PLPBP mutations cause variable phenotypes of developmental and epileptic encephalopathy

1Hiroshi Shiraku, ‡§†Mitsuko Nakashima, ¶Saoko Takeshita, #Chai-Soon Khoo, **Muzhirah Haniffa, **Gaik-Siew Ch’ng, *Kazuma Takada, *Keisuke Nakajima, *Masayasu Ohta, ††Tohru Okanishi, ††Sotaro Kanai, ‡‡Ayataka Fujimoto, §Hirotomo Saitsu, ‡Naomichi Matsumoto, and §§Mitsuhiro Kato

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SUMMARY

Objective: Vitamin B₆-dependent epilepsies are treatable disorders caused by variants in several genes, such as ALDH7A1, PNPO, and others. Recently, biallelic variants in PLPBP, formerly known as PROSC, were identified as a novel cause of vitamin B₆-dependent epilepsies. Our objective was to further delineate the phenotype of PLPBP mutation.

Methods: We identified 4 unrelated patients harboring a total of 4 variants in PLPBP, including 3 novel variants, in a cohort of 700 patients with developmental and epileptic encephalopathies. Clinical information in each case was collected.

Results: Each patient had a different clinical course of epilepsy, with seizure onset from the first day of life to 3 months of age. Generalized tonic–clonic seizures were commonly noted. Myoclonic seizures or focal seizures were also observed in 2 patients. Interictal electroencephalography showed variable findings, such as suppression burst, focal or multifocal discharges, and diffuse slow activity. Unlike previous reports, all the patients had some degree of intellectual disability, although some of them had received early treatment with vitamin B₆, suggesting that different mutation types influence the severity and outcome of the seizures.

Significance: PLPBP variants should be regarded as among the causative genes of developmental and epileptic encephalopathy, even when it occurs after the neonatal period. Early diagnosis and proper treatment with pyridoxine or pyridoxal phosphate is essential to improve the neurologic prognosis in neonates or young children with poorly controlled seizures.

KEY WORDS: Pyridoxine, Pyridoxal phosphate, Vitamin B₆, Development, Electroencephalography.
Developmental and epileptic encephalopathies or early onset epileptic encephalopathies (EOEEs) are characterized by refractory seizures starting in early infancy, mainly during the first year of life, followed by developmental impairment, with characteristic age-dependent seizure types and electroencephalography (EEG) findings. Recent advances in gene analysis using next-generation sequencing have facilitated detection of mutations in the causative genes of EOEEs, as 67 genes and phenotypic series are listed for early infantile epileptic encephalopathy in the Online Mendelian Inheritance in Man (OMIM) database (https://omim.org/phenotypicSeries/PS308350). Some EOEEs, known as vitamin B₆-dependent epilepsy, respond to specific treatments, such as vitamin B₆.

The known biologic mechanisms underlying vitamin B₆-dependent epilepsies are incomplete formation or transport of pyridoxal 5'-phosphate (PLP) or its inactivation by metabolites. Four genes have been identified to be responsible for vitamin B₆-dependent epilepsy. Variants of ALDH7A1, which codes for antiquitin or α-aminoadipic semialdehyde dehydrogenase (AASA) dehydrogenase, were the first genes found in patients with vitamin B₆-dependent epilepsy. Patients with ALDH7A1 variants generally present with seizures soon after birth. They show multiple types of seizures associated with a variety of EEG abnormalities. In most patients, administration of pyridoxine or PLP results in cessation of seizures within minutes, accompanied by depressed amplitude on EEG. AASA, piperidine-6-carboxylate (P6C), and pipecolic acid in the plasma, urine, and cerebrospinal fluid (CSF) serve as specific biomarkers of vitamin B₆-dependent epilepsy caused by ALDH7A1 mutations. AASA dehydrogenase deficiency caused by ALDH7A1 mutations or pyridoxin-dependent epilepsy is a lysine catabolism defect, and a lysine-restricted diet and high-dose arginine supplementation are additional options to improve developmental outcome and epilepsy control. Hyperprolinemia type II (HP II) is caused by defective delta-pyrroline 5-carboxylate (P5C) dehydrogenase encoded by ALDH4A1 and is the second defect leading to increased utilization of PLP. Overdose of L-D-P5C inactivates PLP as a result of a Knoevenagel condensation, and ultimately causes generalized seizures in late infancy or childhood and intellectual disability. In many patients with HP II, seizures are triggered by fever and may be controlled with general anticonvulsants. Severe infantile hypophosphatasia, which is a rare metabolic disease with the hallmark finding of deficient activity of serum tissue nonspecific alkaline phosphatase (TNSALP) encoded by the ALPL gene, can present with pyridoxine-responsive seizures. Pyridoxine can cross the blood–brain barrier, but PLP cannot. TNSALP is necessary for converting PLP to pyridoxine. Defective TNSALP activity results in a deficiency of PLP in the brain; patients with TNSALP deficiency have intractable seizures responsive to pyridoxine but not to PLP. Once enzyme replacement therapy is initiated, patients with TNSALP deficiency can stop pyridoxine supplementation without recurrence of seizures.

PLP-dependent epilepsy is caused by PNPO mutations. This gene encodes PLP oxidase, which converts pyridoxine phosphate and pyridoxamine phosphate into PLP. Patients with PNPO deficiency present with neonatal seizures up to 2 weeks of age, showing myoclonic seizure and status epilepticus, and often become encephalopathic, with various abnormal neurologic presentations. The seizures are resistant to common anticonvulsants and pyridoxine, but administration of PLP leads to prompt cessation of the seizures. These disorders are vitamin B₆-responsive epilepsies, and it should be noted that each disorder demonstrates a different potency between pyridoxine and PLP, as mentioned earlier. More than 150 enzymes are PLP dependent, and most are expressed and function in the central nervous system. Furthermore, there are many cases with vitamin B₆-dependent epilepsy that remain to be resolved.

Recently, Darin et al. found homozygous or compound heterozygous variants of pyridoxal phosphate-binding protein (PLPBP), formerly known as PROSC, proline synthetase co-transcribed (bacterial homolog), designated by HUGO gene nomenclature committee (https://www.genenames.org) in 7 patients of 5 families with vitamin B₆-dependent epilepsy. These variants affect intracellular PLP homeostasis, leading to seizures and altered amino acid or neurotransmitter profiles as a consequence of defective enzyme activity of γ-aminobutyric acid (GABA) transaminase or aromatic L-amino acid decarboxylase (AADC), both of which require PLP as a coenzyme.

We present here clinical and molecular data from 4 unrelated patients harboring a total of 4 variants (3 novel), including homozygous or compound heterozygous variants in PLPBP, identified in a cohort of 700 patients with childhood-onset epileptic encephalopathies.
Methods

Subjects
A total of 700 individuals with developmental and epileptic encephalopathies were analyzed. Among them, 210 individuals were analyzed with their parents. Clinical information was obtained and peripheral blood leukocytes were collected from the patients and their parents after obtaining their written informed consent. DNA was extracted using QuickGene-610L (Fujifilm, Tokyo, Japan) according to the manufacturer’s instructions. The study was approved by the institutional review boards of the Yokohama City University School of Medicine and the Showa University School of Medicine.

Whole-exome sequencing
Whole-exome sequencing (WES) was performed as described previously.7 Patient DNA was captured with SureSelect Human All Exon V4 or V5 kits (Agilent Technologies, Santa Clara, CA, U.S.A.) and sequenced on an Illumina HiSeq2000 or 2500 (Illumina, San Diego, CA, U.S.A.) with 101-bp paired-end reads. Image analysis and base calling were performed by sequence control software real-time analysis and CASAVA software v1.8 (Illumina). Reads were aligned to the human reference genome sequence (UCSC hg19, NCBI build 37) using Novoalign (Novocraft Technologies, Jaya, Malaysia). Polymerase chain reaction (PCR) duplicates were excluded by Picard (http://picard.sourceforge.net/). Single-nucleotide variants (SNVs) and small insertion/deletions (indels) were identified with the Genome Analysis Toolkit UnifiedGenotyper (6) and were filtered according to the Broad Institute best-practice guidelines (version 3). Variants that were selected through the filters were annotated using ANNOVAR.8 Variant pathogenicity was evaluated by SIFT (http://sift.jcvi.org/), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), M-CAP (http://bejerano.stanford.edu/mcap/), and CADD (http://cadd.gs.washington.edu/). Conservation of nucleotides was assessed with GERP (http://mendel.stanford.edu/SidowLab/downloads/GERP/index.html) and PhastCons (http://compgen.cshl.edu/phast/).

Results

Clinical features
Table 1 summarizes the clinical information of the 4 current patients and previously reported cases with PLPBP mutations. As an illustrative case, the clinical history of patient 2 is given here, and the clinical histories of other patients are provided as Appendix S1.

Patient 2
Patient 2 was born at full term without asphyxia. At the age of 3 months, he had an episode of generalized tonic seizure with eye conversion to the upper direction after suddenly crying for several minutes. At 11 months of age, he had repeated tonic seizures, with or without fever. Brain magnetic resonance imaging (MRI) and interictal EEG were normal at that time. Treatment with antiepileptic drugs (AEDs) such as phenobarbital, topiramate, carbamazepine, valproic acid, lamotrigine, and levetiracetam did not ameliorate his seizures. Clobazam was temporarily effective; however, the frequency of the seizures ranged from several times a week to once a month, and he had several episodes of status epilepticus in a year. After exacerbation of seizures, he showed developmental delay (meaningful words at 12 months and walking without support at 2 years and 9 months). After the age of 2 years, brain MRI showed mild cerebral atrophy (Figure S2A).

At the age of 7 years, he had several episodes of status epilepticus every day and needed continuous infusion of midazolam (MDL) for seizures. He also showed episodes of emotional seizures with eye-opening, a frightened expression while saying “scared, scared…,” clinging to his mother, and screaming loudly followed by generalized tonic seizures. Vagus nerve stimulation (VNS) therapy was introduced, and the episodes of status epilepticus ceased for 4 months but then relapsed to the same as before VNS therapy. An intelligence test at that time showed moderate intellectual disability (developmental quotient 39). On the assumption of mid-temporal lobe epilepsy, subdural electrodes were placed, and EEG during his ictal state showed fast activities superimposed on slow waves or 3- to 4-Hz spike-and-slow wave bursts at the right hippocampus and amygdala. He underwent right anterotemporal lobectomy and hippocampal amygdala resection at 8 years, but he had seizures every day, even after the surgery. After a PLPBP mutation was identified, pyridoxine was administered at 8 years of age, and his seizures dramatically disappeared with no AEDs other than pyridoxine.

Variant screening
Using Trio-WES data from 210 families, we searched for variants that were consistent with autosomal dominant and autosomal or X-linked recessive inheritance as described previously.10 We filtered out common variants with minor allele frequencies ≥1% in the single nucleotide polymorphism database 137, the 6,500 exomes of the National Heart, Lung, and Blood Institute exome project, and the Exome Aggregation Consortium (ExAC, Cambridge, MA, U.S.A.),11 and variants found in >5 of our in-house 575 control exomes. In family 1, we found 6 possible variants in 3 genes with autosomal recessive models, but only compound heterozygous PLPBP variants c.122G>A: p.(Arg41Gln) and c.134T>A: p.(Val45Asp), were predicted to be deleterious. Case-only WES in 3 individuals identified 3 homozygous PLPBP variants, c.122G>A: p.(Arg41Gln), c.199G>A: p.(Glu67Lys), and c.614G>A: p.(Arg205Gln), in patients 2, 3, and 4, respectively. All 4

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| Feature                        | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Previous reports |
|-------------------------------|-----------|-----------|-----------|-----------|------------------|
| Gender                        | Male      | Male      | Male      | Male      | Male, female 5    |
| Current age                   | 3 y, 6 mo | 8 y       | 3 y       | 5 y, 5 mo | 4.5 mo to 30 y   |
| Ethnicity                     | Japanese  | Japanese  | Malaysian | Malaysian | Syrian in a family 3, Indian 2, Italian 2, German 2, Arabic 1, Swiss-Italian 1 |
| Consanguinity                 | No        | No        | Yes       | No        | Yes 5 from 3 families, no 6 |
| PLPBP mutation                | c.134T>G>A (p.Val45Asp) and c.122G>A (p.Arg41Gln) | c.122G>A (p.Arg41Gln) | c.199G>A (p.Glu67Lys) | c.614G>A (p.Arg205Gln) and c.122G>A (p.Arg41Gln) | Missense 7, nonsense 1, frameshift 1, splicing 2 |
| Abnormalities during pregnancy| No        | No        | Yes       | No        | Yes 3, no 8     |
| Gestational age in weeks      | 39        | 40        | 33        | 39        | 32–40 (average 37) |
| Fetal distress                | No        | No        | No        | No        | Yes 5, no 6     |
| Birth HC percentage           | 25–50%    | 50%       | 10–50%    | 25–50%    | <10% 4, 25–50% 2, 50% 1, 50–75% 2, 90% 1 |
| Seizure onset                 | 10 days   | 3 mo      | <24 h     | 34 days   | 24 h 6, 2–7 days 3, 9 days 1, 1 mo 1 |
| Seizure type                  | Tonic, clonic, SIA, GTC | Tonic, clonic, GTC, SIA (lip-smacking or grimacing) | GTC, myoclonic | GTC, myoclonic | GTC, myoclonic 4, grimacing 4, eye deviation 2 |
| Interictal EEG findings       | Reduced BGA and multifocal SW activity | Focal discharges | S-B | Diffuse slow polymorphic activity | S-B, 6, reduced BGA 3, focal or multifocal 2, discontinuity 1, abnormal BGA 1, |
| Age at first vitamin B₆ administration | 25 days | 8 y | 5 wk | 5 y, 5 mo | <7 days 2, 9 days 1, 28 days 1, NR 7 |
| Age at first vitamin B₆ administration | 25 days | 8 y | 5 wk | 5 y, 5 mo | <7 days 2, 9 days 1, 28 days 1, NR 7 |
| Type of vitamin B₆ (PN or PLP) | PLP | PN | PN | PN | PN, PL/P, PLP (seizures controlled with PLP) 4 |
| Vitamin B₆ effect             | Improved seizure control and EEG | Prompt cessation of seizures | No apparent improvement | Improved seizure control | Prompt cessation of seizures 10, no effect on EEG 1 |
| Adverse effects of vitamin B₆ | None | None | None | None | None 10, prolonged sleep and muscle hypotonia 1 |
| Vitamin B₆ withdrawal         | Yes, recurrent seizures and irritability at age 1 y, 10 mo | No | No | No | Yes 4, 5, NR 2 |
| Current dose of vitamin B₆    | PLP 200 mg/day | NA | PN 100 mg/day | PN 100 mg/day | 150–450 mg/day |
| Other AEDs                     | CBZ 10 mg/kg/day | No | VPA, PB, CZP, PHT | CZP, TPM | Yes 6, no 4, died 1 |
| Response to AEDs              | Partially effective: PB | No | Partially effective: CLB | Partially effective: CLB, TPM | No to minimal 4, yes 3, better effects with |

Continued
| Feature                          | Patient 1                     | Patient 2                        | Patient 3 | Patient 4                        | Previous reports |
|---------------------------------|-------------------------------|----------------------------------|-----------|----------------------------------|------------------|
| **Course of epilepsy**          | Seizure-free at age 2 y       | Seizure-free after administration of PN | Tonic-clonic seizure once a month | Only 1 febrile seizure after administration of PN | Breakthrough seizure with fever 6, sporadic afebrile seizure 2, photosensitive seizure 1, NR 1, died 1 |
| **Delay of motor development**  | Yes, not delayed in GM        | Yes                              | Yes       | Yes                              | Yes 5, delayed but caught-up 1, no 4, died 1 |
| **Delay of speech development** | Yes                           | Yes                              | Yes       | Yes                              | Yes 5, delayed but caught-up 1, no 4, died 1 |
| **Intellectual disability**     | Yes, mild                     | Yes, moderate                    | Yes       | Yes                              | Yes 6, no 5, died 1 |
| **Acquired microcephaly**       | No                            | No                               | No        | Yes                              | No 4, NA 7 |
| **Brain MRI**                   | Normal                        | Broad gyri and shallow sulci, microcephaly with underdevelopment of white matter | Broad gyri and shallow sulci, microcephaly with underdevelopment of white matter; periventricular cyst | Normal, broad gyri and shallow sulci, microcephaly with underdevelopment of white matter 4, periventricular cyst 3 |
| **Amino acids in plasma before vitamin B6 supply** | Normal | Normal                          | Elevated glycine and threonine | Elevated glycine and threonine | Some amino acids elevated in plasma 1, normal 2, NA 8 |
| **Amino acids in CSF**          | NA                            | NA                               | NA        | NA                               | Some amino acids elevated in CSF 1, normal 2, NA 8 |

AEDs, antiepileptic drugs; BGA, background activity; CBZ, carbamazepine; CLB, clobazam; CSF, cerebrospinal fluid; CZP, clonazepam; EEG, electroencephalography; GM, gross movement; GTC, generalized tonic convulsion; HC, head circumference; LEV, levetiracetam; mo, month(s); MRI, magnetic resonance imaging; NA, not available; NR, no response; PB, phenobarbital; PHT, phenytoin; PLP, pyridoxal phosphate; PN, pyridoxine; S-B, suppression-burst; SIA, seizures with impaired awareness; SW, spike and slow wave; TPM, topiramate; VPA, valproic acid; wk, week(s); y, year(s).
variants were evolutionarily conserved, and 3 variants, c.122G>A: p.(Arg41Gln), c.134T>A: p.(Val45Asp), and c.199G>A: p.(Glu67Lys), were predicted to be deleterious. Although the c.614G>A: p.(Arg205Gln) variant was predicted to be damaging by only one of 4 bioinformatical tools (Table 2), the same variant had been identified in a patient with vitamin B₆-dependent epilepsy. Segregation of PLPB variants was examined by Sanger sequencing on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, U.S.A.), using trio samples (patients and their parents) except for patient 2, because his parental samples were unavailable.

**Table 2. Prediction of pathogenicity of PLPB variants**

| Patient | Mutation | Origin | ExAC | SIFT | PolyPhen2 | HumVar | CADD | phred | M-CAP | GERP | PONOM | Genomic Evolutionary Rate Profiling | M-CAP | Mendelian Clinically Applicable Pathogenicity Score | SIFT: Sorting Intolerant From Tolerant |
|---------|----------|--------|------|------|-----------|--------|------|-------|-------|------|-------|-------------------------------------|-------|-----------------------------------|----------------------------------|
| 1       | c.122G>A: p.(Arg41Gln) | Compound heterozygous (maternal) | 4.061 | 0.978 | 9          | 1      | 36.9 | 0.040 | 0.000 | 0.000 | 1     | 29.3                            | 0.000 | 0.978                              | 36.9                             |
| 2       | c.134T>A: p.(Val45Asp) | Compound heterozygous (paternal) | –     | –     | –          | –      | –    | –     | –     | –    | –     | –                                | –     | –                                 | –                                 |
| 3       | c.199G>A: p.(Glu67Lys) | Homozygous | 4.061 | 0.978 | 9          | 1      | 36.9 | 0.000 | 0.000 | 0.000 | 1     | 35.9                            | 0.000 | 0.978                              | 35.9                             |
| 4       | c.614G>A: p.(Arg205Gln) | Homozygous | 1.218 | 0.959 | 0.000      | 0.000  | 19.5 | 0.054 | 0.000 | 0.000 | 0.000 | 19.59                           | 0.054 | 0.959                              | 19.59                            |

**Discussion**

Up to now, 14 variants in PLPB have been reported in 15 patients from 13 families, including our patients. In this study, WES revealed 4 types of PLPB variants in 4 unrelated patients, including 3 novel variants. One of the 4 patients had compound heterozygous variants in PLPB, and the others had homozygous variants. All patients in this study had missense variants in PLPB, and in silico studies suggest that all variants are highly likely pathogenic. Although functional study of each variant has not been performed, the PLPB variants are considered to be causative of the clinical symptoms in each patient. There are no apparent hot spots for the PLPB variant, but a variant in c.122G>A (p.Arg41Gln) was seen in 2 Japanese patients, and c.260C>T (p.Pro87Leu) and c.614G>A (p.Arg205Gln) variants were found recurrently in 2 unrelated patients with different ethnic backgrounds. Of interest, 4 homozygous variants, namely c.122G>A, c.206A>G, c.260C>T, and c.614G>A, have been identified in nonconsanguineous Japanese, Italian, Arabic, and Malaysian families, respectively. Although c.122G>A and c.206A>G are not registered in 1,000 genome and ExAC databases, 2 carriers of c.260C>T and c.614G>A are registered in ExAC in the European (non-Finnish) population (N = 66,694) and the European (non-Finnish) and South Asian population (N = 16,464), respectively. Each variant seems to be rare among individuals of a specific ethnicity, but it is important to notify the pathogenicity of these variants for genetic counseling.

Neonatal seizure is the most characteristic symptom of vitamin B₆-dependent epilepsy, in particular, of PLP-dependent epilepsy caused by PNPO mutations. Ten of 11 patients with PLPB variants begin to have seizures within the first 10 days of life; only one patient, who had a milder phenotype, presented with the first seizure at 1 month of age. Two of 4 patients in the present study had their first seizures after 1 month of age and lost the chance to receive vitamin B₆ therapy until after the accomplishment of genetic diagnosis. Although the timing of the first administration of vitamin B₆ differs among patients, the severity of the EEG, such as suppression-burst, appears to be correlated.
with the severity of neurologic comorbidities rather than with the period until starting vitamin B₆. Basura et al. reported that 30% of patients with pyridoxine-dependent epilepsy presented with seizures after the neonatal period, and a delay in diagnosis and pyridoxine treatment was not uncommon. Vitamin B₆ should be considered as one of the treatments for postneonatal epilepsy as well as neonatal epilepsy.

Patients with PLPBP variants, as well as patients with other pyridoxine-dependent epilepsies, have various types of seizures, including generalized and focal seizures. As for other pyridoxine-dependent epilepsies, have various types of seizures within 3 months of age in patients with PLPBP variants, because absence and atomic seizures have not been reported. This difference may be related to the onset of seizures within 3 months of age in patients with PLPBP aberrations, because absence and atomic seizures usually occur after 1 year of age. As for focal seizures, seizures with impaired awareness showing lip-smacking or grimacing and eye deviation were seen in 8 of 15 patients, as well as in patients with other pyridoxine-dependent epilepsies. Of interest, no patients with PLPBP variants had epileptic spasms, which are often seen in other pyridoxine-dependent epilepsies and other inherited metabolic epilepsies caused by deficiencies of thiamin, folic acid, and biotin. PLPBP is supposed to be involved in intracellular homeostatic regulation of PLP. Cultured fibroblasts with biallelic PLPBP variants show excessive accumulation of PLP, in contrast to other PLP-dependent epilepsies. It is notable that PLP or pyridoxine is effective in 10–30% of cases of West syndrome, which is characterized by epileptic spasms. The pathologic mechanisms by which the PLPBP variants influence the type of seizures remain to be elucidated.

Patients with pyridoxine-dependent epilepsy show various EEG findings before proper treatment. Our patients also showed a variety of EEG findings, including suppression-burst in one patient. In previous reports, 6 of 11 patients with PLPBP variants showed suppression-burst, and 5 of them began to have seizures within the first 24 h of life. Suppression-burst on EEG is characteristic of neonatal-onset, age-dependent epileptic encephalopathy, particularly of Ohtahara syndrome or early myoclonic encephalopathy. Both show refractory seizures and have a poor neurologic prognosis. However, as far as pyridoxine-dependent epilepsy is concerned, suppression-burst does not necessarily mean a poor prognosis. Plecko et al. reported a patient with suppression-burst in the neonatal period who had almost normal development except for a learning disability.

Darin et al. reported that PLP is more effective than pyridoxine in controlling seizures in some patients with PLPBP variants. On the other hand, Plecko et al. reported that 3 of 4 patients had good seizure control with pyridoxine and showed normal intelligence. In this study, pyridoxine was used in 3 patients and PLP in one patient who displayed a better outcome than the other patients. PLP is only the active coenzyme form of vitamin B₆, whereas pyridoxine requires conversion to PLP to serve as a coenzyme. Although the stability of PLP in solution is lower than that of pyridoxine, the phosphatic forms of vitamin B₆, including PLP, protect them from hydrolysis, and PLP is primarily stored in the body rather than pyridoxine. The cost of pyridoxin is the same or less than that of PLP, depending on the country. In Japan, the price of 30 mg of pyridoxine and PLP is similar, that is, 5.6 yen (0.051 USD or 0.043 Euro). Adverse events associated with pyridoxine and PLP are less frequent compared with those of conventional antiepileptic drugs; however, serious respiratory arrest following injection in neonates with pyridoxine-dependent epilepsy and rhabdomyolysis, diarrhea, vomiting, and an elevation of liver transaminase with high-dose therapy, as well as peripheral neuropathy after long-term use have been observed. It is still debatable which vitamer is more effective for treating seizures and improving the outcome of patients with PLPBP variants.

In conclusion, PLPBP variants should be regarded as among the causative genes of developmental and epileptic encephalopathy, even when it occurs after the neonatal period. Early diagnosis and proper treatment with pyridoxine or PLP is essential to improve the neurologic prognosis in neonates or young children with poorly controlled seizures.

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Disclosure

None of the authors has any conflict of interest to disclose. 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

1. Scheffer IE, Berkovic S, Capovilla G, et al. ILAE classification of the epilepsies: position paper of the ILAE Commission for Classification and Terminology. Epilepsia 2017;58:512–521.
2. Campistol J, Plecko B. Treatable newborn and infant seizures due to inborn errors of metabolism. *Epileptic Disord* 2015;17:229–242.

3. Gospe Jr SM. Neonatal vitamin-responsive epileptic encephalopathies. *Chang Gung Med J* 2010;33:1–12.

4. Farrant RD, Walker V, Mills GA, et al. Pyridoxal phosphate de-activation by pyrroline-5-carboxylic acid. Increased risk of vitamin B6 deficiency and seizures in hyperprolinemia type II. *J Biol Chem* 2001;276:15107–15116.

5. Whyte MP, Greenberg CR, Salman NJ, et al. Enzyme-replacement therapy in life-threatening hypophosphatasia. *N Engl J Med* 2012;366:904–913.

6. Darin N, Reid E, Prunetti L, et al. Mutations in PROSC disrupt cellular pyridoxal phosphate homeostasis and cause vitamin-B6-dependent epilepsy. *Am J Hum Genet* 2016;99:1325–1337.

7. Mizuguchi T, Nakashima M, Kato M, et al. *PARS2* and *NARS2* mutations in infantile-onset neurodegenerative disorder. *J Hum Genet* 2017;62:525–529.

8. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res* 2010;38:e164.

9. Plecko B, Zweier M, Begemann A, et al. Confirmation of mutations in PROSC as a novel cause of vitamin B 6 -dependent epilepsy. *J Med Genet* 2017;54:809–814.

10. Minase G, Miyatake S, Nabatame S, et al. An atypical case of SPG56/CYP2U1-related spastic paraplegia presenting with delayed myelination. *J Hum Genet* 2017;62:997–1000.

11. Lek M, Karzweski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016;536:285–291.

12. Plecko B. Pyridoxine and pyridoxalphosphate-dependent epilepsies. *Handb Clin Neurol* 2013;113:1811–1817.

13. Basura GJ, Hagland SP, Wiltse AM, et al. Clinical features and the management of pyridoxine-dependent and pyridoxine-responsive seizures: review of 63 North American cases submitted to a patient registry. *Eur J Pediatr* 2009;168:697–704.

14. Schmitt B, Baumgartner M, Mills PB, et al. Seizures and paroxysmal events: symptoms pointing to the diagnosis of pyridoxine-dependent epilepsy and pyridoxine phosphate oxidase deficiency. *Dev Med Child Neurol* 2010;52:e133–e142.

15. Toribe Y. High-dose vitamin B(6) treatment in West syndrome. *Brain Dev* 2001;23:654–657.

16. Naasan G, Yabroudi M, Rahi A, et al. Electroencephalographic changes in pyridoxine-dependant epilepsy: new observations. *Epileptic Disord* 2009;11:293–300.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Appendix S1.** Case reports.

**Figure S1.** Interictal electroencephalography findings in patients 1 and 3.

**Figure S2.** Magnetic resonance imaging findings in patient 2 at 6 years (a), patient 3 at 2 years (b), and patient 4 at 9 months (c) of age.