

## BRIEF COMMUNICATION

# Homozygous *PLCBI* deletion associated with malignant migrating partial seizures in infancy

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### SUMMARY

Malignant migrating partial seizures in infancy (MMPEI) is an early onset epileptic encephalopathy with few known etiologies. We sought to identify a novel cause of MMPEI in a child with MMPEI whose healthy parents were consanguineous. We used array comparative genomic hybridization (CGH) to identify copy number variants genome-wide and long-range polymerase chain reaction to further delineate the breakpoints of a deletion found by CGH. The proband had an inherited homozygous deletion of chromosome 20p13, disrupting the promoter region and first three coding exons of the

gene *PLCBI*. Additional MMPEI cases were screened for similar deletions or mutations in *PLCBI* but did not harbor mutations. Our results suggest that loss of PLCβ1 function is one cause of MMPEI, consistent with prior studies in a *Plcb1* knockout mouse model that develops early onset epilepsy. We provide novel insight into the molecular mechanisms underlying MMPEI and further implicate *PLCBI* as a candidate gene for severe childhood epilepsies. This work highlights the importance of pursuing genetic etiologies for severe early onset epilepsy syndromes.

**KEY WORDS:** Focal epilepsy, Migrating partial seizures in infancy, Genetics, Phospholipase C beta 1.

Malignant migrating partial seizures in infancy (MMPEI) is a rare, severe early infantile-onset epileptic encephalopathy (Coppola et al., 1995). The syndrome is associated with virtually continuous multifocal seizures on electroencepha-

logram (EEG) that migrate between cortical regions and hemispheres. Magnetic resonance imaging (MRI) and standard neurometabolic evaluations do not reveal an etiology. Seizures in MMPEI are refractory to conventional treatment with antiepileptic drugs (AEDs), and overall developmental prognosis is poor.

MMPEI is a genetically heterogeneous disorder with few known etiologies. Both deletion and point mutation of the voltage-gated sodium channel gene *SCN1A* are associated with MMPEI (Carranza Rojo et al., 2011; Freilich et al., 2011). A case of MMPEI is also described with duplication of 16p11.2 (Bedoyan et al., 2010). Here we identify an inherited homozygous deletion of the gene *phospholipase C beta 1 (PLCBI)* in a child with MMPEI.

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## SUBJECTS AND METHODS

The proband was evaluated at Children's Hospital Boston (CHB). Additional MMPEI cases were ascertained from Australia, the United Kingdom, Saudi Arabia, Sweden, the United States, and New Zealand. All subjects had MMPEI as described by Coppola et al., (1995). Research was performed in accordance with the institutional review board of CHB; written informed consent was obtained from all participants and their guardians.

Blood samples were collected, and DNA was extracted using standard methods. DNA from the proband and parents was fragmented, labeled, and hybridized to an oligonucleotide-based array for chromosomal microarray analysis (CMA) (CHB DNA Diagnostic Lab version 1.4, Agilent 244K platform; Agilent, Santa Clara, CA, U.S.A.), which detects copy number variants (CNVs) as small as 150 kB.

Delineation of the *PLCB1* deletion was performed using long-range polymerase chain reaction (PCR) (Kurian et al., 2010). Additional MMPEI cases were evaluated for *PLCB1* mutations and intragenic deletions by direct sequencing using PCR primers directed against each exon of *PLCB1*. Primers were designed using PRIMER 3 software (Rozen & Skaletsky, 2000) (sequences available on request).

## RESULTS

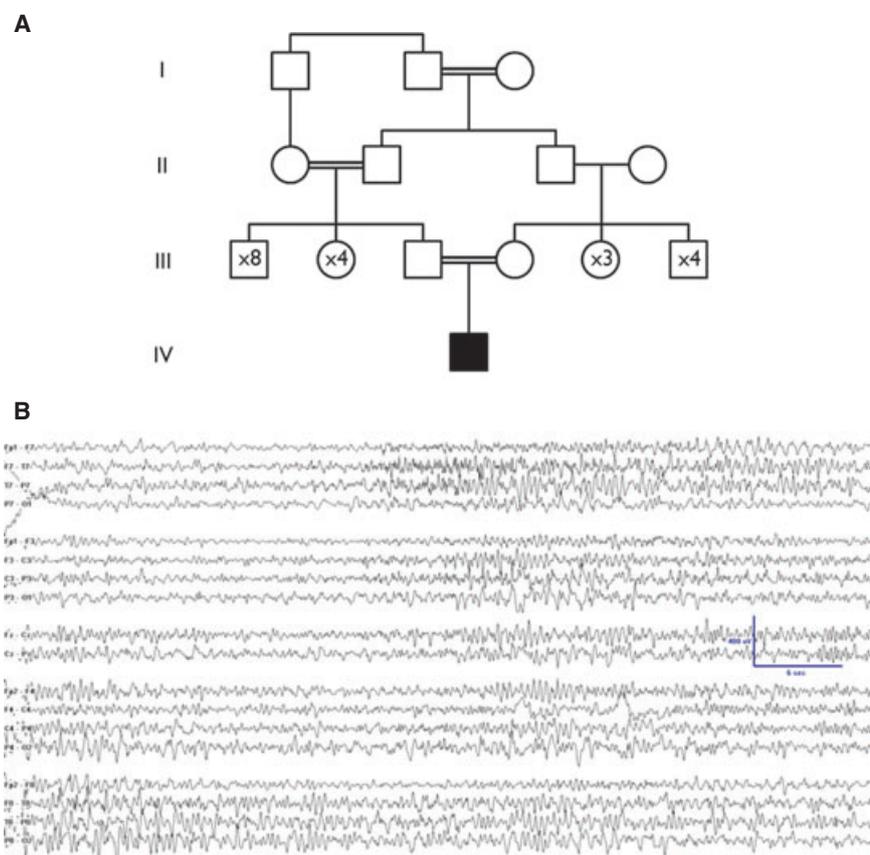
### Clinical Presentation

The patient was born at 42 weeks of gestation after a normal pregnancy. He is the first child of healthy parents who are first cousins of Palestinian descent (pedigree shown in Fig. 1A). Seizures began at 6 months with perioral cyanosis, limpness, mouth automatisms, eyelid fluttering, and at times desaturation with oxygen levels as low as 35–55%; seizures lasted from 10 s to 2 min. Eventually, some seizures consisted only of staring and activity arrest with eye deviation to the right or to the left. Before seizure onset, development was delayed but progressing; he was babbling and bringing objects together but not yet rolling or sitting. Once seizures began, he made only guttural sounds, did not fix or follow objects, and had limited voluntary movements of the limbs. At presentation at 6 months of age, his neurologic examination was notable for marked truncal and appendicular hypotonia. EEG showed multifocal interictal spikes and abundant seizures arising from the right and left temporal lobes independently, at times with migration from one hemisphere to the other within a seizure (Fig. 1B). In the first 4 months of hospitalization, from age 6 months to 10 months, he had an average of 27 electrographic seizures

**Figure 1.**

(A) The four-generation pedigree is notable for multiple consanguineous relationships. The proband's parents are first cousins. (B) An example of the ictal electroencephalography from the proband demonstrates the migrating nature of the seizures typical of MMPEI. On the left side of the page, a right temporal seizure is shown. On the right, migration of the seizure to include the left temporal region is shown.

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per day (about half with clinical symptoms) when EEG recordings were performed.

Treatment was attempted with multiple AEDs, including fos-phenytoin, phenobarbital, pyridoxine, benzodiazepines (lorazepam, clonazepam, diazepam, clobazam, and infusion of midazolam), levetiracetam, rufinamide, topiramate, lacosamide, triple bromide solution (ammonium bromide, potassium bromide, and sodium bromide), stiripentol, prednisolone, and the ketogenic diet (3.5:1 ratio).

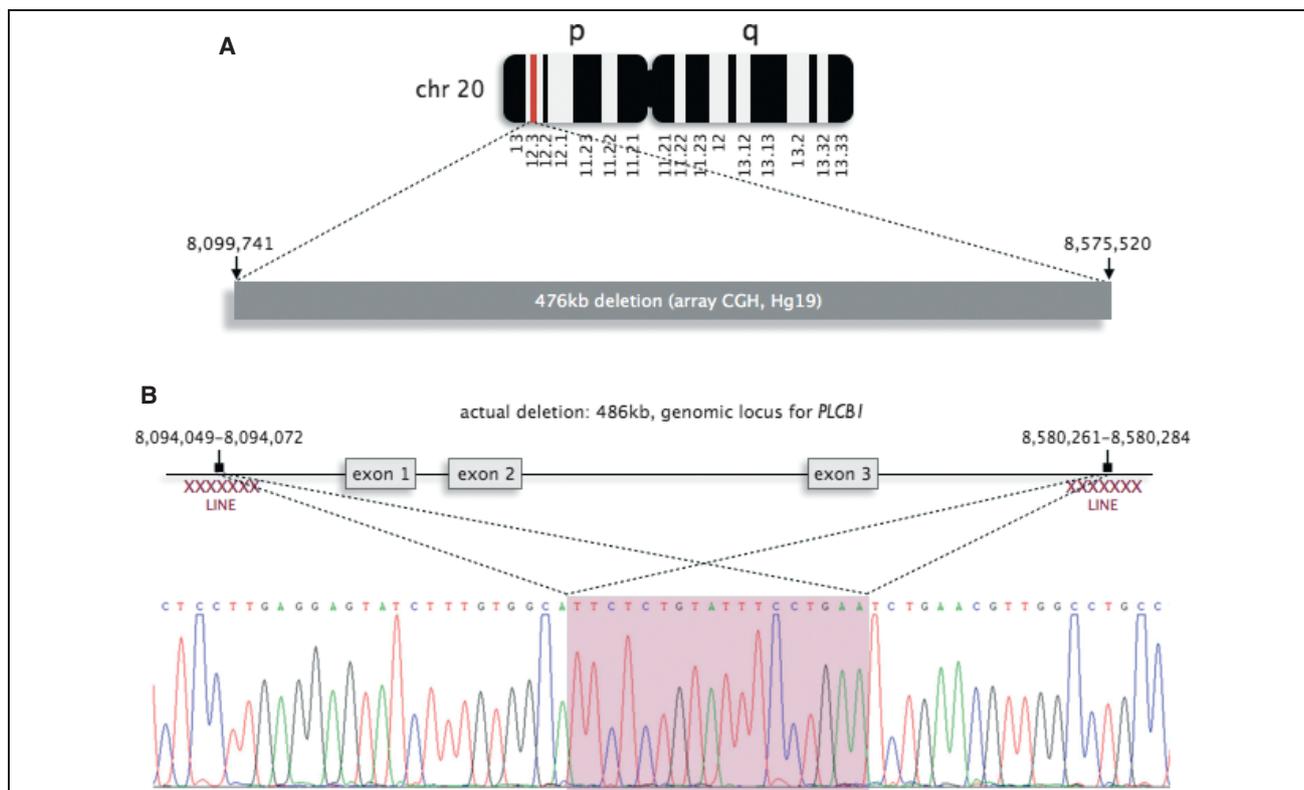
### Diagnostic Evaluation, including CMA

MRI at 6, 7, 8, and 9 months revealed mildly prominent cerebrospinal fluid spaces. MR spectroscopy performed at 9 months was normal. Laboratory investigations for inborn errors of metabolism, neurotransmitter disorders, and *SCN1A* mutations were unrevealing.

Chromosomal microarray analysis of the proband identified three CNVs: (1) homozygous ~476 kb deletion

of chromosome 20p12.3 (0 copies, chr20: 8,099,741-8,575,520 in Human Genome build hg19) (Fig. 2A); (2) heterozygous ~109 kb duplication of chromosome 7p21.3 (three copies); and (3) heterozygous ~125 kb duplication of chromosome 12q24.12 (three copies). Both parents were found to be heterozygous for the 20p12.3 deletion. Of >6,600 patients who have been assessed by comparative genomic hybridization (CGH) in our DNA Diagnostic Laboratory, this is the only family bearing the 20p12.3 deletion. The 7p21.3 and 12q24.12 duplications were maternally inherited variants.

The 20p12.3 deletion includes the first three coding exons of *PLCB1* and 65.6 kilobases (kb) of 5' upstream genomic DNA (Fig. 2B). No other annotated genes or known non-coding RNAs were identified. Using long-range PCR, we localized the deletion breakpoints to chr20: 8,094,049-8,094,072 and chr20: 8,580,261-8,580,284, defining a 486 kb deletion (Fig. 2B). The breakpoints could not be more precisely defined due to a 23-nucleotide sequence



**Figure 2.**

(A) Proband, paternal, and maternal genomic DNA was isolated from peripheral blood and then fragmented, labeled, and hybridized for targeted array comparative genomic hybridization (array CGH). The proband's study revealed a homozygous 476 kb deletion on chromosome 20p12.3 as illustrated in the schematic (red band). This deletion corresponds to coordinates 8,099,741-8,575,520 on chromosome 20 (human genome build hg19). Parental studies revealed that each of the proband's parents is heterozygous for the 20p12.3 deletion. (B) The deletion occurs within the locus of the gene *phospholipase C beta 1* (*PLCB1*). Comparison of genomic DNA with sequenced *PLCB1* cDNAs revealed that the deleted region encompasses the first three coding exons of the gene. More precise deletion breakpoints were identified by long-range PCR of genomic DNA (8,094,049-8,094,072 to 8,580,261-8,580,284) and found to be flanked by repetitive long interspersed elements (LINE, red Xs). The exact deletion boundaries could not be resolved due to 23 nucleotides of 100% sequence homology between 5' and 3' breakpoints (shaded red).

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with 100% homology for both the upstream and the intron 3 sequence (Fig. 2B). The breakpoints lie within two L1 family long interspersed nuclear elements (LINE) L1PA3 and L1PA2 occurring at chr20: 8,089,514-8,095,564 and chr20: 8,575,749-8,581,774.

### Screening of Additional MMPEI Cases

We screened a consanguineous family from Saudi Arabia and two nonconsanguineous families from New Zealand and Sweden, each with two children affected with MMPEI, for additional *PLCB1* mutations or deletions. We also screened 12 MMPEI simplex cases from Australia, two from the United States, and one from the United Kingdom. None exhibited a mutation in *PLCB1*.

## DISCUSSION

We identified a homozygous deletion of *PLCB1* in a patient with MMPEI. Loss of the first three coding exons of the *PLCB1* cDNA and possibly important 5' regulatory elements likely resulted in loss of wild type PLC $\beta$ 1 protein expression and the MMPEI phenotype. This deletion is flanked by repetitive sequences and thus likely arose in the heterozygous state as a result of nonallelic homologous recombination.

*PLCB1* is a novel gene for MMPEI, a rare epilepsy with few identifiable etiologies. Kurian and colleagues previously described a case of early onset epileptic encephalopathy (EOEE) associated with deletion of *PLCB1* (Kurian et al., 2010). Epilepsy onset for our proband occurred at 6 months with focal seizures, an EEG characteristic of MMPEI, and developmental regression. In contrast, the prior case of *PLCB1*-associated epilepsy had onset of tonic seizures at 10 weeks, recurrence at 6 months, and infantile spasms at 8 months; this case had an initially normal EEG and normal development and later developed hypsarrhythmia on EEG and developmental regression (Kurian et al., 2010). Therefore these two patients with *PLCB1* deletions fall into two distinct electroclinical syndromes, with the previous case representing EOEE and our case representing MMPEI.

We demonstrate two phenomena that have become recurring themes in epilepsy genetics: (1) the heterogeneity of the phenotypic presentations of genes associated with early onset epileptic encephalopathies, including *PLCB1*; and (2) the heterogeneity of the genetic etiologies of a well-defined epileptic encephalopathy, namely MMPEI. The lack of *PLCB1* mutations in our additional MMPEI cases further illustrates this genetic heterogeneity.

The enzyme encoded by *PLCB1*, phospholipase C isoform  $\beta$ 1 (PLC $\beta$ 1) generates the intracellular second messengers diacylglycerol and inositol-1,4,5-trisphosphate (Ins-1,4,5,-P<sub>3</sub>, also called IP<sub>3</sub>) from phosphatidylinositol-4,5,-bisphosphonate (PtdIns-4,5-P<sub>2</sub>, also called PIP<sub>2</sub>). A murine model for homozygous *Plcb1* deletion presented

with early generalized seizures and death, underscoring the role of PLC $\beta$ 1 in normal neuronal development and function (Kim et al., 1997). In wild-type rats, the  $\beta$ 1 and  $\delta$ 1 isoforms of the PLC enzyme are expressed postnatally, whereas the  $\gamma$ 1 isoform is expressed prenatally (Shimohama et al., 1998). These observations suggest that disruption of PLC $\beta$ 1 function might not affect the nervous system until after birth, consistent with the observation that both patients with *PLCB1* deletion had normal development reported for several weeks to months. We postulate that this temporal pattern reflects one or more of the following phenomena: (1) a limited role for human PLC $\beta$ 1 in prenatal and early postnatal neuronal development and function; (2) functional redundancy among different neuronal phospholipases expressed during development; and/or (3) the maturation of a neuronal pathway sensitive to PLC $\beta$ 1 deficiency.

Together with other studies, our findings underscore the importance of genome-wide copy number assessment for all unexplained cases of epileptic encephalopathy (Heinzen et al., 2010; Mefford et al., 2010, 2011). The identification of *PLCB1* as the gene associated with MMPEI in our proband opens the possibility of addressing his molecular defect by modification of PLC $\beta$ 1-related pathways. We are optimistic that continued efforts to unravel the molecular mechanisms of early onset epilepsies such as MMPEI may one day translate into clinical interventions to improve the lives of children afflicted with these devastating disorders.

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## DISCLOSURES

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