

De Novo Mutations in *PPP3CA* Cause Severe Neurodevelopmental Disease with Seizures

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Exome sequencing has readily enabled the discovery of the genetic mutations responsible for a wide range of diseases. This success has been particularly remarkable in the severe epilepsies and other neurodevelopmental diseases for which rare, often *de novo*, mutations play a significant role in disease risk. Despite significant progress, the high genetic heterogeneity of these disorders often requires large sample sizes to identify a critical mass of individuals with disease-causing mutations in a single gene. By pooling genetic findings across multiple studies, we have identified six individuals with severe developmental delay (6/6), refractory seizures (5/6), and similar dysmorphic features (3/6), each harboring a *de novo* mutation in *PPP3CA*. *PPP3CA* encodes the alpha isoform of a subunit of calcineurin. Calcineurin encodes a calcium- and calmodulin-dependent serine/threonine protein phosphatase that plays a role in a wide range of biological processes, including being a key regulator of synaptic vesicle recycling at nerve terminals. Five individuals with *de novo PPP3CA* mutations were identified among 4,760 trio probands with neurodevelopmental diseases; this is highly unlikely to occur by chance ($p = 1.2 \times 10^{-8}$) given the size and mutability of the gene. Additionally, a sixth individual with a *de novo* mutation in *PPP3CA* was connected to this study through GeneMatcher. Based on these findings, we securely implicate *PPP3CA* in early-onset refractory epilepsy and further support the emerging role for synaptic dysregulation in epilepsy.

Introduction

Epilepsies are a group of conditions in which recurrent seizures are the defining feature. Seizures arise from a dysregulation of excitatory and inhibitory networks in the brain. While the complex mechanisms of this electrical imbalance are not fully understood, genetic research has identified a large number of epilepsy risk-associated genes that are assumed to play a role in electrical homeostatic mechanisms in the brain.^{1–13} Studies of people with epilepsy and epilepsy mouse models implicate genes governing synaptic vesicle neurotransmitter release and reuptake, a cyclic process that controls the level of neurotransmitters in the synaptic cleft.¹⁴ In humans specifically, mutations in *STXBPI* (MIM: 602926), *STX1B* (MIM: 601485), and *DNM1* (MIM: 602377), each encoding proteins integrally involved in the synaptic vesicle cycle at the nerve terminal, have been securely associated with developmental and

epileptic encephalopathies.^{1,15,16} Epileptic encephalopathies are severe disorders characterized by early-onset epilepsy and developmental delays with or without regression that may be worsened by ongoing epileptic activity.¹⁷ Additionally, there is growing evidence of causative *de novo* mutation in *SNAP25* (MIM: 600322), as two case reports of individuals with neurodevelopmental diseases have been described.^{18,19}

We now implicate *PPP3CA* (MIM: 114105), which encodes a protein involved in synaptic vesicle recycling, in epilepsy and neurodevelopmental disorders, further strengthening the role of synaptic vesicle cycle dysregulation in epilepsy. We report six individuals, each with a *de novo* mutation in *PPP3CA* (GenBank: NM_000944.4); these likely pathogenic variants comprise four missense mutations, one which is recurrent, and a single nonsense mutation (Table 1). Five mutations were identified from a cohort of 4,760 individuals with a range of neurodevelopment

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Table 1. Individuals with PPP3CA Encephalopathy: De Novo PPP3CA Mutations and Clinical Features

	Individual 1 (EG10251B1)	Individual 2 (Igsnd30299is1)	Individual 3 (isrl69xx3)	Individual 4 (T26323)	Individual 5 (Ambry Clinical WES)	Individual 6 (GeneMatcher – GeneDx)
Mutation (GRCh37/hg19)	4-101953430-G-A; NM_000944.4; c.1333C>T (p.Gln445Ter)	4-102030220-T-C; NM_000944.4; c.275A>G (p.His92Arg)	4-101953424-C-T; NM_000944.4; c.1339G>A (p.Ala447Thr)	4-102004360-G-C; NM_000944.4; c.843C>G (p.His281Gln)	4-102004359-C-T; NM_000944.4; c.844G>A (p.Glu282Lys)	4-102004359-C-T; NM_000944.4; c.844G>A (p.Glu282Lys)
Age (gender)	7 y (male)	12.5 y (male)	21.5 y (female)	10 y (female)	22 y (female)	5.5 y (female)
Inheritance	<i>de novo</i>	<i>de novo</i>	<i>de novo</i>	<i>de novo</i>	<i>de novo</i>	<i>de novo</i>
Ethnicity	Korean, European	European	Ashkenazi, Sephardic	Maori, European New Zealander	Pakistani	Eastern European, Ashkenazi, Irish
Presenting Sz (offset)	6 w: MF clonic and M	3 m: FIAS	3.5 y: GTCS	3 m: ES (18 m)	4 y: Ab (10 y)	N/A
Other Sz types (offset)	5 m ES, T; 6 m F	13 m ES (head drop-predominant), T with myoclonic eyelid flutter; 2 y 11 m drop seizures with jackknife flexion of torso & head propulsion to the floor from a seated position; 5 y SE, atypical Ab	T (age unknown)	3 y FIAS (frontal lobe)	5 y M (12 y); 8 y At, FM (12 y); 9 y GTCS, T (13 y)	N/A
Age of first developmental concerns	by 1 m	3 m	2 y	3 m	2 y	birth
Regression	no	5 y with SE	3.5 y	12 m with worsening sz	no	no
Developmental Outcome	profound ID, NV, NA; cortical visual impairment; G-tube and tracheostomy	profound ID, NV, NA (could roll and sit prior to regression), poor visual tracking;	profound ID; limited vocabulary with short sentences (lots of perseverations); autistic features; ataxic gait progressed to wheelchair	profound ID; NA, (unable to sit independently); NV (babble only); G-tube; cortical visual impairment	severe ID, 5 words, unsteady gait; not toilet trained	severe ID, autistic features; walked at 23 m; single words
EEG	5 m: hypsarrhythmia; 8 & 12 m: MFD, GSW; 4 & 5 y: hypsarrhythmia;	5 m: L, R, & bilateral occipital spikes; 17 m: frequent MFD, and PS, abnormal background, burst suppression in sleep, modified hypsarrhythmia; 5 y: MFD, bursts of generalized fast activity, abnormal background	3.5 y: MFD	12 m: MFD, N background; 18 m: MFD, close to hypsarrhythmia in sleep; 2 y: N; 3 y: frequent frontal sharp and spikes; 9 y: frontal fast activity (beta persistent) and occasional frontal spikes	8 y: abnormal background frequent GSW, PS and wave; 14 y: PSW 1-2 Hz bifrontal maximum	20 m: MFD, abnormal background

(Continued on next page)

Table 1. Continued

	Individual 1 (EG10251B1)	Individual 2 (Igsnd30299is1)	Individual 3 (isrl69xx3)	Individual 4 (T26323)	Individual 5 (Amby Clinical WES)	Individual 6 (GeneMatcher – GeneDx)
MRI	6 w: delayed myelination, especially orbitofrontal gyri and anterior temporal lobes; progressive bioccipital white matter loss; progressive bilateral T2 hyper intensity; low NAA to choline ratio	6 m & 5 y: N	5 & 10 y: N	1 y, 2 y & 3 y: mild generalized prominence of subarachnoid spaces	8 y & 9 y: mild cortical sulcal prominence & focal linear hyperintensity in the R centrum semiovale, consistent with a small developmental venous anomaly	2.5 y: N
Epilepsy syndrome	early-onset epileptic encephalopathy	early-onset epileptic encephalopathy; Lennox-Gastaut syndrome	developmental & epileptic encephalopathy	West syndrome, 3 m (not recognized until 12 m), focal (frontal) epilepsy 3 y	developmental & epileptic encephalopathy	none (EEG done to rule out epileptic aphasia)
Neurological examination	severe hypotonia; severe opisthotonus in infancy	marked axial & peripheral hypotonia	spasticity	hypotonia, N growth parameters	mild hypotonia & hyporeflexia	N
AED response	refractory; some response to KD, VGB, PB, TPM, CBD; no response to LEV, RFM, ACTH, PRD	refractory; PB, ZNS, OCBZ, LEV (partial response), VPA, LTG (rash), methsuximide (rash), CZP, CLB, steroids, KD	refractory; VPA, TPM, CBZ, LTG, ZNS, prysoline, PB, OCBZ, felbatol, ETX, LEV, lacosamide; exacerbation on KD; partial response to VNS at 16 y	ES responsive to steroids and VGB; focal epilepsy refractory: VPA, TPM	refractory during childhood; seizure-free on VPA and LTG	NA
Dysmorphic features	none	mild hypertelorism	coarse facial features, thick lips and big tongue (not related to medication), hoarse voice, hypertelorism	none	hypertelorism, prominent nasal tip extending below the columella, extremely short philtrum, small hands and feet, short stature	single palmar crease R hand; micrognathia
Additional features	none	17 m hyperkinetic choreoathetosis & dystonia of limbs	skin puffiness at hands & feet, hoarse voice	frequent laughing spells & rocking behaviors; bilateral talipes	cleft palate, R congenital talipes equinovarus, stereotypical repetitive & self-injurious behaviors	IUGR at 20 w gestation; born at 34 w; tracheostomy until 7 m due to airway difficulties; anxiety, aggression & self-injurious behaviors; stereotypic hand movements

Abbreviations are as follows: Ab, absence seizure; At, atonic seizure; ACTH, adrenocorticotropic hormone; AZD, acetazolamide; CBD, cannabidiol; CBZ, carbamazepine; CLB, clobazam; CZP, clonazepam; EEG, electroencephalogram; ES, epileptic spasms; ETX, ethosuxamide; F, focal seizure; FD, focal epileptiform discharges; FM, focal motor seizure; G-tube, gastrostomy tube; GTCS, generalized tonic-clonic seizure; GSW, generalized spike and slow wave; ID, intellectual disability; IUGR, intrauterine growth retardation; KD, ketogenic diet; L, left; LEV, levetiracetam; LTG, lamotrigine; m, months; M, myoclonic seizure; MFD, multifocal epileptiform discharges; MRI, magnetic resonance imaging; N, normal; NA, nonambulatory; NV, nonverbal; OCBZ, Oxcarbazepine; PB, phenobarbitone; PRD, prednisolone; PS, polyspikes; R, right; RFM, rufinamide; Sz, seizure; SE, status epilepticus; T, tonic seizure; TPM, topiramate; VGB, vigabatrin; VPA, valproate; w, weeks; y, years; ZNS, zonisamide.

disorders and a sixth case was linked to this study through the web-based tool GeneMatcher.²⁰ The majority of individuals (5/6) harboring *de novo* mutations in *PPP3CA* have seizures and all have severe developmental delay. *PPP3CA* encodes an isoform of a subunit of the Ca²⁺ interacting serine/threonine phosphatase, calcineurin. In addition to a prominent role in the activation of T cells,²¹ calcineurin is responsible for the calcium-dependent dephosphorylation of dynamin-1 (encoded by *DNM1*), a critical process regulating endocytosis following depolarization at the nerve terminal.²² These findings show that *PPP3CA* plays a key role in intractable childhood epilepsy and neurodevelopmental disorders. They also illuminate a possible shared pathophysiology with *DNM1*-mediated epilepsy and further support a role for dysregulation of the synaptic vesicle recycling at the nerve terminal in epileptogenesis.

Subjects and Methods

Patient Populations

All individuals or, in the case of minors or those with intellectual disability, their parents or legal guardians gave informed consent for participation in this study. The study was performed according to the standards of the ethics committees and the institutional review boards at each center.

Epilepsy Genetics Initiative (EGI)

Data from 84 trios who had undergone clinical exome sequencing that did not yield a genetic diagnosis were deposited into the EGI repository after informed consent was obtained. Reanalysis at the Institute for Genomic Medicine identified one case subject (EGI0251B1) with a *de novo* *PPP3CA* variant.

Infantile Spasms/Lennox Gastaut Syndrome Cohort

Trio exomes for 356 individuals with epileptic encephalopathies were previously analyzed by the Epi4K and EuroEPINOMICS Consortia to identify *de novo* variants.¹ A single case subject (igsnd30299is1) with a *de novo* *PPP3CA* variant was identified.

Undiagnosed Disease Trios

This cohort consisted of a previously published cohort of 119 trios with undiagnosed genetic disorders. They had a range of phenotypes, including some with neurodevelopmental diseases.²³ Individual isrl69xx3 had a *de novo* *PPP3CA* variant identified from this cohort.

Developmental and Epileptic Encephalopathies Targeted Sequencing Cohort

This cohort consisted of 508 individuals with a diverse range of developmental and epileptic encephalopathy phenotypes that were recruited from the epilepsy clinic through the practices of the investigators, and by referral for epilepsy genetics research internationally after informed consent. Each individual underwent phenotypic analysis with review of medical records, EEG, and MRI imaging when available and was classified into a specific epilepsy syndrome when possible. Targeted sequencing of *PPP3CA* was performed using single-molecule molecular inversion probes. Individual T26323 from this cohort had a *de novo* *PPP3CA* variant.

PPP3CA was selected for targeted sequencing based on the fact that it is found to be co-expressed with other genes known to cause developmental and epileptic encephalopathies (DEE) using the Web-based tool brain co-X.²⁴ To prioritize genes for the tar-

geted sequencing work performed in this study, we used a set of 42 genes where mutations are known to cause DEE (Table S1), 6 publicly available gene expression datasets that are specific to the human brain (Table S2), and an expression data cleaning algorithm (RUV)²⁵ to prioritize 263 unique candidate genes where a *de novo* variant was identified by trio WES sequencing in a cohort of 356 individuals with a diagnosis of infantile spasms or Lennox-Gastaut syndrome.¹ We note that an earlier version of this prioritization method has been previously described²⁶ and did not prioritize *PPP3CA*, but it evaluated a much smaller list of 29 genes where mutations are known to cause DEE and only the Allen Human Brain Atlas Gene expression resource.²⁷

Ambry Genetics

This cohort consisted of 3,693 individuals ascertained sequentially who underwent diagnostic WES sequencing through Ambry Genetics and were reported to have either developmental delay, intellectual disability, epilepsy, and/or autism. One individual with a *de novo* *PPP3CA* variant was identified through trio WES sequencing from this cohort.

Individual 6

This individual underwent diagnostic trio WES sequencing through GeneDx and was connected to our study through the clinician's entry into GeneMatcher. The exact number of WES performed to ascertain this sixth case could not be obtained and thus was not included in the statistical analysis.

Sequencing Analysis

Two samples sequenced at the Institute for Genomic Medicine (IGM) (previously the Center for Human Genome Variation at Duke University) were collected as peripheral blood. Exome enrichment was done using the SureSelect Human All Exon - 65MB, or the SureSelect Human All Exon - 50MB (Agilent), depending on when the trio was sequenced but consistent within the trio at the time. Sequencing was performed in the Genomic Analysis Facility on an Illumina HiSeq 2000. Data for an additional case subject were transferred to the IGM from a clinical exome test performed at the UCLA clinical lab using the Agilent SureSelect Human All Exon 50 Mb XT kit and Illumina HiSeq 2500.

All data from these three trios were analyzed in the same way using our standard bioinformatics pipeline which has been previously described.^{1,23} The pipeline employs GATK best practices, utilizing BWA 0.5.10,²⁸ picard tools 1.59, and the Unified Genotyper from GATK 1.6-11.²⁹⁻³¹ Reads are aligned to the hg19 reference sequence utilized in 1000 Genomes phase 2, which includes EBV-derived decoy sequences. Variant calls are functionally annotated with SnpEff 3.3³² using Ensembl build 73. All samples are processed individually through the bioinformatics pipeline.

Variant calls are loaded into our internal mysql database AnnoDB and further analyzed using ATAV. *De novo* calls are made by comparing proband variant calls to parents and ensuring adequate coverage (10×) to ensure a true homozygous reference call. *De novo* calls are further filtered to be high quality, based on GQ (>20), MQ (>40), QD (>2), QUAL (>50).

One proband was identified through targeted sequencing of *PPP3CA* performed on a cohort of 508 individuals with developmental and epileptic encephalopathies of unknown cause despite previous testing for single-nucleotide and copy number variants prior to this study. A multiplex targeted capture strategy was used to target the coding exons and intron-exon boundaries (>5 bp) (Table S3). Single-molecule molecular inversion probes

(smMIPS) were used as previously described, modified to have a 10-fold increase in the ratio of probe to genomic DNA (2,000:1).^{33,34} A random five-nucleotide molecular tag within the probe allowed for distinction of genomic molecules and a high-confidence consensus call. Sequencing was performed on an Illumina HiSeq 2500 and mapping and processing were performed as previously described.³⁵

Two additional individuals were identified through clinical trio exome sequencing performed by Ambry and GeneDx and matched through GeneMatcher.²⁰ Genomic DNA extraction, exome library preparation, sequencing, bioinformatics filtering, and data analyses were conducted as previously described.³⁶

Variant Prioritization

Variants were considered likely pathogenic if they arose *de novo*, affected the protein sequence, and were not observed in 13,122 internal or external control subjects (NHLBI Exome Sequencing Project Exome Variant Server, Genome Aggregation Database [gnomAD v.2.0], Exome Aggregation Consortium³⁷ [ExAC v.2.0; 123,136 individuals], and the 1000 Genomes project³⁸). Variants were validated by Sanger sequencing.

Statistical Testing

Testing for an excess of *de novo* variants was done by fitDNM.³⁹ fitDNM incorporates gene size, mutation rate, and functional impact to test whether there are more *de novo* observations in the patient cohort than by chance. The p value was then corrected for multiple testing by the number of CCDS genes, 18,668. While one of the individuals did not have full exome sequencing performed, it is a more conservative calculation to correct for all genes even though they were not actually interrogated. The exact number of WES tests performed to ascertain the sixth case subject could not be obtained. The calculated p value is based on the first five case subjects.

Results

Due to the extremely high genetic heterogeneity of the epilepsies, many genes are initially reported in trio-sequencing studies to harbor only one or two individuals with a *de novo* mutation across the entire cohort. Depending on the number of trios studied and the size and mutability of the gene, the number of *de novo* mutations observed in a gene in a single study may not reach the threshold of significance required to robustly implicate the gene as pathogenic. However, it is not uncommon that a subset of these genes harboring *de novo* mutations will emerge as definitive epilepsy genes when studies are combined, allowing for larger cohorts to be evaluated.^{1,7,35} The same is now true for *PPP3CA*. In 2014, the Epi4K Consortium reported the identification of a *de novo* missense mutation in *PPP3CA*.^{1,2} Motivated by the initial observation in the Epi4K cohort and evidence for co-expression of *PPP3CA* with genes previously associated with epileptic encephalopathy²⁶ via brain-coX (Figure S1, Subjects and Methods),²⁴ targeted sequencing of the gene using single-molecule molecular inversion probes was performed in 508 individuals with developmental and epileptic encephalopathies, and one additional case sub-

ject with a *de novo PPP3CA* mutation was identified. In 2015, another individual with a *de novo* missense mutation in *PPP3CA* was identified from exome sequencing of a cohort of individuals with severe congenital disorders.²³ A fourth case subject was identified through the Epilepsy Genetics Initiative, a collaborative effort that seeks to identify novel epilepsy genes through aggregate re-analyses of clinically generated exome-sequence data from individuals who had not received a genetic diagnosis at the time of sequencing. A fifth case subject was identified through clinical exome sequencing at Ambry Genetics, and a sixth case subject was linked to this study through GeneMatcher.²⁰ After compiling these observations, we used fitDNM to calculate the probability of observing five *de novo* mutations in *PPP3CA* by chance across all five cohorts consisting of a total of 4,760 case subjects with neurodevelopmental disease. Considering the site-based mutation rates, the size of the gene, and the size of the overall cohort, observing five *de novo* mutations with these predicted effects on the protein is not expected to occur by chance (uncorrected $p = 1.2 \times 10^{-8}$). This remains statistically significant when correcting for the 18,668 genes targeted in the Consensus Coding Sequence (CCDS v.14) ($p = 2.161 \times 10^{-4}$). While the number of exomes sequenced could not be obtained for the sixth case subject, this finding remains significant if the sixth case subject was found in a population smaller than 15,000. *PPP3CA* also shows extreme depletion of functional variation in the ExAC v.2.0 control population, a pattern consistent with the gene being under purifying selection and associated with severe disease. Of the observed variants in ExAC v.2.0, there is a depletion of variants that are predicted to be damaging: only eight missense variants have PolyPhen 2 HumVar scores⁴⁰ greater than 0.95, and only one stop gain due to an indel and a single splice site variant are reported in 123,136 exomes. Based on the full variant profile of *PPP3CA* in ExAC v.2.0, the calculated residual variation intolerance score (RVIS) is -0.71 translating to being among the 20% most intolerant genes in the genome.⁴¹ *PPP3CA* has a genic constraint score of 3.89,⁴² further supporting the RVIS score that variation predicted to have an effect on protein function is extremely rare in a healthy genome. Given both the significant excess of *de novo* mutations found across our full cohort and the intolerance of functional variation in *PPP3CA* in the control population, we now securely implicate pathogenic variants in *PPP3CA* as a cause of developmental and epileptic encephalopathies.

Of the six case subjects in total, one mutation is predicted to cause a premature stop codon in all Ensembl, RefSeq, and CCDS transcript annotations, while the other four mutations lead to missense changes, including one recurrent mutation (c.844G>A [p.Glu282Lys]; Table 1). Three of the four missense mutations fall within the protein phosphatase 2B (PP2B) catalytic domain (Figure 1). Recently, one additional *de novo* variant in the catalytic domain of calcineurin (c.760A>G [p.Arg254Gly]) was

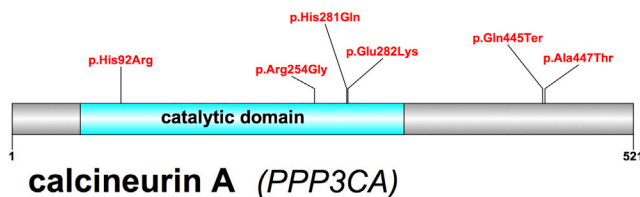


Figure 1. Calcineurin A (GenBank: NM_000944.4) Protein Structure with *De Novo* Mutations Mapped above the Image (Red)

The c.844G>A (p.Glu282Lys) is a recurrent mutation in our cohort. The c.760A>G (p.Arg254Gly) mutation was observed in an individual with an “abnormality of the nervous system” in the Deciphering Developmental Disorders study⁴³ and has not been validated. The catalytic domain (amino acid positions 58–329) is highlighted in blue.

identified in a single individual reported to have an “abnormality of the nervous system” in the Deciphering Developmental Disorders Study.⁴³ The catalytic domain is well conserved among the serine/threonine protein phosphatase family members (PP1, PP2A, PP2B, PP2C) and the low rate of mutation suggests that substitutions in this region may be deleterious.⁴⁴ Furthermore, the active site is coordinated between three histidines (His92, His199, His281), two aspartic acids (Asp90, Asp118), and one asparagine (Asn150),⁴⁵ and 4/6 case subjects have mutations at or adjacent to these amino acid positions.

Clinically, all six case subjects presented with developmental delay (evident from birth to 2 years of age) which was noted by 3 months in four individuals (Table 1). Regression occurred in three children, with all individuals developing severe or profound ID. Three of the six individuals were non-verbal, two had single words, and one spoke in short sentences. Two had autistic features. Four individuals were non-ambulatory.

Epilepsy was present in 5/6 individuals. Three had seizure onset within 3 months of age and had epileptic spasms and focal seizures. In the remaining two case subjects, seizures started later (3.5 and 4 years). All individuals with epilepsy developed multiple seizure types including focal (4/5), epileptic spasms (3/5), tonic (3/5), myoclonic (2/5), generalized tonic-clonic (2/5), and atonic seizures (1/5). On EEG, multifocal epileptiform discharges were seen in all but one individual who had only generalized discharges. Seizures were refractory to antiepileptic drugs in 4/5 children.

The sixth individual (age 5.5) had no seizures, but an EEG performed for abnormal language development showed an abnormal background with multifocal epileptiform discharges (Table 1). Interestingly, individual 6 had the same c.844G>A (p.Glu282Lys) variant as individual 5 (age 22 years) who had the mildest epilepsy which resolved by 16 years. Individual 5 presented with absence seizures at 4 years followed by myoclonic seizures (5 years), atonic and focal seizures (8 years), and generalized tonic-clonic and tonic seizures (9 years).

Hypotonia was noted in four individuals and spasticity in one. In terms of dysmorphic features, hypertelorism was observed in 3/6; one of these individuals also had coarse facial features, thick lips, a large tongue, and a hoarse voice.

MRI was normal for 3/6 probands, with the other individuals having mild abnormalities of uncertain significance (Table 1).

Discussion

We present evidence that mutations of *PPP3CA* are associated with developmental and epileptic encephalopathies. *PPP3CA* encodes the protein calcineurin A. Calcineurin is a highly conserved heterodimer consisting of a catalytic subunit (calcineurin A) and a regulatory subunit (calcineurin B). Calcineurin A is responsible for the interaction of calcineurin with calmodulin.^{46,47} Calcineurin in the brain plays an important role in synaptic transmission via regulation of the signaling response to calcium ions. Specifically it has been shown to suppress long-term potentiation,⁴⁸ play an important role in synaptic plasticity through the regulation of retinoic acid,⁴⁹ and regulate the activity of N-methyl-D-aspartate (NMDA) receptor channel gating.⁵⁰

While the role of calcineurin in synaptic transmission is not fully understood, it is involved specifically with synaptic vesicle recycling, a critical process in neurotransmission. The synaptic vesicle cycle begins with cellular depolarization that occurs following the influx of calcium ions through voltage-gated calcium channels. With this depolarization, neurotransmitter is released into the synaptic cleft through a process of exocytosis. Endocytosis, also regulated by intracellular calcium levels, is the process by which these vesicles are recycled at the plasma membrane to enable refilling with neurotransmitters for the next cellular depolarization/neurotransmission event.¹⁴ Dynamin-1, encoded by *DNM1*, is a nerve terminal phosphoprotein with intrinsic guanosine triphosphatase (GTPase) that serves a critical role in endocytosis. During endocytosis, dephosphorylated dynamin molecules form tetramers that pinch off empty vesicles for recycling when they hydrolyze GTP.⁵¹ The activity of dynamin is regulated by calcineurin, the phosphatase that has been shown to dephosphorylate dynamin-1.²²

Pathogenic variants in dynamin-1 are a known cause of developmental and epileptic encephalopathy that were first implicated when a spontaneous missense mutation was found in a mouse model of epilepsy.⁵² Several years after this discovery, disease-causing *de novo* mutations were identified in a cohort of individuals with epileptic encephalopathies.¹ *DNM1* mutations implicated in epilepsy reduce endocytosis.⁵³ Based on the mechanistic link between calcineurin (encoded in part by *PPP3CA*) and dynamin-1 (encoded by *DNM1*), one possibility is

that the mutations in *PPP3CA* may result in epilepsy by reducing DNMI-mediated endocytosis. Further supporting this conjecture, *PPP3CA*- and *DNMI*-mediated developmental and epileptic encephalopathies have overlapping clinical features. Both diseases are characterized by hypotonia, present with developmental delay, and lead to severe to profound impairment. Seizure onset is in the first year of life with infantile spasms in *DNMI*-related epilepsy, which were also seen in three of our six individuals with *PPP3CA* encephalopathy. In both diseases, individuals develop multiple seizure types and in the majority, the seizures are intractable.

In addition to *DNMI*, mutation in other genes involved in synaptic vesicle cycling include *STXB1*, *STX1B*, and *SNAP25* which have been implicated in epileptic encephalopathies.^{18,19,54,55} *STXB1*, *SNAP25*, and *STX1B* encode proteins that form part of the SNARE complex that mediates docking of synaptic vesicles in the process of exocytosis.⁵⁴ The identification of pathogenic variants in *PPP3CA* adds to this growing body of evidence supporting the role of dysregulation of synaptic vesicle cycling in epilepsy pathophysiology.

Supplemental Data

Supplemental Data include one figure, three tables, and Supplemental Acknowledgments and can be found with this article online at <http://dx.doi.org/10.1016/j.ajhg.2017.08.013>.

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Web Resources

brain-coX, <http://shiny.bioinf.wehi.edu.au/freytag.s/>
CURE's Epilepsy Genetics Initiative, <https://www.cureepilepsy.org/egi/>
GeneMatcher, <https://genematcher.org/>
NHLBI Exome Sequencing Project (ESP) Exome Variant Server, <http://evs.gs.washington.edu/EVS/>
OMIM, <http://www.omim.org/>

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