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CASE REPORT

PNPO Deficiency and Cirrhosis: Expanding the Clinical Phenotype?

D. Coman · P. Lewindon · P. Clayton · K. Riney

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Abstract We report the case of a 4-year-old boy with pyridoxamine 5-phosphate oxidase deficiency, now the second reported case to develop hepatic cirrhosis. He presented with an encephalopathy in the first 1.5 h of life and received a first dose of PLP at 40 h of life. *PNPO* gene sequencing identified homozygosity for a novel variant in exon 7, c.637C>T (p.Pro213Ser). Persistent elevations in alanine transferase and aspartate transferase combined with an echogenic liver on ultrasound prompted performance of a liver biopsy which demonstrated hepatic cirrhosis. This is the second reported case of hepatic cirrhosis in PNPO deficiency. The pathogenesis is unclear but may be related to epigenetic activation of purinergic signaling in the hepatic stellate cells. PNPO deficiency may in time prove to be a suitable candidate for consideration of therapeutic orthotropic liver transplantation in select patients.

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D. Coman (✉) · K. Riney
Neuroscience Department, The Lady Cilento Children's Hospital, Brisbane, QLD, Australia
e-mail: david.coman@health.qld.gov.au

D. Coman · P. Lewindon · K. Riney
School of Medicine, The University of Queensland, Brisbane, QLD, Australia

D. Coman
School of Medicine, Griffith University, Gold Coast, QLD, Australia

P. Lewindon
Gastroenterology Department, The Lady Cilento Children's Hospital, Brisbane, QLD, Australia

P. Clayton
UCL Institute of Child Health, London, UK

Introduction

Pyridoxamine 5-phosphate oxidase (PNPO) deficiency (OMIM 610090) is a rare autosomal recessive inborn error of metabolism (IEM). To date there have been less than 50 cases reported in the medical literature (Veerapandiyan et al. 2011; Mills et al. 2005, 2014; Hoffmann et al. 2007; Clayton 2006; Bagci et al. 2008; Ruiz et al. 2008; Schmitt et al. 2010; Ware et al. 2014; Porri et al. 2014). The initial reported clinical phenotype of PNPO deficiency included prematurity, early-onset neonatal encephalopathy, and seizures resistant to conventional anticonvulsants and pyridoxine. Those who have survived the neonatal period have had significant neurodevelopmental disorders in the form of ongoing seizures, developmental delay, and microcephaly (Mills et al. 2005; Hoffmann et al. 2007; Clayton 2006; Bagci et al. 2008; Ruiz et al. 2008; Schmitt et al. 2010). As is often the case with rare diseases, the clinical phenotype broadens as more cases are diagnosed with time, as demonstrated in recent reports of PNPO deficiency patients whose seizures respond to pyridoxine and who have seizure onset as late as 5 months of age (Mills et al. 2014; Plecko et al. 2014). We now report the second case of a patient with PNPO deficiency in whom hepatic cirrhosis has been identified in early childhood.

Case Report

Our patient is currently 4 years of age and was briefly been reported in the literature (Mills et al. 2014). He was delivered at term via emergency lower uterine segment cesarean section under general anesthetic after a failed induction of labor for preeclampsia at 37 weeks' gestation. There was no documented fetal distress. He was cyanosed

and bradycardic at birth but responded well to initial bag and mask ventilation and had APGAR scores of 5, 8, and 8 (at 1, 5, and 10 min). Birth weight was 3.39 kg, length 50 cm, and head circumference 34 cm.

At 1.5 h of age, he was noted to be encephalopathic and in status epilepticus. He required intubation and ventilation. Loading doses of phenobarbitone, phenytoin, and midazolam did not result in seizure control. The EEG showed a burst suppression pattern and multifocal sharp wave activity. Intravenous pyridoxine 100 mg was administered during this EEG recording (at 40 h of age) with no effect on EEG or seizure activity. A trial of oral pyridoxal 5′-phosphate (PLP) 100 mg TDS (30 mg/kg/day) was commenced at 40 h of age. In the 12 h following PLP administration, the EEG showed ongoing ictal events, the majority manifesting clinically as abnormal eye movements and multifocal, erratic myoclonic jerks. EEG improvement was seen by 3 days of treatment with PLP; seizures were controlled and neurological examination was normalized.

Withdrawal of PLP was attempted at 4 months of age. On reducing the dose to 50 mg once daily, seizure activity returned. The *PNPO* gene (OMIM 603287) was sequenced utilizing automated florescence sequencing methods and showed homozygosity for a novel variant in exon 7, c.637C>T (p.Pro213Ser). Both parents were also heterozygous for this change. Software analysis predicted this to be a pathogenic alteration in the *PNPO* gene (http://sift.jcvi.org, http://genetics.bwh.harvard.edu/pph/).

At 2 years of age, the PLP dose required to prevent seizures was 50mg/kg/day divided into four doses and given at carefully managed, specific intervals (200 mg at 05:00 h, 125 mg at 11:45 h, 125 mg at 16:00 h, and 250 mg at 19:15 h). The PLP has been prepared in a consistent fashion; 50 mg capsules have been opened to release the powder within which was then dissolved in apple juice, with immediate administration. However, seizures that have included apnea continued to be observed during dosing interruption, e.g., vomiting illnesses, or even with delayed dose delivery during "steady state." On follow-up at 4 years of age, this patient is developing well, with a normal neurological examination.

Prior to the performance of a percutaneous liver biopsy, a FibroScan liver stiffness measure was performed returning an abnormal result of 8.5 Kpa. Persistently elevated serum transaminases were noted from 2 years of age. Serial measurements demonstrated alanine transferase (ALT: 205, 132, 89 U/L, reference range 5–20) and aspartate transferase (AST: 47, 75, 112 U/L, reference range <48). Alkaline phosphatase, gamma-GT, and bilirubin levels were always normal. Indices of hepatic synthetic function remained normal, including blood glucose, albumin, and coagulation studies. An abdominal ultrasound at 3 years of age demonstrated a mildly enlarged liver and spleen and mild increase in liver parenchymal echogenicity.

Percutaneous liver biopsy demonstrated early cirrhosis with disturbed hepatic architecture, portal–central linking fibrosis, early nodule formation, and marked sinusoidal fibrosis. There was no evidence of inflammation or steatosis. Occasional hepatocytes demonstrated an induced (i.e., ground glass) appearance. There was no stainable iron, alpha1-antitrypsin globules, copper-associated protein, or hepatitis B surface antigen.

Discussion

PNPO deficiency is a rare disorder with less than 50 reported cases in the literature. Common clinical features in reported cases thus far include (1) neonatal encephalopathy with seizures resistant to multiple anticonvulsants (Mills et al. 2005; Schmitt et al. 2010), (2) burst suppression EEG pattern (Veerapandiyan et al. 2011), (3) nonresponsiveness to pyridoxine (Clayton 2006), (4) complete or partial responsiveness to PLP (Pearl et al. 2013), (5) prematurity (Veerapandiyan et al. 2011), and (6) neonatal lethality if the diagnosis was not suspected and P5P administered (Khayat et al. 2008). Recently three groups of PNPO deficiency clinical phenotypes have been postulated: (1) early-onset neonatal seizures responsive to PLP, (2) infantile spasms in infancy, and (3) seizures beginning in the first three months of life responsive to pyridoxine (Mills et al. 2014). The early diagnosis and treatment with PLP has been linked with improved neurodevelopmental outcomes (Hoffmann et al. 2007).

Recently an 8-year-old Australian patient with PNPO deficiency was described with hepatic cirrhosis and early portal hypertension (Sudarsanam et al. 2014). He was initially managed with 100 mg/kg/day of PLP. At 2 years of age, similar hepatic derangement was observed to that seen in our patient. AST and ALT in this patient decreased numerically with PLP dose reduction to 50–60 mg/kg/day. Hepatic levels of B6 vitamers, pyridoxal and pyridoxic acid, were 40 times greater than two control samples. The authors postulated that cirrhosis might have developed secondary to high doses of PLP (~100 mg/kg/day) or chemical instability of PLP when delivered in the aqueous form. The parents of our patient reported that exposure of the PLP to light and sunlight led to discoloration of the medication and that they had learned to administer it immediately after it was prepared. Mild elevations in AST and ALT have been reported in another patient with PNPO deficiency being treated with 50 mg/kg/day of PLP, although that patient has not been reported to have undergone a liver biopsy (Porri et al. 2014).

Liver cirrhosis is an end point of multiple potential insults such as infection, toxin ingestion, drug-induced liver injury, autoimmune inflammation, and IEM. Despite the heterogeneity in potential triggers and potentiators, the molecular pathways that lead to fibrosis remain constant. The hepatic stellate cell (HSC) is the main fibrogenic cell type which orchestrates the deposition of extracellular matrix material (ECM) in the liver (Friedman 2000). HSCs are located in the perisinusoidal cells in the subendothelial space between hepatocytes and sinusoidal endothelial cells (Blomhoff and Wake 1991). After a fibrogenic stimulus, HSCs are activated into a myofibroblast-like state which upregulates the secretion of collagen, fibronectin, and extracellular matrix proteins (Dranoff et al. 2007).

The process of HSC activation has been demonstrated to be under epigenetic control including aberrant DNA methylation, noncoding RNA expression, and histone posttranslational modification (Yao and Li 2015; Bian et al. 2013; Mann 2014). Collagen and procollagen gene expression can be regulated by a number of signal pathways. The dominant stimulus occurs via the cytokine, transforming growth factor beta (TGF-B) (Friedman 2000). Other signaling pathways leading to fibrosis include Toll-like 4 receptor (Wnt, Ying Ying), peroxisome proliferator-activated receptor gamma, and purinergic receptors (Yao and Li 2015). Upregulation of the purinergic receptors P2Y on the HSC has been linked with collagen production, suggesting these receptors may be appropriate targets for antifibrotic agents (Dranoff et al. 2004, 2007). Of interest, pyridoxal-phosphate-6-azophenyl-2',4'-disulfonate (PPADS) is a synthetic inhibitor of P2 receptors (Dranoff et al. 2007). In vitro studies of PPADS demonstrated their ability to reduce HSC proliferation and fibrogenesis. PPADS analogues have been proven to act on P2 receptors in both antagonist and agonist roles, for example, PLP and pyridoxine-alpha 4,5-monophosphate, respectively (Brown et al. 2001). Aberrant purinoceptor activation of HSC due to PLP metabolites or degradation products may be a potential cause of hepatic fibrosis in PNPO deficiency.

Our case is now the second patient with PNPO deficiency in whom hepatic cirrhosis has been demonstrated. Our patient has never received "high dose" PLP, rather a dosage range of 30–50 mg/kg/day. These two cases highlight the broadening phenotype of PNPO deficiency and the occurrence of significant liver disease, cirrhosis, in early childhood. Surveillance for evidence of cirrhosis should be part of the clinical management of these patients. However, the most suitable form of monitoring is unclear. Cystic fibrosis liver disease (CFLD) is a common cause of morbidity and mortality in children with cystic fibrosis. Clinical modalities employed to monitor for CFLD, such as hepatic ultrasound and biochemical indices, are not predictive for their risk of clinically significant hepatic fibrosis (Lewindon et al. 2011). Clinically significant CFLD can exist in the presence of normal biochemical parameters, AST, ALT, and ultrasound. The gold standard test for identifying hepatic fibrosis is a liver biopsy but is not without clinical risk (Lewindon et al. 2011). To date only two PNPO deficiency patients have undergone a liver biopsy, so the true incidence of hepatic fibrosis is unknown in this cohort; however, reasonable hepatic cirrhosis surveillance in the PNPO deficiency cohort may be yearly ultrasound examinations and liver function testing.

These two cases of cirrhosis have led to consideration of the role of orthotopic liver transplantation (OLT) in the future management in our patient, but also as a potential treatment alternative for the IEM, as is the case for numerous IEM. The urea cycle defects and organic acidurias are the most suitable for OLT (Mazariegos et al. 2014) in carefully selected patients. OLT can generate a metabolic level of protection from the morbidity and mortality associated with a metabolic decompensation. Increasing reports of semi-elective OLT have emerged for "treatable IEM" such as glycogen storage disease type 1a where the disease burden associated with the nutritional management has a major impact on the quality of life (Boers et al. 2014). Crigler-Najjar disease is a non-life-threatening defect in bilirubin conjugation in which OLT is now an established option to improve the quality of life, reducing the requirement for phototherapy that is typically required for >16 h/day (Tu et al. 2012). PLP is a cofactor for numerous enzymes associated with neurotransmitter function, i.e., aromatic amino acid decarboxylase (synthesis of dopamine and 5-hydroxytryptamine), branched chain amino acid 2-oxoglutarate aminotransferase (synthesis of glutamate), glutamate decarboxylase (conversion of glutamate to GABA), GABA transaminase (breakdown of GABA and regeneration of glutamate), glycine cleavage system (catabolism of glycine), L-serine racemase (formation of D-serine), and histamine decarboxylase (synthesis of histamine) (Clayton 2006). The principal manifestation of the disorder is thus likely to be due to a deficiency of PLP in the brain leading to disordered neurotransmission and hence seizures, although we cannot be absolutely certain that accumulation of other vitamers such as pyridoxamine phosphate in the brain does not contribute to impaired function of PLP-dependent enzymes. A liver transplant should result in normal conversion of dietary pyridoxine, pyridoxamine, and their phosphates to PLP in the liver and thus lead to correction of circulating PLP deficiency. However, it will not correct the abnormality of the salvage pathway in the brain, which may be important for conversion of pyridoxamine phosphate back to PLP in that organ. On balance, it seems likely that OLT would lead to improved seizure control and a reduced requirement for PLP and/or the possibility of giving extra B6 in the form of

pyridoxine with possibly a lower risk of liver damage/cirrhosis in the graft.

In some PNPO patients, there is an emerging phenotype of normal neurodevelopmental outcomes (Mills et al. 2014), but this comes with a high disease burden and the need for constant vigilance. Insufficient P5P (e.g., dose interruption or delay) can result in life-threatening seizures and severe neurological morbidity. In our patient, even a delay of 15 min in usual dose timing has led to life-threatening seizures with apnea. The "round-the-clock" medication requirement to reduce the risk of catastrophe produces a significant disease burden for the family. While an OLT in PNPO deficiency would not cure the disease, we propose that a steady-state production of hepatic-derived PLP might lower the risk of seizures with delayed dosing and minimize the potential risk of long-term P5P-related complications. To this end we postulate that PNPO deficiency may be a suitable IEM for the consideration of OLT in carefully selected cases.

Compliance with Ethics Guidelines

Conflict of Interest

David Coman, Peter Lewindon, Peter Clayton, and Kate Riney declare that they have no conflicts of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

Author Contributions

A/Prof David Coman is a metabolic physician and involved in the care of the patients and has coordinated the manuscript development and design.

A/Prof Peter Lewindon is a pediatric gastroenterologist who is involved in the patients' care and has been involved in the manuscript development.

Professor Peter Clayton is a metabolic physician who has provided invaluable clinical advice on the patients and has been involved in the manuscript development.

Dr. Kate Riney is a pediatric neurologist, is the primary caregiver for the patient, and has been involved in the manuscript development.

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