

6-Pyruvoyl-tetrahydropterin Synthase Deficiency with Mild Hyperphenylalaninemia

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Severe 6-pyruvoyl-tetrahydrobiopterin synthase deficiency is a tetrahydrobiopterin deficiency disorder that presents in infancy with developmental delay, seizures, and abnormal movements associated with hyperphenylalaninemia usually detectable by neonatal phenylketonuria screening programs. We describe an 8-year-old girl with delay, seizures, and dystonia with mild hyperphenylalaninemia detected in late childhood. The diagnosis of 6-pyruvoyl-tetrahydrobiopterin synthase deficiency was made by analysis of pterins in urine, pterins and neurotransmitters in cerebrospinal fluid, and enzyme assay. The patient improved clinically taking oral tetrahydrobiopterin, levodopa/carbidopa, and 5-hydroxytryptophan. This treatable condition may not always be detected by routine population screening for hyperphenylalaninemia.

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6-Pyruvoyl-tetrahydrobiopterin synthase (PTPS) deficiency is a recessively inherited neurometabolic condition identified as the most common cause of tetrahydrobiopterin (BH₄) deficiency. It is the cause of approximately 60% of all cases of BH₄ deficiencies as listed in the BIoDEF International database of Tetrahydrobiopterin Deficiencies (<http://www.bh4.org/biodef1.html>).¹ BH₄ is a cofactor for phenylalanine hydroxylase, enzymes involved in biosynthesis of neurotransmitters, and brain nitric oxide synthase. BH₄ disorders include deficiency of enzymes involved in biosynthesis (guanosine triphosphate cyclohydrolase I

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[GTPCH] or PTPS) or regeneration (pterin-4 α -carbinolamine dehydratase or dihydropteridine reductase [DHPR]) of BH₄ (Fig). Patients with BH₄ deficiency usually develop hyperphenylalaninemia (HPA) and neurological symptoms mainly caused by lack of catecholamines and serotonin. Some forms of BH₄ deficiency, namely dominantly inherited GTPCH deficiency² and sepiapterin reductase deficiency,³ present without HPA. The former also has masqueraded as cerebral palsy in some reported cases.^{4–6}

In PTPS deficiency, the majority of patients have the severe form that presents in infancy with HPA, developmental delay, seizures, disturbance of tone, movement disorder, drowsiness, and hypersalivation. HPA usually is detectable by neonatal phenylketonuria screening programs.⁷ The mild form of PTPS deficiency is associated with moderate or transient HPA, usually no neurological involvement, and normal cerebrospinal fluid (CSF) neurotransmitter levels.^{7,8} In severe PTPS deficiency, the neopterin/biopterin ratio is increased in both urine and CSF; and the CSF neurotransmitter metabolites 5-hydroxy-indoleacetic acid and homovanillic acid are low. Diagnosis is confirmed with enzyme studies of PTPS.

The treatment of a BH₄ deficiency involves controlling the HPA with oral BH₄ supplementation or low-phenylalanine diet, and correction of neurotransmitter deficiencies with L-dopa/carbidopa and 5-hydroxytryptophan.⁷ Therefore, treatment for severe PTPS, GTPCH (autosomal recessive form), and DHPR deficiency includes both oral neurotransmitter precursors; and BH₄ supplementation or low-phenylalanine diet for severe DHPR deficiency; and treatment for mild PTPS and DHPR deficiency requires only BH₄ supplementation or low-phenylalanine diet, respectively.⁷

Treatment monitoring involves regular clinical evaluations, periodic CSF measurements of concentrations of homovanillic acid and 5-hydroxy-indoleacetic acid, or both.⁷ At our center, we use a combined approach with regular assessments by our metabolic clinic and CSF neurotransmitter measurements.

In this article, we present a case demonstrating that severe PTPS deficiency may be associated with mild HPA and may be misdiagnosed as cerebral palsy. We also compare this patient with the other cases of severe PTPS deficiency (N = 223) registered in the BIoDEF database¹ and described in the literature.

Case Report (BIODEF No. 465)

This patient was referred at 8 years of age with a diagnosis of cerebral palsy, intellectual disability, and seizures. She was born in South Korea after a normal pregnancy and delivery to nonconsanguineous parents. She was hypotonic at birth with a birth weight at the 5th percentile. Generalized tonic-clonic seizures began at 2 months of age. At 8 years of age, her seizures were char-

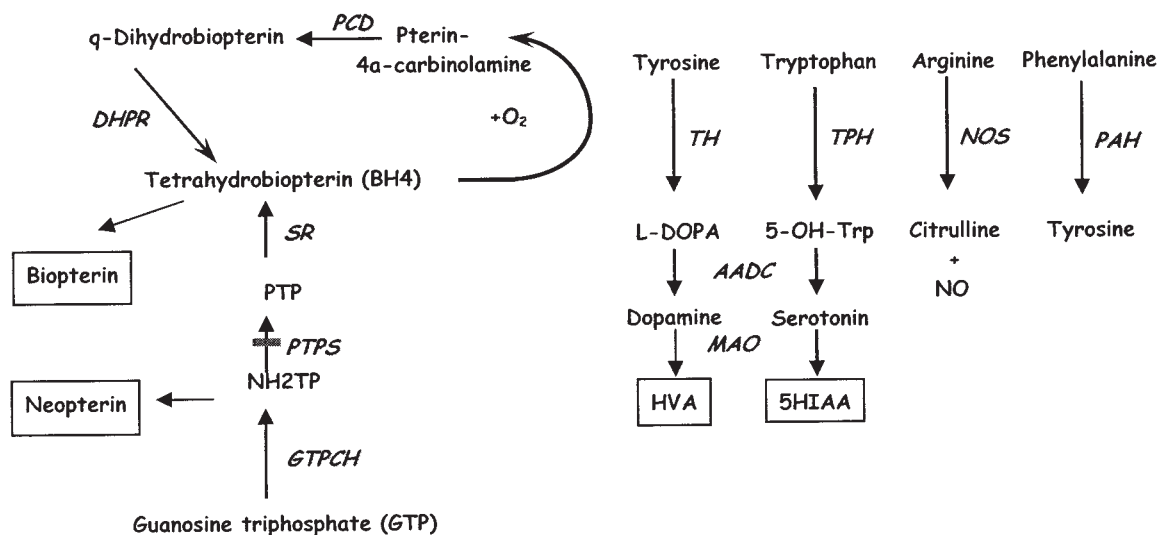


Fig. Tetrahydrobiopterin biosynthetic and regeneration pathway, and catecholamine and serotonin metabolism. AADC = aromatic L-amino acid decarboxylase; DHPR = dihydropteridine reductase; GTPCH = guanosine triphosphate cyclohydrolase I; 5-HTP = 5-hydroxytryptophan; HVA = homovanillia acid; 5-HIAA = 5-hydroxy indoleacetic acid; MAO = monoamine oxidase; NH₂TP = dihydroneopterin triphosphate; NOS = nitric oxide synthase; PAH = phenylalanine hydroxylase; PCD = pterin-4 α -carbinolamine dehydratase; PTP = 6-pyruvoyltetrahydropterin; PTPS = 6-pyruvoyltetrahydropterin synthase; SR = sepiapterin reductase; TH = tyrosine hydroxylase; TPH = tryptophan hydroxylase.

acterized by head drops, tonic seizures, or generalized tonic-clonic seizures, which occurred weekly. Lamotrigine resulted in improvement in seizure control. She also had episodes of drowsiness and irritability since birth. Developmentally, she walked at 21 months old and first spoke at 3 years old. At a chronological age of 8 years, her developmental age equivalent was 3 years. Physical examination demonstrated normal head circumference, hypersalivation, and truncal eczematous-like rash. Neurological examination indicated truncal hypotonia, increased limb tone, generalized dystonia, brisk deep-tendon reflexes, and limb ataxia.

Amino acid analysis reportedly screened negative for HPA (Table 1) 2 years of age. Retrospective enquiry showed that the analysis was likely done by the semi-quantitative bacterial inhibition assay (Guthrie method) commonly used by newborn screening programs.

At referral, quantitative plasma amino acid analysis by automated anion-exchange chromatography showed persistent mild HPA (see Table 1). Urine neopterin/biopterin ratio was increased (Table 2). In CSF, the neurotransmitter metabolites homovanillic acid and 5-hydroxy-indoleacetic acid were reduced. BH₄ was reduced, whereas neopterin was increased (see Table 2). PTPS enzyme activity in red blood cells was reduced, confirming the diagnosis of PTPS deficiency (see Table 2).

The patient was administered L-dopa/10% carbidopa (1.0mg/kg/day), 5-hydroxytryptophan (4.3mg/kg/day), and BH₄ (3.5mg/kg/day) therapy at 10 years old. She

has had no further seizures without antiepileptic medication. The dystonia has also improved, together with her motor skills and episodes of fatigue. Her plasma phenylalanine levels normalized on treatment (see Table 1).

Discussion

We present a unique case of severe PTPS deficiency associated with mildly increased plasma phenylalanine levels first recognized at 8 years of age. This patient was clinically symptomatic from infancy. Plasma phenylalanine at 2 years of age screened negative for HPA at a level of approximately 120 μ mol/L. Although we are unable to obtain records from Korea, it is likely that she also had a negative neonatal screen for HPA at birth. When reevaluated at 8 years old, she had already been labeled as having cerebral palsy with no obvious

Table 1. Plasma Phenylalanine (μ mol/L)^a

Age (yr)	Patient
<i>Pretreatment</i>	
2	120 (ref. range <120)
8	166, 156 (ref. range 38–76)
10	226 (ref. range 38–76)
<i>Posttreatment</i>	
10 to current	46–62 (mean 52; N = 8; ref. range 38–76)

^a6-Pyruvoyl-tetrahydrobiopterin deficiency is typically associated with plasma phenylalanine levels of 240 to 2,500 μ mol/L.⁷

Table 2. Pterins (urine and CSF), Neurotransmitters (CSF), and PTPS Activity in Red Blood Cells

Measure	Patient	Control
Urine pterins		
Neopterin	8.1	1–7.9mmol/mol Cr
Biopterin	0.22	0.5–4.6mmol/mol Cr
% Biopterin ^a	2.6	46–77 %
CSF pterins		
Neopterin	53	7–40nM
BH ₄	7	9–40nM
CSF neurotransmitters		
HVA	73	218–852nM
5-HIAA	11	66–338nM
PTPS activity		
Activity in RBCs	1.8	17.6 (11–29) uU/gm Hb
% of control mean	10%	100%

^a% Biopterin = 100 × biopterin/(neopterin + biopterin).

CSF = cerebrospinal fluid; PTPS = 6-pyruvoyl-tetrahydrobiopterin; HVA = homovanillic acid; 5-HIAA = 5-hydroxyindoleacetic acid; RBC = red blood cell.

cause, but plasma phenylalanine level was persistently yet mildly increased when repeated. Urine pterins and CSF neurotransmitter and pterin levels suggested PTPS deficiency, which was confirmed by reduced PTPS enzyme activity in her red blood cells. She still benefited from treatment first introduced at aged 10 years.

Our case is unique because unlike some recognized forms of BH₄ deficiency that are not associated with HPA (dominantly inherited GTPCH deficiency² and sepiapterin reductase deficiency³), PTPS deficiency typically is associated with plasma phenylalanine levels of 240 to 2,500 μmol/L; thus, the HPA usually can be detected through neonatal PKU screening programs.⁷ A review of the BIODDEF database¹ for registered severe PTPS patients (N = 223), as well as review of published literature,⁹ shows that there are two patients (BIODEF no. 4 and no. 250) whose initial plasma phenylalanine at 5 days old was not increased (80 and 60 μmol/L, respectively). However, repeat measurements, initiated because of neurological symptoms in the first patient at 6 months old and the second patient at 17 months old, showed markedly increased phenylalanine levels at 1,200 and 1,331 μmol/L, respectively. This supports the possibility of a negative neonatal HPA screen occurring in severe PTPS deficiency, which we suspect to have occurred in our patient. However, the subsequent marked increases of phenylalanine levels in the other two patients stand in striking contrast with the persistence of only mild HPA in our patient.

There are also three other patients in the BIODDEF database¹ with severe PTPS deficiency associated with mild HPA. No results from newborn screening were available on any of these patients. Only one patient (BIODEF no. 348) has been reported in the litera-

ture.¹⁰ This patient had generalized dystonia with marked diurnal fluctuation, seizures, and mental retardation associated with a phenylalanine level of 169 μmol/L at 41 years old. Urinary percentage of neopterin was high, but CSF neurotransmitter levels were normal. She had a marked and sustained positive response to L-dopa. There is limited information available for the remaining two patients in the BIODDEF database.¹ For one patient (BIODEF no. 350), his plasma phenylalanine level was 180 μmol/L at 5 years old, and his diagnosis was confirmed with evidence of reduced enzyme activity. The other patient (BIODEF no. 267) had mental retardation, hypotonia, and swallowing difficulties. She had an affected sibling and family history of consanguinity. Investigations initiated at 8 years of age showed a plasma phenylalanine level of 176 μmol/L and an increased urine neopterin/biopterin ratio. Her symptoms improved after receiving L-dopa and BH₄ therapy at 8 years old.

Plasma phenylalanine levels also have been reported to be transiently increased in PTPS deficiency. Blau and colleagues¹¹ described an isolated central (severe) form of PTPS deficiency associated with transient HPA. The child developed irritability and slowing of motor development when receiving dietary treatment for HPA (1,636 μmol/L), which was found on her neonatal metabolic screen. Urine pterin and enzyme studies were consistent with PTPS deficiency, and CSF monoamines also were reduced. Neurological symptoms resolved on L-dopa/carbidopa and 5-hydroxytryptophan. Plasma phenylalanine continued to be slightly increased (130–190 μmol/L), and then normalized at 6.5 years of age on a normal diet. Transient HPA may also occur in the mild or peripheral form of PTPS deficiency.⁸

Our patient demonstrates that routine population screening for HPA may not suffice to detect all patients with PTPS deficiency. Our case and others indicate that a negative newborn screen for HPA can occur in severe PTPS deficiency.^{1,9} Furthermore, subsequent measurements in childhood may also be less than the cutoff values routinely used in newborn population screening (typically 120–180 μmol/L). This was seen in our case; however, the values were clearly greater than the age-related (±2 standard deviations) reference range established for the use of quantitative amino acid analysis in metabolic patient investigations. Physicians need to be aware that laboratories differ in their analytical methods and in the thresholds chosen for reporting results as abnormal. The HPA may also be transient as described in the isolated central form of PTPS deficiency.¹¹ Blau and colleagues⁷ recommend screening for a BH₄ deficiency in all newborns with plasma phenylalanine levels greater than 120 μmol/L and in older children with consistent neurological symptoms and signs. This

may include patients with cerebral palsy without a causative factor. This case also demonstrates that treatment should still be initiated independent of age at diagnosis.

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