Molecular analysis and long-term follow-up of patients with different forms of 6-pyruvoyl-tetrahydropterin synthase deficiency

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Abstract The outcome of 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency, the most common form of tetrahydrobiopterin (BH₄) deficiency, depends on factors such as severity of the disease, type of mutation, time of diagnosis, and mode of treatment. We investigated five patients from four different families, four of them presenting with the severe form of PTPS deficiency and one with the mild peripheral form. In this study, missense (L26F, T67M, P87L, V124L, D136G, D136V) and nonsense (R15–16ins) mutations were detected by reverse transcriptase polymerase chain reaction and sequence analysis. Two patients with the severe form were compound heterozygotes (T67M/P87L and D136G/R15–16ins), two siblings were homozygous for the D136V mutation, and in the patient with the mild form, heterozygous L26F/V124L mutations were present. Two patients are on combined therapy with l-dopa/carbidopa/5-hydroxytryptophan plus BH₄, the siblings are on monotherapy with BH₄, and the patient with the mild form is now off treatment, presenting with normal plasma phenylalanine levels.

Conclusion Long-term follow-up shows that the outcome of 6-pyruvoyl-tetrahydropterin synthase deficiency benefits from treatment started in the first months of life and that the phenotype may change with age. Additionally, depending on the type of mutations, prenatal damage to the fetus may multiply the clinical abnormalities and thus worsen the prognosis of the disease. In patients initially diagnosed with the mild peripheral form of the disease, therapy with tetrahydrobiopterin should be stopped after some time to test whether hyperphenylalaninaemia was only a transient condition.

Key words Hyperphenylalaninaemia · Mutation analysis · Neurotransmitters · Tetrahydrobiopterin

Abbreviations BH₄ tetrahydrobiopterin · 5HIAA 5-hydroxyindoleacetic acid · HPA hyperphenylalaninaemia · 5HPT 5-hydroxytryptophan · HVA homovanillic acid · PTPS 6-pyruvoyl-tetrahydropterin synthase
Introduction

Hyperphenylalaninaemia (HPA) due to tetrahydrobiopterin (BH₄) deficiencies represents a heterogeneous group of progressive neurological disorders caused by autosomal recessively inherited mutations affecting enzymes in the biosynthesis or regeneration of BH₄ [13]. BH₄ is the natural cofactor for phenylalanine (Phe), tyrosine, and tryptophan hydroxylase as well as for all three forms of nitric oxide synthase [29, 34] (Fig. 1). It is synthesised in a three-step pathway from GTP by the enzymes GTP cyclohydrolase 1 (EC 3.5.4.16), 6-pyruvoyl-tetrahydropterin synthase (PTPS; EC 4.6.1.10), and sepiapterin reductase (EC 1.1.1.153). After coupling as an active cofactor to the aromatic amino acid hydroxylases, it is regenerated by pterin-4a-carbinolamine dehydratase (EC 4.2.1.96) and dihydropteridine reductase (EC 1.6.99.7) [53].

In almost all cases, BH₄ deficiency presents with neurological signs linked to impaired catecholamines and serotonin synthesis. Symptoms may become evident in the first weeks of life, but are mostly seen at an average age of 4 months [20, 9]. Most infants are born small for gestational age [46]. Abnormal signs in the neonatal period may include poor sucking, impaired tone, and microcephaly. Frequent symptoms of PTPS deficiency, the most common form of BH₄ deficiency, resemble Parkinson disease, indicating a lack of dopamine in the basal ganglia [3]. Extrapyramidal signs 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. Ataxia, hyperreflexia, hypothermia as well as episodes of hyperthermia (in the absence of infections), drowsiness, irritability, disturbed sleep patterns, and convulsions (grand mal or myoclonic) are often seen. In addition, ptosis and pinpoint pupils, presumably due to dysfunction of the oculosympathetic pathway, are observed [8, 39].

Among the most prevalent variants of PTPS deficient HPA, at least two different phenotypes are described. The more common severe central (typical) form is accompanied by the above mentioned abnormalities of biogenic amines in CSF, as assessed by CSF measurement of catecholamines and serotonin metabolites [22]. These patients usually require a combined treatment with BH₄ and the neurotransmitter precursors L-dopa/carbidopa and 5-hydroxytryptophan (5HTP) [1]. In contrast, the rare mild peripheral (atypical) form of PTPS deficiency is characterised by normal neurotransmitter homeostasis and moderate or transient HPA [36]. These patients need monotherapy with BH₄ in order to maintain normal plasma Phe levels.

Reports about the long-term treatment and outcome of patients with BH₄ deficiency are still scarce [2, 44, 47, 49]. Many of the patients diagnosed late are retarded and some diagnosed in the first weeks of life did not respond clinically to different treatment protocols. About 9% of all PTPS-deficient patients registered in the international BIODEF database died [8]. Although 33 different mutations have been detected [12, 27, 33, 51, 52, 54], only some have been tested for functionality and in only a few cases data on phenotype-genotype correlation are available [11, 38, 43]. In this paper we describe new mutations and long-term follow-up in five PTPS-deficient patients with various clinical outcomes.

Fig. 1 Biosynthesis, regeneration, and functions of tetrahydrobiopterin. (AADC aromatic amino acid decarboxylase, AR aldose reductase, CR carbonyl reductase, DHPD dihydropteridine reductase L-dopa 3,4-dihydroxyphenylalanine, GTPCH GTP cyclohydrolase 1, SHAA 5-hydroxyindoleacetic acid, HVA homovanillic acid, 5-OH-Trp 5-hydroxytryptophan, NOS nitric oxide synthase, PAH phenylalanine-3-hydroxylase, PCD pterin-4a-carbinolamine dehydratase, SR sepiapterin reductase, TH tyrosine-4-hydroxylase, TPH tryptophan-5-hydroxylase, PTPS 6-pyruvoyl-tetrahydropterin synthase)