Two Filipino Patients with 6-Pyruvoyltetrahydropterin Synthase Deficiency

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ABSTRACT

Hyperphenylalaninemia can result from defects in either the phenylalanine hydroxylase (PAH) enzyme or in the synthesis or recycling of the active pterin, tetrahydrobiopterin (BH4), which is an obligate co-factor for the PAH enzyme, as well as tyrosine hydroxylase and tryptophan hydroxylase. One of the most common causes of BH4 deficiency is a defect in the synthesis of 6-pyruvoyltetrahydropterin synthase (PTPS) enzyme. Patients present with progressive neurological disease such as mental retardation, convulsions and disturbance of tone and posture despite strict adherence to diet and good metabolic control. The authors report the first two cases of PTPS deficiency in the Philippines. Both are females with initial phenylalanine levels of more than 1300 umol/L who continued to develop neurologic deterioration despite good metabolic control and strict adherence to diet. Further investigation showed that they both had PTPS deficiency. Treatment was started with BH4, L-dopa/carbidopa, and 5-hydroxytryptophan (5HT) with concomitant significant improvements in their neurologic and developmental outcomes.

Key Word: hyperphenylalaninemia, 6-Pyruvoyltetrahydropterin synthase, tetrahydrobiopterin

Introduction

Phenylalanine (PHE) is an essential aromatic amino acid, mainly metabolized in the liver by the PHE hydroxylase (PAH) system. The PAH enzyme requires a cofactor, the active pterin, tetrahydrobiopterin (BH4), which is also an obligate co-factor for tyrosine hydroxylase and tryptophan hydroxylase[1] (Figure 1). Defects in either PAH or the synthesis or recycling of BH4 may result in hyperphenylalaninemia, as well as deficiency of tyrosine, L-dopa, dopamine, melanin, catecholamines, and 5-hydroxytryptophan.[1]

Severe PAH deficiency results in classical phenylketonuria (PKU) with blood PHE levels more than 1200 μmol/L when individuals are on a normal protein intake. Milder defects are termed hyperphenylalaninemia.[1] Tetrahydrobiopterin deficiency, a variant of hyperphenylalaninemia, may be caused by deficiency of one of the following enzymes: guanosine triphosphate cyclohydrolase (GTPCH), 6-pyruvoyltetrahydropterin synthase (PTPS), dihydropteridine reductase (DHPR) and pterin-4a-carbinolamine dehydratase (PCD). The first two enzymes are involved in the biosynthesis of tetrahydrobiopterin, the last two in its regeneration.[3] A third enzyme in the biosynthesis of BH4 is sepiapterin reductase, but its deficiency is not associated with hyperphenylalaninemia [1] (Figure 1).

Clinical manifestations for a severe PAH defect or BH4 synthesis/recycling defect can be similar, with patients presenting with progressive neurological impairment during infancy. Since management of these patients will depend on the etiology, determining the correct cause for the hyperphenylalaninemia is important.

Diagnostically, defects in BH4 synthesis/recycling would show either a decreased DHPR activity or an abnormal pterin high performance liquid chromatography (HPLC) analysis such as accumulation of neopterin in biopterin-synthetase deficiency; high levels of pterins in DHPR deficiency; and low biopterin and high neopterin levels in PTPS deficiency. PAH enzyme deficiency on the other hand, will have normal DHPR activity and high levels of pterins.[4,5]

Here we present two cases of patients detected to have elevated PHE, initially treated as cases of classical PKU, which on further testing were found to have BH4 synthesis defects.

Clinical reports

Patient 1

Patient 1 is a 1 year old girl, third child of non-consanguineous parents. She was first seen on the 19th day of life, after her newborn screening showed elevated phenylalanine levels (1400 μmol/L). At that time, the patient was noted to be slightly hypertonic with increased sleeping time. She was started on a protein restricted diet and phenylalanine free formula with regular follow-up at our metabolic clinic.

Despite good compliance with the recommended diet and phenylalanine levels less than 400 μmol/L, the patient developed seizures at 4 months of life and subsequently presented with developmental regression, loss of head control and hypotonia. These were initially attributed to the possible effects of the increased PHE levels in the neonatal period. She was then referred to a neurologist and started on anti-convulsant therapy.

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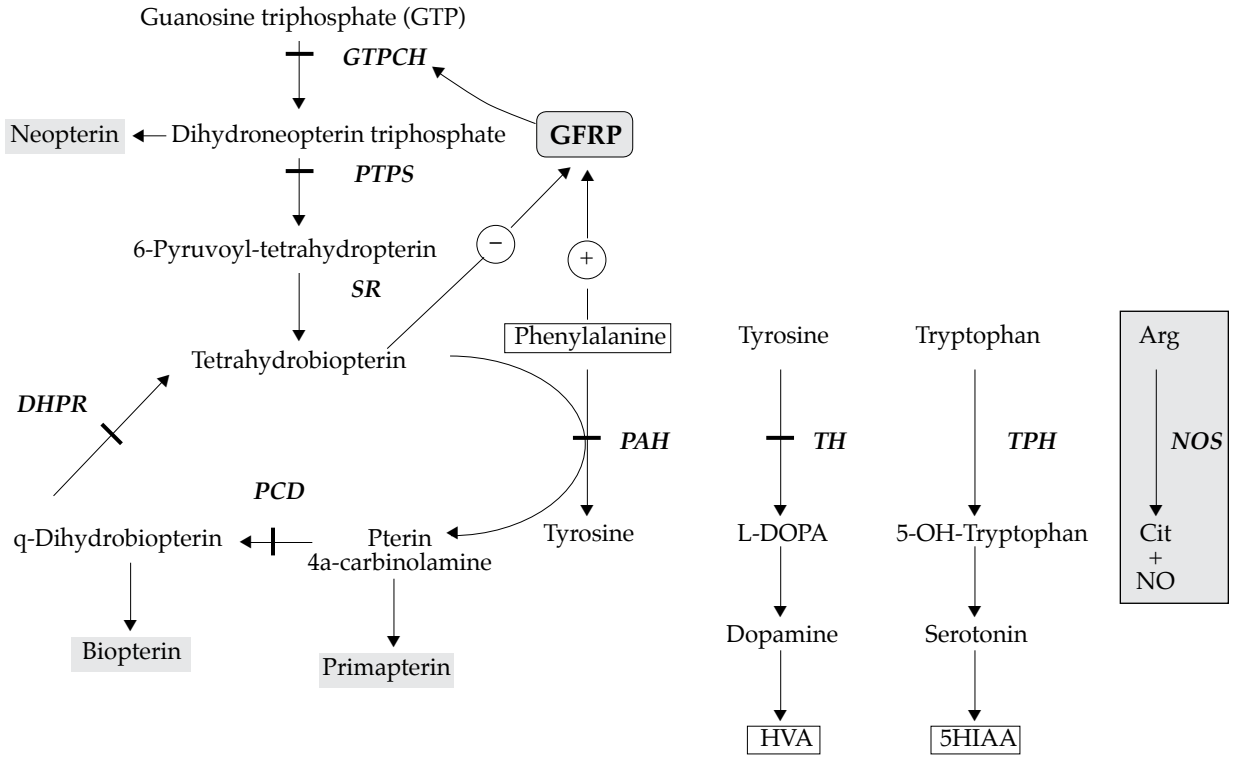

Figure 1. Aromatic amino acid hydroxylases and function of tetrahydrobiopterin. GTPCH - GTP cyclohydrolase I, PTPS - 6-pyruvoyltetrahydropterin synthase, SR - sepiapterin reductase, DHPR - dihydropteridine reductase, PCD – pterin-4a-carbinolamine dehydratase, GFRP - GTPCH feedback regulatory protein, PAH - phenylalanine hydroxylase, TH - tyrosine hydroxylase, TRH - tryptophan hydroxylase, NOS - nitric oxide synthase, HVA - homovanillic acid, 5HIAA - 5-hydroxyindoleacetic acid[2]

At approximately 7 months, BH4 deficiency was considered due to the persistence of seizures and developmental delay. Investigations included DHPR activity, which was normal (done at The Children's Hospital at Westmead, Sydney Australia), and pterin levels which revealed PTPS deficiency (done at Taipei Veterans General Hospital, Taiwan). Prolactin levels also showed significantly elevated results at 1124.5 μIU/mL (n.v. 80-500 μIU/mL).

She was initially started only on levodopa/carbidopa because of the unavailability of the BH4 and 5HT tablets. The patient was started at a dose of 1 mg/kg/day and increased weekly by 1 mg/kg/day with no adverse reactions noted. One month after starting levodopa treatment, the patient was noted to have improvement in motor skills, with slight head control, and better muscle tone. At this time, 5HT tablets were also started at 1 mg/kg/day. However, the patient developed loose bowel movements with increased frequency. Increments were decreased to 0.3mg/kg/day weekly until 5 mg/kg/day was achieved. Three weeks later, patient was also started on BH4 tablets at a dose of 3 mg/kg/day. No untoward reactions were noted after intake of BH4 tablets. Within 1 month after starting BH4 tablets, the seizure episodes disappeared, recurring only after phenobarbital maintenance was abruptly stopped by the parent. Seizures have been noted to decrease in frequency following re-administration of the phenobarbital.

Repeat prolactin level showed 710.3 μIU/mL, which was still mildly elevated, thus levodopa was increased to 6mg/kg/day.

At the time of this writing, she is maintained on levodopa/carbidopa at 6 mg/kg/day, 5HT at 5 mg/kg/day, and BH4 tablets at 3 mg/kg/day. Anti-convulsants (oxcarbazepine and phenobarbital) have been slowly tapered with seizures occurring less frequently. The patient has been off protein restriction for two months now with PHE levels remaining within the normal range. There is still some developmental delay in expressive language with the patient only able to utter single syllables. However, the rest of the developmental skills are now within the acceptable standard for age.

Patient 2

Patient 2 is a girl, now 2 years of age. She is the second child of non-consanguineous parents. The older sibling has mental retardation. Newborn screening was not done at birth. At 3 months of life, she had spasticity and seizures,

thus newborn screening was recommended which showed elevated PHE levels (1300 μmol/L). She was diagnosed to have classical PKU, referred to our metabolic clinic and started on protein restricted diet. Although there was regular follow-up, metabolic control was poor, which was attributed to limited and irregular availability of the phenylalanine-free milk. Seizures were uncontrolled even with anti-convulsant therapy (topiramate and phenobarbital) and patient had poor developmental progress with persistent head lag and hypotonia.

Starting at 1 year 9 months of age, PHE-free formula supply became more regular. Although there was better metabolic control (PHE levels ranged from 400-800 μmol/L), patient still had uncontrolled seizures despite very slight improvement in development. A possible BH4 deficiency was considered. Investigations showed normal DHPR enzyme activity (done at Children's Hospital at Westmead, Sydney Australia) and pterin analysis revealed PTPS deficiency (done at Taipei Veterans General Hospital, Taiwan). Prolactin was elevated (1051.2 μIU/mL). The patient was initially started on levodopa/carbidopa at 1 mg/kg/day, followed a week later with 5HT tablets. No untoward reactions were noted with these two medications.

As of this writing, at 2 years of age, there has been improvement with her developmental skills with the levodopa and 5HT (at 5 mg/kg/day for each). BH4 tablets have yet to be procured by the family. Seizures were also noted to have decreased in frequency. She however, continues to have hypotonia and head lag. Developmental assessment still shows gross developmental delay.

Discussion

The diagnostic and management approach of patients confirmed to have elevated PHE levels on newborn screening should include delineation of the etiology. Although both PAH defects and BH4 synthesis/recycling defects will produce elevated PHE results and possibly similar clinical manifestations, the management for each differs. PAH enzyme defects may require (depending on the severity of mutation) protein restriction in combination with a PHE-free formula. BH4 synthesis/recycling defects on the other hand, may require exogenous BH4 administration apart from levidopa/ carbidopa and 5HT supplementation.[4]

The clinical courses of these patients showed the need to carry-out further testing to define between PAH defects and BH4 synthesis/recycling defects. Clinically, a BH4 synthesis/recycling defect may be seen in patients who continue to develop neurologic problems despite strict adherence to diet and good metabolic control,[1] as was seen in patient 1 with phenylalanine levels below 400 μmol/L. This was primarily due to the neurotransmitter deficiency secondary to the deficiency of the co-factor BH4, an obligate co-factor for tyrosine hydroxylase and tryptophan hydroxylase in the production of dopamine and serotonin, respectively. Although PKU may also manifest with deficiency of dopamine and serotonin, this is only seen in cases where there is poor metabolic control. The increased level of PHE results in an imbalance of other large neutral amino acids within the brain, resulting in decreased brain concentrations of tyrosine and serotonin[6]. Therefore, the finding of persistent neurologic manifestations despite normal to near normal levels of PHE points more to a BH4 synthesis/recycling defect, as seen in patient 1. Similarly, Patient 2 was suspected with this disorder after careful evaluation of the metabolic status (regular milk supply and improving PHE levels).

Both patients described here were confirmed to have PTPS deficiency. In the International Database of BH4 Deficiencies, PTPS deficiency represents 59% of all BH4 deficiencies.[7] PTPS deficiency results in a decrease in the biosynthesis of BH4, thereby reducing the activity of PAH which needs BH4 as a cofactor in the metabolism of PHE.[1] PTPS deficiency in humans may not only produce the typical phenylketonuric phenotype but may also be the source of neurological signs and symptoms due to impaired syntheses of levodopa and serotonin.[4] Without treatment, the natural history for PTPS deficiency is poor with progressive neurological disease and early death. The outcome with treatment depends upon the age of diagnosis and phenotypic severity. Most children have some degree of learning difficulties despite satisfactory control.[1]

Although the Philippine newborn screening laboratory does not have facilities to carry out DHPR and pterin testing, outsourcing to foreign laboratories has made it possible to determine the cause of elevated PHE levels in our patients. It is therefore recommended that patients identified with elevated PHE levels on newborn screening should immediately undergo a BH4 loading test. This would indicate either a BH4 synthesis/recycling defect or a BH4 responsive PAH defect. Early correct diagnosis of the cause of the elevated PHE is important for proper management of these patients and crucial for their successful growth and development.

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