# Clinical and genetic features in pyridoxine-dependent epilepsy: a Chinese cohort study

XIANRU JIAO | JIAO XUE | PAN GONG | YE WU | YUEHUA ZHANG | YUWU JIANG | ZHIXIAN YANG

Department of Pediatrics, Peking University First Hospital, Beijing, China.

Correspondence to Zhixian Yang, Department of Pediatrics, Peking University First Hospital, No.1, Xi'anmen Street, Xicheng District, Beijing 100034, China. E-mail: zhixian.yang@163.com

**This article is commented on by Coughlin II on page 268 of this issue.**

**PUBLICATION DATA**
Accepted for publication 18th September 2019.
Published online 18th November 2019.

**ABBREVIATIONS**

| | |
|---|---|
| α-AASA | α-aminoadipic semialdehyde |
| AED | Antiepileptic drugs |
| P6C | Piperideine-6-carboxylic |
| PDE | Pyridoxine-dependent epilepsy |
| PLP | Pyridoxal 5′-phosphate |

**AIM** To characterize the clinical and genetic characteristics of a large cohort of patients with pyridoxine-dependent epilepsy (PDE).
**METHOD** We retrospectively collected clinical and genetic information of 33 (15 males, 18 females; mean [SD] age 4y 11mo [2y 5mo]; 1y 3mo–10y 4mo) patients with PDE from 31 unrelated families at a single centre.
**RESULTS** There were many types of seizures, with focal seizures in 32 cases. Dravet syndrome was suspected clinically in two patients. Electroencephalogram (EEG) was normal in seven patients at the initial stage and then in 17 patients during pyridoxine maintenance therapy. Genetic studies revealed 26 kinds of variants in *ALDH7A1* and four in *PLPBP* with 18 variants unreported previously, and 48 *ALDH7A1* variants were located in exon 11, 12, 14, and 17 or intron 9 and 11. In addition, three patients carried different exons deletion. Among these, seizures could be controlled for several years in one patient by levetiracetam monotherapy. Another patient remained seizure free for up to 7 months without therapy. All patients received oral pyridoxine treatment, with only one case (with exon 8–13 deletion) showing poor control.
**INTERPRETATION** This study illustrates the range of clinical presentations and genetic causes in PDE, as well as responsiveness to antiepileptic drugs. A relationship between EEG and pyridoxine therapy could be seen in many cases. Seizure control was seen in all with pyridoxine monotherapy except for one patient.

Pyridoxine-dependent epilepsy (PDE) is an autosomal recessive disorder of pyridoxine metabolism.[1] Intractable neonatal epileptic encephalopathy is the classical presentation with the hallmark feature that seizures respond uniquely to high dose of pyridoxine, and not to antiepileptic drugs (AEDs).[2] *ALDH7A1* was first identified as a pathogenic gene for PDE in 2006.[3] Mutations deactivate the α-aminoadipic semialdehyde (α-AASA) dehydrogenase in the lysine catabolism pathway, which destabilizes the balance between α-AASA and piperideine-6-carboxylic (P6C), further deactivating pyridoxal 5′-phosphate (PLP).[3] Subsequently, mutations in *PLPBP* (previously called *PROSC*) encoding PLP binding protein were identified in 2016, resulting in impaired cellular PLP homeostasis.[4] To date, more than 280 patients with PDE-*ALDH7A* and 27 with PDE-*PLPBP* have been reported.[4–16]

Here, we report the clinical, electrophysiological, biochemical, and genetic spectrum of 33 patients with PDE from 31 unrelated families, including 12 patients (patient 1–6, patient 8–13) who were reported previously.[17–19] Thirty-one patients were PDE-*ALDH7A1*, and the other two patients were PDE-*PLPBP*.

## METHOD

### Patients and materials

Thirty-three patients from 31 unrelated families were recruited. We adopted the method of liquid chromatography-mass spectrometry described previously to detect the concentrations of α-AASA, P6C, and pipecolic acid.[20] Fifteen patients underwent liquid chromatography-mass spectrometry screening for α-AASA, P6C, and pipecolic acid. Biochemical testing, blood urinary metabolic screening, electroencephalogram (EEG), and brain magnetic resonance imaging (MRI) were performed on some of the patients, and genetic studies performed on all. Neurodevelopmental assessment was performed according to intelligence tests (Wechsler or Gesell intelligence scales) or clinical judgement and parents' questionnaires.

### Genetics analysis

Genetic analysis of *ALDH7A1* was performed as described previously.[18] Genetic analysis of *PLPBP* was performed as described by Plecko et al.[5]

The study was approved by the Biomedical Research Ethical Committee of Peking University First Hospital.

Written permissions were obtained from all patients' parents or guardians.

## RESULTS

### Phenotypic spectrum

Demographic and clinical characteristics are summarized in Table S1 (online supporting information). The 33 patients (15 males, 18 females; mean [SD] age 4y 11mo [2y 5mo]; 1y 3mo–10y 4mo) were from 31 unrelated families (two affected siblings in two families). Patient 13 had a sibling who had neonatal seizures and died at the age of 5 years. Perinatal abnormalities were observed in 11 cases, including asphyxia in four patients, meconium-stained amniotic fluid in three, postpartum hypoxia in two, abnormal fetal movement and threatened preterm labour in one. Convulsive seizures occurred within 24 hours of life in six patients, 1 to 10 days in 12, 1 to 3 months in seven, and 3 to 6 months in seven. Patient 14 had a later onset, at the age of 13 months. Focal seizures occurred in 32 out of 33 patients. Other seizure types included: epileptic spasms in five patients, focal to bilateral tonic-clonic seizures (secondarily generalized seizures) in five, generalized tonic-clonic seizures, and myoclonic seizures in two. These different seizures types could exist simultaneously in the same individual. In addition, 13 cases had a history of status epilepticus.

The phenotype of two patients was similar to Dravet syndrome. For patient 12, a febrile, focal to bilateral tonic-clonic seizure occurred at the age of 5 months. Then, febrile or afebrile seizures occurred and sometimes attained status epilepticus, which could not be controlled by multiple AEDs. Single *SCN1A* test was negative and the neurological gene panel revealed *ALDH7A1* mutation. At the age of 21 months, she took pyridoxine monotherapy persistently (150mg/d) and remained seizure free for about 5 years. For patient 18, seizures during febrile diseases occurred at 3 months manifesting as unilateral or bilateral limb convulsions, sometimes lasting for more than half an hour or showing clusters within a few hours. At 6 months old, various AEDs were combined or taken in turn, but showed no significant effect. After a genetic diagnosis at the age of 4 years and 9 months, she began to be treated with pyridoxine (210mg/d) and remained seizure free for nearly 6 years.

### EEG, MRI, biochemical, and metabolic investigations

EEG were obtained in 30 patients (Table S2, online supporting information). Before or after pyridoxine, the EEG was normal in seven patients, and showed focal or multifocal discharges in 17, generalized discharges in three, burst suppression in one, hypsarrhythmia in one, and generalized slow wave rhythm in one. At the last follow-up, EEG normalized in 17 out of 20 patients, with 16 cases returning to normal within 28 days to 16 months and remaining normal. The EEG of patient 1 returned to normal at the age of 5 months with pyridoxine treatment for 3 months, but showed focal discharges once again after 2 years, without seizure recurrence. By increasing the maintenance dose of pyridoxine, the EEG returned to normal again. For patient 3, despite being treated with pyridoxine for nearly 5 years without seizures, the EEG was still abnormal with no significant improvement in severity. Before and after using pyridoxine, the EEG of patient 20 still showed interictal multifocal discharges with seizures. In addition, the EEG showed functional centrotemporal spikes at the age of 7 years in patient 31.

**What this paper adds**

- There is a parallel relationship between electroencephalogram and pyridoxine therapy in many patients.
- Patients with pyridoxine-dependent epilepsy may respond well to low-dose pyridoxine.

Among the 28 patients with brain MRI results (Table S2), 19 showed normal MRI. The MRI abnormalities included enlarged ventricles (3/13), abnormal white matter signals (2/13), subarachnoid widened (2/13), posteior horn of lateral ventricle delayed myelination (1/13), corpus callosum hypoplasia (2/13), diffusely delayed myelination (1/13), cerebral white matter dysplasia (1/13), and brain atrophy (1/13). These abnormalities could exist simultaneously in the same individual.

Fifteen patients underwent the search for biomarkers during pyridoxine treatment between the age of 16 months and 8 years (Table 1). The concentrations of the sum of α-AASA and P6C (α-AASA-P6C) in plasma and urine were significantly increased in all 15 patients. Pipecolic acid concentrations in plasma and urine were elevated in 10 patients, but normal in five patients. Abnormal biochemical results were found in 7 out of 11 patients, including electrolyte disturbance, lactic acidosis, elevated lactate dehydrogenase, or elevated glutamic oxalate transaminase. The amino acids in blood were elevated in patients 23 and 27, including arginine, threonine, leucine, phenylalanine, methionine, and ornithine. Only patient 15 had elevated galactose in the urine organic acid testing.

### Gene mutation analysis

All patients underwent genetic sequencing (Table S2). Thirty-one patients carried *ALDH7A1* mutation and two carried *PLPBP* mutation. *PLPBP* complex heterozygous mutations were detected in patient 32 (*c.119 C>T* [*p.P40L*] and *c.207+1 G>T* [*IVS2+1 G>T*]) and patient 33 (*c.45C>A* [p.C15X] and *c.338T>C* [*p.M113*]). Twenty-six kinds of variants were identified in *ALDH7A1* mutations (Fig. 1), including 17 missense mutations, two nonsense mutations, three splicing site mutations, four deletion mutations. Among them, two cases (patient 20 and patient 25) were identified as homozygous mutations in *ALDH7A1*. However, when testing the proband's parents, only one paternal mutation site was detected. Subsequently, two different kinds of maternal deletion of exons were validated in both patients by quantitative polymerase chain reaction: exons 8 to 13 and exon 6. In addition, patient 29 underwent quantitative polymerase chain

**Table 1:** Biochemical markers in 15 patients with pyridoxine-dependent epilepsy

| Patient ID/Sex | Age at detection (y:mo) | Duration of treatment with pyridoxine (y:mo) | Pipecolic acid | | α-AASA-P6C | |
|---|---|---|---|---|---|---|
| | | | Plasma (μmol/L) | Urine (μmol/mmol Cr) | Plasma (μmol/L) | Urine (μmol/mmol Cr) |
| 1/F | 3:0 | 3:0 | 8.1221 | 0.2048 | 21.0868 | 122.0004 |
| 3/M | 3:10 | 3:0 | 5.6205 | 0.3781 | 16.6715 | 56.0896 |
| 4/M | 5:6 | 3:9 | 4.2038 | 0.0315 | 15.2904 | 42.5998 |
| 5/M | 3:4 | 3:0 | 4.9616 | 0.1501 | 4.398 | 37.5832 |
| 6/F | 6:1 | 5:2 | 5.281 | 0.0950 | 13.4936 | 32.3642 |
| 9/M | 5:8 | 5:1 | 6.6532 | 0.1604 | 11.3282 | 67.0984 |
| 10/M | 2:6 | 2:0 | 2.1296 | 0.0393 | 5.4767 | 23.2588 |
| 12/F | 5:3 | 3:5 | 6.1772 | 0.3562 | 24.1705 | 109.9488 |
| 14/F | 5:4 | 1:4 | 3.9366 | 0.0201 | 10.1806 | 18.0173 |
| 15/F | 4:5 | 4:0 | 5.054 | 0.0567 | 9.6614 | 15.0009 |
| 16/F | 1:4 | 0:11 | 2.2946 | 0.0366 | 4.9316 | 18.4633 |
| 17/M | 4:9 | 0:2 | 1.4934 | 0.0750 | 5.3217 | 3.2157 |
| 18/M | 8:7 | 4:0 | 3.0904 | 0.1298 | 7.7447 | 46.7611 |
| 19/F | 4:5 | 0:4 | 2.932 | 0.1542 | 6.7307 | 18.1117 |
| 20/F | 4:3 | 3:10 | 4.8373 | 0.0456 | 6.5793 | 18.7264 |

Reference range:[20] α-aminoadipic semialdehyde (α-AASA)-piperideine-6-carboxylate (P6C), plasma: 4.40–24.17μmol/L, urine: 3.22–122.00μmol/L; pipecolic acid, plasma: 1.49–8.12μmol/L, urine: 0.02–0.38μmol/L.

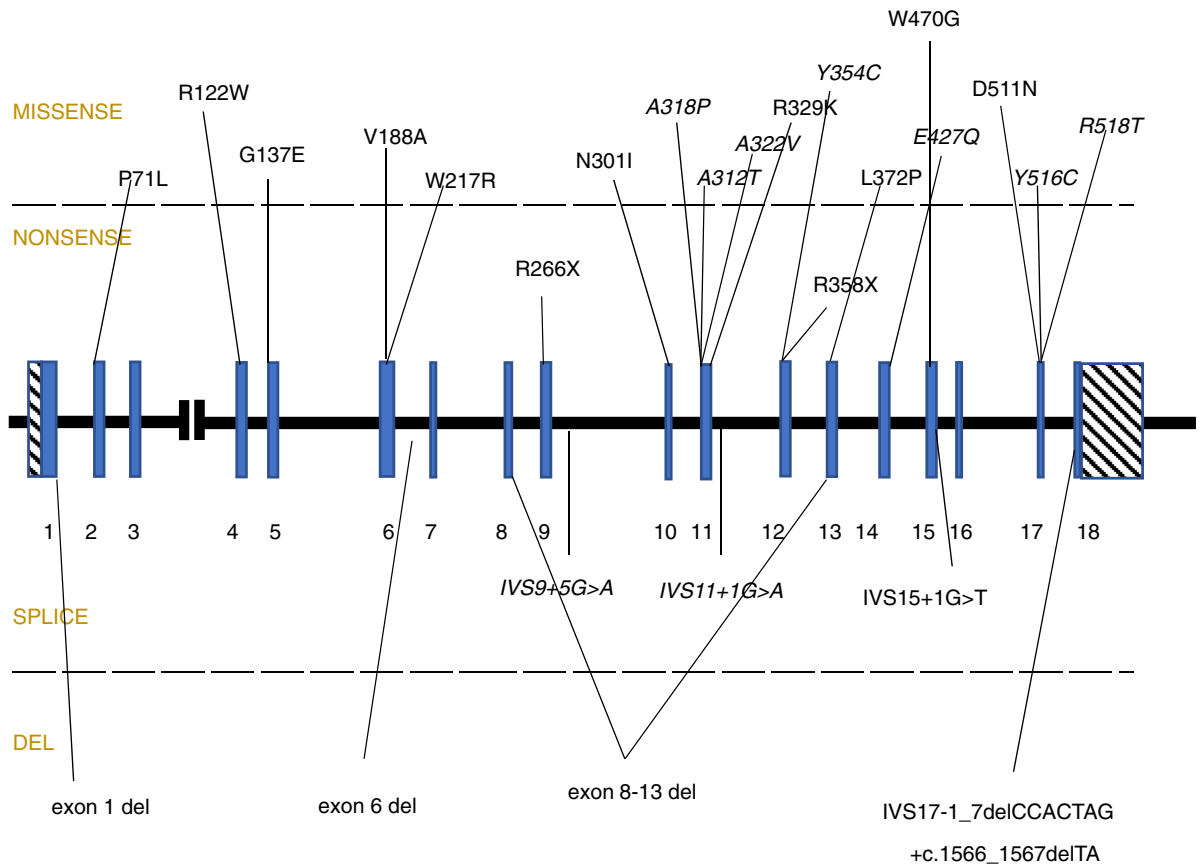


**Figure 1:** All mutations identified in *ALDH7A1* in 31 patients. Mutations appearing more than twice are shown in italics. Mutations are organized as missense, nonsense, splice, and deletion. The 5′ and 3′ noncoding regions are shown with diagonal stripes. [Colour figure can be viewed at wileyonlinelibrary.com]

reaction at the initial genetic testing, with one paternal missense mutation and one heterozygous deletion in exon 1, while her mother showed wild type.

## Treatment outcome

Before pyridoxine administration, 26 patients were treated by multiple AEDs. The duration of seizures decreased or

the interval between seizures increased in 20 patients. In four patients (patients 21, 23, 26, and 27), AEDs combined with pyridoxine were initially used, so the response to AEDs alone could not be observed. The treatment before pyridoxine was unknown in two patients (patients 22 and 25). When patient 7 developed convulsions on the ninth day after birth, pyridoxine without AEDs was used initially because of family history. There were particular treatment experiences in two patients. For patient 31, since onset at 5 months, the seizures could be controlled well for 2 to 3 years with levetiracetam therapy. Seizures relapsed because of febrile diseases at the age of 3 and 5 years. The patient was genetically diagnosed with PDE at the age of 5 years and 9 months. From then on, he was treated with pyridoxine monotherapy and had no seizures for nearly 2 years, despite the occurrence of febrile diseases. For patient 14, focal seizures occurred at 13 months of age. Untreated with AEDs or pyridoxine, she had no seizures for 7 months. Seizures lasted for several minutes with clusters, and the frequency was not changed by the combination of AEDs. After genetic diagnosis at the age of 4 years, pyridoxine was added (180mg/d), and no seizures occurred for nearly 4 years.

After diagnosis, all patients received pyridoxine therapy. The duration of pyridoxine maintenance therapy ranged from 15 days to 6 years. The length of recurrence interval after pyridoxine withdrawal ranged from 3 to 100 days. Thirty-two patients treated with pyridoxine monotherapy were controlled well. In the remaining patient (patient 20), the seizures were still uncontrolled with both pyridoxine at the maximum dose of 360mg/day and a combination of three AEDs.

### Follow-up

At last follow-up, 33 patients were aged from 2 months to 9 years. All patients except for one (patient 20) had good control of seizures under pyridoxine monotherapy, with dosages ranging from 30mg/day to 240mg/day. Among them, the therapeutic dose of pyridoxine ranged from 1.6mg/kg/day to 4.7mg/kg/day in seven patients, 5.0mg/kg/day to 7.9mg/kg/day in 14, and 8.1mg/kg/day to 12.8mg/kg/day in 11. Seizures relapse occurred in eight patients because of febrile diseases. In two of them, the seizures were controlled by doubling the dose of oral pyridoxine. Five patients were controlled by intravenous pyridoxine. For the remaining patient, neither oral dose-doubling nor intravenous pyridoxine could control the seizures, which only responded to diazepam.

Up to the last follow-up, the neurological development of 15 out of 33 patients was within typical limits. The others showed varying degrees of developmental delay that was considered mild in nine cases, moderate in four, and severe in five. In five patients (patient 3, 5, 14, 17, and 21) formal testing occurred (Wechsler or Gesell intelligence scales), while others were assessed by clinical judgement or parents' questionnaires (child development testing).

### Antenatal diagnosis

Five families with PDE (families of patients 1, 5, 10, 15, and 20) underwent antenatal diagnosis procedure for a second pregnancy. One case (family 10) was wild type and three cases (family 1, 5, and 20) had heterozygous mutations. The result of another case (family 15) was the same as proband, so abortion was induced. One family (patient 6 and 7) followed doctors' advice to accept intrauterine pyridoxine therapy on patient 7 because of a rejection of antenatal diagnosis. As for another family (patient 27 and 28), because of late diagnosis in the first child, the second child unfortunately also presented with PDE without antenatal diagnosis.

## DISCUSSION

Here we report a cohort study of 33 patients with PDE, showing 26 disease-causing variants in *ALDH7A1* and four variants of *PLPBP*. The largest number of cases reported in previous literature was 30, with a focus on analysing the imaging findings rather than phenotype and genotype.[21] van Karnebeek et al.[7] focused on six cases with atypical manifestations among 266 cases reported in 49 articles. Coughlin et al.[22] reviewed the genetics of 185 cases from previous reports. Therefore, this study involved the largest cohort of patients with PDE in a single centre reported to date, with analysis of both clinical and genetic features.

Up until now, 27 patients with PDE-*PLPBP* had been reported. Compared with the reported cases with *ALDH7A1* mutations, patients with PDE-*PLPBP* showed high preterm birth rate, early onset, seizures mainly in the form of generalized tonic-clonic seizures and lactic acidosis in more than 50 per cent of patients.[4–6,8] In our cohort, no specific clinical features except the elevation of lactic acid of PDE-*PLPBP* were found to distinguish these two cases from patients with PDE-*ALDH7A1*.

In the literature summarized by Mills et al.,[23] abnormal fetal movement (33%) and fetal distress (29%) were found, as well as postnatal respiratory distress (33%). In this study, 33 per cent had abnormal pregnancy or birth history, and one case had been found to have abnormal fetal movements, indicating the possibility of intrauterine seizures. In previous reports, convulsions appeared in 89 per cent of patients within 1 month.[23] In atypical form, seizures might appear later, sometimes as late as at 3 years.[3] In our cohort, seizures of 61 per cent of patients ($n$=20) occurred within the neonatal period, while 39 per cent ($n$=13) had a later age at onset, up to 13 months. In their review, Basura et al.[24] concluded that there were various types of seizures, focal seizures being the most common (30%). The latter were present in 97 per cent of patients ($n$=32) in our cohort. In addition, two of our patients presented with clinical features that were similar to Dravet syndrome, suggesting that PDE had an extensive clinical spectrum.

Previous reports showed that burst suppression was the most common EEG feature (53%) among patients with PDE-*PLPBP*.[4–6] Owing to only two PDE-*PLPBP* cases in our study, the EEG did not show this feature. According

to previous literature, the EEG of patients with PDE included slow background activity with multifocal or generalized discharges (58%), burst suppression (16%), and normal EEG (11%).[23] In our cohort, EEG outcomes were abnormal in 77 per cent ($n$=23) of patients with burst suppression only in one. During the follow-up, the EEG normalized in 85 per cent of patients ($n$=17). For patient 3, frequent convulsions and even status epilepticus occurred during the neonatal period without being treated by pyridoxine correctly, after which the patient's development was severely impaired. The EEG of this patient also remained abnormal. In our cohort, a parallel relationship between EEG improvement and long-term pyridoxine treatment could be seen to some degree. Notably, before or after pyridoxine, the EEG was normal in 23 per cent ($n$=7) of patients and normalized in 85 per cent ($n$=17) of patients at the last follow-up. Interestingly, in one patient, the EEG returned to normal again when increasing the dose of pyridoxine without seizure recurrence, suggesting that EEG monitoring could guide the clinical medication during the course of PDE. In this series, normality and nonspecific abnormalities of brain MRI were observed, as previously reported.[23] But in our cohort, the proportion of patients who had normal MRI seems particularly high (68%, $n$=19). For example, Mills et al.[23] found normal brain MRI in 6 out of 19 patients and Bok et al.[25] in only 1 out of 9 patients. It was reported that long-term high doses of pyridoxine could cause cerebral demyelinating, so regular brain MRI monitoring was recommended.[26] Unfortunately, because of several factors such as patients' poor compliance, the follow-up brain MRI is lacking at present.

Elevated concentrations of α-AASA, P6C, and pipecolic acid in blood, urine, and cerebrospinal fluid could be used as biomarkers for the diagnosis.[26] However, the detection method of biomarkers is currently available in only a few laboratories in some countries. Using the method established in our hospital in 2018, biomarkers were detected in 15 patients during pyridoxine therapy. Literature suggested that pipecolic acid levels could be normalized with age or after pyridoxine treatment for months to years.[24] In our study, the high concentrations of pipecolic acid in two-thirds of patients and α-AASA-P6C in all 15 patients suggested that pyridoxine monotherapy was insufficient to improve the level of biomarkers, and triple therapy with pyridoxine, arginine supplementation, and dietary lysine restriction might be adopted in the future.[27] To our knowledge, at present, triple therapy has been carried out in no more than 20 patients in the world. Some reports suggest further reduced toxic metabolites in the blood, urine, and cerebrospinal fluid, and possible improvement of neurodevelopmental outcomes, but this has not been confirmed.[16,27] Consistent with previous reports,[7] abnormal biochemical and plasma amino acids and urine organic acids test results were not found to be specific in this series.

Genetic studies in our cohort identified 26 kinds of variants in *ALDH7A1* and four in *PLPBP*, with missense mutations being the most common. Among them, 15 *ALDH7A1* variants and three *PLPBP* variants were unreported previously. In a review of the literature, *p. E427Q* was the most common mutation presenting in 26 per cent of all alleles.[22] This 'hot spots' mutation accounted for only 5 per cent in our study, but *p. Y516C* (16%), *IVS11+1G>A* (15%), and *p. Y345C* (13%) had high frequency. Among them, *p. Y345C* and *IVS11+1G>A* has been reported in China only, while *p. Y516C* was reported in a few patients from other countries.[28] Mills et al.[23] found that exons 4, 6, 9, 11, and 14 seemed to be 'hot spots' in *ALDH7A1* mutations, existing in 60 per cent of white patients. In our 31 Chinese patients, 77 per cent variants in *ALDH7A1* were located in exon 11, 12, 14, and 17 or intron 9 and 11. These data suggest that ethnic differences exist in *ALDH7A1* mutation. The available genetic data showed deletion of one or more exons in *ALDH7A1*, including exons 8 and 9, exons 12 to 18, exons 14 to 17, and exons 16 and 17.[22] In our study, there were three patients carrying a different deletion of exons, which were not shown by initial genetic studies in two of them. For another patient with exon deletion mutation, the variant was considered de novo. The above indicated that suspected patients with negative results of gene sequencing or only one mutation site should undergo quantitative polymerase chain reaction or multiplex ligation-dependent probe amplification testing.

Lin et al. reported that the most common AEDs with initial response in patients with PDE was phenobarbital.[29] In our cohort, 77 per cent ($n$=20) of patients had transient response to commonly used AEDs, including phenobarbital. One of our patients was treated by levetiracetam alone for several years without seizures. Before therapy, another patient was seizure free for 7 months. In previous reports, seizures commonly recurred within 1 to 51 days after the withdrawal of pyridoxine, or patients could remain seizure free for a maximum of 5.5 months.[23] Our patients were also treated with pyridoxine intermittently before definite diagnosis and relapsed within 3 to 100 days. Therefore, PDE should not be excluded based on response to AEDs and the delay recurrence of pyridoxine after withdrawal. Seizure freedom was reported in 87 per cent of patients with PDE-*ALDH7A1* on pyridoxine monotherapy,[24] but only in about 30 per cent of patients with PDE-*PLPBP*.[4–6] In our cohort, 30 patients with PDE-*ALDH7A1* (97%) and two patients with PDE-*PLPBP* became seizure free on pyridoxine monotherapy (i.e. much more than previously reported). There has been no uniform standard for the maintenance dose of pyridoxine; the recommended dosage changes between 15mg/kg/day and 30mg/kg/day in infants or up to 200mg/day in neonates and 500mg/day in adults.[26] In our patients, maintenance dosage was mostly between 30mg/kg/day and 240mg/day (i.e. 1.6–12.8mg/kg/day), which is lower than in the literature.[26] It seemed that patients with PDE responded well to a lower dose of pyridoxine. The large individual dose differences indicate that further research is needed to recommend individualized

long-term treatment. Notably, one patient who carried a deletion of exons 8 to 13 had the worst response to pyridoxine at a dose of 360mg/day combined with multiple AEDs. This might result from the large fragments deletion of *ALDH7A1*, resulting in severe loss of protein function.

It has been reported that when patients with PDE had acute febrile diseases, breakthrough convulsion could be prevented or controlled by increasing the dosage of pyridoxine,[17] which appeared to be confirmed in our study. However, oral pyridoxine dose-doubling failed to prevent recurrence and then control seizures in six of our patients, whereas intravenous pyridoxine or diazepam achieved control, possibly owing to quicker availability of pyridoxine by intravenous injection and sedative effect of diazepam. Based on this observation, we would suggest use of intravenous pyridoxine with or without diazepam if oral dose-doubling pyridoxine fails to prevent recurrence during febrile diseases.

According to a previous report, 71 per cent of patients with PDE had developmental delay.[25] In our cohort, 55 per cent ($n$=18) of patients showed different degrees of neurodevelopmental delay, which was mild in nine cases, moderate in four cases, and severe in five cases. Among the patients, there were affected siblings in two families. In one family, development was within normal limits in both affected children. In another family, because of the large difference in diagnosis time and therefore treatment, one patient had a severe delay and the other was nearly typical. Nevertheless, two patients with the same genotype from different families also showed completely different phenotypes. For delayed diagnosis, the mean lag time was reported as 311 days, and the longest was nearly 9 years.[24] In our study, the lag period ranged from 15 days to 6 years. One patient with typical development received a delayed diagnosis by about 6 years, while the other with delayed diagnosis for 2 months still showed severe intellectual disability. Another three patients with delayed diagnosis for 9 months showed different prognosis. This confirms that the relationship between phenotype and genotype is complex. In addition to genotype and delayed diagnosis, age at onset, and drug maintenance dose also affects outcomes.[23]

The prenatal screening of *ALDH7A1* for affected family members had been proposed.[30] In our study, five families with PDE performed antenatal diagnosis for a second pregnancy and a further family with a positive result chose to induce abortion. In two families with two affected siblings in whom the antenatal diagnosis had not been performed, the second patient with PDE was born. In addition, for family 13, the first dead child might also have had PDE. This suggests that diagnosis in the first fetus could impact management decisions about termination or (very) early treatment to avoid poor prognosis, arguing in favour of antenatal diagnosis and timely (intrauterine) treatment.

## CONCLUSION

This study involved the largest cohort of patients with PDE from a single centre. We described the details of the clinical and genetic alterations of 33 patients with PDE, with some atypical symptoms, extending the phenotypic and genotypic spectrum. In addition, we described a parallel relationship between EEG and long-term pyridoxine therapy to some extent. In terms of genetics, we identified high frequency mutation sites and exons or introns in a Chinese population. Given the neurodevelopmental outcome issues, triple therapy should be studied in this group, especially for patients with uncontrolled seizures and developmental delay.


## ACKNOWLEDGEMENTS

We thank the patients and their families for participating. This work was financed by National Nature Science Foundation of China (81771393) and Beijing Municipal Science & Technology Commission (Z171100001017125). The authors have stated that they had no interests which might be perceived as posing a conflict or bias.


## SUPPORTING INFORMATION

The following additional material may be found online:

**Table S1:** Summary of demographics and clinical characteristics in 33 patients

**Table S2:** Summary of EEG, brain MRI findings, and molecular genetics studies in 33 patients

## REFERENCES


1. Hunt AD Jr, Stokes JJ, McCrory WW, Stroud HH. Pyridoxine dependency: report of a case of intractable convulsions in an infant controlled by pyridoxine. *Pediatrics* 1954; **13**: 140–5.
2. Baxter P. Pyridoxine-dependent and pyridoxine-responsive seizures. *Dev Med Child Neurol* 2001; **43**: 416–20.
3. Mills PB, Struys E, Jakobs C, et al. Mutations in antiquitin in individuals with pyridoxine-dependent seizures. *Nat Med* 2006; **12**: 307–9.
4. 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**: 1325–37.
5. Plecko B, Zweier M, Begemann A, et al. Confirmation of mutations in PROSC as a novel cause of vitamin B6-dependent epilepsy. *J Med Genet* 2017; **54**: 809–14.
6. Johnstone DL, Al-Shekaili HH, Tarailo-Graovac M, et al. PLPHP deficiency: clinical, genetic, biochemical, and mechanistic insights. *Brain* 2019; **142**: 542–59.
7. van Karnebeek CD, Tiebout SA, Niermeijer J, et al. Pyridoxine-dependent epilepsy: an expanding clinical spectrum. *Pediatr Neurol* 2016; **59**: 6–12.
8. Shiraku H, Nakashima M, Takeshita S, et al. *PLPBP* mutations cause variable phenotypes of developmental and epileptic encephalopathy. *Epilepsia Open* 2018; **3**: 495–502.
9. Toldo I, Bonardi CM, Bettella E, et al. Brain malformations associated to Aldh7a1 gene mutations: report of a novel homozygous mutation and literature review. *Eur J Paediatr Neurol* 2018; **22**: 1042–53.
10. Haidar Z, Jalkh N, Corbani S, Fawaz A, Chouery E, Mégarbané A. Atypical pyridoxine dependent epilepsy resulting from a new homozygous missense mutation, in ALDH7A1. *Seizure* 2018; **57**: 32–3.

11. Navarro-Abia V, Soriano-Ramos M, Núñez-Enamorado N, et al. Hydrocephalus in pyridoxine-dependent epilepsy: new case and literature review. *Brain Dev* 2018; **40**: 348–52.
12. Wang S, Sun J, Tu Y, Zhu L, Feng Z. Clinical and genetic characteristics of pyridoxine-dependent epilepsy: case series report of three Chinese patients with phenotypic variability. *Exp Ther Med* 2017; **14**: 1989–92.
13. Coci EG, Codulti L, Fink C, et al. Novel homozygous missense mutation in ALDH7A1 causes neonatal pyridoxine dependent epilepsy. *Mol Cell Probes* 2017; **32**: 18–23.
14. Samanta D. A 15-year-old with seizures: late diagnosis of pyridoxine-dependent epilepsy. *Acta Neurol Belg* 2016; **116**: 667–9.
15. Marguest F, Barakizou H, Tebani A, et al. Pyridoxine-dependent epilepsy: report on three families with neuropathology. *Metab Brain Dis* 2016; **31**: 1435–43.
16. Yuzyuk T, Thomas A, Viau K, et al. Effect of dietary lysine restriction and arginine supplementation in two patients with pyridoxine-dependent epilepsy. *Mol Genet Metab* 2016; **118**: 167–72.
17. Xue J, Qian P, Li H, Wu Y, Liu XY, Yang ZX. A cohort study of pyridoxine-dependent epilepsy and high prevalence of splice site IVS11+1 G>A mutation in Chinese patients. *Epilepsy Res* 2015; **118**: 1–4.
18. Yang ZX, Yang XL, Wu Y, et al. Clinical diagnosis, treatment, and ALDH7A1 mutations in pyridoxine-dependent epilepsy in three Chinese infants. *PLoS ONE* 2014; **9**: e92803.
19. Xue J, Yang ZX, Wang S, Zhang YH. A case of atypical pyridoxine-dependent epilepsy. *Chin J Pediatr* 2016; **54**: 861–2.
20. Yuzyuk T, Liu A, Thomas A, et al. A novel method for simultaneous quantification of alpha-aminoadipic semialdehyde/piperideine-6-carboxylate and pipecolic acid in plasma and urine. *J Chromatogr B Analyt Technol Biomed Life Sci* 2016; **1017–18**: 145–52.
21. Friedman SD, Ishak GE, Poliachik SL, et al. Callosal alterations in pyridoxine-dependent epilepsy. *Dev Med Child Neurol* 2014; **56**: 1106–10.
22. Coughlin CR 2nd, Swanson MA, Spector E, et al. The genotypic spectrum of ALDH7A1 mutations resulting in pyridoxine dependent epilepsy: a common epileptic encephalopathy. *J Inherit Metab Dis* 2019; **42**: 353–61.
23. Mills PB, Footitt EJ, Mills KA, et al. Genotypic and phenotypic spectrum of pyridoxine-dependent epilepsy (ALDH7A1 deficiency). *Brain* 2010; **133**: 2148–59.
24. Basura GJ, Hagland SP, Wiltse AM, Gospe SM Jr. 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.
25. Bok LA, Halbertsma FJ, Houterman S, et al. Long-term outcome in pyridoxine-dependent epilepsy. *Dev Med Child Neurol* 2012; **54**: 849–54.
26. Stockler S, Plecko B, Gospe SM Jr, et al. Pyridoxine dependent epilepsy and antiquitin deficiency clinical and molecular characteristics and recommendations for diagnosis, treatment and follow-up. *Mol Genet Metab* 2011; **104**: 48–60.
27. Coughlin CR 2nd, van Karnebeek CD, Al-Hertani W, et al. Triple therapy with pyridoxine, arginine supplementation and dietary lysine restriction in pyridoxine-dependent epilepsy: neurodevelopmental outcome. *Mol Genet Metab* 2015; **116**: 35–43.
28. Nam SH, Kwon MJ, Lee J, et al. Clinical and genetic analysis of three Korean children with pyridoxine-dependent epilepsy. *Ann Clin Lab Sci* 2012; **42**: 65–72.
29. Lin J, Lin K, Masruha MR, Vilanova LC. Pyridoxine-dependent epilepsy initially responsive to phenobarbital. *Arq Neuropsiquiatr* 2007; **65**: 1026–9.
30. Bennett CL, Chen Y, Hahn S, Glass IA, Gospe SM Jr. Prevalence of ALDH7A1 mutations in 18 North American pyridoxine-dependent seizure (PDS) patients. *Epilepsia* 2009; **50**: 1167–75.

## 目的

总结33例吡哆醇依赖性癫痫（pyridoxine-dependent epilepsy, PDE）患者的临床特征及遗传学特点。

## 方法

回顾性收集33例PDE患者的临床资料（15位男性，18位女性；平均年龄[SD]：4岁11个月[2岁5个月]；1岁3个月~10岁4个月），所有患者均进行遗传学检测。

## 结果

癫痫发作类型多样，32例患者出现局灶性发作。2例患者在未经遗传学确诊前临床疑诊为Dravet综合征。7例患者初次脑电图正常，随后，在吡哆醇维持治疗期间，17的患者脑电图恢复正常。基因检测结果提示有26种不同的*ALDH7A1*突变，4种*PLPBP*突变。其中，18种突变国际上未见相关报道。48个*ALDH7A1*突变位于外显子11、12、14和17或内含子9和11。此外，3例患者携带不同的外显子缺失。33例患者中，1例经左乙拉西坦单药治疗可控制数年无发作，另1例在接受治疗前，可7个月无癫痫发作。所有患者均口服吡哆醇治疗，仅有1例携带8-13号外显子缺失的患者经吡哆醇单药治疗后，发作未控制。

## 结论

本研究总结了33例PDE患者临床及遗传学特征，以及抗癫痫药的治疗效果。本研究揭示了脑电图与长期吡哆醇维持治疗之间的一种平行关系。另外，在本研究中，32例患者可被吡哆醇单药控制。