Case Report

6-Pyruvoyltetrahydropterin Synthase Deficiency Two-Case Report

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6-Pyruvoyltetrahydropterin Synthase (PTPS) deficiency is the most common cause of hyperphenylalaninemia due to tetrahydrobiopterin deficiency. The presenting symptoms of PTPS deficiency are mental retardation, convulsions, disturbance of tone and posture, drowsiness, irritability, abnormal movements, hypersalivation, and swallowing difficulties[1-3]. The authors reported the first two cases of PTPS deficiency in Thailand. Both cases were male infants who showed phenylalanine levels of 25.23 mg/dl and 23.4 mg/dl respectively. The urinary pterins analysis showed low biopterin and high neopterin. The percentage of urinary biopterin was also found to be very low. The mutation analysis of the first case revealed a point mutation of exon 4, a homozygous C to T transition at nucleotide 200 in codon 67 (T67M), and the second case showed a compound heterozygous of exon 4, C to A transition at nucleotide 200, and exon 5, C to T transition at nucleotide 259 of the PTS gene confirming that they had PTPS deficiency. Treatment was started with neurotransmitters and a low phenylalanine diet. Family carriers were detected by means of urinary pterins determination and mutation analysis.

Keywords: *Hyperphenylalaninemia, 6-Pyruvoyltetrahydropterin synthase, Tetrahydrobiopterin*

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Hyperphenylalaninemia detected by neonatal screening is caused by a deficiency of either phenylalanine hydroxylase or its cofactor, tetrahydrobiopterin (BH_4), which is required for aromatic amino acids hydroxylation. Hyperpheny-lalaninemia due to BH_4 deficiencies is caused by autosomal recessively inherited mutations affecting enzymes in the biosynthesis or regeneration of BH_4 which is required as a cofactor by various enzymes such as phenylalanine-4-hydroxylase, tyrosine-3-hydroxylase and tryptophan-5-hydroxylase. The latter two are key enzymes in the biosynthesis of neurotransmitters, dopamine and serotonin. BH_4 is synthesised in a three-step pathway from guanosine triphosphate by the enzyme guanosine triphosphate cyclohydrolase I (GTPCH), 6-pyruvoyltetrahydropterin synthase (PTPS) and sepiapterin reductase (SR). After coupling as an active cofactor to the aromatic amino acid hydroxylases, it is regenerated by pterin-4a-carbinolamine dehydratase (PCD) and dihydropteridine reductase (DHPR). A deficiency of BH_4 leads to a variant form of hyperphenylalaninemia accompanied by severe monoamine neurotransmitter shortage that results in progressive mental retardation due to limiting cofactor availability for the tyrosine and tryptophan hydroxylases[1,4,5]. A majority of the patients recognized early were diagnosed because they developed progressive cerebral deterioration despite an early neonatal diagnosis of hyperphenylalaninemia and effective dietary control of the levels of phenylalanine in blood. The frequency of BH_4 deficiency is about 1-2% of all hyperphenylalaninemic cases in Caucasians but is higher in Turkey, Taiwan and Saudi Arabia[6-8]. About 60% of all BH_4 deficiencies are caused by defects in the PTPS enzyme[7,9].

6-Pyruvoyltetrahydropterin synthase deficiency is an autosomal recessively inherited presenting with neurological signs due to impaired catechola-

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mines and serotonin synthesis. Symptoms may become evident in the first week of life but are mostly seen at an average age of 4 months[10]. A decrease in activity and loss of head control may herald the onset of a progressive neurological degenerative disease. Frequent symptoms of PTPS deficiency include characteristic truncal hypotonia, increased limb tone, postural instability, hypokinesia, choreatic or dystonic limb movements, gait difficulties, hypersalivation due to swallowing difficulties and oculogyric crises.

The gene encoding the PTPS enzyme (PTS) has been cloned and found to span ~8 kb and consist of six exons. The gene maps to chromosome 11q 22.3-q 23.3 and encodes the 145 amino acids of each subunit of the homohexameric enzyme[1,3,11-14]. 38 mutations including missense mutations, splicing mutations and small deletions spreading through six exons of the PTS gene have been described. More than 420 cases have now been reported in an international database of tetrahydrobiopterin deficiencies (2005)[7,11]. These are the first cases of PTPS deficiency reported in Thailand.

Case Report

Case 1

A one-month-old male infant, was born at term as the first child to nonconsanguineous parents after an uneventful pregnancy and delivery. Birth weight was 2,540 g and no abnormal symptoms were observed post-partum. Newborn screening at the age of 3 days revealed elevated plasma phenylalanine level of 5.3 mg/dl. Repeated study at 15 days of age showed phenylalanine of 25.23 mg/dl. He was treated with a low phenylalanine diet. At the age of 4 months, he manifested muscular hypotonia and poor head control. Electroencephalogram (EEG) and computerized tomography (CT) scan of the brain were normal. A metabolic work up was performed because of progressive psychomotor retardation. The urinary pterins analysis showed low biopterin (0.38 mmol/mol creatinine) and high neopterin (22.9 mmol/mol creatinine). The total biopterin ratio (B%) [biopterin / (biopterin + neopterin)%] was also found to be very low (1.6%). The urinary pterins and B% of the patient, his parents (obligate heterozygotes) and grandparents are shown in Table 1. The mutation analysis of this patient was found to have a point mutation of exon 4, a homozygous C to T transition at nucleotide 200 in codon 67 (T67M) of the PTS gene (Fig 1). The diagnosis of PTPS deficiency was made at the age of 5 months and therapy with L-dopa/carbidopa, 5-hydroxytrytophan in addition to a phenylalanine restricted diet was introduced. The boy is now 2 years old with microcephaly, truncal hypotonia with

Table 1. Biochemical parameters

Case	Phenylalanine (mg/dl)	Neopterin (mmol/mol creatinine)	Biopterin (mmol/mol creatinine)	B%	Diagnosis
Case 1					
1. Patient	25.23	22.9	0.38	1.6	Affected
2. Father	1.01	0.65	1.06	62.1	Heterozygote
3. Mother	1.51	0.48	0.47	49.7	Heterozygote
4. Paternal grandfather	1.19	1.43	0.91	38.9	Heterozygote
5. Paternal grandmother	1.12	0.50	2.26	82.0	Normal
6. Maternal grandfather	0.93	0.75	1.79	70.5	Normal
7. Maternal grandmother	1.24	0.93	0.81	46.5	Heterozygote
Case 2					
8. Patient	23.4	18.9	0.10	0.5	Affected
9. Father	1.07	0.41	0.36	46.4	Heterozygote
10. Mother	1.29	0.47	0.50	51.6	Heterozygote
11. Paternal grandmother	1.10	0.24	0.40	62.5	Heterozygote
12. Maternal grandfather	1.21	0.30	0.67	69.0	Heterozygote
13. Maternal grandmother	0.89	0.28	0.63	69.3	Normal
Reference range					
2 M-6 M	<2	0.9-7.49	1.73-3.68	26.2-68.4	
Adult	<2	0.15-0.52	0.38-1.14	53.3-75.8	

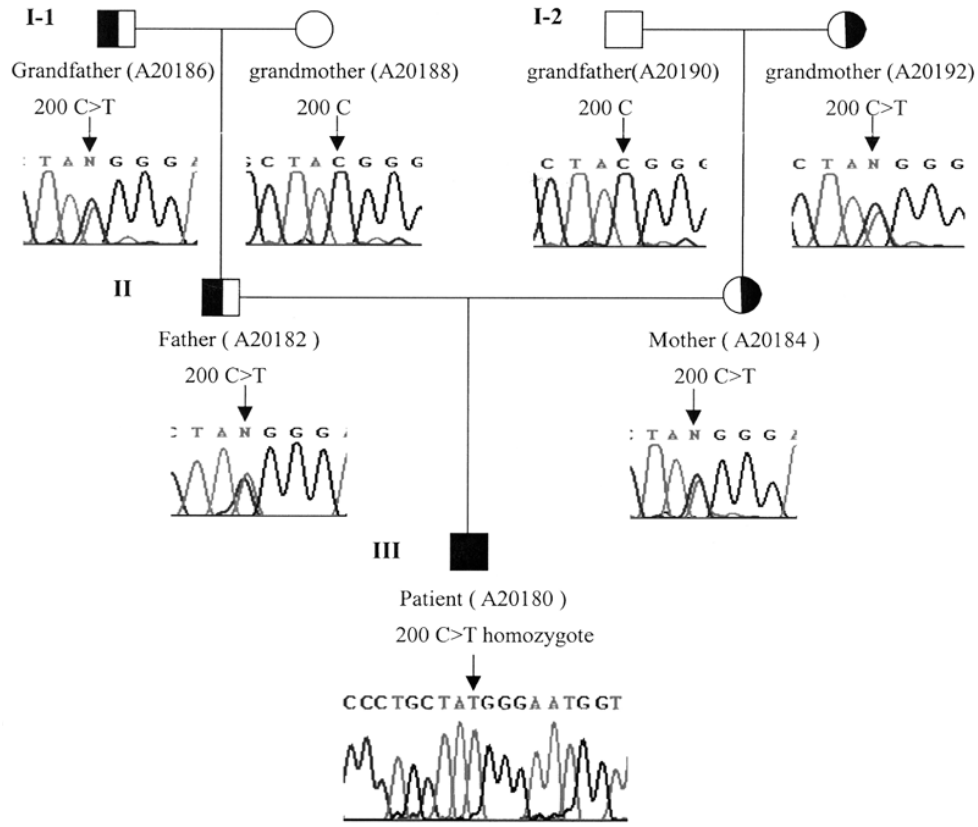

Fig. 1 Mutation identified in the PTS gene of case 1 and his family

hypertonia of the extremities, poor speech and moderately developmental delay.

Case 2

A one-month-old male infant was born to consanguineous parents (the third cousins). Birth weight was 2,700 g product of a term uncomplicated pregnancy, labor and delivery. Newborn screening for PKU was done on the third day of life. His first blood sample test showed phenylalanine level of 8 mg/dl and the second blood sample showed phenylalanine level of 23.4 mg/dl. The urinary pterins analysis showed low biopterin (0.10 mmol/mol creatinine) and high neopterin (18.9 mmol/mol creatinine) with low total biopterin ratio (0.5%). The urinary pterins and B% of the patient, his parents (obligate heterozygotes) and grandparents are shown in Table 1. The mutation analysis of the patient was found to have a compound heterozygous of exon 4, C to A transition at nucleotide 200, and exon 5, C to T transition at nucleotide 259 in codon 87 (P87S) of the PTS gene (Fig 2). He was treated with L-dopa/carbidopa, 5-hydroxytryptophan combine with a phenylalanine restricted diet. He is currently 5 months old with mild delayed development.

Discussion

The authors present 2 cases of PTPS deficiency associated with increased plasma phenylalanine levels first detected from the newborn screening program. Hyperphenylalaninemia, which is the initial observation in PTPS deficiency, is also a feature of other metabolic disorders, including phenylketonuria (PKU), guanosine triphosphate cyclohydrolase I deficiency, and dihydropteridine reductase deficiency. Since the treatment of BH_4 deficiency is different from that of PKU, the differential diagnosis of BH_4 deficiency is essential for newborns with hyperphenylalaninemia. Two laboratory diagnostic tests are commonly used to differentiate between all BH_4 deficiencies, including the analysis of the pterins in urine to differentiate BH_4 synthesis defects and PCD deficiency and the measurement of DHPR activity in blood to detect DHPR deficiency[1,2,6,10,15]. The analysis of pterins in urine of both patients were found that urinary biopterin and the total

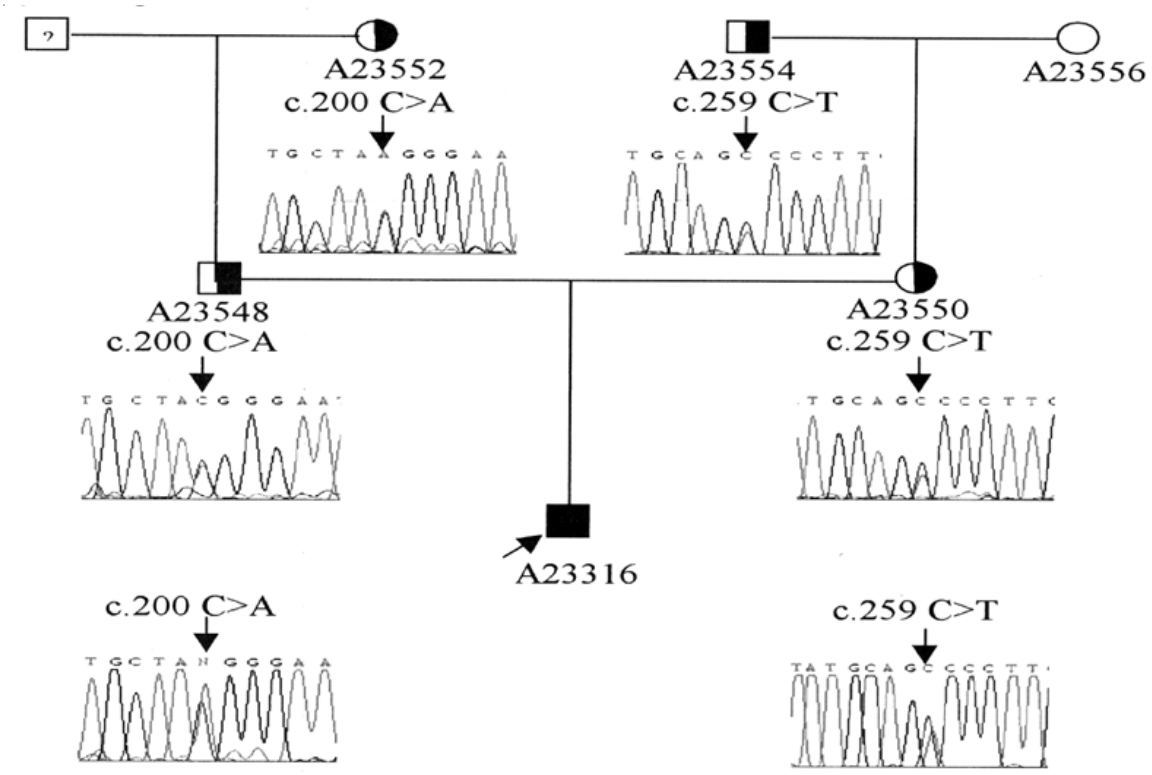

Fig. 2 Mutation identified in the PTS gene of case 2 and his family

biopterin (B%) were very low and urinary neopterin was very high the results of which were consistent with the PTPS deficiency.

The mutation analysis of case 1 was found to have a point mutation of exon 4, a homozygous C to T transition at nucleotide 200 in codon 67 (T67M) of the PTS gene. In this case, the paternal allele is a C to T mutation inherited from his grandfather and the maternal allele is C to T mutation which is inherited from his grandmother. The mutation analysis of case 2 was found to have a compound heterozygous of exon 4, C to A transition at nucleotide 200, and exon 5, C to T transition at nucleotide 259 in codon 87 (P87S) of the PTS gene. From the mutation analysis, the paternal allele is a C to A mutation inherited from his grandmother and the maternal allele is C to T mutation inherited from his grandfather. A review of the BIOMDB database was found that the C to A transition at nucleotide 200 was a novel mutation detected in the PTS gene which had not been reported previously[11].

Blood and urine of their family members found that the phenylalanine level, urinary neopterin and biopterin were within normal range. The B% values of the heterozygotes were within normal control and much higher than that of the PTPS deficiency patients. The molecular analysis of the parents, paternal grandfather and maternal grandmother of case 1 and parents, paternal grandmother and maternal grandfather of case 2 were compatible with heterozygotes of PTPS deficiency.

Both patients were diagnosed to have PTPS deficiency rather late because screening for BH_4 deficiencies was not an integral part of the newborn screening for PKU in Thailand. After they received treatment with neurotransmitters (L-dopa/carbidopa, 5-hydroxytrytophan) with a phenylalanine restricted diet, the clinical conditions had a positive response especially the hypertonicity of case 1, which was a gradual decrease. The present study emphasizes that early detection and treatment are very important for BH_4 deficiencies to prevent mental retardation.

Conclusion

The neonatal screening program is beneficial for patients in early detection and treatment especially in the prevention of mental retardation. Screening for BH_4 deficiencies should be included in every hyperphenylalaninemic infant. Mutation analysis at the

DNA level can be used as a confirmation method to detect heterozygote in families whose mutation type has been identified.

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โรคที่เกิดจากการขาดเอนไซม์ 6-pyruvoyltetrahydropterin synthase: รายงานผู้ป่วย 2 ราย

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การขาดเอนไซม์ 6-pyruvoyltetrahydropterin synthase เป็นสาเหตุที่พบบ่อยที่สุดของภาวะเฟ็นนิลอะลานีนสูงในเลือดที่เกิดจากการขาดสาร tetrahydrobiopterin อาการแสดงของผู้ป่วยโรคนี้ได้แก่ภาวะปัญญาอ่อน ชัก มีความผิดปกติของการตึงตัวของกล้ามเนื้อ ซึม หงุดหงิดง่าย การเคลื่อนไหวที่ผิดปกติ น้ำลายไหล และกลืนอาหารลำบาก ได้รายงานผู้ป่วยจำนวน 2 รายที่เกิดจากการขาดเอนไซม์ 6-pyruvoyltetrahydropterin synthase เป็นครั้งแรกในประเทศไทยโดยทั้งคู่ได้รับการตรวจพบว่ามีระดับของสารเฟ็นนิลอะลานีนในเลือดที่สูงผิดปกติจากการตรวจกรองทารกแรกเกิด ผลการตรวจหาระดับของสารเฟ็นนิลอะลานีนในผู้ป่วยทั้ง 2 รายได้ค่าเท่ากับ 25.23 mg/dl และ 23.4 mg/dl ตามลำดับ จากการตรวจวัดระดับของสาร pterins ในปัสสาวะพบว่าระดับของ biopterin มีค่าอยู่ในระดับที่ต่ำ ส่วน neopterin มีค่าอยู่ในระดับที่สูง และจากการคำนวณหาค่าร้อยละของ biopterin ในปัสสาวะพบว่ามีค่าที่ต่ำมาก จากการตรวจวิเคราะห์หาการผ่าเหล่าโดยวิธีทางอณูวิทยา ในผู้ป่วยรายแรกพบว่ามีการผ่าเหล่าของยีน PTS ชนิด homozygous บนแอกซอนที่ 4 นิวคลีโอไทด์ตำแหน่งที่ 200 โดยเปลี่ยนจาก C เป็น T ส่วนในผู้ป่วยรายที่ 2 พบว่ามีการผ่าเหล่าชนิด compound heterozygous บนแอกซอนที่ 4 นิวคลีโอไทด์ตำแหน่งที่ 200 โดยเปลี่ยนจาก C เป็น A และบนแอกซอนที่ 5 นิวคลีโอไทด์ที่ 259 โดยเปลี่ยนจาก C เป็น T ผู้ป่วยทั้ง 2 รายได้รับการรักษาด้วยอาหารที่มีสารเฟ็นนิลอะลานินต่ำร่วมกับการให้สารส่งผ่านประสาทและได้ทำการตรวจหาพาหะในครอบครัวโดยการตรวจวัดระดับของสาร pterins ในปัสสาวะร่วมกับการตรวจหาการผ่าเหล่าโดยวิธีทางอณูวิทยา