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Complexities of pyridoxine response in PNPO deficiency

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ABSTRACT

Pyridox(am)ine- 5- phosphate Oxidase deficiency (PNPO) is a rare cause of neonatal metabolic encephalopathy associated with refractory status epilepticus. We report a case of a premature neonate presenting with drug-resistant seizures beginning at 2 hours of life. The baby showed initial transient response to pyridoxine followed by recurrence. Genetic report confirmed the diagnosis of PNPO deficiency. A literature review on phenotypic variants in terms of response to pyridoxine is also presented along with a proposed algorithm to manage a case of suspected vitamin responsive epilepsy. This case highlights our limited understanding of why variation in response to treatment exists in children with PNPO deficiency.

Pyridox (am)ine- 5- phosphate oxidase (PNPO) deficiency is a vitamin-responsive epilepsy syndrome that poses significant challenges in management. Traditionally, pyridoxal phosphate (PLP) was considered to be the treatment of choice; however, a considerable proportion of cases respond to pyridoxine (PN) [1]. Even in these infants whose seizures respond to pyridoxine, the effect is variable and unpredictable [2,3]. The genotype and phenotype correlation relative to a ssuccessful response to treatment is poor. Here, we describe a preterm infant with refractory status epilepticus who showed a transient response to pyridoxine therapy but no effect on addition of PLP. The infant was later diagnosed to have a novel pathogenic mutation in the PNPO gene. This case highlights our limited understanding of why variation in response to treatment exists in children with PNPO deficiency.

Case report

A 31-week preterm 1500 g male newborn was born to non-consanguineous parents by caesarean section due to maternal pre-eclampsia and fetal distress. Poor respiratory efforts with severe hypotonia were observed at birth with Apgar scores of 5 at 1 minute, and 7 at 5 minutes. The cord blood gas analysis was suggestive of mixed acidosis with high lactate levels (5.1 mmol/L). Surfactant was administered immediately after delivery. At 2 hours of life, he developed multifocal myoclonic jerks, eyelid twitching and facial grimacing with ictal crying. The EEG showed a burst suppression pattern; the generalized spike and sharp wave paroxysms correlating with myoclonic jerks (Fig. 1A). His seizures were resistant to phenobarbital and levetiracetam. Intravenous pyridoxine (100 mg) was given (4th hour of life), resulting in cessation of seizures. Repeat EEG after seizure cessation showed generalized suppression with no epileptiform discharges (Fig. 1B).

Investigations revealed normal MRI and metabolic profile (including CSF lactate, glycine, sugar, blood ammonia, lactate, acylcarnitine profile, and urine organic acids). Blood sample was sent for whole exome sequencing. Similar seizures however, recurred on the fourth day of life after 36 hours of seizure freedom. This prompted us to start trials of other vitamins namely PLP (30 mg/kg/day), biotin (10 mg/day), and folinic acid (3 mg/kg/day) together along with continuing pyridoxine. As no benefit was noted, the vitamins were stopped on day 12 of life. His seizures remained drug-resistant despite the use of multiple anti-seizure

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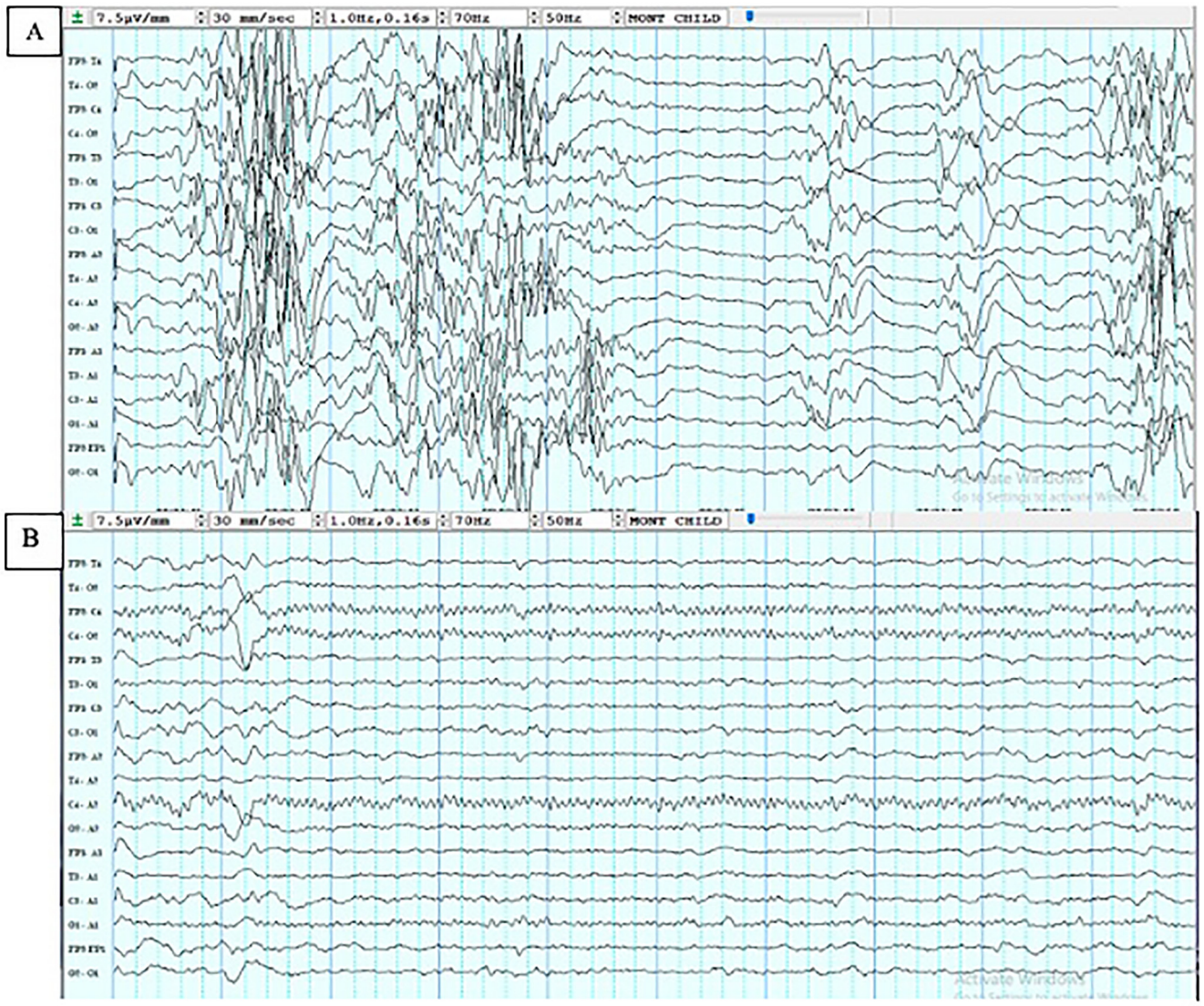

Fig. 1. (A) Electroencephalographic (EEG) sample on day 1 (3rd hour of life) shows a burst of rhythmic spike and sharp waves with background attenuation (B) EEG sample day 2 on (30 hours later than 1A) shows continuous low amplitude background with the absence of epileptiform discharges.

medications including phenobarbital, levetiracetam, clobazam and topiramate. He was discharged upon parental request on day 25. At discharge he was severely encephalopathic and required nasogastric feeds. He continued to have numerous daily seizures at home.

The exome sequence analysis identified a homozygous missense variation in exon 5 of the *PNPO* gene (c.482 g > A) that results in the amino acid substitution of Histidine for Arginine at codon 161 (p.Arg161His) on day 42. The parents did not consent for further testing. Oral PN (30 mg/kg/day) was then restarted on an outpatient basis considering PN responsive PNPO deficiency disorder. Significant seizure control was noted after seven days, but encephalopathy was still persistent. Seizures soon recurred even on therapy, and the child finally expired at 56 days of life during sleep. There was no peri-mortem fever, fast breathing, or history suggestive of aspiration. A verbal autopsy could not elicit a definite cause of death.

Discussion

PNPO deficiency is a rare neurometabolic disorder with less than 100 genetically proven cases reported in literature [1]. Clinical markers include a history of infertility, miscarriages, and excessive fetal movements, and preterm birth. Many affected infants require resuscitation at birth and are profoundly encephalopathic [1,2]. Seizures develop within hours of birth in most babies (66% on the first day; 83% within the first week) [2]. Typical seizure semiology includes multifocal myoclonia, abnormal eye movements, facial grimacing, inconsolable cry, generalized tonic, epileptic spasms [3]. Our patient had a history of excessive fetal movements, prematurity, encephalopathy at birth, and drug-resistant epilepsy, with typical seizure types commencing from the second hour of life. The clinical differentials considered were hypoxic ischemic encephalopathy, structural brain malformations, or inherited metabolic disorders like biotinidase deficiency, non-ketotic hyperglycinemia, and vitamin responsive epilepsies. The detailed workup ruled out most of the structural and metabolic etiologies. The final diagnosis was obtained by genetic analysis.

PNPO plays an important role in pyridoxine metabolism pathway. Dietary pyridoxine and pyridoxamine is converted to its active form pyridoxal-5-phosphate (PLP) by this enzyme. Theoretically, PLP should be the sole treatment option for PNPO, but it responds in a substantial number of cases (44%) only to PN [1]. Response to therapy with PN is subject to the presence of residual functional enzyme activity which enables its conversion to PLP.

Other possible mechanism of PN response is by virtue of its chaperone effect inhibiting the premature damage of PNPO enzyme [4].

Pyridoxine response is guided by certain specific mutations, notably R225H, D33V, R116Q/P as reported earlier. It involves replacement of highly sustained arginine residues as reported in pyridoxine sensitive cases [3,5]. The current case also consists of replacing the arginine residue by histidine due to a novel mutation (c.482G > A, p.Arg161His), thus adding to existing literature. Given that cases with similar mutations are known to have the variable therapeutic response from complete to partial, other environmental parameters like prematurity, age at therapeutic trial, riboflavin status, and pyridoxine levels in mothers might be additionally contributory [4]. Since, PNPO is flavin mono nucleotide(FMN) dependent enzyme, addition of riboflavin may also be of benefit. [10].

Therapeutic variants of PNPO deficiency may be classified as: prompt responders to PN, late responders to PN, partial responders to PN, worsening variant on the addition of PLP to PN, vitamin combination responders [3–10] (Table 1). These variants of pyridoxine responsiveness make the treatment complex and unpredictable. Our patient was treated twice with pyridoxine. The response was prompt but short-lasting on both occasions. Partial responsiveness to pyridoxine in PNPO deficiency is well known [6,7]. PLP was added in our case on day 4 after seizure recurrence which demonstrated no benefit and hence stopped after 7 days. Since, inconsistency in therapeutic response is often observed in

Table 1
Variants of Pyridoxine responsive PNPO deficient cases.

Category	Genetic mutation	Reference	Treatment given	Outcome	Proposed hypothesis
Prompt responders (within two weeks) [3,4,5,8] (n = 9)	c.98A > T, p.D33V c.347G > A, p.R116Q c.674G > A, p.R225 H	Mills[4] et al 2014 (n = 3)	Initial dose 18–55 mg/kg/day Maintenance 6–26 mg/kg/day	Mild developmental delay (3)	-Partial residual PNPO enzyme activity -Chaperone effect of PN on PNPO preventing its damage
	c.674G > A, p.R225 H	Plecko[8] et al 2014 (n = 4)	PN (17–50 mg/kg/day] along with anti-seizure medications	Normal development (3) Spastic paraparesis (1)	
	c.421C > T, p.R141				
	c.481C > T; p.R161C c.674G > A, p.R225 H	Jaeger[5] et al 2016 (n = 1)	Initial 100 mg stat followed by 15 mg/kg/day single dose	Normal development (2)	
		Lugli[3] et al 2019 (n = 1)	Initial 100 mg IV B6 followed by 20 mg/kg/day		
Late responders (two weeks upto 6 months)[3,5,9] (n = 8)	c.674G > A, p.R225 Hc.674G > A, p.R225 H	Plecko[8] et al* 2014 (n = 2)	Gradual response to B6; Status epilepticus within 12 hours of switch to PLP.	Normal development, gait instability (1) Global delay (1) mild GDD	No clear mechanism proposed
	c.(98A > T) p.(D33V)	Mills[4] et al 2014 (n = 5)	Time taken to seizure control 6 months	Spastic quadriplegia (1) Mild delay (2) Severe delay (1) Asperger syndrome (1)	
	c.674G > A, p.R225H	Levtova[9] et al 2015	Brief treatment at D7 and between 7 month-12 month (100 mg BD)	Seizure-free during the period on pyridoxine. Died at 14 month due to refractory status epilepticus	
Partial responders[6,7] (n = 2)	c.352G > A p.G118R,	Pearl[6] et al 2012	Complete seizure control with PN for 6 weeks followed by breakthrough seizure which responded to PLP	Seizure-free, mild developmental delay	Leaky mutation; Partial residual PNPO enzyme activity
	c.674G > A, p.R225H;	Ware[7] et al 2014	Prompt response in the neonatal period. Partial responsive to PN until 7 years with occasional seizures. Worsening of seizures with the sudden withdrawal of B6 along with initiation of PLP @44 mg/kg/day	Seizure-free, Autism spectrum disorder	
	c.482G > A, p.R161H	Current case	Transient response from day1-3; stopped at D12 due to non-response. Restart at day 45, seizure control over 7 days	Died at 56 days of life due to refractory status epilepticus	
Paradoxical worsening[4,8] (n = 5)	Not specified	Mills[4] et al 2014 (3/8)	Response to PN was present but symptoms deteriorated with a change to PLP	Individual details not available	-High dose of PLP may cause seizures-Impaired inhibition of PNPO by PLP thus increasing the risk of toxic levels of PLP - Build-up of Pyridoxamine phosphate that may have an adverse effect
	c(674G > A) p(R225 H)	Plecko[8] et al 2014* (2)	Gradual response to PN; Status epilepticus within 12 hours of switch to PLP. Pyridoxine (150–200 mg) at age 8–9 years	GDD, occasional seizures	
Combination vitamins[4,10] (n = 2)	D33V + R225C + R116Q	Mills[4] et al 2014 (1)	Time taken to control 3.5 months. Seizure control by adding a multivitamin to pyridoxine	Dyslexia/Aspergers	-Riboflavin (FMN) act as a cofactor to PNPO hence aids in the synthesis of PLP
	c.352G N A p.Gly118R	Mohanlal[10] et al 2020 (1)	B6 + Riboflavin + Thiamine + 4 ASM	Developmental delay/ spastic diplegia	

GDD: Global developmental delay, PLP: Pyridoxal 5 phosphate, PN: Pyridoxine.
*Same cases had a late response and paradoxical worsening.

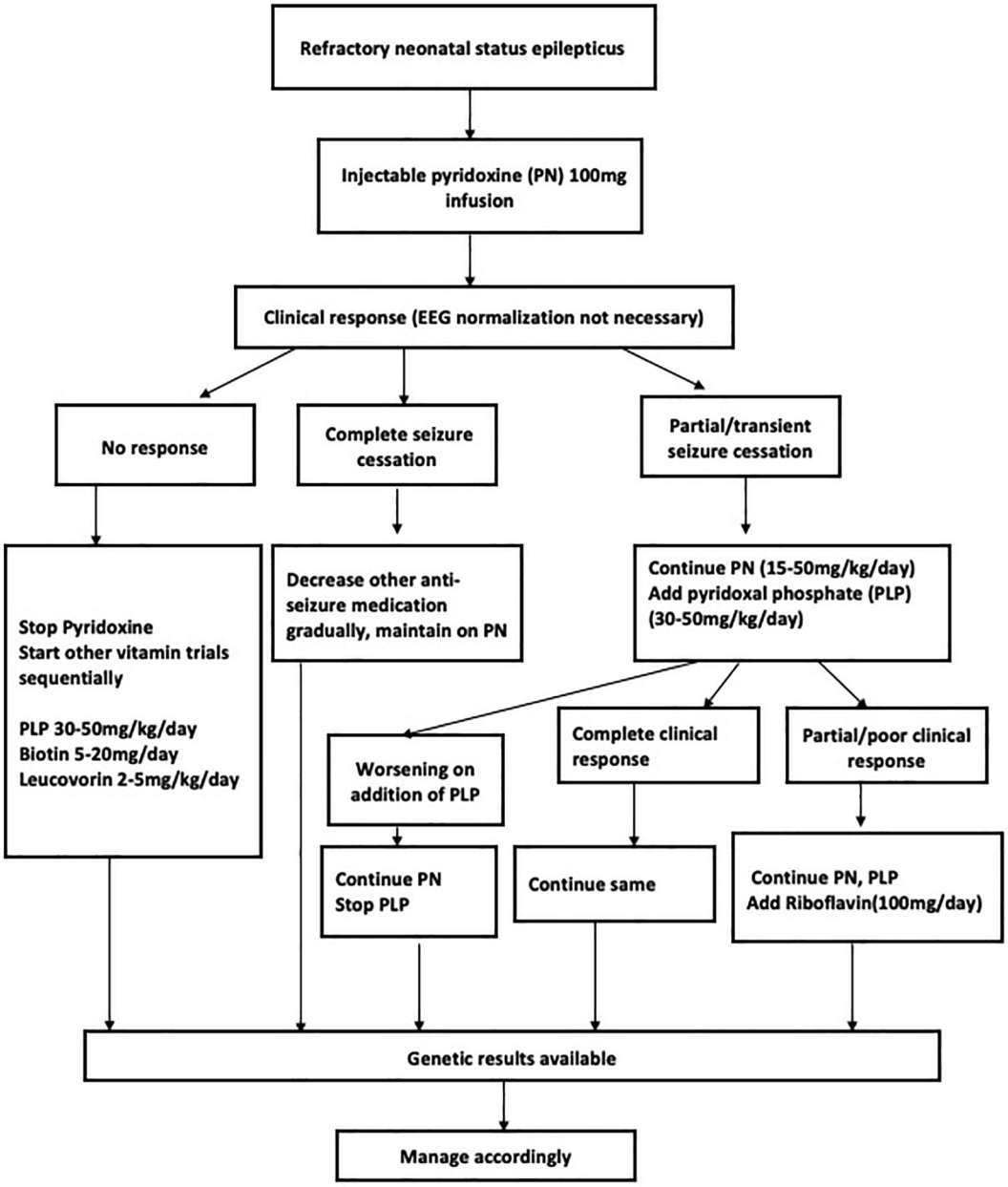

Fig. 2. Flow chart for management of refractory neonatal status epilepticus with suspected vitamin responsive epilepsy.

this disorder, it is advisable to continue the empirical vitamin treatments (PN, PLP) if any clinical response is observed till the genetic results are available [2]. We propose an algorithm for such situations (Fig. 2).

Our patient succumbed fourteen days after final genetic diagnosis of PNPO deficiency even after restarting PN. The specific cause of death was not clearly identified, but he was a fragile, malnourished (weight at discharge was 1375 grams), encephalopathic infant and was hence at risk of sepsis, hypoglycemia, hypothermia and aspiration. Sudden unexpected death in epilepsy (SUDEP) could also be a possibility. However, this cannot be diagnosed in the absence of an autopsy and additional information. Prolonging the PLP treatment along with pyridoxine after day 12 and the addition of riboflavin might have made the difference in outcome.

Though, it is considered to be a treatable disorder, the neurodevelopmental outcome is not always favourable despite seizure control. In a large series of 87 patients with PNPO deficiency, the authors reported mortality in 25%, developmental delay in 50 %, and normal development in the rest of the patients. Prematurity, early-onset seizures, and delay in treatment initiation have been associated with a bad prognosis [1].

In conclusion, our case and the accompanying review highlights the complexity of treatment with pyridoxine and PLP in conveying a favorable response in infants with PNPO deficiency. Vitamin treatment should continue until the results of molecular genetic testing are available. The need for early and continued treatment is crucial for overall survival as well as neurodevelopmental outcome in potentially treatable cases of neonatal metabolic encephalopathy.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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