J Inherit Metab Dis (2009) 32 (Suppl 1):S279–S282
DOI 10.1007/s10545-009-1221-x

CASE REPORT

Novel mutation affecting the pterin-binding site of *PTS* gene and review of *PTS* mutations in Thai patients with 6-pyruvoyltetrahydropterin synthase deficiency

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Received: 16 April 2008 / Submitted in revised form: 30 July 2009 / Accepted: 25 August 2009 / Published online: 13 October 2009
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Summary Tetrahydrobiopterin (BH_4) deficiency comprises heterogeneous disorders resulting in hyperphenylalaninaemia (HPA) and lack of monoamine neurotransmitters. Among these, 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency is the most common disorder. We report a female Thai patient with PTPS deficiency who was initially detected by newborn screening for HPA, and later treated by supplements of BH_4, L-dopa/carbidopa, and 5-hydroxytryptophan. Monitoring of serum prolactin representing dopamine sufficiency is used for optimizing the dosage of L-dopa. She showed a remarkable progress of development despite delayed treatment at 5 months of age. Mutation analysis revealed two heterozygous missense mutations of the *PTS* gene: c.259C>T (p.P87S) inherited from the father; and c.147T>G (p.H49Q) inherited from the mother. The latter is a novel mutation that affects the pterin-binding site of the PTPS enzyme. This novel mutation expands the mutation spectrum of PTPS deficiency. Notably, some *PTS* mutations have been reported in both Thai and Chinese patients. Whether these common mutations are the result of a founder effect with common ancestors of Thai and Chinese people or intermarriage between Thai and Chinese descents in Thailand remain unclear. In conclusion, severe neurological impairment from BH_4 deficiency could be prevented by newborn screening for HPA and proper metabolic management. However, pterin analysis for early diagnosis of BH_4 deficiency is still not available in most developing countries.

Communicating editor: Eva Morava

Competing Interests: None declared

References to electronic databases: BIODEF database: http://www.bh4.org. Homologene database: http://www.ncbi.nlm.nih.gov/homologene/. SNP database: http://www.ncbi.nlm.nih.gov/projects/SNP/. Conserved Domains database: http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml. 6-Pyruvoyltetrahydropterin synthase deficiency: OMIM 261640.

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Abbreviations

BH_4	tetrahydrobiopterin
HPA	hyperphenylalaninaemia
5-HTP	5-hydroxytryptophan
PAH	phenylalanine hydroxylase
PTPS	6-pyruvoyltetrahydropterin synthase

Introduction

Hyperphenylalaninaemia is caused by a deficiency of either phenylalanine hydroxylase (PAH) or one of the four enzymes involved in biosynthesis or regeneration of its cofactor, tetrahydrobiopterin (BH_4) (Blau et al. 2001). BH_4 is also the cofactor for tyrosine hydroxylase and tryptophan hydroxylase, which are required for the biosynthesis of catecholamines and serotonin, respectively (Kaufman 1993). Thus, BH_4 deficiency leads to severe shortage of monoamine neurotransmitters resulting in progressive mental retardation despite effective dietary control of the levels of phenylalanine. The patients with BH_4 deficiency are apparently normal in the first 2–3 months of life; thereafter the patients present with truncal hypotonia, increased limb tone, bradykinesia, episodic rigidity, and oculogyric crisis (Blau et al. 2001). Infants affected with BH_4 deficiency could be detected before they developed symptoms by newborn screening for hyperphenylalaninaemia (HPA) and then confirmed by measurement of pterins in urine or a dried blood spot (Zurflüh et al. 2005).

PTPS deficiency (OMIM 261640) is the most common cause of BH_4 deficiency, approximating 60% of the BH_4-deficient patients (BIODEF database: http://www.bh4.org). PTPS-deficient patients usually require a combined treatment with BH_4 and the precursors of neurotransmitters, L-dopa/carbidopa and 5-hydroxytryptophan (McInnes et al. 1984), and the outcome may be quite variable (Jäggi et al. 2008). Here we report a novel mutation and successful treatment in a Thai patient with PTPS deficiency.

Case report

The patient is a girl who was born at term to parents of Thai and Chinese ethnic origins. Her birth weight was 3530 g. She was referred to our hospital at 1 month due to positive newborn screening of HPA (phenylalanine 600 μmol/L, reference range <120 μmol/L). Her initial plasma phenylalanine level was 1006 μmol/L (reference range 25–75 μmol/L), and she was diagnosed with moderate phenylketonuria. She had been treated with dietary phenylalanine restriction since diagnosis. Her follow-up phenylalanine levels after phenylalanine restriction were between 85 and 317 μmol/L. In spite of good phenylalanine control, she developed truncal hypotonia and intermittent dystonia at 3 months. Further investigations to rule out BH_4 deficiency revealed very high serum prolactin (67 ng/ml, reference range 4.04–15.2); and pterin analysis in the dried blood spots revealed no biopterin detectable (<0.01 nmol/g Hb, reference range 0.15–2.91), normal neopterin (2.43 nmol/g Hb, reference range 0.31–4.45), and <0.5% biopterin (%biopterin=100×biopterin/(neopterin+biopterin), reference range 3.9–78.3%). A very low percentage of biopterin was indicative of PTPS deficiency.

The combined treatment with BH_4 supplementation (5 mg/kg per day) (Schircks Laboratories, Switzerland) and L-dopa (5 mg/kg per day, combined with carbidopa) was started at 5 months. The prolactin level decreased to 17.4 ng/ml within 1 month after treatment (Fig. 1a). After 2 months of treatment, dystonia disappeared and hypotonia was much improved. The phenylalanine levels decreased to the normal range despite a regular protein diet (Fig. 1b). The BH_4 was gradually increased to 7.5 mg/kg per day, and L-dopa/carbidopa to 10 mg/kg per day by 14 months. 5-Hydroxytryptophan (5-HTP) was slowly introduced to 5 mg/kg per day at 12 months. At 13 months, she started cruising and speaking a single word. At the most recent visit (18 months), she could walk alone, and speak a couple of meaningful words.

PTS mutation analysis

Molecular analysis was performed after the parents' informed consent had been obtained. The genomic DNA of the index patient and the parents was isolated from the dried blood spots collected on filter paper as described elsewhere (Liu et al. 1998). PCR amplification and DNA sequencing of six exons of the *PTS* gene were performed as previously described (Liu et al. 1998). The mutation analysis of the *PTS* gene revealed two heterozygous missense mutations: c.259C>T (p.P87S) in exon 5, and c.147T>G (p.H49Q) in exon 2 (data not shown). The p.P87S mutation is inherited from the father and is one of the known *PTS* mutations (Thöny and Blau 2006). The p.H49Q mutation is inherited from the mother and has not previously been reported (BIODEF database: http://www.biopku.org). We compared the PTS protein sequences between several species and found that the histidine at codon 49 is located at the pterin-binding site, which is 100% conserved across all species (Homologene database: http://www.ncbi.nlm.nih.gov/homologene/). The absence of the novel missense mutation in 100 alleles from unrelated control individuals also supported the conclusion that this nucleotide change is a pathogenic mutation (data not shown).

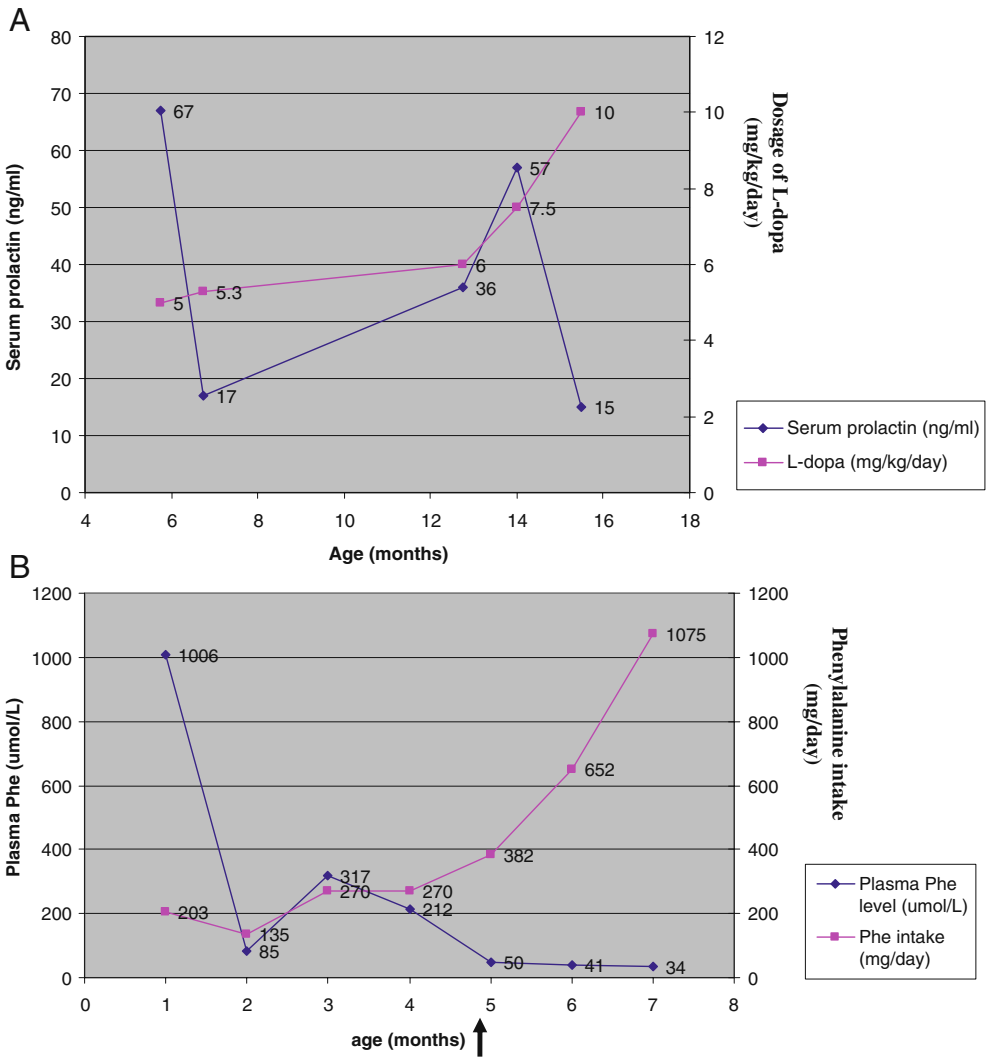

Fig. 1 (**a**) The correlation between the serum prolactin levels (ng/ml) and dosage of L-dopa (mg/kg per day). (**b**) The plasma phenylalanine levels (μmol/L) and phenylalanine intake (mg/day). Note the marked increase of phenylalanine tolerance after the introduction of BH_4 supplementation. The arrow denotes the time that the BH_4 was started

Discussion

This is the first report of successful treatment in a Thai PTPS-deficient patient. The patient was detected for HPA from the first month of life but she was not tested for BH_4 deficiency owing to lack of availability of pterin analysis and supply of BH_4 for loading test in Thailand at that time. Initial diagnosis was done using a dried blood specimen analysis of pterins. Although, the elevation of neopterin in blood was not as high as reported in other PTPS-deficient patients, the fact that there was no biopterin detectable and thus an extremely low percentage of biopterin was highly diagnostic. She was started on the treatment at 5 months, which is slightly delayed. Monitoring of serum prolactin representing dopamine sufficiency is used for optimizing the dosage of L-dopa (Ogawa et al. 2008) (Fig. 1a). The rapid decrease of the prolactin level correlated with markedly improved neurological features in our patient. The BH_4 supplementation normalized the patient's phenylalanine tolerance (>1000 mg/day) (Fig. 1b).

We identified two heterozygous missense mutations in this patient: the c.259C>T (p.P87S) mutation, which is one of the common Asian *PTS* mutations (Imamura et al. 1999; Liu et al. 2001; Pangkanon et al. 2006); and the c.147T>G (p.H49Q) mutation, which is a novel mutation. This mutation is hypothesized to alter the ability of the pterin-binding site of the PTPS enzyme to bind zinc which is a key element of catalytic activity (Conserved Domains database: http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml). The effect of this mutation was demonstrated in the p.H48L-mutant rat (the histidine residue at codon 48 in rats corresponds with the histidine residue at codon 49 in humans)

leading to no residual PTPS activity (Bürgisser et al. 1995). So far three *PTS* mutations have been identified in the two previously reported Thai patients with PTPS deficiency: c.200C>T (p.T67M), c.200C>A (p.T67K), and c.259C>T (p.P87S) (Pangkanon et al. 2006). Notably, the p.T67M and p.P87S mutations have also been reported in Chinese patients with PTPS deficiency (Liu et al. 2001). Whether these common mutations are the result of a founder effect with common ancestors of Thai and Chinese people or intermarriage between Thai and Chinese descents in Thailand remains unclear. More patients and haplotype analysis of sharing flanking markers are required to elucidate the origin of the common mutations.

In conclusion, severe neurological impairment from BH_4 deficiency could be prevented by newborn screening for HPA and proper metabolic management. Although newborn screening programmes for hyperphenylalaninaemia are established in many developing countries, pterin analysis for early diagnosis of BH_4 deficiency is still not available in most countries. The BH_4 loading test could be an alternative screening test, with a cost of 17–23 USD for each test (using a single dose of 20 mg/kg). However, this test cannot discriminate between patients with BH_4 deficiency and those with BH_4-responsive phenylketonuria. Lifelong BH_4 supplement is the mainstay of treatment but remains very expensive for developing countries.

Acknowledgements We are indebted to our patient, her family, and the referring physician for their cooperation. This study is supported in part by the Swiss National Science Foundation grant no. 3100A0-1199852/1 (to N.B.).

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