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RESEARCH REPORT

Early-onset infant epileptic encephalopathy associated with a de novo *PPP3CA* gene mutation

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Abstract Epileptic encephalopathies are severe seizure disorders accompanied by intellectual disability. Whole-exome sequencing technology has enabled the discovery of genetic mutations responsible for a wide range of diseases, and severe epilepsy and neurodevelopmental diseases are often associated with rare de novo mutations. We identified a novel de novo frameshift mutation in the *PPP3CA* gene encoding calcium-dependent protein phosphatase (calcineurin) catalytic subunit A (c.1255_1256del, p.Ser419Cysfs*31) in an 11.5-mo-old female with early-onset refractory epilepsy and developmental delay. This finding expands the list of *PPP3CA* mutations associated with early-onset severe neurodevelopmental disease with seizures and provides further details on clinical features.

[Supplemental material is available for this article.]

INTRODUCTION

Epilepsy is the most common central nervous system (CNS) disease in childhood, with an incidence of 70.1 per 100,000 children younger than 2 yr of age (Eltze et al. 2013). Severe epilepsies of infancy are devastating disorders associated with developmental delay (DD). Recent studies of early-onset epileptic encephalopathies using next-generation sequencing have revealed several new disease genes, many harboring de novo mutations with autosomal dominant inheritance, such as *GABRB3*, *ALG13*, *KCNB1*, *CHD2*, *DNM1*, *GNAO1*, *GRIN2A*, and *GRIN2B* (Epi4K Consortium et al. 2013; Suls et al. 2013; EuroEPINOMICS-RES Consortium et al. 2014; Mastrangelo 2015). Myers et al. studied 4760 trio families with neurodevelopmental disease in the probands and detected five individuals with de novo *PPP3CA* mutations (Myers et al. 2017). Another de novo mutation in *PPP3CA* was identified in an individual with severe undiagnosed developmental disorder by exome sequencing of 4293 families (Deciphering Developmental Disorders Study 2017). In addition, Mizuguchi et al. reported de novo mutations of the *PPP3CA* gene associated with epileptic encephalopathies (Mizuguchi et al. 2018).

The *PPP3CA* gene encodes the protein phosphatase 3 catalytic subunit α (PPP3CA: MIM 114105), which forms part of the Ca^{2+}-interacting serine/threonine protein phosphatase calcineurin (CN). CN couples intracellular Ca^{2+} signals to various cellular responses, such as T-cell activation, vesicular trafficking, cell growth, and apoptosis. Although widely distributed, CN

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exhibits particularly high expression in the brain and in lymphocytes. The zebrafish *Ppp3ca* gene is expressed in the cerebellum, hindbrain, tectum olfactory epithelium, and skin as early as 72 h postfertilization. Further, the spatial and temporal expression patterns of the five CN genes generally detected in the CNS—three encoding catalytic subunits (*PPP3CA–C*) and two encoding regulatory subunits (*PPP3R1* and *PPP3R2*)—also implicate CN in neuronal development (Hammond and Udvadia 2010). Indeed, *Ppp3ca*$^{-/-}$ mice show defects in CNS development as well as reduced bone mineral density (Kayyali et al. 1997; Sun et al. 2007).

Proper neural circuit development is essential for both cognitive function and maintaining excitatory–inhibitory balance, which is disrupted in epilepsy. Thus, it is not surprising that various de novo mutations in CN subunit genes are associated with early-onset epilepsy accompanied by neurodevelopment delay. In this study, we identified a novel de novo frameshift mutation of *PPP3CA* in an infant female with early-onset refractory epilepsy. We also review previous cases with de novo *PPP3CA* mutations.

RESULTS

Clinical Presentation and Family History

The female case patient was first diagnosed with seizures at 2 mo of age, and at presentation was experiencing seizures roughly 10 times per day without fever. Routine blood cell and biochemical examinations were normal. However, head magnetic resonance imaging (MRI) revealed dilation of lateral ventricles. Sleep electroencephalograph (EEG) showed multiple spike waves, spike, sharp-slow, and spike-slow waves on left central and posterior temporal lobes with hemispheric asymmetry, under the background activity of 4–5 Hz θ wave and low-to-moderate 2–3 Hz δ waves. She was treated by levetiracetam 0.5 ml q12h, but seizures persisted, presenting as sudden blinks, upper limb inflections, and periodic leg shaking in clusters of one to 20 times, with clusters occurring five to 20 times per day. Levetiracetam was increased to 1.75 ml q12h, and combined with sodium valproate, and valproic acid SR tablets 1–2.5 ml q12h, and topiramate tablets 3.125–25 mg gradually, but epileptic seizures still occurred. She was then treated with a ketogenic diet. On admission, abnormalities in head MRI were observed, including brain dysplasia with cystic right basal ganglia, thin corpus callosum, and widened brain interval (Fig. 1A–D). Hippocampal MRI also revealed a relatively small hippocampus. The X-ray images showed low bone density in the left hand and slightly elevated bone density of the metaphysis at the distal end of the ulnar radius. Head magnetic resonance spectroscopy (MRS) showed reduced peak *N*-acetylaspartate (NAA) in the bilateral basal ganglia and thalamus, indicating neuronal lesions. The ketogenic diet was added to 180 ml q6h, with antiepileptic drug treatment because of intractable seizures. She was able to maintain normal blood glucose/ketone during the ketogenic diet. The patient subsequently visited at 8.5, 9.5, and 11.5 mo old, but epileptic seizures remained and developmental quotient (DQ) was <20. The patient was diagnosed with intractable epilepsy and DD. As of the last follow-up by phone at 16 mo old, the patient still could not raise her head and exhibited frequent seizures (epilepsy every 15 min) under drug control (Fig. 2). There was no similar medical history in her family.

Genomic Analysis

The mean depth of the sequencing data in this family was ~120× (Supplemental Table S1). We identified a novel frameshift mutation (c.1255_1256del, p.Ser419Cysfs*31) in *PPP3CA* (Fig. 3A). The mutation predicted a truncated protein with a 30-amino acid addition from position 419 (Fig. 3B,C). The mutation was not detected in her parents; therefore, it was classified as a de novo mutation (Table 1). The affected region is located near the calmodulin-

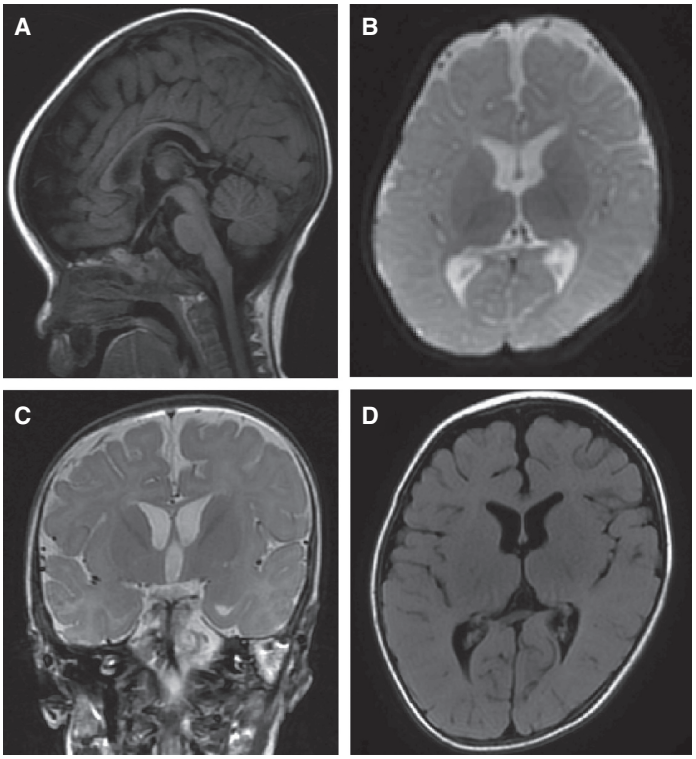

Figure 1. Brain magnetic resonance images of the case patient. (*A*) Sagittal T1 FLAIR image showing brain hypoplasia and thin corpus callosum. (*B*) Axial diffusion-weighted image showing cystic basal ganglia. (*C*) Coronal fast spin echo T2 image. (*D*) Axial T2 FLAIR image showing brain interval enlargement.

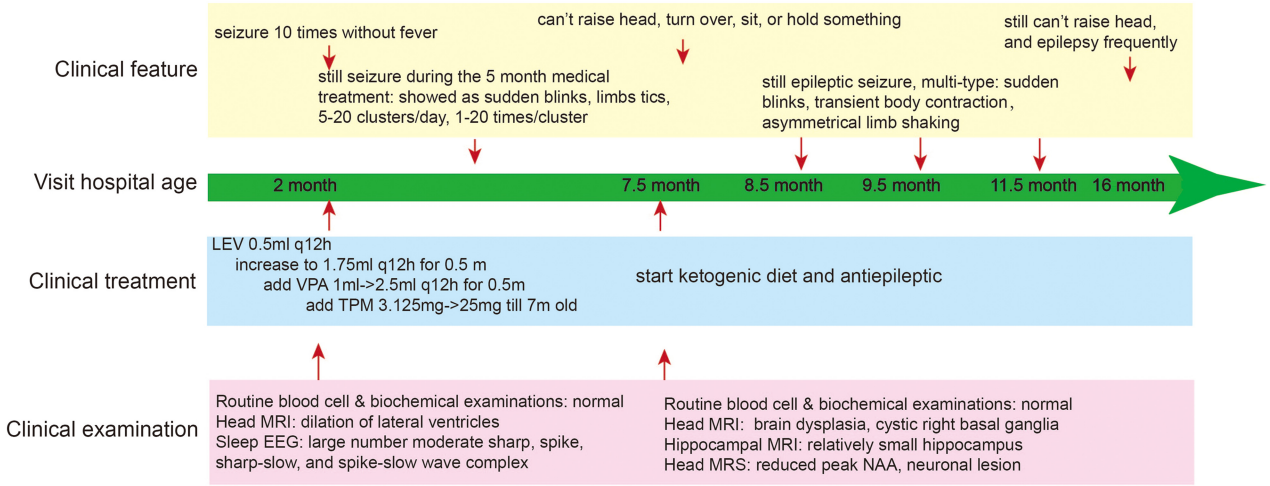

Figure 2. Clinical summary of the case patient. Age at visits is shown by the thick green axis with arrows. The clinical features are described in yellow blocks. Clinical examination results are summarized in the pink blocks at the corresponding age. Clinical treatment is summarized in the sky blue blocks.

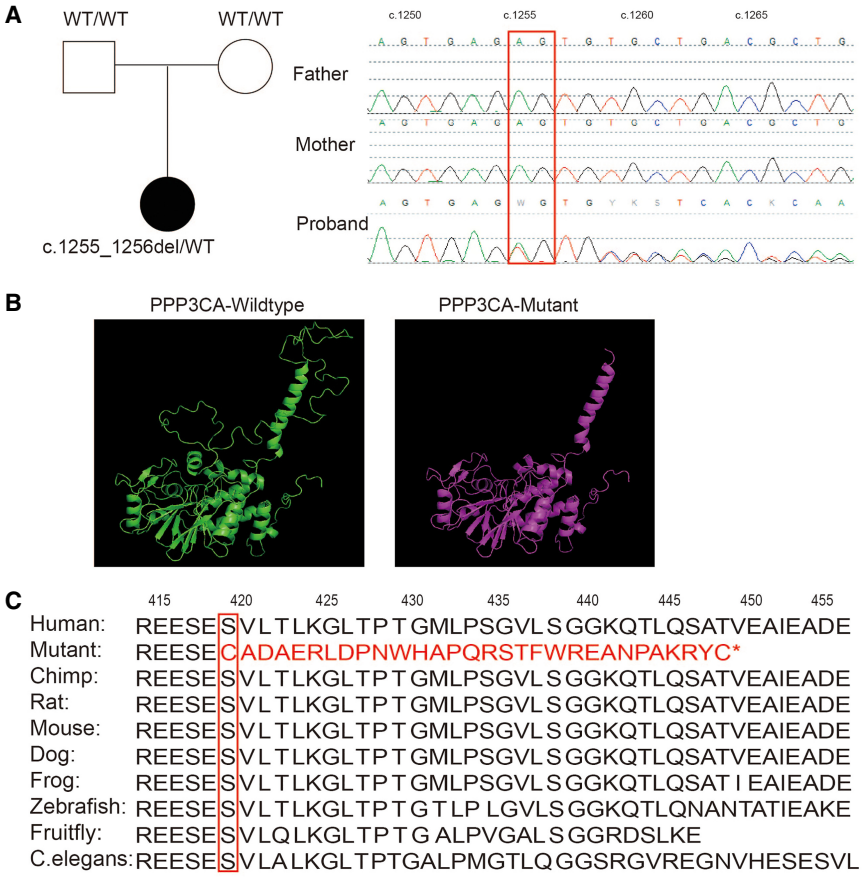

Figure 3. Rare de novo frameshift mutation in *PPP3CA*. (*A*) The trio family and the de novo frameshift mutation detected by whole-exome sequencing and confirmed by Sanger sequencing. (*B*) The predicted structure of *PPP3CA*. The structure of wild-type *PPP3CA* is shown in green, and the predicted structure of the mutant is shown in purple. The frameshift mutation encodes a truncated 449-amino acid protein. (*C*) The mutation region is conserved among human, chimp, rat, mouse, dog, frog, zebrafish, fruit fly, and *Caenorhabditis elegans*.

binding domain and is conserved among species, including human, chimp, rat, mouse, dog, frog, and zebrafish (Fig. 3C). The sequence change predicts loss of the autoinhibitory (AI) domain (Fig. 4). The mutation was not reported in the 1000 Genome Project (1KG), Exome Aggregation Consortium (ExAC), Human Genome Mutation Database (HGMD), or PubMed and is not recorded in our laboratory internal database of 15,000 pediatric patients, including 2000 epilepsy patients.

Table 1. *PPP3CA* variant in this study

Gene	Chr: position GRCh 37(hg19)	HGVS	Variant type	Effect[a]	Genotype	Origin	Clinvar ID
PPP3CA	Chr 4:101953506	NM_000944:c.1255_1256delAG NP_000935.1:p.Ser419Cysfs*31	Deletion	Pathogenic	Heterozygous	De novo	SCV000692475

HGVS, Human Genome Variation Society.
[a]Evaluated according to ACMG guidelines.

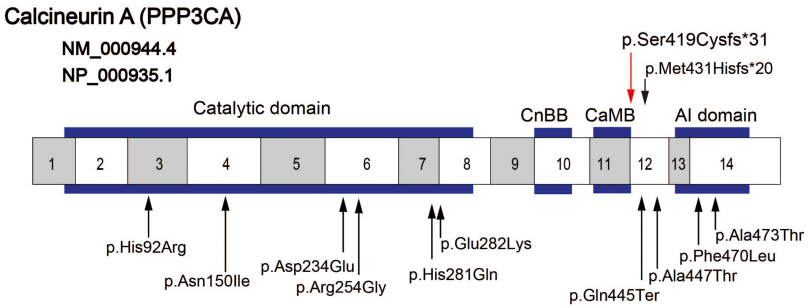

Figure 4. The previously reported mutations of *PPP3CA*. Boxes in gray/white with numbers are the exons. The dark blue frames demarcate the catalytic, calcineurin B-binding (CnBB), calmodulin-binding (CaMB), and autoinhibitory (AI) domains. The mutations indicated by black arrows were reported in previous papers, and that indicated by the red arrow was detected in the present study.

DISCUSSION

The calcium-dependent CN is an important regulator of synaptic vesicle recycling, a critical process controlling neurotransmission (Arendt et al. 2015). The *PPP3CA* gene encodes one isoform of the catalytic subunit (calcineurin A), which forms a functional isodimer with the regulatory subunit (calcineurin B).

Recent studies have identified de novo mutations of *PPP3CA* associated with epileptic encephalopathies (Myers et al. 2017; Mizuguchi et al. 2018). Including this study, a total of 12 *PPP3CA* mutations have been documented in 14 patients (Cases 2 and 3 share the same mutation as do Cases 9 and 10) (Table 2). The three deleterious mutations in Case 1, 2, and 11, including two frameshift mutations (c.1255_1256delAG and c.1290dupC) and one nonsense mutation (c.1333C > T, p.Gln445Ter), may delete the AI domain, which in turn would alter CN activity. These three patients all presented with early-onset refractory seizures and severe DD.

The pLI value of *PPP3CA* is 1, indicating extreme loss-of-function (LoF) intolerance, possibly due to nonsense-mediated mRNA decay (NMD) and ensuing haploinsufficiency. However, Mizuguchi et al. reported that one frameshift mutation (c.1290dupC) escaped NMD (Mizuguchi et al. 2018). In our study, we found that in 293 cells transfected with a plasmid encoding the c.1255_1256del mutant of *PPP3CA*, the mRNA expression levels were similar to the wild type, but the mutant protein cannot be detected by western blot, even after repeated experiments (Supplemental Fig. S1). Thus, we speculated that the frameshift mutation (p.Ser419Cysfs*31) confers haploinsufficiency because of an unstable protein. In contrast, two missense mutations (p.Phe470Leu and p.Ala473Thr) located in the AI domain were classified as gain of function as enzyme activity was maintained (Mizuguchi et al. 2018). Both of these patients (Cases 13 and 14) showed severe skeletal abnormalities and moderate/severe DD, and Case 14 also exhibited tractable seizures. In the current case, low bone density of the left hand and slightly raised density in the metaphysis at the distal end of the ulnar radius were revealed by X-ray imaging. This suggests that the *PPP3CA* gene may also disrupt normal bone metabolism when the enzyme active is affected. In addition, MRI revealed a relatively small hippocampus, a phenotype also observed in *Ppp3ca*$^{-/-}$ mice (Kayyali et al. 1997).

The catalytic domain is well conserved across species, so substitutions in this region may be deleterious. Mizuguchi et al. reported that four missense mutations identified in this domain, p.His92Arg (Cases 3 and 4), p.Asn150Ile (Case 5), p.Asp234Glu (Case 6),

Table 2. Genotype and clinical features of 14 patients with *PPP3CA* mutations, including the current case patient (case 1)

Case number (PMID)	Case 1 (this study)	Case 2 (29432562)	Case 3 (28942967)	Case 4 (29432562)	Case 5 (29432562)	Case 6 (29432562)	Case 7 (28135719)
Age/gender	11.5 m/F	2 y/F	12.5 y/M	4 y/F	19 y/F	2 y/M	NA/F
Variant (NM_000944.4)	c.1255_1256del p.Ser419Cysfs*31	c.1290dupC p.Met431Hisfs*20	c.275A>G p.His92Arg	c.275A>G p.His92Arg	c.449A>T p.Asn150Ile	c.702C>G p.Asp234Glu	c.760A>G p.Arg254Gly
Variant type	Frameshift	Frameshift	Missense	Missense	Missense	Missense	Missense
Domain	/	/	Catalytic	Catalytic	Catalytic	Catalytic	Catalytic
Inheritance	De novo	De novo	De novo	De novo	Not maternal	De novo	De novo
Seizure onset	2 m	6 m	3 m	22 m	8 m	7 m	NA
EEG	Sharp, spike, sharp-slow, spike-slow wave	Hypsarrhythmia, repetitive polyspike-slow waves	Bilateral occipital spikes; MFD, PS	Occipital polyspike-slow waves	Hypsarrhythmia, spike-slow waves, polyspike-slow waves	Hypsarrhythmia, multifocal epileptic discharge	NA
DD (onset)	7.5 m	NA	3 m	16 m	5 m	6 m	NA
MRI: ventricle/ white matter/ hippocampal	Dilation/N/Might be small	NA/NA/NA	N/N/N	Normal at 4 y	Normal at 8 y	NA/NA/NA	NA/NA/NA
MRS	Basal ganglia and hypothalamic NAA peak decrease, neuronal lesion	NA	NA	NA	NA	NA	NA
Skeletal	Low bone density in the left hand, and the density of the metaphysis at the distal end of the ulnar radius raised slightly	N	NA	N	N	N	NA
Treatment	Refractory; some response: KD; no response: LEV, VPA, TPM	Refractory; partial response: VPA; no response: TPM, et al.	Refractory; partial response: LEV	Refractory; no response: VPA; partial response: LTG	Refractory; no response: VPA, et al.	Refractory; partial response: VPA; no response: TPM, et al.	NA

Continued on following page.

Table 2. *Continued*

Case number (PMID)	Case 8 (28942967)	Case 9 (28942967)	Case 10 (28942967)	Case 11 (28942967)	Case 12 (28942967)	Case 13 (29432562)	Case 14 (29432562)
Age/gender	10 y/F	22 y/F	5.5 y/F	7 y/M	21.5 y/F	5 y/F	7 y/M
Variant (NM_000944.4)	c.843C > G p.His281Gln	c.844G > A p.Glu282Lys	c.844G > A p.Glu282Lys	c.1333C > T p.Gln445Ter	c.1339G > A p.Ala447Thr	c.1408T > C p.Phe470Leu	c.1417G > A p.Ala473Thr
Variant type	Missense	Missense	Missense	Nonsense	Missense	Missense	Missense
Domain	Catalytic	Catalytic	Catalytic	/	/	AI	AI
Inheritance	De novo	De novo	De novo	De novo	De novo	De novo	De novo
Seizure onset	3 m	4 y	NA	6 w	3.5 y	/	9 m
EEG	MFD; frontal sharp and spikes	GSW, PS and wave, abnormal background	MFD, abnormal background	Hypsarrhythmia; MFD, GSW	MFD	/	Abnormal background without epileptic discharge
DD/ID (onset)	3 m	2 y	Birth	1 m	2 y	At birth	10 m
MRI: ventricle/ white matter/ hippocampal	NA/NA/NA	NA/NA/NA	N/N/N	NA/Loss/NA	N/N/N	NA/NA/NA	Enlargement/ change/NA
MRS	NA	NA	NA	Low NAA to choline ratio	NA	NA	NA
Skeletal	NA	NA	NA	NA	NA	Gracile bones, fractures, arthrogryposis	Arthrogryposis
Treatment	ES responsive: steroids, VGB; focal epilepsy refractory: VPA, TPM	Refractory during childhood; seizure-free: VPA, LTG	NA	Refractory; some response: KD, et al.; no response: LEV, et al.	Refractory; partial response: VNS	NA	Tractable; response: LTG

AI, auto-inhibitory; DD, developmental delay; EEG, electroencephalogram; ES, epileptic spasms; ExAC, exome aggregation consortium; GSW, generalized spike and slow wave; KD, ketogenic diet; LEV, levetiracetam; LTG, lamotrigine; m, month; MRI, magnetic resonance imaging; MFD, multifocal epileptiform discharges; MRS, magnetic resonance spectroscopy; NAA, N-acetylaspartate; N, normal; NA, not mentioned; PS, polyspikes; TPM, topiramate; VGB, vigabatrin; VPA, valproate; w, week; m, month; y, year; 1KG, 1000 genome; /, not in any domain.

and p.His281Gln (Case 8), were LoF (Mizuguchi et al. 2018). These patients were diagnosed with West syndrome or Lennox–Gastaut syndrome, and all suffered from refractory seizures and profound/severe intellectual disability (ID) (Myers et al. 2017; Mizuguchi et al. 2018). However, Mizuguchi et al. found that another missense mutation in this domain, p.Glu282Lys, did not affect CN signaling in a yeast assay (Mizuguchi et al. 2018). Two patients (Cases 9 and 10) with the mutation p.Glu282Lys did not present with refractory seizures. Rather, Case 9 had mild epilepsy from age 4 to 9 yr that resolved by age 16, whereas Case 10 had no epilepsy until age 5.5 yr, although EEG showed abnormal background activity with multifocal epileptiform discharges. However, both cases suffered from severe ID. The patient with the p.Arg254Gly mutation (Case 7) reported in the Deciphering Developmental Disorders study (Deciphering Developmental Disorders Study 2017) was described with an "abnormality of the nervous system," but there was no mention of epileptic seizures. This mutation has not been subjected to functional study. The missense mutation p.Ala447Thr in Case 12 is outside the functional domain, but nonetheless was associated with refractory seizures and profound ID.

In summary, all 14 patients with de novo *PPP3CA* mutations documented to date have presented with obvious DD and ID regardless of the domain mutated. Therefore, rare mutations in the catalytic domain and other domains are associated with clinically severe DD and ID. In this study, we describe a novel frameshift mutation of *PPP3CA* related to early-onset severe neurodevelopmental disease with seizures. This description further expands our knowledge of the relationships among *PPP3CA* mutations and neural deficits.

METHODS

Whole-Exome Sequencing

Genomic DNA was extracted from whole blood using the QIAamp DNA Blood Mini Kit (Catalog no. 51106). Whole-exome capture was performed using the Agilent SureSelect Human All Exon 50Mb Kit followed by sequencing as 150-bp paired-end runs on an Illumina XTen platform. More than 10 GB of raw data was generated for each sample. Variants were called using the Genome Analysis Toolkit Best Practices Pipeline (Version 3.2.2) (Van der Auwera et al. 2013), and data filtering, variants prediction, and interpretation followed ACMG guidelines and our previous work (Richards et al. 2015; Chen et al. 2018). Segregation of the *PPP3CA* mutation within the family was confirmed by Sanger sequencing on the ABI 3730 Genetic Analyzer (Applied Biosystems).

Protein Structure Analysis

The secondary structures of PPP3CA in wild type and mutants were analyzed by UniProt (http://www.uniprot.org/), Mutalyzer (https://www.mutalyzer.nl/), and SWISS-MODEL (https:// www.swissmodel.expasy.org). We downloaded the FASTA sequence of PPP3CA from UniProt (ID: Q08209) and selected 4orb.1.A as the protein structure template (Li et al. 2016). We used MODELLER automated modeling (Marti-Renom et al. 2000). Protein structures were visualized by PyMOL 1.7.4.

ADDITIONAL INFORMATION

Data Deposition and Access

The pathogenic *PPP3CA* mutation has been submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) with submission number SCV000692475. Patient family permission to deposit the trio-family whole-exome sequencing data was not obtained.

Ethics Statement

This study was approved by the ethics committees of Children's Hospital, Fudan University (2015-130 and 2016-235). The pretest counseling was performed in the clinic. Oral consent was obtained from patient's parents for research.

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Author Contributions

Y.Q. and H.W. drafted this manuscript. B.W., Y.L., and X.D. oversaw the clinical genetic diagnosis, Q.Q. analyzed the head MRI data, and W.Z. guided the clinic form information.

Competing Interest Statement

The authors have declared no competing interest.

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