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Short Communication

Pyridoxine responsive epilepsy caused by a novel homozygous PNPO mutation☆

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ABSTRACT

We report a patient with anti-epileptic treatment refractory neonatal seizures responsive to pyridoxine. Biochemical analysis revealed normal markers for antiquitin deficiency and also mutation analysis of the *ALDH7A1* (*Antiquitin*) gene was negative. Mutation analysis of the *PNPO* gene revealed a novel, homozygous, presumed pathogenic mutation (c.481C>T; p.(Arg161Cys)). Measurements of B_6 vitamers in a CSF sample after pyridoxine administration revealed elevated pyridoxamine as the only metabolic marker for PNPO deficiency. With pyridoxine monotherapy the patient is seizure free and neurodevelopmental outcome at the age of 14 months is normal.

1. Introduction

Pyridoxal-phosphate dependent epilepsy (PLP-DE) is caused by mutations in the *PNPO* gene leading to a deficiency of pyridox(am)ine-5′-phosphate oxidase (PNPO), which converts pyridoxine-5′-phosphate and pyridoxamine-5′-phosphate into pyridoxal-5′-phosphate (PLP), the active form of vitamin B6 (Fig. 1).

The majority of patients with classical pyridoxine dependent epilepsy (PDE) have mutations in the *ALDH7A1* (Antiquitin) gene encoding alpha-aminoadipic semialdehyde (α-AASA) dehydrogenase [1]. Deficiency of α-AASA dehydrogenase results in accelerated loss of PLP and accumulation of metabolites of the lysine degradation pathway, i.e. α-AASA, Δ^1-piperideine-6-carboxylate and pipecolic acid [2].

Patients with PNPO deficiency and antiquitin deficiency present with neonatal or early-infantile onset drug-resistant seizures [3–5].

Previously it was thought that patients with PNPO deficiency respond solely to PLP and patients with antiquitin deficiency respond to both pyridoxine and PLP. However, recently patients with PNPO deficiency and pyridoxine responsiveness have been reported [6–8].

We report a neonate with a novel, homozygous and presumed pathogenic mutation in the *PNPO* gene with pyridoxine responsive seizures. Biomarkers for antiquitin deficiency and sequencing of the *ALDH7A1* gene were normal.

2. Case report

A boy was born after an uneventful pregnancy and delivery at 37 4/7 weeks' gestation to consanguineous parents of Pakistani ethnicity. Pregnancy occurred after previous miscarriages. There was no history of excessive intrauterine movements. Family history was negative for epilepsy.

One day after birth he developed focal seizures responsive to phenobarbital (20 mg/kg). On day 3 he developed generalized tonic–clonic seizures with apnea. EEG revealed a burst suppression pattern and centrally located rhythmic sharp and slow waves, consistent with status epilepticus. Initiation of bursts corresponded with an apneic event.

Therapeutic doses of phenobarbital, midazolam and lidocaine did not resolve seizure activity. Full septic workup was negative, cranial MRI was normal.

Abbreviations: CSF, cerebrospinal fluid; PDE, (classic) pyridoxine dependent epilepsy; PLP, pyridoxal-5′-phosphate; PLP-DE, pyridoxal-phosphate dependent epilepsy; PNPO, pyridox(am)ine-5′-phosphate oxidase; α-AASA, alpha-aminoadipic semialdehyde dehydrogenase.

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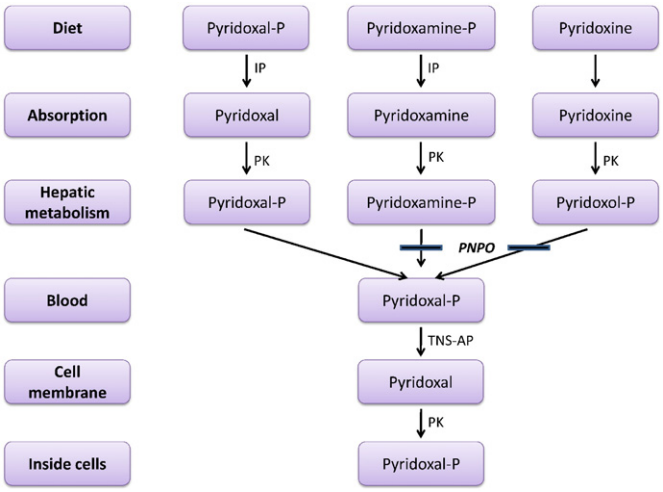

Fig. 1. Conversion of dietary vitamin B6 to intracellular pyridoxal 5′-phosphate cofactor. IP: intestinal phosphatases; P: 5′-phosphate; PK: pyridoxal kinase; PNPO: pyridox(am)ine phosphate oxidase; TNS AP: tissue nonspecific alkaline phosphatase. A solid bar indicates an enzyme block [22].

A pyridoxine trial (100 mg/iv, in one dose) on day 4 did not lead to immediate improvement of the EEG. Pending the results of biochemical analysis, pyridoxine was continued in a single dose of 15 mg/kg/day. Other anticonvulsants were tapered off. EEG a day after pyridoxine challenge showed a continuous background activity. Within days his clinical condition improved and seizures disappeared.

Biochemical testing had to be performed after pyridoxine administration because no samples were taken before treatment. Levels of pipecolic acid in cerebrospinal fluid (CSF) and plasma, as well as α-AASA in urine were normal (Table 1).

As biomarkers for antiquitin deficiency were found normal, pyridoxine was withdrawn. At this point the patient was in good clinical condition. Forty-eight hours after withdrawal he developed status epilepticus, refractory to a combination of anti-epileptic drugs but resolving after reinitiating pyridoxine.

Meanwhile, sequencing of the *ALDH7A1* gene was performed, which did not identify any mutations.

Subsequently, DNA sequence analysis of the *PNPO* gene was carried out and revealed a novel, homozygous missense mutation (c.481C>T; p.(Arg161Cys)). Both parents were found to be carriers of the mutation, confirming homozygosity in the patient. The mutation was predicted to be pathogenic, based on the conservation of the amino acid and the in silico analysis.

In order to look for functional confirmation of PNPO deficiency, CSF and plasma amino acid profiles were analyzed, showing no significant abnormalities, apart from a slightly decreased homocarnosine in CSF. Also neurotransmitter analysis revealed a normal pattern of the biogenic amine metabolites.

However, measurement of B_6 vitamers in the CSF sample revealed a strongly increased concentration of pyridoxamine. Pyridoxamine was also measured in CSF samples of two patients with antiquitin deficiency during pyridoxine treatment for comparison. In these samples only modest elevations were found (Table 1).

Apart from a breakthrough seizure during a viral infection with fever the patient is seizure-free with pyridoxine monotherapy (15 mg/kg/day; once daily). Neurodevelopmental outcome at the age of 14 months is normal.

Table 1
Biochemical profile of the patient and of two patients with antiquitin deficiency during pyridoxine treatment for comparison. α-AASA: alpha-aminoadipic semialdehyde dehydrogenase; 5-HIAA: 5-hydroxyindole-3-acetic acid; HVA: homovanillic acid; MHPG: 3-methoxy-4-hydroxyphenylglycol.

	Patient	Antiquitin patient 1	Antiquitin patient 2	Reference value
Urine (mmol/mol kreat)				
AASA	0.5			0.0–2.0
CSF (nmol/l)				
Pipecolic acid	0.07			0.00–0.10
Pyridoxal 5′-phosphate	21	178	44	11–46
Pyridoxal	297	11,107	3667	5–106
Pyridoxine	13,000	1324	14,101	5–11
Pyridoxamine	5100	64	13	n.d.
Amino acids (μmol/l)				
Glycine	8.2			3.0–8.3
Threonine	52.6			15.0–130.0
Homocarnosine	4.3			5.5–12.0
Neurotransmitters (nmol/l)				
HVA	1091			445–2228
5-HIAA	729			593–1653
MHPG	104			85–306
3-O-methylDOPA	299			108–506
Plasma				
Pipecolic acid, μmol/l	1.0			0.1–7.0
Lactate, mmol/l	0.9			0.0–2.3
Pyruvate, mmol/l	0.06			0.00–0.13

3. Discussion

We describe a neonate with anti-epileptic drug treatment refractory seizures responsive to pyridoxine. Antiquitin biomarkers and *ALDH7A1* sequencing were normal. The homozygous mutation found in the *PNPO* gene, has to our knowledge not been reported in other patients with neonatal-onset PDE. However, other pathogenic missense mutations, often involving an arginine, have been reported in PNPO deficiency [7].

Mutations in the *PNPO* gene lead to absent or reduced PNPO activity resulting in low levels of PLP. Administration of PLP controls seizures in the majority of patients [9].

Patients with (partial) PNPO deficiency and a transient or complete response to pyridoxine instead of PLP have been described before [7, 10, 11]. Remarkably some of these patients developed even worsening of seizure control when given PLP [6]. The majority of these pyridoxine responsive PNPO deficient patients have early onset therapy resistant seizures. Additional reported features include prematurity, low Apgar scores, irritability, respiratory distress and a parental history of infertility [7].

Plecko et al. described 11 patients with homozygous or compound heterozygous, novel PNPO mutations in a group of 31 patients with pyridoxine-responsive seizures. These patients had normal biomarkers for antiquitin deficiency and normal sequencing of the *ALDH7A1* gene.

Patients with antiquitin deficiency often show an immediate response to pyridoxine administration and frequently need anti-epileptic co-medication to control seizures [5]. Our patient had a delayed pyridoxine response and is seizure free on pyridoxine monotherapy. Forty-four percent of the patients described by Plecko et al. also showed an immediate pyridoxine response and 66% of patients were seizure free with pyridoxine monotherapy [6]. The rapid recurrence of seizures after withdrawal of pyridoxine described here is highly unusual in antiquitin deficiency and was also observed in one of the patients with PNPO deficiency responsive to pyridoxine described by Plecko [5, 12].

Biochemical analysis in PNPO deficiency may reveal a reduction of PLP and pyridoxal [9, 13]. Most likely due to earlier administration of pyridoxine, we found normal values in CSF. Aromatic amino acid decarboxylase, threonine dehydratase and glycine cleavage system enzymes are PLP-dependent enzymes. Therefore secondary amino acid deficiencies in CSF like elevated glycine and threonine can be expected, but were absent in our patient. Only homocarnosine was slightly decreased,