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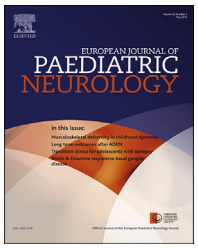

Review Article

# PLCB1 epileptic encephalopathies; Review and expansion of the phenotypic spectrum

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ABSTRACT

*Background:* Biallelic loss-of-function mutations of phospholipase C-β1 (*PLCB1*) have been described in three children with an early onset epileptic encephalopathy (EE). In two of them a homozygous deletion of the promotor and first three coding exons was found. The third patient had an almost identical heterozygous deletion in combination with a heterozygous splice site variant. All patients had intractable epilepsy and a severe developmental delay.

*Methods and results:* We present the case of a boy with an infantile EE starting at the age of four months with a fever induced status epilepticus, modified hypsarrhythmia and developmental regression. The epilepsy was reasonably controlled with corticoids and valproate whereupon generalized tonic-clonic seizures appeared only each 3–4 months. However, only a slow developmental progress was seen hereafter, resulting in a severe intellectual disability with absent speech, motor delay and autistic features. We identified a novel homozygous partial deletion of *PLCB1*, affecting exons 7–9.

*Conclusions:* This report emphasizes the role of *PLCB1* haploinsufficiency in severe EE. We demonstrate a phenotypic variability in patients with a *PLCB1*-associated EE. In addition, our findings underscore the importance of microarray analysis in all patients with an EE of unknown etiology.



## Contents



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## 1. Introduction

An epileptic encephalopathy (EE) is a condition in which the epileptic activity itself is believed to contribute to severe cognitive and behavioral impairment above and beyond what might be expected from the underlying pathology.[1] An EE can present at any age but is most common and most severe in infancy and early childhood, probably due to the more vulnerable developing brain.[2] In infancy, several epilepsy syndromes like Ohtahara syndrome (early infantile epileptic encephalopathy – EIEE), early myoclonic epileptic encephalopathy (EMEE), West syndrome, Dravet syndrome and Malignant Migrating Partial Seizures in Infancy (MMPSI) can present with features of an EE. The underlying etiology of an EE can be structural, metabolic, acquired and/or genetic, but in the majority of cases it is not identifiable.[3,4] However, in the last decade many genetic causes were identified.[5,6] Analysis of copy number variations (CNVs) and sequencing of genes associated with epileptic encephalopathies are therefore often routinely performed when other causes are ruled out.[7] Since 2010, homozygous or compound heterozygous loss-of-function mutations of phospholipase C-β1 (*PLCB1*) have been described in three children with an early onset EE, strongly suggesting a causal relationship.[8–10] Here we present a novel patient with biallelic loss of function of *PLCB1* with a different genetic and phenotypic spectrum.

A written informed consent for publication of this case study was obtained from both parents.

## 2. Case study

### 2.1. Clinical history

The index case was first seen in our pediatric neurology department at the age of 3 years, presenting with a fever-induced generalized tonic-clonic status epilepticus. He was intubated and sedated shortly with cessation of the status epilepticus. At that time he took no anti-epileptic drug (AED) since 3.5 months, although he had been treated with carbamazepine and valproate in the past.

He is the first child of healthy consanguineous refugees from Syria. The pregnancy was uneventful. He was born at term with a normal birth weight (3.250 kg) and had a normal development during the first months. Early developmental milestones were normal. At the age of four months, the first febrile status epilepticus was noted during a viral infection. Hereafter a developmental regression was seen together with the onset of multiple seizures, comprising epileptic spasms, focal seizures of mainly the left arm and generalized tonic-clonic seizures. The electroencephalography (EEG) showed a modified hypsarrhythmia. Treatment with valproate, carbamazepine and corticoids was initiated. The seizures were difficult to control but the frequency decreased after the age of 11 months. However, generalized tonic-clonic seizures were still noted every three–four months. Since the improved seizure control, his development gradually progressed. At that time parents, left Syria and arrived several months later in Belgium. During their travel the stock of AED had run out forcing them to stop his anti-epileptic treatment. After the withdrawal he was seizure free for 3.5 months, whereupon he developed a febrile status epilepticus. No further regression was seen after the status epilepticus.

Evaluation at the age of 4 years revealed a severe intellectual disability and autistic features. Bayley Scales of Infant Development-II (BSID-II) showed a motor development of 11 months at the age of 37 months. He is not able to speak and has limited visual interest. He started walking at the age of 3 years, although this is currently still broad based and limited to short distances. He had some mild dysmorphic features with a short, sloping forehead, brachycephaly and a high palate (Fig. 1) (head circumference (−3SD), weight (−1.5SD), length (−1.9SD)). Neurologic examination showed a mild axial hypotonia with normal tone in the extremities. No social interaction and only short visual fixation of objects was seen. Grasping was limited and only shortly with the palm of his hand. Self-mutilating behavior was present with hand biting and hand flapping.

After the status epilepticus at 3 years, a treatment with valproate was initiated with control of seizures during the follow-up period of one year. EEG at 4 years of age showed slowing of the background without epileptic activity. Central imaging with magnetic resonance imaging (MRI) at the age of 3 and 3.5 years showed mild generalized atrophy. Metabolic work-up, brainstem evoked response auditory test (BERA) and fundoscopy were also normal.

Family history (Fig. 2) revealed the presence of a sister who was born prematurely at a gestational age of 6 months and died soon after birth. Prenatal ultrasound had shown enlarged ventricles and structural brain abnormalities in this girl. No autopsy was performed in Syria and medical records were unavailable. In addition, the daughter of the father's sister had intractable seizures and severe psychomotor delay. She died at the age of 3.5 years. No genetic analysis was performed. Of note, her mother was married to her maternal cousin.

### 2.2. Molecular analysis

Routine analysis comprising a genome-wide single nucleotide polymorphism (SNP) array using the Cyto-SNP12v2.1 chip showed a pathogenic 32 kb homozygous deletion at chromosome 20p12 (arr 20p12.3(8645677x2,8647972-8679884x0,8683411x2) dn hg19).[11] This small deletion includes exon 7, 8 and 9 of *PLCB1* gene (ENST00000338037) and no other coding genes. The breakpoints are intronic. The parents were heterozygous carriers for the same deletion. In addition, the patient had two duplications of unknown significance; a duplication 3q29 that was paternally inherited (arr 3q29(197509098x2,197521147-197606228x3,197612020x2) pat)

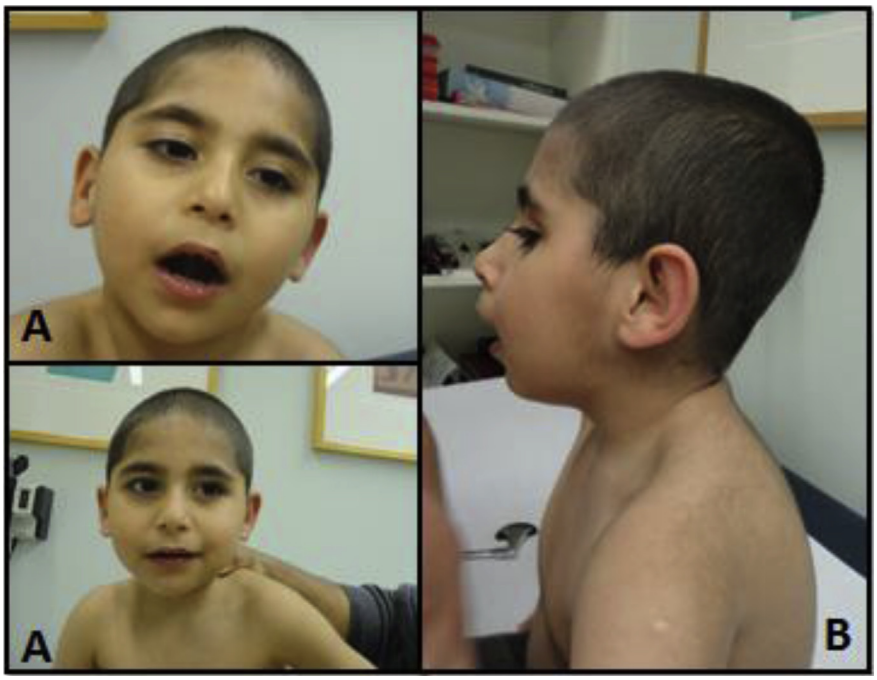


**Fig. 1 – Pictures of the index case (at age of 4 years) indicating the mild dysmorphic features. A: Frontal view showing low implanted frontal hairline and thick arched eyebrows. There is lack of eyecontact and drooling due to underlying hypotonia. B: Lateral view showing brachycephaly, sloping forehead and racial hypertrichosis.**

and a duplication 11p15.4, that was maternally inherited (arr 11p15.4(3613163x2,3624237-3756686x3,3768719x2) mat). As the abnormalities were both inherited form a non-affected parent, we consider their contribution to the clinical presentation of our patient less likely.

## 3. Discussion

We describe a patient with an infantile EE, febrile induced status epilepticus and developmental regression leading to severe intellectual disability and autistic features due to a novel homozygous deletion op exons 7–9 of *PLBC1* detected by SNP-array.

The *PLCB1* gene encodes one of the phosphoinositide-specific phospholipase C (PLC) enzymes that plays an essential role in the transduction of a variety of extracellular signals across the cell membrane.[12] Currently thirteen different mammalian PLC isozymes have been identified which are classified into six families; β (1–4), γ (1–2), δ (1,3,4), ε, ζ and η (1–2) based on their biochemical properties.[13] PLCβ1 is activated by $G_q$ protein-coupled neurotransmitter receptors, especially the muscarinic acetylcholine receptors.[14] Activated PLCβ1 hydrolyzes phosphatidylinositol 4,5-biphosphate ($IP_2$) to generate two important second messengers; 1,4,5-triphosphate ($IP_3$) and diacylglycerol (DAG).[12] In general $IP_3$ releases $Ca^{2+}$ from intracellular stores and DAG stimulates protein kinase C (PKC), a phosphorylating enzyme. Through this cascade many important biological responses, including proliferation, differentiation, survival and secretion, are initiated. PLCβ1 is most prominently expressed in the brain, especially in the cerebral cortex and hippocampus.[12] Plcβ1$^{-/-}$ mice displayed a growth retardation and low viability.[14] Most of them displayed a selective impairment of muscarinic acetylcholine signaling in the hippocampus resulting in epileptic seizures and death. Since cholinergic stimulation of the hippocampus normally decreases its excitability through the activation of inhibitory interneurons,[15] loss of the cholinergic signaling in the Plcβ1$^{-/-}$ hippocampus can explain the seizure activity associated with *Plcb1* loss-of-function mutations in mice and humans.

Heterozygous *PLCB1* variants have been suggested to play a role in the onset of epilepsy and neurocognitive and neuropsychiatric disorders like autism[16] and schizophrenia.[17] Lal et al. searched in 1366 patients with genetic generalized epilepsy for microdeletions. In one of them, a boy with childhood absence epilepsy, a heterozygous deletion (exon 1–3) in *PLCB1* was found.[18] Girirajan et al. found an enrichment of deletions and duplications involving *PLCB1* in a group of patients with autism.[19] Lo Vasco et al. studied the orbito-frontal cortex of 15 schizophrenic patients and found 3 patients with a *PLCB1* deletion of one allele (in all nuclei) and 1 patient with a somatic *PLCB1* deletion of both alleles (in 76/100 analyzed nuclei). None of the control patients had a *PLCB1* deletion.[17] Although the *PLCB1* deletion of our index case was inherited, no cases of psychiatric illness or autism are reported in family members of our patient.

Since 2010, three cases of autosomal recessive *PLCB1*-associated EE have been described.[8–10] Details on the previously described *PLCB1* mutations and the clinical phenotypes of these patients are summarized in Table 1. Our case differs from the previously reported cases with respect to the genotype and phenotype. The first two cases had a homozygous deletion of the promotor region and the first three coding

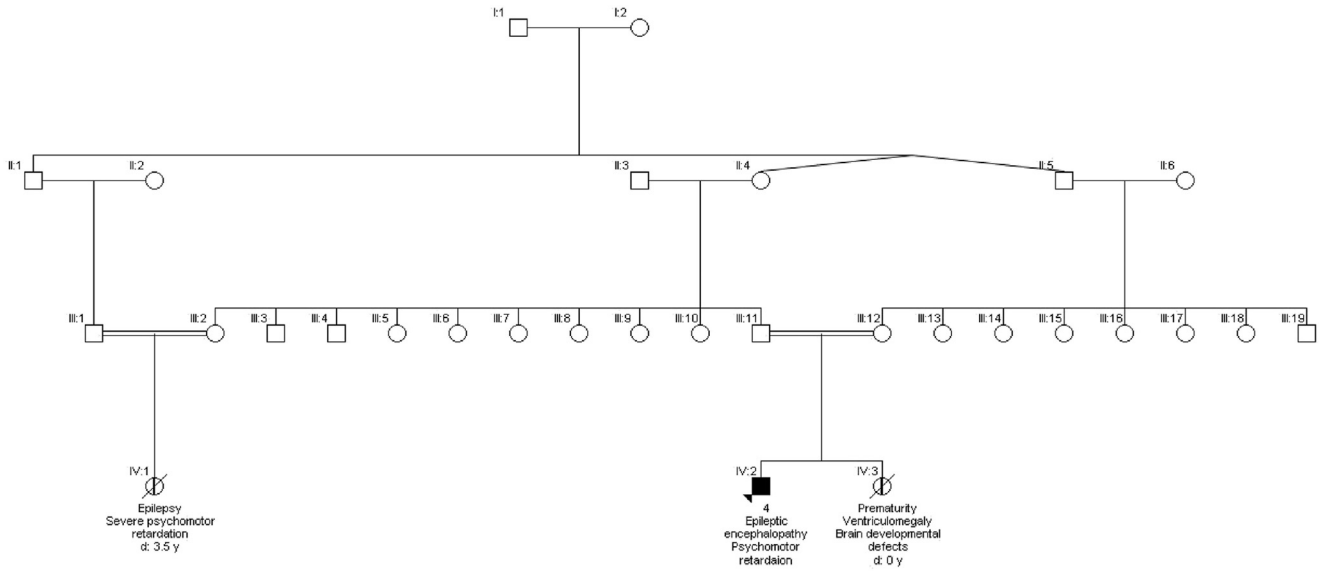


**Fig. 2 – Four generation consanguinous pedigree of the index case showing the relationship with his niece (IV:1) who died at the age of 3.5 y due to an EE of unknown cause.**

exons. The parents in both cases were consanguineous but from different origins (respectively Bangladesh and Pakistan). The third case had a heterozygous deletion (with almost identical breakpoints as the second case) in combination with a heterozygous splice site variant affecting the first base after exon 1. The latter was predicted to cause a deleterious splicing leading to a nonsense-mediated decay or a truncated protein product. In our case a homozygous deletion including exon 7, 8 and 9 of *PLCB1* was found, which has not been reported yet. Next to the genotypic differences, a slight difference can be noted in the clinical presentation in all cases. The first patient presented at the age of 10 weeks with tonic seizures evolving in a West syndrome at the age of 8 months. After two months the seizures changed again to therapy resistant focal and generalized tonic-clonic seizures. At the age of 2.5 years his development was estimated between 0 and 3 months. The second boy was diagnosed with malignant migrating partial seizures in infancy at the age of six months. The third case presented with a catastrophic seizure onset at the age of 10 months characterized by bilateral upper limb jerking, eye deviation and staring. At the age of three year she still has 2–3 seizures daily. Our case presented at the age of four months with a febrile status epilepticus whereupon he developed an EE which could be controlled at the age of 11 months. The EE was difficult to classify since he showed a mixture of epileptic spasm, focal seizures and generalized tonic-clonic seizures in combination with a modified hypsarrhythmia. In contrast to the three other cases his seizures were relatively easy to control both during the EE period as thereafter when generalized tonic clonic seizures were seen each 3–4 months. At the age of 3 years he presented to our hospital with a generalized febrile status epilepticus after being seizure-free for over a year using valproate monotherapy. Both in terms of development and epilepsy our patient thus seems to have a better outcome than the other cases. However all cases share an infantile EE and developmental regression. Since the concept of an EE implies that the epileptic activity is responsible for the cognitive and behavioral decline, the better outcome in our patient could be explained by the shorter duration of his 'epileptic encephalopathic' period. This is however still a point of discussion.[20] Especially in genetic EEs it is difficult to distinguish to what extent the underlying genetic defect or the epilepsy itself is responsible for the negative impact on the development. Nevertheless like in many other genetic encephalopathies a phenotypic variability is possible, which may be related to genetic modifiers or environmental factors.

A niece of our proband died at the age of 3.5 years with the clinical picture of an early onset EE without an identified cause. She had three healthy siblings. Since her parents were consanguineous and related to both parents of our patient it is possible that she also had a *PLCB1* related EE. Unfortunately there is little information concerning this girl and parents are not genetically tested yet. Another question is whether the sister of our index case had the same homozygous deletion. Prenatal echography's showed abnormal brain development with enlarged ventricles. She was born prematurely and died after birth in Syria where no autopsy, central imaging or genetic testing was performed postnatal. Considering that all four cases with confirmed *PLCB1* EE had no major structural brain malformations (see Table 1) and PLCβ1 mainly seems to have a role in postnatal cortical development in mice,[21] it is likely that another etiology contributed to the abnormal brain development of this girl.

In conclusion, we present the case of the fourth patient with a *PLCB1* associated early onset EE and reviewed the literature. This paper highlights the genotypic and phenotypic variability of this severe genetic epilepsy syndrome. With the increased availability of genetic tests, additional cases are expected to be found, leading to a further elucidation of the phenotypic spectrum of this severe genetic epilepsy syndrome. Meanwhile it is recommended to perform microarray in patients with EE of unknown etiology.

**Table 1 – Review of the literature.**

| Case | Mutation | Seizure onset | Epilepsy course | Development | Magnetic resonance imaging (MRI) of the brain |
|---|---|---|---|---|---|
| Case 1 (Kurian et al., 2010)[8] | Homozygous deletion (8034441–8520723) including promotor region and exon 1–3 | 10 w with Ts | Start: early onset EE with West syndrome<br>At FU: therapy resistant epilepsy (frequent Ts and TCs) | Regression from 8 m<br>At the age of 2.5 y functioning at 0–3 m, could not lift his head, inconsistent visual fixation, axial hypotonia and spastic quadriparesis<br>Died at the age of 2.9 y due to cardiorespiratory failure in the course of a complicated infection | Normal at 5 m and 13 m |
| Case 2 (Poduri et al., 2012)[9] | Homozygous deletion (8099741–8575520) including promotor region and exon 1–3 | 6 m with focal seizures | Start: malignant migrating partial seizures in infancy<br>At 10 m still therapy resistant epilepsy | Slightly delayed prior to seizure onset<br>Regression with onset of seizures. No visual fixation. No speech development, only guttural sounds. Limited voluntary movements with truncal and appendicular hypotonia. | Mild enlarged cerebrospinal fluid spaces at 6, 7, 8 and 9 m. |
| Case 3 (Ngoh et al., 2014)[10] | Heterozygous deletion (8099252–8575333) including promotor region and exon 1–3<br>Heterozygous intron 1 splice site variant (c.99 + 1 G > A) | 10 m with catastrophic onset of seizures (bilateral upper limb jerking, eye deviation and staring) | Start: early infantile EE<br>At 3 y therapy resistant epilepsy with 2–3 TCs/day | Concerns about developmental delay and hypotonia prior to seizure onset.<br>At 3 y of age severe global neurodevelopmental delay. Unable to sit, does not grasp, some visual fixation. Gastrostomy. | Mild global reduction of supratentorial cerebral volume and mild hypoplastic corpus callosum at 11 m. |
| Current case | Homozygous deletion (8647972–8679884) including exon 7–8 | 4 m with febrile status epilepticus | Start: early infantile EE (4 –11 m)<br>At 3 y febrile status epilepticus<br>At 4 y seizure free for over one year with VPA monotherapy | Regression at seizure onset.<br>At 4 y severe global developmental delay. Mild axial hypotonia. Able to walk independently (broad-based). Short visual contact. Limited grasping and social interaction. | Mild generalized atrophy at 3 y and 3.5 y |

Legend: w: weeks; m: months; Ts: tonic seizures; TCs: tonic-clonic seizures; VPA: valproate; EE: epileptic encephalopathy; FU: follow-up.

## Conflict of interest

No conflict of interest.

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