Epilepsia, 53(8):e146–e150, 2012
doi: 10.1111/j.1528-1167.2012.03538.x

BRIEF COMMUNICATION

Homozygous *PLCB1* deletion associated with malignant migrating partial seizures in infancy

*†[1]Annapurna Poduri, †‡§[1]Sameer S. Chopra, †§Edward G. Neilan, *P. Christina Elhosary, ¶#Manju A. Kurian, ¶Esther Meyer, **Brenda J. Barry, *†[3]Omar S. Khwaja, ††Mustafa A. M. Salih, ‡‡Tommy Stödberg, §§Ingrid E. Scheffer, ¶¶Eamonn R. Maher, *†##***Mustafa Sahin, †††Bai-Lin Wu, †§##Gerard T. Berry, †§**##Christopher A. Walsh, †§‡‡‡[2]Jonathan Picker, and *†[2]Sanjeev V. Kothare

*Department of Neurology, Children's Hospital Boston, Boston, Massachusetts, U.S.A.; †Harvard Medical School, Boston, Massachusetts, U.S.A.; ‡Department of Internal Medicine, Brigham and Women's Hospital, Boston, Massachusetts, U.S.A.; §Division of Genetics, Children's Hospital Boston, Boston, Massachusetts, U.S.A.; ¶Neurosciences Unit, Institute of Child Health, University College London, London, United Kingdom; #Department of Neurology, Great Ormond Street Hospital, London, United Kingdom; **Howard Hughes Medical Institute, Chevy Chase, MD, U.S.A.; ††Division of Pediatric Neurology, College of Medicine and King Khalid University Hospital, King Saud University, Riyadh, Saudi Arabia; ‡‡Department of Neuropediatrics, Astrid Lindgren Children's Hospital, Karolinska Hospital, Stockholm, Sweden; §§Department of Medicine and Paediatrics, University of Melbourne, Austin Health and Royal Children's Hospital, Melbourne, Victoria, Australia; ¶¶Department of Medical and Molecular Genetics, Centre for Rare Diseases and Personalised Medicine, University of Birmingham School of Medicine, Institute of Biomedical Research, Birmingham, United Kingdom; ##Manton Center for Orphan Disease Research, Children's Hospital Boston, Boston, Massachusetts, U.S.A.; ***F.M. Kirby Neurobiology Center, Children's Hospital Boston, Boston, Massachusetts, U.S.A.; †††Department of Laboratory Medicine, Children's Hospital Boston, Boston, Massachusetts, U.S.A.; and ‡‡‡Department of Child and Adolescent Psychiatry, Children's Hospital Boston, Boston, Massachusetts, U.S.A.

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 *PLCB1*. Additional MMPEI cases were screened for similar deletions or mutations in *PLCB1* 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 *PLCB1* 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 electroencephalogram (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* (*PLCB1*) in a child with MMPEI.

Accepted April 17, 2012; Early View publication June 12, 2012.
Address correspondence to Annapurna Poduri, Division of Epilepsy and Clinical Neurophysiology, Department of Neurology, Fegan 9, Children's Hospital Boston, 300 Longwood Avenue, Boston, MA 02115, U.S.A. E-mail: annapurna.poduri@childrens.harvard.edu
[1]These authors contributed equally to the manuscript.
[2]These senior authors contributed equally to the manuscript.
[3]Present address: F. Hoffmann-La Roche AG, Basel, Switzerland.

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

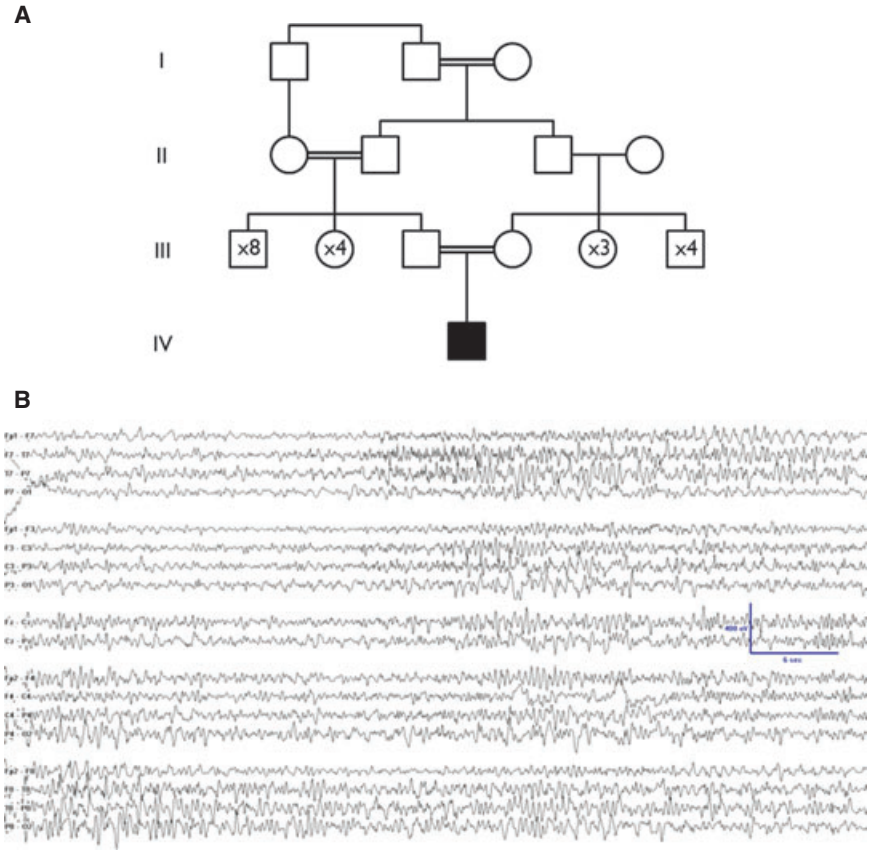

Figure 1.
(**A**) The four-generation pedigree is notable for multiple consanguineous relationships. The proband's parents are first cousins. (**B**) An example of the ictal electroencephalography from the proband demonstrates the migrating nature of the seizures typical of MMPEI. On the left side of the page, a right temporal seizure is shown. On the right, migration of the seizure to include the left temporal region is shown.
Epilepsia © ILAE

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

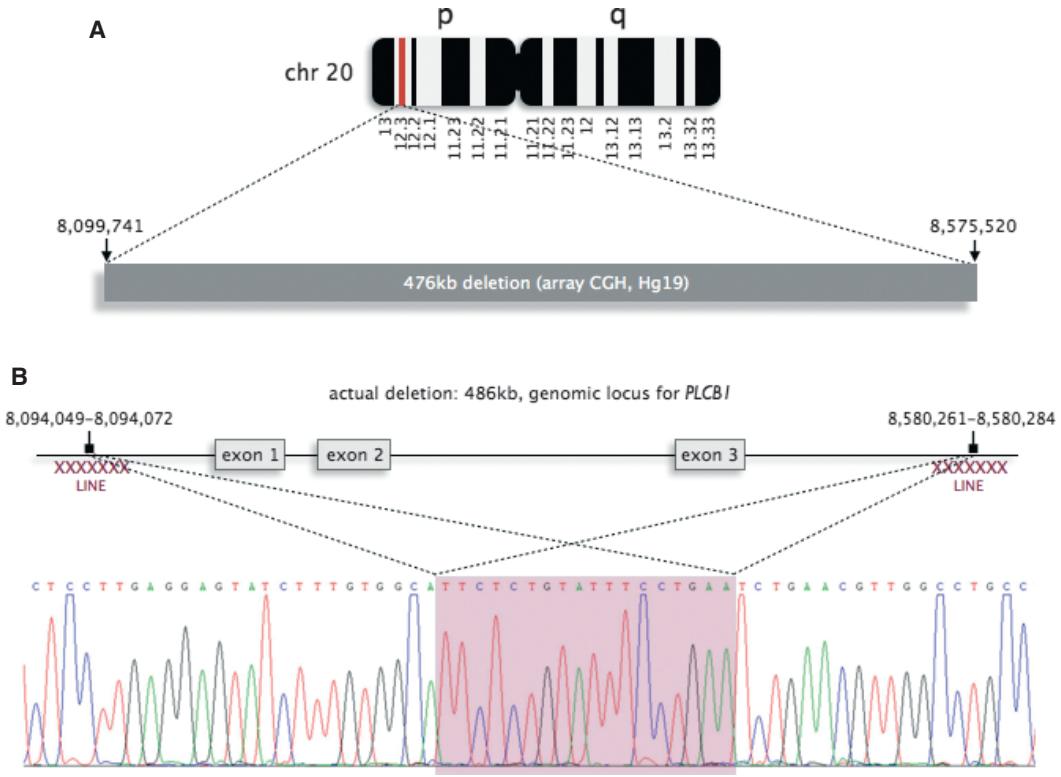

Figure 2.
(**A**) Proband, paternal, and maternal genomic DNA was isolated from peripheral blood and then fragmented, labeled, and hybridized for targeted array comparative genomic hybridization (array CGH). The proband's study revealed a homozygous 476 kb deletion on chromosome 20p12.3 as illustrated 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).

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 PLCβ1 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 β1 (PLCβ1) generates the intracellular second messengers diacylglycerol and inositol-1,4,5-trisphosphate (Ins-1,4,5,-P_3, also called IP_3) from phosphatidylinositol-4,5,-bisphosphonate (PtdIns-4,5-P_2, also called PIP_2). A murine model for homozygous *Plcb1* deletion presented with early generalized seizures and death, underscoring the role of PLCβ1 in normal neuronal development and function (Kim et al., 1997). In wild-type rats, the β1 and δ1 isoforms of the PLC enzyme are expressed postnatally, whereas the γ1 isoform is expressed prenatally (Shimohama et al., 1998). These observations suggest that disruption of PLCβ1 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 PLCβ1 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 PLCβ1 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 PLCβ1-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.

Acknowledgments

We thank the families of the children with MMPEI who participated in this research study. Annapurna Poduri was supported by the NINDS (K23NS069784). Manju Kurian's research is funded by Action Medical Research and Great Ormond Street Children's Charities. Mustafa A. Salih was supported by the College of Medicine Research Center (CMRC Project No. 07-581), College of Medicine, King Saud University, Riyadh, Saudi Arabia. Mustafa Sahin is an investigator of the CHB Translational Research Program and was also supported by John Merck Fund, Nancy Lurie Marks Family Foundation, and Manton Center for Orphan Disease Research. Ingrid E. Scheffer receives/has received research support from the National Health and Medical Research Council of Australia, Health Research Council of New Zealand, The University of Melbourne, American Epilepsy Society, the Jack Brockhoff Foundation, the Shepherd Foundation and the Perpetual Charitable Trustees. Eamonn Maher's research was funded by Action Medical Research. Bai-Lin Wu is supported by the Chinese National "973" project on Population and Health (2010CB529601) and the Science and Technology Council of Shanghai (09JC1402400). Christopher A. Walsh is an investigator of the Howard Hughes Medical Institute and was also supported by the NINDS (R01NS035129, R01NS035129S), the NIMH (NIMH1RC2MH089952), the Manton Center for Orphan Disease Research, the Dubai Harvard Foundation for Medical Research, the Simons Foundation, and the Nancy Lurie Marks Family Foundation.

Disclosures

Dr. Neilan was supported by Pharming Group and the Luke O'Brien Foundation. Dr. Scheffer has served on scientific advisory boards for UCB and Janssen-Cilag EMEA, received speaker honoraria from Athena Diagnostics, UCB, Biocodex and Janssen-Cilag EMEA, and received funding for travel from Athena Diagnostics, UCB, Biocodex, and Janssen-Cilag EMEA. Dr. Sahin's research has been supported by Novartis

(NCT00789828). The other authors report no disclosures. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

References

Bedoyan JK, Kumar RA, Sudi J, Silverstein F, Ackley T, Iyer RK, Christian SL, Martin DM. (2010) Duplication 16p11.2 in a child with infantile seizure disorder. *Am J Med Genet A* 152A:1567–1574.

Carranza Rojo D, Hamiwka L, McMahon JM, Dibbens LM, Arsov T, Suls A, Stödberg T, Kelley K, Wirrell E, Appleton B, Mackay M, Freeman JL, Yendle SC, Berkovic SF, Bienvenu T, De Jonghe P, Thorburn DR, Mulley JC, Mefford HC, Scheffer IE. (2011) De novo SCN1A mutations in migrating partial seizures of infancy. *Neurology* 77:380–383.

Coppola G, Plouin P, Chiron C, Robain O, Dulac O. (1995) Migrating partial seizures in infancy: a malignant disorder with developmental arrest. *Epilepsia* 36:1017–1024.

Freilich ER, Jones JM, Gaillard WD, Conry JA, Tsuchida TN, Reyes C, Dib-Hajj S, Waxman SG, Meisler MH, Pearl PL. (2011) Novel SCN1A mutation in a proband with malignant migrating partial seizures of infancy. *Arch Neurol* 68:665–671.

Heinzen EL, Radtke RA, Urban TJ, Cavalleri GL, Depondt C, Need AC, Walley NM, Nicoletti P, Ge D, Catarino CB, Duncan JS, Kasperaviciūte D, Tate SK, Caboclo LO, Sander JW, Clayton L, Linney KN, Shianna KV, Gumbs CE, Smith J, Cronin KD, Maia JM, Doherty CP, Pandolfo M, Leppert D, Middleton LT, Gibson RA, Johnson MR, Matthews PM, Hosford D, Kälviäinen R, Eriksson K, Kantanen AM, Dorn T, Hansen J, Krämer G, Steinhoff BJ, Wieser HG, Zumsteg D, Ortega M, Wood NW, Huxley-Jones J, Mikati M, Gallentine WB, Husain AM, Buckley PG, Stallings RL, Podgoreanu MV, Delanty N, Sisodiya SM, Goldstein DB. (2010) Rare deletions at 16p13.11 predispose to a diverse spectrum of sporadic epilepsy syndromes. *Am J Hum Genet* 86:707–718.

Kim D, Jun KS, Lee SB, Kang NG, Min DS, Kim YH, Ryu SH, Suh PG, Shin HS. (1997) Phospholipase C isozymes selectively couple to specific neurotransmitter receptors. *Nature* 389:290–293.

Kurian MA, Meyer E, Vassallo G, Morgan NV, Prakash N, Pasha S, Hai NA, Shuib S, Rahman F, Wassmer E, Cross JH, O'Callaghan FJ, Osborne JP, Scheffer IE, Gissen P, Maher ER. (2010) Phospholipase C beta 1 deficiency is associated with early-onset epileptic encephalopathy. *Brain* 133:2964–2970.

Mefford HC, Muhle H, Ostertag P, von Spiczak S, Buysse K, Baker C, Franke A, Malafosse A, Genton P, Thomas P, Gurnett CA, Schreiber S, Bassuk AG, Guipponi M, Stephani U, Helbig I, Eichler EE. (2010) Genome-wide copy number variation in epilepsy: novel susceptibility loci in idiopathic generalized and focal epilepsies. *PLoS Genet* 6:e1000962.

Mefford HC, Yendle SC, Hsu C, Cook J, Geraghty E, McMahon JM, Eeg-Olofsson O, Sadleir LG, Gill D, Ben-Zeev B, Lerman-Sagie T, Mackay M, Freeman JL, Andermann E, Pelakanos JT, Andrews I, Wallace G, Eichler EE, Berkovic SF, Scheffer IE. (2011) Rare copy number variants are an important cause of epileptic encephalopathies. *Ann Neurol* 70:974–985.

Rozen S, Skaletsky H. (2000) Primer3 on the WWW for general users and for biologist programmers. In Krawetz S, Misener S (Eds). *Bioinformatics methods and protocols: methods in molecular biology*. Humana Press, Totowa, NJ, pp. 365–386.

Shimohama S, Sumida Y, Fujimoto S, Matsuoka Y, Taniguchi T, Takenawa T, Kimura J. (1998) Differential expression of rat brain phospholipase C isozymes in development and aging. *Biochem Biophys Res Commun* 243:210–216.