marked by infantile spasms.

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INTRODUCTION

Infantile spasms, also known as West syndrome, accompanied by developmental and intellectual delay are among the most severe types of epilepsy syndromes. Epileptic spasms attack infants in the first 2 years of life, most commonly between 4 and 8 mo. Genetic defects in the etiology of infantile spasms have been increasingly understood, including *TSC1/TSC2* mutations causing tuberous sclerosis (O'Callaghan et al. 2004), as well as mutations in the genes *ARX* (Kato et al. 2003), *CDKL5* (Paciorkowski et al. 2011), and *STXBP1* (Otsuka et al. 2010; Saitsu et al. 2010). Most recently, cyclin-dependent kinases 19 (*CDK19*) pathogenic variants have been identified in four patients with a neurodevelopmental syndrome involving intellectual disability, hypotonia, dysmorphic features, and infantile spasms (Chung et al. 2020; Sugawara et al. 2020).

CDK19 encodes a serine/threonine-specific kinase working in partnership with cyclin C as the regulatory subunit to achieve kinase activity (Lim and Kaldis 2013; Calpena et al. 2019). To fulfill the regulation on RNA polymerase II (RNA Pol II)-based transcription, the CDK19/cyclin C complex forms a multisubunit CDK module by recruiting MED12L and MED13L (Bourbon 2008; Lim and Kaldis 2013; Pelish et al. 2015). Highly enriched expression in the brain implicates that *CDK19* acts as a hub gene that controls the expression of other genes and contributes to neurodevelopment. Thus, the disruption of CDK19 would cause detrimental neurological symptoms (Wang et al. 2017; Davie et al. 2018).

Here we report a fifth case of a 10-mo-old male patient clinically manifesting with neurodevelopmental syndrome characterized by infantile spasms from whom a novel de novo *CDK19* pathogenic variant was identified. The protein structure modeling was presented to evaluate the pathogenic effect of *CDK19* variant in this case.

RESULTS

Clinical Presentation

The male proband experienced full-term normal delivery at 40 wk with no special family history. At 4 mo of age, he developed infantile spasms occurring in clusters and in the form of nodding and huddling. The symptoms always occurred shortly after falling asleep or soon after waking up. Oral treatment with topiramate alleviated the attack to some extent but failed to control the seizures completely. Before seizure onset, his development was normal. However, when evaluated at the age of 10 mo, his developmental milestones were delayed. He could flip over but was unable to sit up on his own; he could grasp objects actively but had poor eye contact with people. He had an overall development quotient of 43 with mild delay in language, social behavior, and gross motor skills, but a severe delay in adaptive behavior and fine motor skills. His weight was 11.5 kg (99th percentile), and his head circumference was 41 cm (third percentile). Additionally, the individual had some dysmorphic features including hypertelorism, a prominent nose with a bulbous tip and low nasal bridge, and high-arched palate. A neurologic exam was significant for hypotonia. His brain magnetic resonance imaging (MRI) showed that part of the cerebral gyrus of the cerebral lobe of the brain was widened, sulci was deepened, and both lateral ventricles and the bilateral frontotemporal subarachnoid space were enlarged (Fig. 1A). Video electroencephalogram (vEEG) detected interictal hypsarrhythmia with diffuse and multifocal sharp wave discharges and frequent spikes with slowing in the background during sleep and wake. vEEG showed ictal epileptic spasms along with extensive medium-high-amplitude 1.5- to 2-Hz spike slow wave in bilateral leads and complex slow wave bursts with total conductive voltage reduction and low amplitude fast wave activity in the background (Fig. 1B). Electromyogram showed myoelectric burst at the same time. The results of laboratory examinations including liver, kidney, and thyroid function tests, electrolytes, blood ammonia, lactic acid, urine, and/or blood organic, and amino acid screening were normal.

Genomic Analyses

Trio-based whole-exome sequencing (WES) identified a novel de novo heterozygous variant in exon 1 of *CDK19* (NM_015076.5 c.92C > A (p.Thr31Asn)) in the patient (Table 1). The variant in the proband and the wild type of the *CKD19* gene in healthy parents were confirmed by Sanger sequencing (Fig. 2A). The variant was classified as likely pathogenic based on ACMG 2015 guideline, the lines of evidence included being a confirmed de novo variant but not highly specific phenotype (PS2_Moderate), being absent from gnomAD population database (PM2_Supporting), being located in the critical protein kinase domain (PM1), with missense *Z*-score > 3 from gnomAD (PP2) and being predicted to be deleterious by multiple bioinformatic software (PP3), including damaging (score = 0.001) using SIFT, probably damaging (score = 0.999) using PolyPhen, and scored 26.8 in CADD. There were no additional variants of interest considered compatible with the phenotype of the proband.

Protein Modeling

We built a CDK19 protein model in complex with cyclin C with the presence of ATP using the program PyMOL (Schrödinger, LLC). The affected amino acid residue, Thr31, located in the

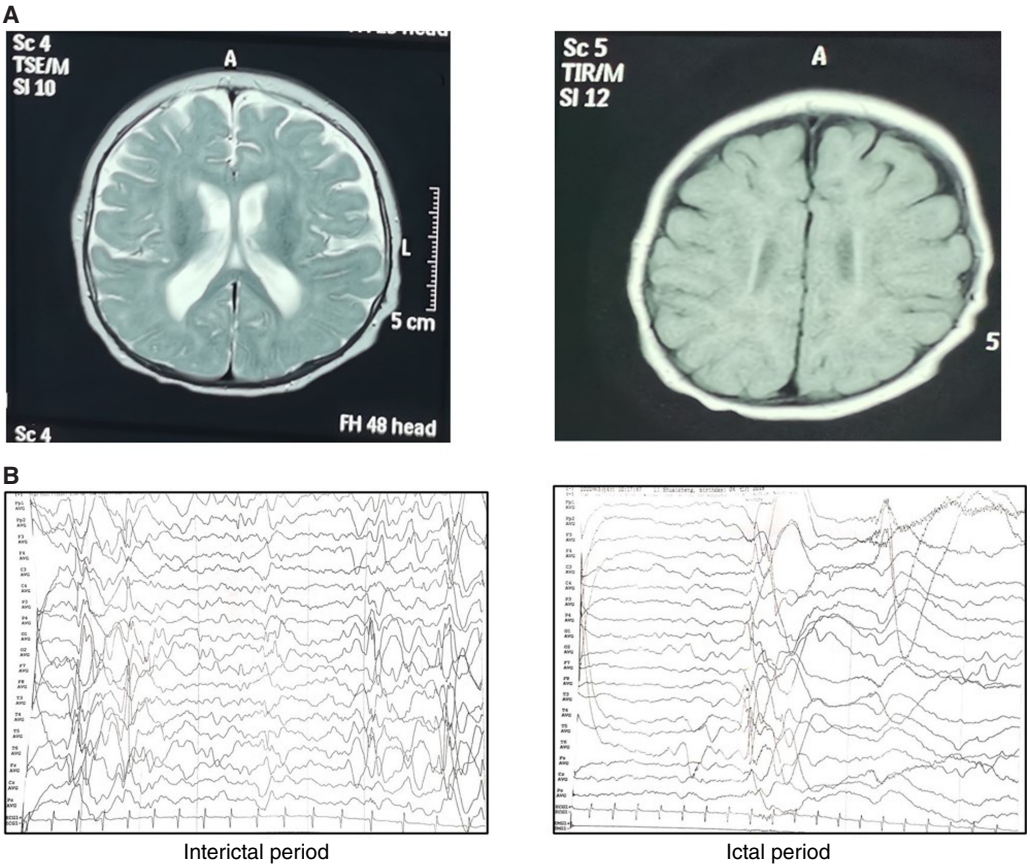

Figure 1. Clinical features of the patient. (*A*) Brain MRI images showed widened cerebral gyrus, deepened sulci, and enlarged lateral ventricles. (*B*) EEGs showed hypsarrhythmia during interictal period and epileptic spasms along with myoclonic burst during the ictal period.

glycine-rich loop, is evolutionarily conserved among species (Fig. 2B). The protein modeling displays that the variant p.Thr31Asn occurs at the upper surface of the ligand binding pocket surrounding the ATP-binding (Fig. 2B).

DISCUSSION

Recent reports on four cases revealed that variants harbored in a novel gene *CDK19* resulted in the neurodevelopmental syndromes involving intellectual disability and epilepsy encephalopathy in humans (Chung et al. 2020; Sugawara et al. 2020). The syndrome was newly defined as developmental and epileptic encephalopathy 87 in OMIM (DEE87; MIM: 618916).

Table 1. Variant table

Gene	Chromosome	HGVS DNA reference	HGVS protein reference	Variant type	Predicted effect	dbSNP/ dbVar ID	Genotype	ClinVar ID
CDK19	6	c.92C>A	p.Thr31Asn	Missense	Substitution		Heterozygous	VCV000973853.1

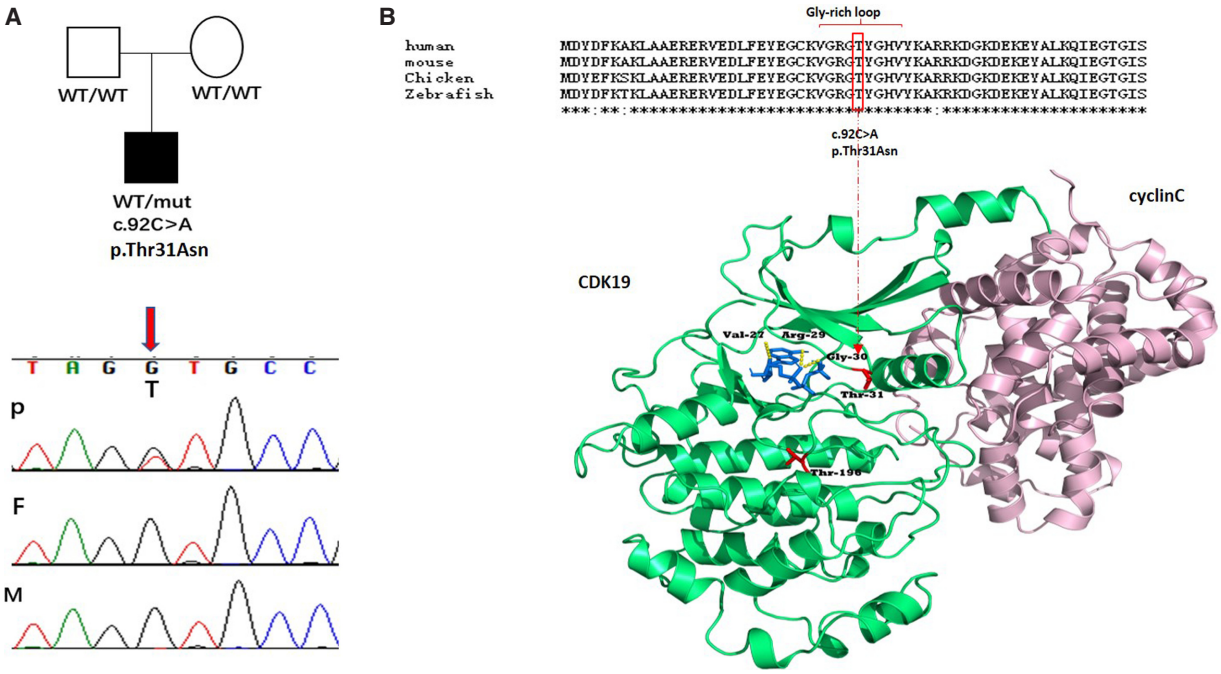

Figure 2. *CDK19* variants and CDK19 protein model. (*A*) Family pedigree and electropherograms of Sanger sequencing. The red arrowhead indicates the de novo *CDK19* variant (c.92C > A, p.Thr31Asn) in our proband. (*B*) Clustal alignment of CDK19 and protein modeling. The amino acid residue threonine in the red box at position 31 is fully conserved among different species. The red arrowhead points to the affected amino acid (p.Thr31Asn) in the protein structure modeling. The blue molecular structure is ATP; the yellow dotted line shows hydrogen bonding between ATP and amino acid.

Here, we encountered a fifth affected individual with the clinical phenotypes similar to the previously reported patients (Table 2). Notably, the head circumference of our patient was found to be small in the fetal period during pregnancy, suggesting that the developmental abnormality occurred at a very early stage of life. Among the five patients reported so far, our patient developed infantile spasms at the earliest age—4 mo. As epileptic spasms occurred frequently, his development concerning motor, language, and social behavior halted globally or even regressed. The patient's laboratory examination results differentiated his diagnosis from inborn metabolic disorders and mitochondrial diseases that could also cause infantile epilepsies. His hypsarrhythmia EEG and seizure onset at 4 mo old could also help to differentiate the diagnosis of Ohtahara syndrome, which often occurs in infants younger than 3 mo old with a burst-suppression EEG pattern. In the previous study, various antiepileptic drugs were unable to control the seizures for three patients. For our proband, oral treatment with topiramate was able to alleviate seizures for a few months. However, his seizures became refractory and drug-resistant on our latest follow-up at the patient's age of 17 mo old. The alterations of antiepileptic drugs are being considered currently and the prognosis is under observation.

The possible pathogenic effects of all *CDK19* variants was assessed using protein structure modeling. The crystal structure model displayed that within the glycine-rich loop, the variant p.Thr31Asn lies closely to the amino acid residues p.Arg29 and p.Gly30, which forms hydrogen bonding with the phosphates of ATP. Consistent with other known transcriptional CDKs, the glycine-rich loop of CDK19 in the protein kinase domain consists of a highly conserved sequence GRGTYGHV, with arginine and glycine serving as the

Table 2. Clinical features of affected individuals with *CDK19* variants

	Chung et al. (2020)			Sugawara et al. (2020)	This study
Age/Sex	Proband1 25 yr/female	Proband2 2 yr/male	Proband3 1 yr/male	Proband4 7 yr/female	Proband5 10 mo/male
Variant	c.586A > G (p.Thr196Ala)	c.586A > G (p.Thr196Ala)	c.94T > C (p.Tyr32His)	c.94T > C (p.Tyr32His)	c.92C > A (p.Thr31Asn)
De novo	Yes	Yes	Yes	Yes	Yes
Brain MRI	Borderline microcephaly	Mild atrophy	Delayed myelination	Brain atrophy and white matter abnormalities	Widened cerebral gyrus, deepened sulci and enlarged lateral ventricles
Facial dysmorphism	Not applicable	Yes	Yes	Yes	Yes
Hypotonia/motor delay	Yes	Yes	Yes	Yes	Yes
Intellectual disability	Yes	Not applicable	Not applicable	Yes	Not applicable
Epilepsy	Early infantile epileptic encephalopathy	Yes/infantile spasms	Yes/infantile spasms	Yes/infantile spasms	Yes/infantile spasms
Seizure onset age	15 mo	6 mo	9 mo	6 mo	4 mo
Seizure types	Generalized tonic–clonic seizures; complex partial seizures	Generalized tonic–clonic seizures; epileptic spasms	Atonic seizures	Epileptic spasms; tonic seizures	Epileptic spasms
Developmental delay	Yes	Yes	Yes	Yes	Yes

bone residue for ATP positioning (Grant et al. 1998; Okur et al. 2016). p.Thr31, although not in direct contact with ATP, was speculated to play an indispensable role in structure to assist the key residues, thus ensuring proper ATP binding and a normal phosphoryl-transfer reaction (Hemmer et al. 1997; Grant et al. 1998; Okur et al. 2016; Hamilton et al. 2018). The perturbation of p.Thr31Asn might alter the conformation of the loop and further depress ATP affinity, thus abolishing CDK19 kinase activity. The same theory may apply to the neighbor p.Tyr32His. Previous studies showed that p.Thr196Ala was located in the loop associated with substrate binding and suggested that the variant might greatly reduce the substrate affinity and inhibit substrate phosphorylation (Jeffrey et al. 1995; Hemmer et al. 1997; Hamilton et al. 2018). To initiate transcription, the carboxyl-terminal domain (CTD) of RNA polymerase II was phosphorylated by the CDK19/cyclin C complex as a targeted substrate (Bourbon 2008; Lim and Kaldis 2013; Calpena et al. 2019; Chung et al. 2020). We assumed that the disruption of *CDK19* kinase activity by pathogenic variants impaired the total transcriptional activity for the CDK module, thereby leading to detrimental pathologies due to an aberrant downstream transcriptional profile. Further analysis is needed to explicitly verify how *CDK19* variants were responsible for the related pathologies.

As next-generation sequencing prevails in clinical diagnosis, many genes have been identified to be genetically associated with infantile spasms. However, variants in *CDK19* have not been detected until recently, suggesting that it may be a rare causation for the syndrome. A loss-of-function (lof) mechanism for *CDK19* is controversial. On one hand, a high pLI score of 1 and a low o/e score of 0.03 (both scores reflect the tolerance

of a given gene to lof variants including frameshift, splice donor/acceptor, stop-gain variants) in gnomAD imply that *CDK19* is severely constrained for lof variation; however, there are a few lof variants including both stop-gain and frameshift variants in gnomAD. On the other hand, the missense *Z*-score of *CDK19* is 3.56, indicating that there is general selection against coding variation. Consequently, whether lof is the disease mechanism is questionable. Furthermore, the expression of missense variants significantly reduced viability of wild-type *Drosophila* and the mutant protein failed to rescue the lethality or neurologic phenotypes observed in the flies that lost *Cdk8* (*CDK19* homolog in fly). These findings support that nonsynonymous variants in *CDK19* might cause dominant negative effects (Chung et al. 2020). It is therefore unlikely that the phenotype observed in the patients results from a simple loss of function of *CDK19*, but suggests rather that a dominant negative mechanism is largely responsible.

CDK19 has only been associated with DEE87 very recently (updated on OMIM in 10/2020). Currently, the clinical validity of gene–disease association can only be classified as moderate with limited experimental data and case-level genetic evidence published so far (Chung et al. 2020; Sugawara et al. 2020). Following a ClinGen gene curation protocol (https://clinicalgenome.org/docs/gene-disease-validity-standard-operating-procedure-version-8/), our case of a rare missense, presumed dominant negative variant (starting score = 0.1 point) found to be de novo (additional 0.4 point) would be scored at 0.5 point. More case-level genetic evidence will be necessary for a future upgrade of *CDK19*-related DEE87 to a strong gene–disease relationship.

In summary, using trio-based WES, we identified a new de novo heterozygous missense variant in *CDK19* associated with a neurodevelopmental disorder involving intellectual disability, hypotonia, dysmorphic features, and infantile spasms. The variants that have been identified so far are all located in the kinase domain of the CDK19 protein, which may bring about detrimental pathological effects through disabling the normal transcription activity. Further efforts are needed to delineate the underlying pathomechanisms. Nonetheless, our case further confirmed the gene–disease association of *CDK19*-related epileptic encephalopathy.

METHODS

Genetic Testing and Variant Interpretation

Genomic DNA was extracted from the peripheral blood of the proband and his parents. WES was carried out at the patient's age of 8 mo old. The sequencing libraries were prepared, and the xGen Exome Research Panel probes (IDT) were used to enrich the target sequences (Supplemental Table 1). Variant analysis was performed using a Sentieon pipeline (Sentieon) with alignment to a reference genome GRCh38. Sequence variants were checked with population databases gnomAD (http://gnomad.broadinstitute.org/) and evaluated using PolyPhen-2, MutationTaster, and SIFT. Variant pathogenicity was interpreted according to the American College of Medical Genetics (ACMG) guidelines (Richards et al. 2015). The variants were further confirmed by Sanger sequencing.

ADDITIONAL INFORMATION

Data Deposition and Access

The *CDK19* variant was submitted to ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) and can be found under accession number VCV000973853.1.

Ethics Statement

Written informed consent was obtained from both of the patient's legal guardians (his parents) to participate in this study. This study was approved by the human ethics committees of The Children's Hospital, Capital Institute of Pediatrics, Beijing.

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Competing Interest Statement

The authors have declared no competing interest.

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Authors Contributions

Q.C. and X.W. proposed the meaning and concept of the study and designed the plan for the case. Q.C. and S.Y. made contributions to data collection and analysis. W.Y., Q.C., and X.W. drafted and revised the manuscript. All of the authors read and approved the final manuscript to be published and agreed to be responsible for the accuracy of the data and details.

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