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GUANOSINE TRIPHOSPHATE CYCLOHYDROLASE I DEFICIENCY*
A Rare Cause of Hyperphenylalaninemia

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Tetrahydrobiopterin (BH_4) deficiencies are a heterogeneous group of disorders caused by a defect in two of the three enzymes involved in its biosynthesis or in the two recycling enzymes. Except for the deficiency of dehydratase, an enzyme catalyzing a reaction in the recycling pathway, all other variants of BH_4 deficiency are characterized by developmental delay, progressive neurological deterioration, hypokinesis, drooling, swallowing difficulty, truncal hypotonia, increased limb tone, myoclonus and brisk deep tendon reflexes.

A deficiency of guanosine triphosphate cyclohydrolase I (GTPCH), the first enzyme in the biosynthetic pathway of BH_4, is described in a 14-month-old male infant with hyperphenylalaninemia, developmental delay, hypertonia of the extremities, seizures, feeding difficulties, and vomiting. Urinary pteridine screening revealed very low levels of neopterin and biopterin which was highly suggestive of GTPCH deficiency. Low cerebrospinal fluid concentrations of 5-hydroxyindoleacetic acid (5HIAA) and homovanillic acid concentrations, together with no detectable neopterin and decreased concentrations of biopterin and folate, agreed with the diagnosis of GTPCH deficiency. Subsequently measured neopterin and biopterin synthesis in cytokine-stimulated skin fibroblasts confirmed GTPCH deficiency, albeit indirectly. The patient showed marked improvement on a low-protein low-phenylalanine diet with neurotransmitter precursor administration. The favorable outcome in this patient clearly shows that not only newborns with elevated phenylalanine levels but also older children with neurological signs and symptoms should be secreened for a BH_4 deficiency in order to have maximum benefit of the treatment. *Key words: hyperphenylalaninemia, guanosine triphosphate cyclohydrolase I deficiency, neopterin, biopterin, neurotransmitters, neonatal screening.*

Primary forms of hyperphenylalaninemia (HPA) result from a deficiency of either the apoenzyme, phenylalanine hydroxylase (PAH), or its cofactor, tetrahydrobiopterin (BH_4), which are required for the conversion of phenylalanine (Phe) to tyrosine (Tyr). BH_4 is the cofactor required not only by PAH but also

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by tyrosine and tryptophan hydroxylases, and its deficiency leads to defective catecholamines and serotonin synthesis in the central nervous system. Unlike patients with PAH deficiency due to neurotransmitter deficiency, BH_4-deficient patients usually do not experience a good neurological outcome on a low-Phe diet alone[1-3].

Variant forms of phenylketonuria (PKU) and HPA may be due to genetic defects in either the recycling or the de novo synthesis of BH_4. This cofactor is synthesized in vivo from purine nucleotide guanosine triphosphate (GTP). Conversion of GTP to a phosphorylated neopterin derivative, the first reaction in the BH_4 synthetic pathway, is catalyzed by guanosine triphosphate cyclohydrolase I (GTPCH)[1-3].

Compared to classical PKU (1:10,000), defects in BH_4 metabolism are rare, with an incidence of ≈1:500,000 to 1:1 million births; GTPCH deficiency is even more rare[1,3]. Considering its rare occurrence, we wish to present detailed clinical and biochemical features of a new case.

Case Report

A 14-month-old male infant was referred for evaluation of developmental delay, seizures, feeding difficulty, and vomiting. He was born at term to a 24-year-old gravida 1, para 1. The birth weight was 2,500 g. The parents were first cousins. His medical history was remarkable for noticeably increased muscle tone in the extremities and frequent seizure episodes starting at four months of age. He was treated with phenobarbital and valproic acid with almost no decrease in the frequency of seizures.

Physical examination revealed a spastic infant with normal vital signs. His weight was 6,150 g ($< 5^{th}$ percentile); length 68 cm ($< 5^{th}$ percentile), and head circumference, 44 cm ($< 5^{th}$ percentile). Neurological examination revealed spasticity of the extremities, hypotonicity of the trunk, and increased deep tendon reflexes. He was unable to control his head, to walk, or to speak. The remainder of the physical examinatilon results were unremarkable.

On laboratory evaluation complete blood count, routine urinalysis and blood chemistry were all within normal ranges. Hyperphenylalaninemia was detected by amino acid chromatography. Serum Phe concentration was 7.7 mg/dl (465 mol/L). The rather low urinary neopterin and biopterin concentrations, with a neopterin to biopterin ratio of approximately 1 as measured by HPLC on samples collected on filter paper[4], suggested a defect in GTPCH. After a loading test with BH_4 (20 mg/kg), serum Phe and Tyr concentrations were as follows: 0-hour Phe: 5.7 mg/dl (343 µmol/L), Tyr: 3.1 mg/dl (172 µmol/L); 4-hour Phe: 1.56 mg/dl (94 µmol/L), Tyr: 2.4 mg/dl (131 µmol/L): and 8-hour Phe: 1.0 mg/dl (61 µmol/L), Tyr: 2.7 mg/dl (147 µmol/L). Low cerebrospinal fluid (CSF) concentrations of 5-hydroxyindoleacetic acid (5HIAA) and homovanillic acid (HVA) concentrations, together with no detectable neopterin and decreased concentrations of biopterin and folate, agreed with the diagnosis of GTPCH deficiency

(Table I). Measurement of neopterin and biopterin synthesis in cytokine-stimulated skin fibroblasts, an indirect method of measuring GTPCH activity[5], confirmed the diagnosis (Fig. 1). Electroencephalography (EEG), cranial computerized tomography (CT), visual evoked potential (VEP), and brain-stem auditory evoked potential (BAEP) yielded normal findings.

Table I: Some of the Laboratory Data of the Patient

URINE	
Neopterin (mmol/mol creatinine) normal: 1.1-4.0	0.14
Biopterin (mmol/mol creatinine) normal: 0.5-3.0	0.17
PLASMA	
Phenylalanine (µmol/L) normal: < 120	465
RED BLOOD CELLS	
DHPR activity (mU/mg Hb) normal: 2-5	2.5
FIBROBLASTS*	
Biopterin (pmol/mg protein) controls: 160-350	22
Neopterin (pmol/mg protein) controls: 20-70	8
CSF	
5HIAA (nmol/L) normal: 224 (114-326)	127
HVA (nmol/L) normal: 660 (295-932)	79
3OMD (nmol/L) normal: < 50	112
5OHTrp (nmol/L) normal: < 10	8
L-dopa (nmol/L) normal. < 25	< 5
5MTHF (nmol/L) normal: 63-111	24
Neopterin (nmol/L) normal: 9-30	0
Biopterin (nmol/L) normal: 10-40	9

* stimulated with interferon-γ/tumor necrosis factor-α[5].
DHPR: dihydropteridine reductase, GTPCH: guanosine triphosphate cyclohydrolase I, CSF: cerebrospinal fluid, 5HIAA: 5-hydroxyindoleacetic acid, HVA: homovanillic acid, 3OMD: 3-O-methyldopa, 5OHTrp: 5-hydroxytryptophan, 5MTHF: 5-methyltetrahydrofolic acid.

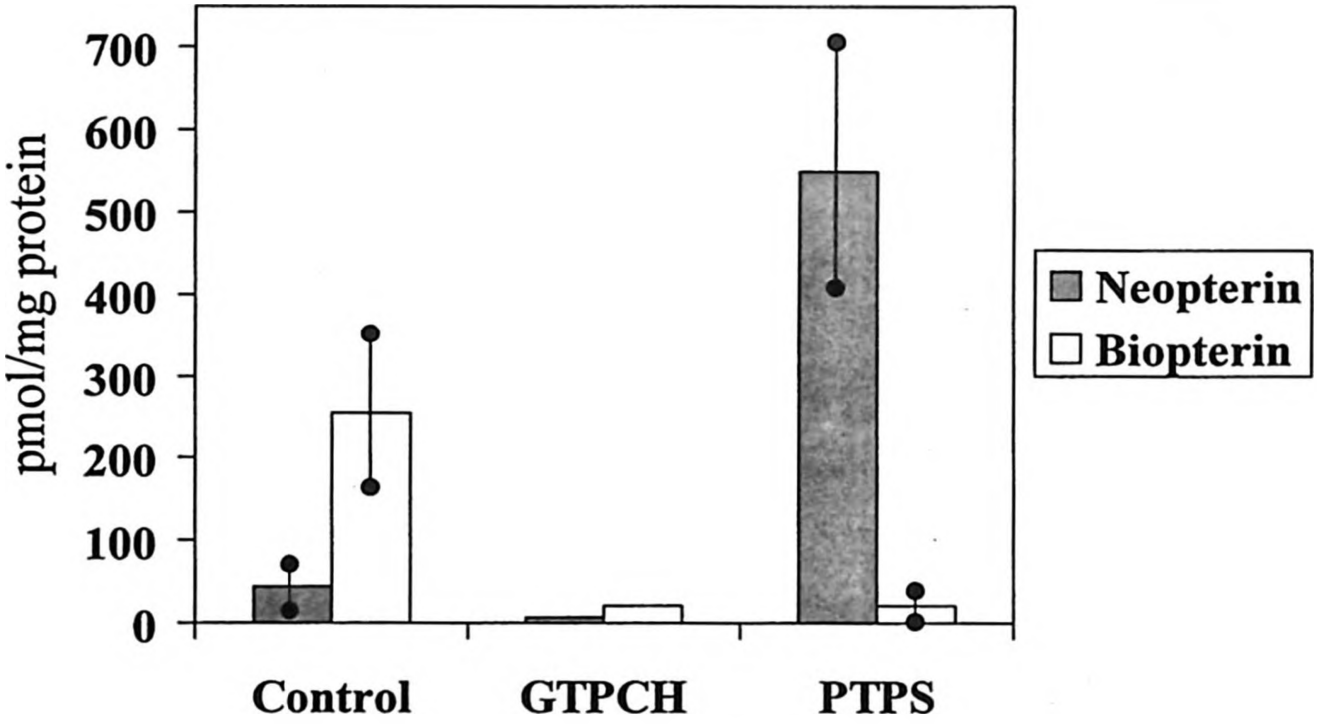

Fig. 1: Neopterin and biopterin concentrations in fibroblasts from controls, our patient with the GTPCH deficiency, and from PTPS-deficient patients after 24 hours stimulation with interferon-γ/tumor necrosis factor-α. The graph shows median values and the range. Neopterin and biopterin production is an indirect measure for the GTPCH activity[5].

A Phe-restricted diet was introduced along with neurotransmitter precursors replacement therapy consisting of L-dopa/carbidopa (7.5-10 mg/kg/day) and 5-hydroxytrytophan (7.5-10 mg/kg/day). Synthetic BH_4 was unfortunately not available. Doses of neurotransmitter precursors were adjusted according to serum prolactin levels (initial: 18.8 ng/ml; after substitution with 7.5 mg/kg/day L-dopa/carbidopa and 5-hydroxytryptophan: 9.3-15.0 ng/ml; after short time withdrawal: 21.0 ng/ml; normal 2.5-17.0 ng/ml). The patient is now $2^1/_2$ years old and is able to walk and speak a few words, and experiences almost no seizure activity.

Discussion

The frequency of various inborn errors of metabolism is quite high in Turkey, where the rate of consanguineous marriages continues to be high[6,7]. Aminoacidopathies, particularly PKU (1:6000) and other forms of HPAs (1:12,500), are the most common metabolic disorders[6,8]. Based on the internationally gathered data, BH_4 deficiencies account for one to three percent of all HPAs[9]. Although we do not routinely screen all hyperphenylalaninemic newbrons for BH_4 deficiencies, the data of the international registry points to a relatively high incidence (15%) in Turkey[9]. According to the international database most of the patients with BH_4 deficiency suffer from 6-pyruvoyl-tetrahydropterin synthase

(PTPS) deficiency (58%), followed by dihydropteridine reductase (DHPR) deficiency (35%), GTPCH deficiency (3%), and "primapterinuria" (4%)[2, 9]. Turkey, with a high number of cases with DHPR deficiency, has a different pattern of BH_4 deficiencies[10]. As can easily be seen from the international data, GTPCH deficiency is the rarest form. Since its first description in 1984 by Niederwieser et al.[11], there have been 16 cases recorded (including the present case) in the database (BIODEF, http://www.unizh.ch/~blau/bh4.html). To the best of our knowledge, this is the first documented case from Turkey.

Patients with BH_4 deficiency, except for those with pterin-4a-carbinolamine dehydratase (PCD) deficiency, exhibit a clinical picture that is characterized by developmental delay and progressive neurological deterioration, tremors, hypokinesis, drooling, drowsiness, irritability, swallowing difficulty, oculogyric crises, recurrent episodes of hyperthermia in the absence of infections, truncal hypotonia, increased limb tone, myoclonus or dystonic limb movements, and very brisk deep tendon reflexes. These symptoms, resulting from the biogenic amine deficiency, fluctuate diurnally in their intensity. Affected patients usually present with one or more of the signs and sympdoms listed above within the first few months of life (the median age at which clinical signs become evident is 4-5 months)[1-3, 9, 10]. Increased limb tone, seizures, developmental delay, and feeding difficulty and vomiting, most probably due to difficulty in swallowing, were noticed by our patient's parents at around four months of age. Most of the patients with BH_4 deficiency are born with a low birth weight as was the case in the presented patient[9]. Untreated patients develop progressive microcephaly with bioelectric activity abnormalities on EEG tracings and cerebral atrophy on CT scan or magnetic resonance imaging (MRI)[3]. Our patient was microcephalic with no EEG, CT, VEP, or BAEP abnormalities.

Defects involving the BH_4 metabolic pathway can be differentially diagnosed by measurement of the urinary neopterin and biopterin levels[2, 3, 9]. Guanosine triphosphate cyclohydrolase I deficiency is characterized by neopterin and biopterin deficiency with a neopterin to biopterin ratio of ≈ 1. In CSF, neopterin, biopterin, as well as the neurotransmitter metabolites such as HVA and 5HIAA are generally low[2, 3, 9, 12]. Our patient met all these laboratory criteria, and had responded to BH_4 loading with a decrease in serum Phe concentrations following cofactor administration, as is expected in BH_4 synthesis defects. Normal activity of dihydropteridine reductase in erythrocytes excluded the possibility of a regeneration defect. Based on the results of urinary pteridine measurements and of the BH_4 loading test, GTPCH deficiency was suspected in the present case and was confirmed by indirect measurement of enzyme activity in cultured fibroblasts (Table I; Fig. 1).

Untreated severe deficiency of neurotransmitters in the central nervous system may lead to death in some of the BH_4-deficient patients[1-3, 9]. Accurate and early diagnosis of such cases is, therefore, essential. Treatment is aimed at decreasing blood Phe levels by putting the patient on a low-protein and low-Phe diet or, even better, on BH_4 substitution, and normalizing low CSF levels of the neurotransmitters by oral administration of the neurotransmitter precursor, L-dopa and 5-hydroxytryptophan, together with a decarboxylase inhibitor (carbidopa). The efficiency of treatment should be monitored by regular analysis of neurotransmitter metabolites in CSF[3, 9]. Such an investigation, however, requires frequent lumbar punctures and thus cannot be easily repeated. Recently, the serum prolactin level was found to be a good indicator of L-dopa. If there is no effective inhibition on prolactin by L-dopa[13], serum prolactin levels increase[14]. Our patient's therapeutic regimen consisted of a low-protein and low-Phe diet, L-dopa/carbidopa, and 5-hydroxytryptophan. Doses of neurotransmitter precursors were increased to levels maintaining the serum prolactin concentration within normal limits. The dietary tolerance of Phe is higher in BH_4 deficiency (300-700 mg/day) than in classical PKU, and the dietary Phe tolerance increases markedly with age in these patients[3]. The present patient currently tolerates 680 mg Ph/day.

Treatment is much more effective when initiated as soon as possible after birth[3, 9]. Although in our patient treatment started at 14 months of age, he was doing well neurologically as evidenced by improvement in muscle tone, and being more active, able to walk and speak, and almost free of seizures. No doubt, the patient would have been doing better neurologically if his illness had been detected shortly after birth. Therefore, it is recommended that every newborn with even slight but persistent HPA be tested for a BH_4 deficiency[3, 9]. Recently, it has been shown that some variants of BH_4 deficiency may present without HPA and that some newborns may be missed by the PKU newborns screening program[15, 16]. Establishment of early and correct diagnosis is essential not only for effective treatment but also for giving parents the chance of prenatal diagnosis in a subsequent pregnancy. In GTPCH deficiency, affected families may benefit from prenatal diagnosis by the measurement of amniotic fluid neopterin and biopterin concentrations, though these are less reliable[2, 3, 9], and by the more accurate study of the GTPCH gene, which is mapped to chromosome 14q22.1-q22.2[17]. The family of the presented case will be offered this service since their only child thus far is affected by GTPCH deficiency.

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