

# Further Delineation of Pyridoxine-Responsive Pyridoxine Phosphate Oxidase Deficiency Epilepsy: Report of a New Case and Review of the Literature With Genotype-Phenotype Correlation

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## Abstract

In recent years, the clinical spectrum of pyridoxine phosphate oxidase (PNPO) deficiency has broadened. There are a growing number of patients with a transient or lasting response to pyridoxine in addition to cases that respond more traditionally to pyridoxal-phosphate. However, among pyridoxine-responsive patients with *PNPO* gene mutation, there are only a few reports on electroencephalogram (EEG) ictal/interictal patterns, and data regarding the outcomes are inconsistent. We describe a case of neonatal onset epilepsy with missense mutation c(674G>A) p(R225 H) in *PNPO* gene and pyridoxine responsiveness. Comparing this patient with 24 cases of previously described pyridoxine-responsive pyridoxine phosphate oxidase deficiency epilepsy, we found that patients carrying the missense mutation c(674G>A) p(R225 H) of the *PNPO* gene might have a more severe epileptic phenotype, possibly because of their lower residual PNPO activity. Indeed, pyridoxine-responsive pyridoxine phosphate oxidase deficiency epilepsy remains a challenge, with neurodevelopmental disabilities occurring in about half of the cases.

## Keywords

pyridoxine phosphate oxidase deficiency, pyridoxine-dependent epilepsy

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Pyridoxine-dependent epilepsy (PDE) and pyridoxine phosphate oxidase deficiency (PNPO) are rare diseases that need rapid diagnosis and appropriate treatment. As biochemical and genetic analyses require time, the administration of pyridoxine and pyridoxal-5-phosphate is still the best method to make a preliminary diagnosis and to give empirical therapy.<sup>1</sup> The first described patients with pyridoxine phosphate oxidase deficiency had neonatal seizures that did not respond to treatment with pyridoxine, but they responded to pyridoxal-5-phosphate.<sup>2</sup> The underlying genetic defect in the *PNPO* gene was identified in 2005 by Mills, who sequenced the *PNPO* gene in patients with biochemistry suggestive of pyridoxine phosphate oxidase deficiency and identified different mutations reducing pyridoxine phosphate oxidase activity.<sup>3</sup> In recent years, the clinical spectrum of pyridoxine phosphate oxidase deficiency has broadened because there are a growing number of patients with a transient or lasting response to pyridoxine, in addition to cases that more traditionally respond to pyridoxal-5-phosphate.<sup>4</sup> However, this could be an overlap area, as it is likely that some patients could be responsive to either or to both, and some anecdotal cases seem to yield optimal results with a

combination of pyridoxine and pyridoxal-5-phosphate.<sup>5-7</sup> Among patients with pyridoxine phosphate oxidase deficiency epilepsy and partial pyridoxine responsiveness, some cases had seizure relapse on pyridoxine therapy and achieved a better long-term neurologic outcome with pyridoxal-5-phosphate.<sup>4</sup> Specific genotypes (R225H/C and D33 V), however, are more likely to result in seizures with long-lasting pyridoxine response and some patient's conditions could even worsen after changing from pyridoxine to pyridoxal-5-phosphate.<sup>5-7</sup> Clinical and EEG manifestations of pyridoxine phosphate oxidase deficiency are similar to PDE epilepsy. Clinical seizures are characterized by multifocal and myoclonic jerks intermixed with tonic seizures or subtle movements. Rapid erratic eye movements and facial grimacing are frequently described in

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pyridoxine phosphate oxidase deficiency epilepsy.<sup>1,4,7,8</sup> Seizures are often accompanied by neurologic symptoms, such as irritability, sleeplessness, apnea, staring, and startle reaction. EEG features are variable and include slow wave background, absent sleep features, and burst suppression patterns. The electroclinical correlation can be unclear, and it may be difficult to distinguish interictal background from ictal EEG discharge.<sup>1,7,8</sup>

There are few reports on EEG ictal/interictal patterns among patients with *PNPO* gene mutation and pyridoxine responsiveness. Furthermore, data on outcomes are inconsistent.<sup>5,6</sup> To further characterize pyridoxine-responsive pyridoxine phosphate oxidase deficiency epilepsy, we described electroclinical patterns and outcomes of a neonate affected by pyridoxine-responsive epilepsy with missense mutation c(674G>A) p(R225 H) of the *PNPO* gene. To delineate a possible genotype-phenotype correlation, we compare our patient to cases with pyridoxine-responsive epilepsy with pyridoxine phosphate oxidase deficiency.

## Case Report

This case concerns the first child of healthy parents coming from an Albanian city. The child had an unremarkable family history. The male infant was born at term by vaginal delivery with meconium-stained amniotic fluid. Apgar score was 6, 8, and 9 at the first, second, and third minute, respectively. Birth weight was 2740 g, length was 48 cm, and cranial circumference was 34 cm. In the first hours of life, the newborn presented with respiratory distress and seizures characterized by repetitive generalized myoclonic jerks with synchronous adduction of the upper and lower limbs, often associated with grimaces of the face. A video EEG recorded subcontinuous generalized high-voltage poly spikes and slow-wave discharges (1-2 seconds), which occurred during the 4 limb myoclonic jerks. EEG depression (2-3 seconds) followed the electroclinical paroxysms and corresponded to motor arrest. This paroxysmal and flattening ictal pattern was the only recognizable pattern and corresponded to an electroclinical status epilepticus (Figure 1A,B). During status epilepticus, the clinical condition worsened, severe lactic acidosis developed, and mechanical ventilation was needed. Antiepileptic therapy was administered according to local protocol for neonatal seizures: phenytoin (intravenous, loading dose 20 mg/kg, maintenance 5 mg/kg/d), given along with midazolam (intravenous, loading dose 200 µg/kg, maintenance 200 µg/kg/h). The clinical seizures ceased, but the electrical discharges continued (Figure 1C). Intravenous pyridoxine (100 mg) was then administered and electrical seizures stopped within 30 minutes. After seizures were controlled, the EEG showed a unique and severely discontinuous background pattern with a long-lasting depressed interburst interval (suppression-burst) (Figure 1D). Phenytoin and midazolam were gradually decreased and discontinued on day 6 of life. The baby then maintained seizure control (Figure 1E) through pyridoxine monotherapy (20 mg/kg/d). Brain magnetic resonance imaging (MRI) at 7 days of life showed mild hyperintense periventricular white matter. In the second week

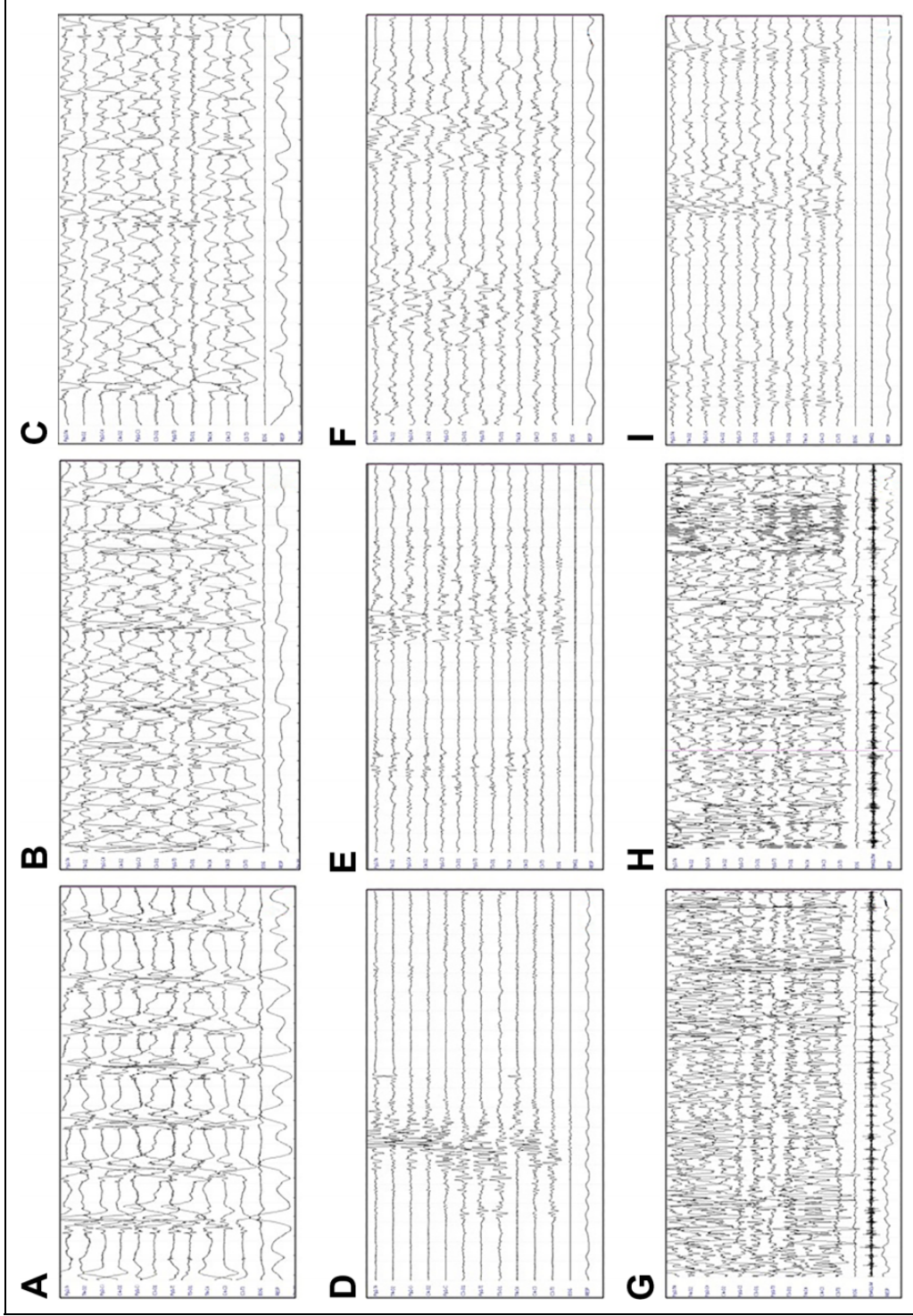
of life, the baby showed normal sleep state cycles and EEG pattern (Figure 1F). The infant was discharged home on pyridoxine therapy (20 mg/kg/d) at age 20 days. After 1 month, the infant was readmitted because of irritability, startles, and vomiting. Parents reported that in the days immediately preceding readmission, symptoms occurred just before pyridoxine intake and disappeared after administration; therefore, a pyridoxine shortage was suspected. To confirm pyridoxine dependent seizures, a withdrawal trial was performed. Twenty-four hours after pyridoxine withdrawal, a video EEG showed motor staring, erratic eye movements, and rowing and pedaling without electric discharges. Afterward, a multifocal clonic electroclinic seizure was recorded, lasting a few minutes (Figure 1G,H). The seizure was interrupted by oral pyridoxine administration (Figure 1I). Subsequently, pyridoxine therapy (15 mg/kg/dose given twice daily) led to seizure control and the symptoms disappeared. Brain MRI performed in the second month of life was within a normal range as well as the neurodevelopmental follow-up at age 18 months.

Next-generation sequencing for epilepsy showed a homozygous missense mutation c(674G>A) p(R225 H) in *PNPO* gene (Hom.pR225 H). Parents were heterozygous for the same mutation. Because of the severity of the clinical findings and the need to promptly administer pyridoxine therapy at birth, we could not perform metabolic analyses before administering B6. However, at 4 months of life, levels of pipercolic acid in urine, plasma, and cerebral spinal fluid were low, whereas cerebral spinal fluid pyridoxal-5-phosphate (108.5 nmol/L), 5-methyl tetrahydrofolate, neurotransmitters, and amino acids were within a normal range.

The c(674G>A) p(R225 H) mutation in the *PNPO* gene has already been described in 13 pyridoxine-responsive children (Table 1). Table 2 compares the group of 14 patients carrying the c(674G>A) p(R225 H) mutation of the *PNPO* gene with 11 additional pyridoxine-responsive patients carrying different types of *PNPO* mutations.

## Discussion

This single case report provides a detailed electroclinical description of an infant with pyridoxine-responsive pyridoxine phosphate oxidase deficiency before and after B6 administration. In this case, a prompt response to pyridoxine was documented by clinical findings and EEG recordings. The response was long-lasting and confirmed by normal neurodevelopmental outcome at age 18 months. In our case, clinical and EEG findings before and after treatment were similar to those reported by Nabbout et al<sup>13</sup> for PDE. The EEG findings described a continuous, bilateral, high-voltage rhythmic slow wave activity that was synchronous with myoclonic jerks. Moreover, our patient showed seizure control and discontinuous background EEG after pyridoxine therapy, as often reported in patients with PDE.<sup>13,14</sup> However, genetic testing did not confirm the typical aldehyde dehydrogenase 7A1 gene (*ALDH7A1*) mutation but rather revealed a missense mutation c(674G>A) p(R225 H) of the *PNPO* gene.



**Figure 1.** (A,B) Subcontinuous generalized high-voltage polyspike and slow-wave discharges lasting 1-2 seconds (during the 4 limb myoclonic jerks), followed by a discontinuous EEG pattern (2-3 seconds) corresponding to motor arrest. (C) High-voltage slow wave and spike-slow wave discharges (persisting after phenytoin and midazolam therapy). (D) EEG background 1 hour after pyridoxine administration showing severely discontinuous EEG pattern with long-lasting suppression (10-15 second) and pathologic medium voltage background with sharps, theta, and sub-alpha rhythms (burst suppression). (E) Seizure free and moderately discontinuous EEG pattern on pyridoxine monotherapy (day 6 of life). (F) (39 weeks postmenstrual age): Tracé alternant during quiet sleep in the second week of life. (G,H) Electroclinical seizure during pyridoxine withdrawal characterized by sharp and sharp-slow wave discharge predominant in the right hemisphere with superimposed faster rhythms. (I) (44 weeks postmenstrual age): The EEG recorded 8 hours after pyridoxine readministration showed no seizures and tracé alternant with an excessively flat inter-burst period (lasting 2-5 seconds).

**Table 1.** Patients Carrying the Homozygous Missense Mutation c(674G>A) p(R225 H) of the PNPO Gene.

Patient number (references)	Gestational age/fetal distress	Origin	Seizure onset, type	EEG	First pyridoxine administration	Pyridoxine response	Pyridoxine treatment dose	MRI	Outcome (18-24 mo)
1. Ware et al 2014 <sup>9</sup>	38 wk; no	Greece	1 d; multifocal myoclonic jerks and eye movements	Excess of multifocal sharps activity	7 d	Prompt	15 mg/kg/d <sup>a</sup>	Normal	Occasional seizures, autism spectrum disorder
2. Mills et al 2014 <sup>5</sup>	NR	NR	24 h; generalized tonic clonic seizures	NR	6 mo	Yes	200 mg/d	NR	Spastic quadriplegia, seizure free
3. Mills et al 2014 <sup>5,c</sup>	NR	Caucasian	30 min/NR	BS	2 wk	Yes	30 mg/kg/d	Bright areas in the peritrigonal white matter	Minimal delay, occasional seizures
4. Mills et al 2015 <sup>5,c</sup>	36 wk; no	Kosovo	10 h/NR	BS	5 d	Yes	12.3 mg/kg/d	NR	Global developmental delay
5. Plecko et al 2014 <sup>6</sup>	35 wk; yes	Kosovo	24 h/NR	BS	6-9 d	Yes	150-200 mg/d	Normal	Global developmental delay, seizure free
6. Plecko et al 2014 <sup>6</sup>	Term; no (green amniotic fluid)	Albania	3 h; NR	BS	2 d	Improved <sup>c</sup>	33 mg/kg/d	Normal	Global developmental delay, occasional seizures
7. Plecko et al 2014 <sup>6</sup>	35 wk; yes	Albania	2 d; NR	BS	2 d	Improved	30 mg/kg/d	Normal	Normal, seizure free
8. Plecko et al 2014 <sup>6</sup>	36 wk; yes	Kosovo	NR	BS	6 wk	Prompt response	20 mg/kg/d	Bilateral	Spastic tetraparesis, occasional seizures
9. Plecko et al 2014 <sup>6</sup>	Term; no (RDS after birth)	Kosovo	11 h; NR	Discontinuous pattern	1 d	Prompt response	13 mg/kg/d	Normal	Normal, seizure free
10. Plecko et al 2014 <sup>6</sup>	Term; yes	Serbia	6 d; NR	Discontinuous pattern	6 d	Prompt response	30 mg/kg/d	Normal	Normal, seizure free
11. Plecko et al 2014 <sup>6</sup>	34 wk; no (abdominal distension)	Unknown	2 d; NR	NR	7 mo	Delayed response	50 mg/kg/d	Mild brain atrophy	Spastic tetraparesis, occasional seizures
12. Plecko et al 2014 <sup>6</sup>	Term; no	Former Yugoslavia	3 h; NR	Discontinuous pattern	1 d	Prompt response	17 mg/kg/d	Normal	Normal, seizure free
13. Levtova et al 2015 <sup>10</sup>	33 wk; no	Canada	2 h (possible in utero seizures); myoclonic seizures	Discontinuous pattern	7 d (stopped at 14 d); from 7-12 mo	Delayed response	100 mg x 2 from 7 to 12 mo	Normal	Died at 14 mo for refractory status epilepticus
14. Our patient	37 wk; no (RDS after birth)	Former Yugoslavia	12 h generalized spasm/multifocal clonic seizures	BS	1 d	Prompt response	20 mg/kg	Normal	Normal at 18 mo, seizure free

Abbreviations: BS, burst suppression; EEG, electroencephalograph; MRI, magnetic resonance imaging; NR, not reported; PLP, pyridoxal-5-phosphate; PNPO, pyridoxine phosphate oxidase; RDS, respiratory distress syndrome.

<sup>a</sup>After diagnosis of PNPO deficit, pyridoxine was replaced by PLP 44 mg/kg/d.

<sup>b</sup>Hom.p.R225H→p.R116Q genotype.

<sup>c</sup>Status epilepticus when switched to PLP.

**Table 2.** Comparison Between Pyridoxine Responsive Patients With Different Type of PNPO Mutations.

Type of mutation	References	Number of cases	Burst suppression pattern (n = 9)	Prompt response to pyridoxine (n = 19)	Normal neuroimaging (MRI/US) (n = 15)	Seizure free during pyridoxine therapy (n = 17)	Normal neurologic outcome <sup>a</sup> (n = 8)	Severe neurologic disabilities <sup>b</sup> (n = 5)
c.(674GA) p.(R225H)	Mills et al 2014 <sup>5</sup> ; Plecko et al 2014 <sup>6</sup> ; Guerin et al 2015 <sup>7</sup> ; Ware et al 2014 <sup>9</sup> ; Levtova et al 2015 <sup>10</sup>	14	7/14	9/14	9/14	7/14	5/14	4/14
c.(98A>T) p.(D33V)	Mills et al 2014 <sup>5</sup> ; Guerin et al 2015 <sup>7</sup>	4	NR	3/4	NR	4/4	0/4 <sup>c</sup>	1/4
c.(347G>A) p.(R116Q)	Mills et al 2014 <sup>5</sup> ; Guerin et al 2015 <sup>7</sup>	1	NR	1/1	0/1 <sup>§</sup>	1/1	0/1 <sup>d</sup>	0/1
c.(421C>T) p.(R141C)	Plecko et al 2014 <sup>6</sup> ; Guerin et al 2015 <sup>7</sup>	1	0/1	1/1	1/1	1/1	1/1	0/1
c.(347G>A) p.(R116P)	Di Salvo et al 2017 <sup>11</sup>	3	0/3	3/3	3/3	3/3	1/3	0/3
c.(481C>T) p.(R161C)	Jaeger et al 2016 <sup>12</sup>	1	1/1	1/1	1/1	1/1	1/1	0/1
c(325G>A) p(G118R)	Pearl et al 2013 <sup>4</sup>	1	1/1	1/1	1/1	0/1	0/1	0/1
Total number of tested cases		25	20	25	21	25	25 <sup>e</sup>	25

Abbreviations: MRI, magnetic resonance imaging; NR, not reported; PNPO, pyridoxine phosphate oxidase; US, ultrasonography.

<sup>a</sup>18-24 months of age.

<sup>b</sup>Spastic quadriplegia or severe intellectual deficiency.

<sup>c</sup>2 of 4 mild had developmental delay.

<sup>d</sup>MRI changes consistent with hypoxic ischemic encephalopathy and mild developmental delay.

<sup>e</sup>Other 6 of 25 patients had only mild developmental delay.

The p.R225 H mutation affects the pyridoxal-5-phosphate binding site in a strongly conserved region of the PNPO protein and dramatically reduces the activity of pyridoxine phosphate oxidase enzyme to 8% of residual activity. Expression studies in CHO-K1 cell lines show that transfection with DNA containing the p.R225 H mutation result in undetectable enzyme activity.<sup>5</sup> However, the in vivo observed pyridoxine response suggests some residual enzymatic activity for p.R225 H, presumably sufficient to allow synthesis of pyridoxal-5-phosphate from pyridoxine. Alternatively, pyridoxine could have a chaperone effect and preserve the mutant PNPO protein from premature decay.<sup>5,6</sup> This mutation has been already reported in the literature (Table 1).<sup>5,6,7,9,10</sup>

Ware et al<sup>9</sup> reported a newborn infant with myoclonic jerks, an obvious pyridoxine dependency and normal *ALDH7A1* gene sequencing. A subsequent study of the *PNPO* gene revealed a homozygous missense mutation c(674G>A). In this case, pyridoxine discontinuation with simultaneous administration of increasing amounts of pyridoxal-5-phosphate led to sufficient long-term seizure control, although autism spectrum disorder had been diagnosed.<sup>9</sup>

Mills and Plecko described the same c(674G>A) homozygous missense mutation in 11 children with pyridoxine-responsive seizures who had normal biomarkers for antiquitin deficiency and normal sequencing of the *ALDH7A1* gene.<sup>5,6</sup> The same mutation probably affected 2 siblings who died

before having a genetic diagnosis and before pyridoxine therapy was administered.<sup>6</sup> A delayed response to pyridoxine in the neonatal period was reported by Levtova et al.<sup>10</sup> B6 therapy was initially discontinued and then readministered from age 7 to 12 months. Because of insufficient seizure control, B6 therapy was definitely discontinued. Of note, the child died because of refractory status epilepticus at age 14 months.<sup>10</sup> Therefore, this pyridoxine-responsive *PNPO* mutation cannot be regarded as mild, because children, as reported by Plecko and Levtova, have died because their epileptic encephalopathy was not treated continuously with pyridoxine.<sup>6,10</sup>

Patients with a p(R225 H) *PNPO* mutation had additional clinical features similar to PDE epilepsy, such as fetal distress or respiratory problems.<sup>6</sup> Nevertheless, patients with pyridoxine phosphate oxidase deficiency epilepsy are more likely to be premature; 6 of 14 patients with a p(R225 H) *PNPO* mutation were born before 37 weeks of pregnancy.<sup>3,5</sup> Interestingly, all of the patients were Caucasian, and half of them (as in our case), came from former Yugoslavia, suggesting a founder mutation. The onset of seizure was within the first day of life in the majority of children (Table 1).

This study is one of the few to report a patient with a full EEG recording, whereas in previously described cases, clinical charts report that burst suppression pattern or discontinuous background EEG activity were quite common. Except for patient 1 and 13 reported in Table 1,<sup>9,10</sup> all the remaining

patients were undergoing pyridoxine therapy with some degree of responsiveness at the time of the last evaluation. Seven children were seizure-free during pyridoxine treatment, whereas the remaining patients had occasional seizure relapse. Interestingly, 3 patients experienced status epilepticus<sup>6</sup> or seizure relapse<sup>9</sup> when switched to pyridoxal-5-phosphate. This finding may suggest that the PNPO protein carrying the mutation p.R225 H might be completely inhibited by the administration of exogenous pyridoxal-5-phosphate,<sup>2,13</sup> but further in vitro studies are needed to confirm this hypothesis.<sup>6</sup> Indeed, in the current case, a pyridoxal-5-phosphate trial was not performed because of normal metabolic status and pyridoxine seizure control.

MRI findings of patients reported in table 1 range from normal to white matter hyperintensity, mild brain atrophy, and encephalomalacia. Overall neurodevelopmental outcome was quite poor and similar to the previously reported pyridoxine phosphate oxidase deficiency epilepsy. Only 5 of 14 children had a normal outcome, whereas the others experienced varying degrees of disabilities, including mild intellectual deficiency and spastic quadriplegia. All 3 patients with severe neurologic outcomes started pyridoxine therapy after the first month of life and the female who had died was not under B6 supplementation.<sup>10</sup> Moreover, 4 of the 5 patients with normal neurologic outcomes showed a prompt clinical and EEG response to pyridoxine therapy, similar to the child examined herein.<sup>5-7</sup>

Among all reported cases of genetically confirmed pyridoxine phosphate oxidase deficiency epilepsy, further types of *PNPO* gene mutations displayed clinical responsiveness to pyridoxine treatment. To establish a genotype-phenotype correlation, we compared patients carrying the c(674G>A) p(R225 H) missense mutation of the *PNPO* gene with patients carrying different types of pyridoxine-responsive *PNPO* mutations (Table 2). Within this group, the missense mutation D33 V, which reduces pyridoxine phosphate oxidase activity to 44%, was quite common and generally associated with prompt pyridoxine responsiveness. Another previously reported mutation is R116Q, which decreases pyridoxine phosphate oxidase activity by 20%. This polymorphism might be a common contributor or susceptible allele to epilepsy and is present in tandem with R225 H, R225C, and D33 V.<sup>5</sup> The recently described pyridoxine-responsive *PNPO* mutation c.(347G>A) p(R116P) is associated with a later onset of symptoms and a milder epilepsy phenotype.<sup>11</sup> The missense mutation c(674G>A) p(R225 H) of the *PNPO* gene seems to have a more severe epileptic phenotype than other types of mutations (eg, D33 V, R116Q, R141C mutations), as seizure control was obtained in 7 of 14 total cases, possibly because of a lower residual pyridoxine phosphate oxidase activity.<sup>5,6,7,12</sup> Of interest, the c(325G>A) p(G118 R) mutation shows an initial transient response to pyridoxine, followed by seizure relapse and a better long-term neurologic outcome with pyridoxal-5-phosphate.<sup>4</sup> This case emphasizes the difficulty in choosing whether to use pyridoxine or pyridoxal-5-phosphate. Partial pyridoxine responsiveness may be a clue to the diagnosis, but should not be taken as proof that patients would benefit by using pyridoxine

exclusively instead of pyridoxal-5-phosphate. The critical issue would be to distinguish patients with pyridoxine responsiveness whose outcome may still improve with pyridoxal-5-phosphate, as opposed to patients with pyridoxine responsiveness that actually do worse on pyridoxal-5-phosphate. Even if 3 patients with R225 H mutation experienced status epilepticus or seizure relapse when switched to pyridoxal-5-phosphate, the deterioration on pyridoxal-5-phosphate could not be considered specific to the R225 H group, as in most cases (like ours), a pyridoxal-5-phosphate trial was not performed.

Although uninterrupted pyridoxine therapy in responding to pyridoxine phosphate oxidase deficiency epilepsy could be beneficial, additional therapeutic strategies (including pyridoxal-5-phosphate or both pyridoxal-5-phosphate and pyridoxine) should be considered in the long-term treatment of most cases. Pyridoxine-responsive pyridoxine phosphate oxidase deficiency epilepsy is a challenging and severe disease, with neurodevelopmental disabilities occurring in about a half of cases.<sup>13,15</sup>

In conclusion, this case report has several important implications for clinical practice. First, the detailed electroclinical description of our case with a c(674G>A) p(R225 H) missense mutation of the *PNPO* gene has shown that this type of pyridoxine phosphate oxidase deficiency epilepsy is indistinguishable by PDE epilepsy, both by clinical and EEG findings. Second, patients with relative pyridoxine-responsive epilepsy associated with mutations in the *PNPO* gene might be a prevalent group considering that 25 cases of epilepsy, which is half of the total reported pyridoxine phosphate oxidase deficiency epilepsies, have been reported to date.<sup>7</sup> This evidence shifts the paradigm of exclusive pyridoxal-5-phosphate responsiveness of patients with pyridoxine phosphate oxidase deficiency. Therefore, considering the high preponderance of birth asphyxia in antiquitin as well as in pyridoxine phosphate oxidase deficiency, a standardized trial with pyridoxine should be performed routinely in all neonates with therapy resistant seizures. Pyridoxal-5-phosphate should be added in case of pyridoxine-unresponsive or partially responsive seizures to facilitate a pyridoxal-5-phosphate synergistic effect occurring in some cases of pyridoxine phosphate oxidase deficiency.<sup>4</sup> Finally, we recommend to test patients for both *ALDH7A1* and *PNPO* mutations when pyridoxine-responsive seizures are suspected. Next-generation sequencing panels for epilepsy, including pyridoxine metabolism genes, can greatly facilitate the diagnostic approach to neonatal epilepsy.

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### Author Contributions

LLug and MCB wrote and prepared the manuscript. LO took care of EEG recording and analysis. LLuc and FF was responsible for clinical

management and follow-up. AB and FF critically reviewed the manuscript.


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### Patient Consent

The investigations were done on a clinical basis. Consent was obtained from the family for the publication of this patient's history.

### Ethical Approval

This case is included the study 67.13, approved by the local ethics committee (approval date 10th of May 2013). This research was conducted according to the World Medical Association Declaration of Helsinki.

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