Pyridoxal in the cerebrospinal fluid may be a better indicator of vitamin B6-dependent epilepsy than pyridoxal 5′-phosphate

Supplementary material 1

Case reports

Case 1

This female patient was born at 35 weeks of gestational age weighing 2266 g. She had irritability, vomiting, and cyanosis immediately after birth and required sedation and mechanical ventilation for 1 day. Thereafter, she was fine until 3 months of age, when she started having focal tonic-clonic seizures that at times evolved to bilateral convulsive seizures. At 4 months of age, she started having jerking seizures (myoclonic seizures or epileptic spasms). Electroencephalogram (EEG) showed multifocal spikes. Brain magnetic resonance imaging (MRI) revealed asymmetric brain atrophy. Phenobarbital, clonazepam, and carbamazepine were ineffective. Pyridoxal 5′-phosphate (PLP) 140 mg/day suppressed the seizures and improved EEG and her mood. At 2 years of age she had convulsive status epilepticus when she had adenovirus infection. This was not controlled by midazolam but was by intravenous pyridoxine 200 mg. She has had no seizures since then. Now 4 years of age, she can walk with support and hold a conversation with her family. She has selective mutism. Genetic analysis of *ALDH7A1* gene (NM_001182.5) revealed compound heterozygosity for a missense mutation c.1292C>T, p.Pro431Leu in exon 14 and a deletion of exons 5–7.

Case 2

This female patient was born at 39 weeks of gestational age weighing 2760 g. She started having convulsive seizures (details unavailable) on her day of birth, which were partially controlled by phenobarbital, diazepam, and midazolam. PLP was effective for suppressing seizures. Brain MRI was normal. Seizures recurred after stopping PLP at 7 months of age and

were suppressed again by restarting PLP. She had a febrile seizure at 2 years, 5 months. She started having focal tonic and clonic seizures at 3 years, 3 months. EEG showed multifocal spikes and evolved to continuous spike-waves during sleep (CSWS) at 6 years of age. Her seizures were eventually controlled at 6 years, 8 months by the addition of valproic acid and ethosuximide. EEG at 10 years old showed focal spikes but no CSWS. After several years, valproic acid and ethosuximide were tapered off without seizure recurrence. EEG at 23 years old was normal. She is currently on PLP 80 mg/day alone. She has mild intellectual disability. Genetic analysis revealed a nonsense mutation c.241C>T, p.Arg81* in exon 2 and a missense mutation c.1061A>G, p.Tyr354Cys in exon 12 of *ALDH7A1* gene. We could not confirm compound heterozygosity because we were unable to analyze parents' samples.

Case 3

This case has been reported elsewhere [1]. This male patient was born at 39 weeks of gestation weighing 2865 g. He had pulmonary hemorrhage and was intubated for 2 days. He also had tonic and clonic seizures. Brain MRI showed a thin corpus callosum, diffuse white matter signal abnormality, and delayed malformation. Seizures were suppressed by midazolam, phenobarbital, and levetiracetam. EEG at 14 days of age showed decreased synchrony but no epileptic discharges. Vitamins, including PLP, were prescribed but the efficacy of PLP was not recognized because his seizures had already been controlled. After stopping the vitamins, he started having epileptic spasms that were confirmed by ictal EEG recording. Intravenous PLP (60 mg) suppressed seizures. Oral PLP 18 mg/kg/day was started and all anti-epileptic drugs were discontinued without seizure recurrence. He had a febrile seizure at 1 year, 9 months old but no seizures have occurred since then. Now 2 years of age, he can walk with support and speak. Genetic analysis of *ALDH7A1* gene revealed compound heterozygosity for a missense mutation c.1196G>T, p.Gly399Val in exon 13 and a splicing

cite mutation c.1200+1G>A at the beginning of intron 13.

Case 4

This case has been reported elsewhere [2]. This male patient was born at 37 weeks of gestational age weighing 2660 g via emergent caesarian section because of maternal infection and fetal distress. Immediately after birth he had irritability and focal or bilateral tonic seizures. EEG at 26 days of age showed suppression-burst. Seizures were suppressed by intravenous thiopental, phenobarbital, midazolam, and then oral valproic acid and clobazam. At 6 months of age, he had status epilepticus after infection. Zonisamide reduced seizures and PLP suppressed seizures. EEG showed multifocal spikes at 7 months of age. Brain MRI revealed diffuse cerebral atrophy and enlarged ventricles. He had ventriculoperitoneal shunt at 3 years, 3 months. Around this time he started having focal asymmetric tonic seizures daily. PLP was stopped at 5 years of age, which caused seizure clustering. Restarting PLP, increasing lamotrigine, and adding topiramate succeeded in suppressing seizures at 5 years, 1 month of age. He is unable to sit alone and has no meaningful words at 6 years old. Genetic analysis of *ALDH7A1* gene at Tokyo Women's Medical University revealed compound heterozygosity for a missense mutation c.974C>T, p.Thr325Ile in exon 11 and a splicing site mutation c.192+3A>T at the beginning of intron 1. This splicing site mutation was confirmed to result in absent expression of the corresponding allele, which could be considered a probable consequence of erroneous splicing [2].

Case 5

This case has been reported elsewhere [3]. This male patient was born at 36 weeks of gestational age weighing 2691 g. Focal clonic and tonic seizures started at 1 day of age. He also had irritability, tremor, and myoclonus. Brain ultrasonography showed right

intraventricular hemorrhage (grade 3) and MRI revealed white matter abnormality. EEG showed bicentral spikes. Seizures were temporarily controlled by phenobarbital and midazolam. After stopping them, tonic seizures and tremor recurred. Phenobarbital and clonazepam were ineffective. Seizures were suppressed and EEG abnormality improved by intravenous pyridoxine 100 mg on 22 days of age. He has had no seizures on oral PLP 30 mg/kg/day since then except for a febrile seizure at 10 months of age. Brain MRI at 1 month of age incidentally revealed cerebral sinovenous thrombosis, which resolved in 2 months on warfarin. He can sit alone but cannot pull himself up at 11 months of age. Genetic analysis of *ALDH7A* gene at Kanagawa Children's Medical Center revealed homozygous missense mutations of c.1292C>T, p.Pro431Leu in exon 14. Homozygosity was confirmed by using different primer sets to amplify exon 14 and by demonstrating the presence of two copies of exon 14 by real-time PCR. The analysis of his parents revealed that they were heterozygous for this mutation.

Case 6

This female patient was born at 41 weeks of gestational age weighing 3835 g. She started having seizures 9 hours after birth. She was irritable and had a high-pitched cry. EEG at 1 day of age was normal. Brain MRI was normal. Phenobarbital and midazolam were ineffective but intravenous vitamin B6 suppressed the seizures at 8 days of age. She has been seizure free since then on oral PLP 75 mg/day. Her intelligence quotient was 71 at 6 years of age and 59 at 10 years of age. Genetic analysis of *ALDH7A1* gene at Shinshu University revealed compound heterozygosity for a splicing site mutation c.871+5G>A in intron 9 and a missense mutation c.923A>G p.Asp308Gly in exon 11.

Case 7

This female patient was born at 41 weeks of gestation weighing 3622 g. There was no asphyxia. She started having clonic seizures involving the right or left upper and lower limbs that frequently evolved to bilateral clonic seizures at 1 day after birth. Her seizures temporarily resolved between 11 months and 3 years old with phenobarbital. After that she started having seizure clustering 3 to 4 times a year when she had infection, which was not completely suppressed by the addition of clobazam and levetiracetam. Her seizures were characterized as unilateral or bilateral tonic seizures involving the upper limbs with impaired consciousness. MRI was normal. Her EEG at 6 years, 9 months old demonstrated possible multifocal spikes. Metabolic work-up demonstrated elevated α-aminoadipic semialdehyde but normal pipecolic acid levels. Genetic analysis of *ALDH7A1* gene revealed a novel missense mutation c.1221T>A, p.Asn407Lys in exon 14. There was no intragenic copy number variations demonstrated by real-time PCR. PLP 40 mg/day was started and she has remained seizure free on PLP and clobazam since then. Her IQ was 86 before PLP therapy but it improved to 101 after an 18 month period.

References

[1] Dowa Y, Shiihara T, Akiyama T, et al. A case of pyridoxine-dependent epilepsy with novel *ALDH7A1* mutations. Oxford Med Case Rep 2020;3:99-103.

[2] Yanagishita T, Yamamoto K, Koike T, et al. Novel compound heterozygous *ALDH7A1* mutation causes the hemi-allelic expression in a patient with pyridoxine-dependent epilepsy. Tokyo Women's Med Univ J 2019;3:73-7.

[3] Kuhara T, Akiyama T, Ohse M, et al. Identification of new biomarkers of pyridoxine-dependent epilepsy by GC/MS-based urine metabolomics. Anal Biochem 2020;604:113739.