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SYMPOSIUM ON NEUROTRANSMITTER DISORDERS

Disorders of biopterin metabolism

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Summary Defects in the metabolism or regeneration of tetrahydrobiopterin (BH_4) were initially discovered in patients with hyperphenylalaninaemia who had progressive neurological deterioration despite optimal metabolic control (malignant hyperphenylalaninaemia). BH_4 is an essential cofactor not only for phenylalanine hydroxylase, but also for tyrosine and two tryptophan hydroxylases, three nitric oxide synthases, and glyceryl-ether monooxygenase. Defective activity of tyrosine and tryptophan hydroxylases explains the neurological deterioration in patients with BH_4 deficiency with progressive mental and physical retardation, central hypotonia and peripheral spasticity, seizures and microcephaly. Five separate genetic conditions affect BH_4 synthesis or regeneration: deficiency of GTP cyclohydrolase I, 6-pyruvoyl tetrahydropterin synthase, sepiapterin reductase, dihydropteridine reductase (DHPR) and pterin-4α-carbinolamine dehydratase. Only the latter of these conditions is relatively benign and is associated with transient hyperphenylalaninaemia. All these conditions can be identified in newborns by an elevated phenylalanine, with the exception of sepiapterin reductase and the dominant form of GTP cyclohydrolase I deficiency that results in biopterin deficiency/insufficiency only in the brain. Diagnosis relies on the measurement of pterin metabolites in urine, dihydropteridine reductase in blood spots, neurotransmitters and pterins in the CSF and on the demonstration of reduced enzyme activity (red blood cells or fibroblasts) or causative mutations in the relative genes. The outcome of BH_4 deficiency is no longer malignant if therapy is promptly initiated to reduce plasma phenylalanine levels and replace missing neurotransmitters. This is accomplished by a special diet and/or BH_4 supplements and administration of L-dopa, carbidopa, 5-hydroxytryptophan, and, in certain cases, a MAO-B inhibitor. Patients with DHPR deficiency also require folinic acid supplements, since DHPR may help in maintaining folate in the tetrahydro form. Several patients with BH_4 deficiency treated since the newborn period have reached adult age with good outcome.

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References to electronic databases: 6-Pyruvoyl-tetrahydropterin synthase: OMIM 261640. Dihydropteridine reductase: OMIM 261630. Dopa-responsive dystonia: OMIM 128230. GTP cyclohydrolase (GTPCH) I deficiency: OMIM 233910, 600225. GTP-cyclohydrolase I feedback regulatory protein: OMIM 602437. GTP-cyclohydrolase I: EC 3.5.4.16. Pterin-4α-carbinolamine dehydratase: OMIM 126090. Sepiapterin reductase: OMIM 182125; EC 1.1.1.153.

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Abbreviations

BH_4	tetrahydrobiopterin
CSF	cerebrospinal fluid
DHPR	dihydropteridine reductase
GFRP	GTP cyclohydrolase I feedback regulatory protein

GTPCH	GTP cyclohydrolase I
5-HIIA	5-hydroxyindoleacetic acid
HVA	homovanillic acid
PCD	pterin-4-α-carbinolamine dehydratase
PTPS	6-pyruvoyl-tetrahydropterin synthase
SR	sepiapterin reductase

History of biopterin

Pterins were initially isolated as yellow purine-like pigments from butterflies (Lepidoptera) (1889–95) (Hopkins 1889, 1942). The structure of pterins was elucidated and their purification was accomplished in the 1930s (summarized in Schopf 1964). After the discovery that phenylketonuria was caused by an abnormality of phenylalanine hydroxylase, the structure of the cofactor required for enzyme activity, tetrahydrobiopterin, was elucidated in 1963 (Kaufman 1963). In the 1970s, tetrahydrobiopterin and precursors were chemically synthesized (Schircks et al 1976) and found to be required for several other hydroxylases including tyrosine and neuronal tryptophan hydroxylase, the rate-limiting steps in the production of the neurotransmitters dopamine and serotonin (reviewed in Hyland 2007). All these enzymes are monooxygenases that incorporate one atom of oxygen from molecular oxygen (O_2) into the substrate and reduce the second atom to water. Tetrahydrobiopterin (BH_4, 2-amino-6-(1,2-dihydroxypropyl)-5,6,7,8-tetrahydro-1*H*-pteridin-4-one, $C_9H_{15}N_5O_3$, MW 241.2) provides the two electrons required for the reduction of the second atom to water and therefore acts as substrate rather than a tightly bound cofactor as several vitamins do with other enzymes.

The medical importance of BH_4 became evident with the discovery of atypical variants of hyperphenylalaninaemia. Hyperphenylalaninaemias result from impaired conversion of phenylalanine to tyrosine. The most common and clinically important is classic phenylketonuria, which is characterized by an increased concentration of phenylalanine in blood, increased concentrations of phenylalanine and its by-products (notably phenylpyruvate, phenylacetate, and phenyllactate) in urine, and severe mental retardation if untreated in infancy. Classic phenylketonuria results from reduced activity of phenylalanine hydroxylase, an enzyme expressed only in the liver in humans. A special diet low in phenylalanine and supplemented with tyrosine initiated within 2 weeks of age can prevent mental retardation in patients with classic phenylketonuria.

In the 1970s, it became evident that a subgroup of patients with hyperphenylalaninaemia developed neurological complications despite prompt dietary treatment (Bartholomé 1974; Rey et al 1976; Smith et al 1975). These patients had atypical forms of phenylketonuria caused by mutations in genes required for tetrahydrobiopterin synthesis or regeneration (Kaufman et al 1978). These atypical forms of phenylketonuria affect 1–3% of the total patients with phenylketonuria with an estimated combined frequency of 1:500 000–1:1 000 000 births. Improved synthetic chemistry lead to the availability of limited amounts of biopterin compounds in the late 1970s (Schircks et al 1976). Administration of this preparation to patients with atypical phenylketonuria rapidly normalized plasma phenylalanine levels, independently from diet (Schaub et al 1978).

Tetrahydrobiopterin is also a cofactor of nitric oxide synthases, increasing their activity and NO production (Schmidt and Alp 2007). Suboptimal concentrations of BH_4 in the endothelium might reduce the biosynthesis of NO, thus contributing to the pathogenesis of vascular endothelial dysfunction (Katusic et al 2009), although no specific abnormalities in this pathway have yet been demonstrated in patients with impaired synthesis or regeneration of tetrahydrobiopterin. Finally, tetrahydrobiopterin is essential for the activity of glyceryl-ether monooxygenase that cleaves lipid ethers into glycerol and the corresponding aldehyde (Watschinger et al 2009), but the physiological relevance of this enzyme has not yet been fully established.

Biosynthesis of tetrahydrobiopterin

The identification of multiple patients with atypical phenylketonuria, knowledge of the synthetic system in lower organisms, the availability of more refined biochemical techniques, and many years of intense study have clarified the biosynthesis of tetrahydrobiopterin in humans (Fig. 1).

The rate-limiting reaction in the synthesis of tetrahydrobiopterin is catalysed by GTP cyclohydrolase I (Niederwieser et al 1984) (GTPCH, OMIM 600225; EC 3.5.4.16). This enzyme is regulated by the GTP cyclohydrolase I feedback regulatory protein (GFRP, GCHFR, OMIM 602437), allowing inhibition of synthesis when there is excess tetrahydrobiopterin and a stimulation of synthesis when phenylalanine levels are increased. Phenylalanine favours the binding of GFRP to GTP cyclohydrolase I and its subsequent activation (Maita et al 2002). The stimulation by phenylalanine

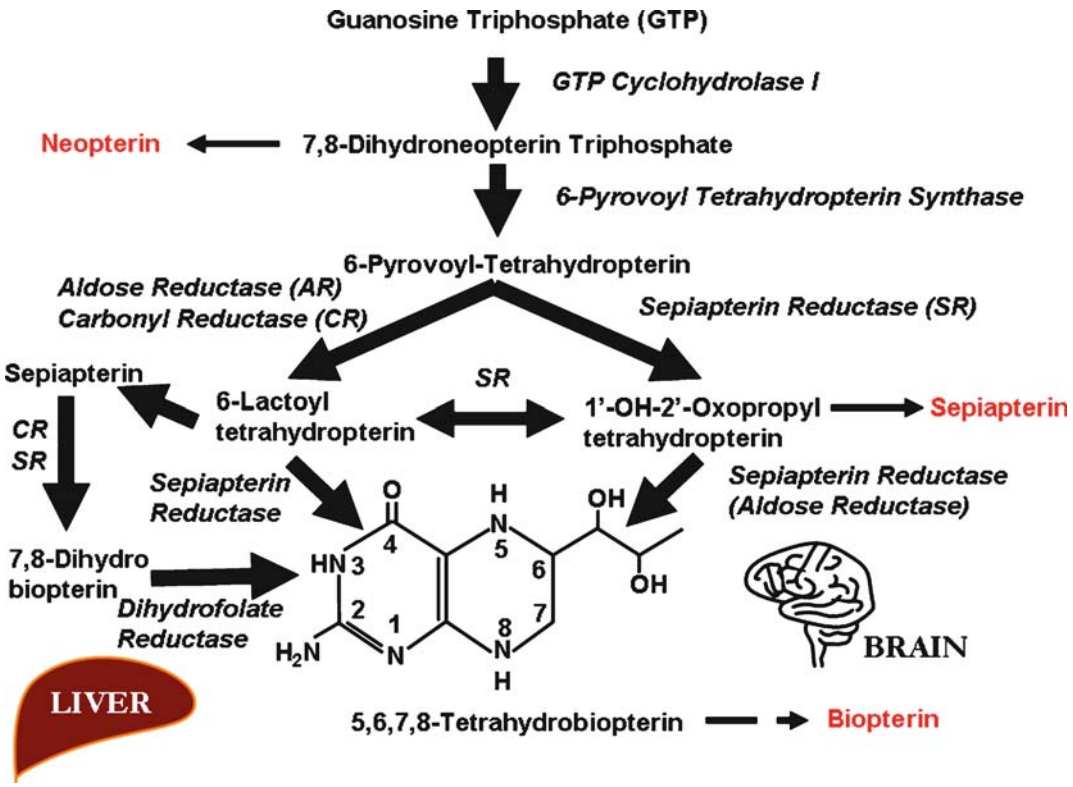

Fig. 1 Biosynthesis of tetrahydrobiopterin (BH_4). BH_4 synthesis requires three different enzymes. Some steps can be performed differently in the brain as compared to the liver. The origin of the commonly measured neopterin, biopterin, and sepiapterin is indicated

explains why levels of neopterin and biopterin are different in patients with classic phenylketonuria (phenylalanine hydroxylase deficiency) compared with controls, even though these patients have normal synthesis of tetrahydrobiopterin.

6-Pyruvoyl-tetrahydropterin synthase (PTPS, MIM 261640) removes triphosphate from 7,8-dihydroneopterin triphosphate and operates an internal redox transfer to generate 6-pyruvoyl-tetrahydropterin that is then converted to BH_4 by sepiapterin reductase (SR, OMIM 182125; EC 1.1.1.153). SR catalyses a two-step reaction and, in physiological conditions, is the only enzyme required to complete the synthesis of BH_4. Aldose reductase (AR), carbonyl reductase (CR), and dihydrofolate reductase can also convert 6-pyruvoyl-tetrahydropterin to BH_4. In fact, sepiapterin can be formed non enzymatically after reactions catalysed by SR, AR or CR, then carbonyl reductase (or SR) can convert it into 7,8-dihydrobiopterin that can be transformed into tetrahydrobiopterin by dihydrofolate reductase (Fig. 1). With SR deficiency, sufficient amounts of tetrahydrobiopterin can be synthesized in the liver (due to different levels of the CR, AR and dihydrofolate reductase enzymes), but not in the brain, explaining why patients with SR deficiency do not have hyperphenylalaninaemia (Bonafe et al 2001) but only the neurological dysfunction.

Regeneration of tetrahydrobiopterin

Tetrahydrobiopterin provides electrons during the reaction required to hydroxylate substrates (phenylalanine, tyrosine and tryptophan, Fig. 2) and, as a result, is oxidized to its hydroxyl compound pterin-4α-carbinolamine. Pterin-4α-carbinolamine dehydratase (PCD, OMIM 126090) converts pterin-4α-carbinolamine to quininoid dihydropterin (q-dihydrobiopterin), which is regenerated to BH_4 by dihydropteridine reductase (DHPR, OMIM 261630). The first reaction can also occur nonenzymatically, probably explaining the milder phenotype (Curtius et al 1990). By contrast, DHPR deficiency was one of the first defects of biopterin metabolism identified in humans that was named "malignant hyperphenylalaninaemia" and that can still results in a severe phenotype despite therapy (Kaufman et al 1975).

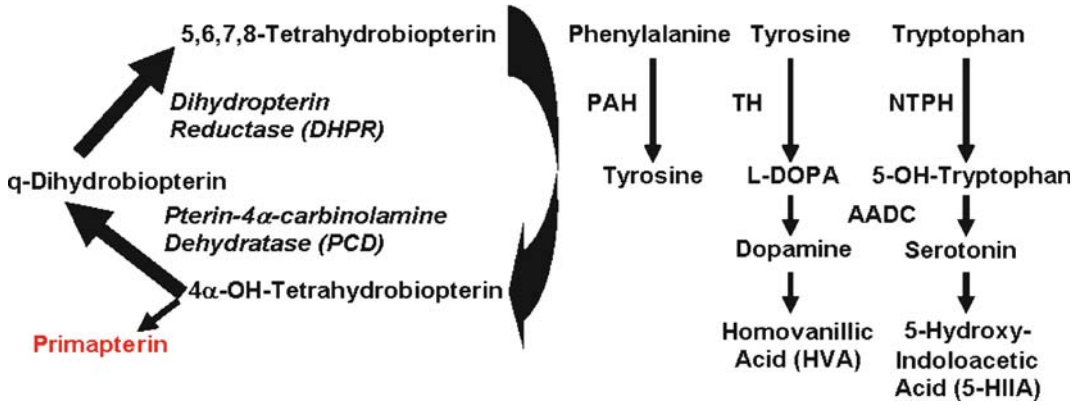

Fig. 2 Regeneration of tetrahydrobiopterin. Tetrahydrobiopterin provides electrons for the hydroxylation of phenylalanine, tyrosine, and tryptophan by the action of phenylalanine hydroxylase (PAH), tyrosine hydroxylase (TH), and neuronal tryptophan hydroxylase (NTPH), respectively. Reduction of 4α-hydroxytetrahydrobiopterin back to the active form requires the sequential action of pterin-4α-carbinolamine dehydratase and dihydropteridine reductase. In the absence of pterin-4α-carbinolamine dehydratase, the substrate is spontaneously converted to primapterin that can be detected in urine

Tyrosine and tryptophan, after the hydroxylation, undergo further metabolism to form stable neurotransmitter metabolites homovanillic (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) that can be measured in the cerebrospinal fluid (Fig. 2).

Disorders of biopterin synthesis and regeneration

Disorders of biopterin synthesis and regeneration were initially identified in patients with phenylketonuria who did not respond to dietary treatment. In fewer than 2% of phenylketonurics, mutations affect the gene for 6-pyruvoyl-tetrahydropterin synthase (60%), dihydropteridine reductase (30%), GTP cyclohydrolase I (5%), and pterin-4-α -carbinolamine dehydratase (5%). In these cases, persistent impairment of hydroxylating activity results not from abnormality in phenylalanine hydroxylase, but from tetrahydrobiopterin deficiency due to blocks in the pathway by which tetrahydrobiopterin is synthesized from GTP or regenerated (deficiency of dihydropteridine reductase). Tyrosine hydroxylase and neuronal tryptophan hydroxylase also require tetrahydrobiopterin and their products (L-dopa and 5-hydroxytryptophan) are essential for the synthesis of neurotransmitters.

Clinical presentation

Although different disorders have peculiar biochemical changes (described below with each disease), the clinical presentation is similar for all of them. For this reason, we report one of our patients as an example. This term baby girl had a normal birth weight (3.632 kg) and was identified with phenylketonuria by newborn screening. Dietary phenylalanine restriction was initiated at 14 days of age with good response of plasma phenylalanine levels that dropped close to the normal range (Fig. 3). At 40 days of age the results of pterin screening indicated normal DHPR activity, but biopterin decreased to 0.8–2.6% (normal 19–60%). Diet was liberalized in preparation of BH_4 loading. She started vomiting for pyloric stenosis that required surgery. After surgery, she received tetrahydrobiopterin (BH_4) at a dose of 10 mg/kg of body weight. BH_4 loading normalized within 6 h plasma phenylalanine levels, despite a completely normal diet. At that time, the infant had normal examination except for irritability and mild head lag. She was maintained on 10 mg/kg BH_4 and she had normal phenylalanine levels for the rest of her life.

At 90 days of age she started having stiffening of the extremities with trunk hypotonia, and her eyes rolled back. Episodes were initially intermittent, but increased

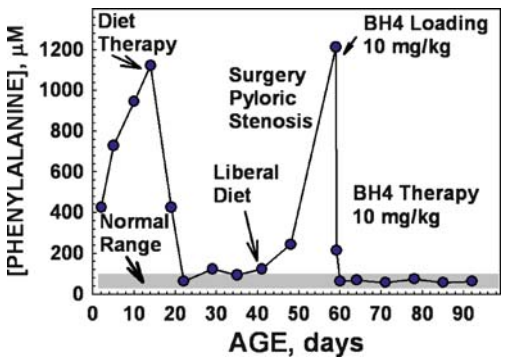

Fig. 3 Plasma phenylalanine levels in a patient with 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency. Different interventions and the normal range (shaded area) are indicated

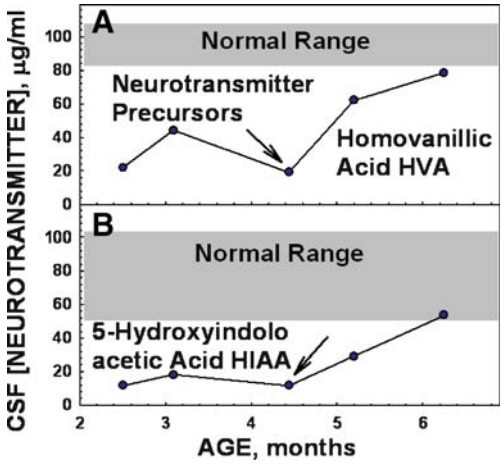

Fig. 4 CSF neurotransmitter metabolites in a patient with 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency. Arrows indicate when therapy with neurotransmitter precursors dopa and 5-hydroxytryptophan was initiated. The shaded areas indicate the normal range

in frequency over time, prompting her admission to the hospital at 4.5 months of age. At that time, her development corresponded to that of a 2-months-old child. EEG was normal. CSF analysis indicated low levels of neurotransmitters (Fig. 3). She was started on dopa/carbidopa and 5-hydroxytryptophan, which initially caused agitation. These were stopped and restarted at a very low dose and increased gradually. CSF analysis indicated a progressive normalization in neurotransmitter metabolites (Fig. 4). Her muscle tone improved. Despite therapy with BH_4, CSF biopterin did not increase (Fig. 5). Neopterin decreased, however, probably reflecting lower levels of phenylalanine and decreased activation of GTP cyclohydrolase. A diagnosis of PTPS deficiency was eventually established in this patient by enzyme assay.

She continued BH_4 at 25 mg three times per day (1.2 mg/kg per day at present) and dopa/carbidopa and 5-hydroxytryptophan at levels that are periodically adjusted. Phenylalanine levels remained normal on unrestricted diet. She required resources in school for comprehension up to 11th grade. She completed normal high school with GPA of 3.35/4.00. At 20 years of age, she appears normal and is attending vocational school before entering college. She continues to have movement problems when she forgets to take her medication on the regular schedule.

GTP cyclohydrolase (GTPCH) I deficiency (OMIM 233910, 600225)

Deficiency of GTP cyclohydrolase I can occur in a recessive and in a dominant form. The dominant form, with mutation in only one of the two alleles for GTP cyclohydrolase I, causes dopa-responsive dystonia (OMIM 128230), characterized by childhood-onset dystonia and a dramatic and sustained response to low doses of levodopa. Patients with the recessive form have mutations in both alleles for GTP cyclohydrolase I and are usually detected because of elevated phenylalanine on newborn screening, although there are exceptions (Horvath et al 2008). Patients present with developmental delays and neurological dysfunction with trunk hypotonia, hypertonia of the extremities, abnormal movements, tremors, convulsions, and sometimes autonomic dysfunction. The *GCH1* gene is composed of 6 exons on 14q22.1-22.2. More than one hundred different mutations have been identified in patients with different forms of GTP cyclohydrolase I deficiency (Thöny and Blau 2006). Only 7 out of 110 mutant alleles are present in a homozygous or compound heterozygous state, causing the autosomal recessive form of GTP cyclohydrolase I deficiency (Thöny and Blau 2006). All the others are present at the heterozygous state and cause dopa-responsive dystonia, in which patients usually present during school years with gait disturbance and progressive neurological involvement with tremors and clumsiness of movements. There is diurnal fluctuation of symptoms with worsening in the evening. Intellectual, cerebellar, sensory, or autonomic disturbances usually do not occur. This latter form is transmitted in a dominant fashion, although some of the parents can have minimal or no symptoms due to reduced penetrance.

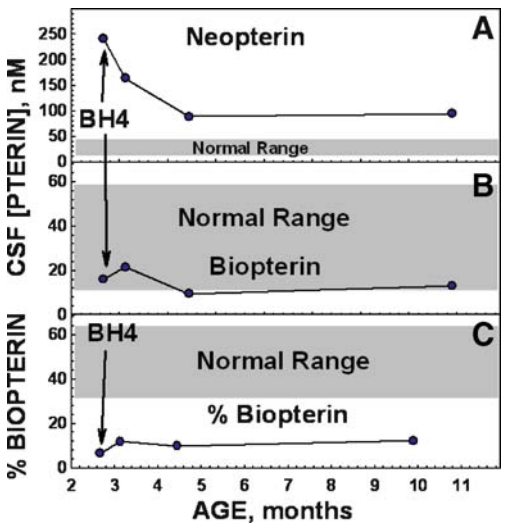

Fig. 5 CSF neopterin and biopterin in a patient with 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency. Initiation of BH_4 therapy decreased neopterin content, likely secondary to reduced synthesis due to the normalization of plasma phenylalanine levels (Fig. 3). High phenylalanine levels stimulate GTP cyclohydrolase I activity. Initiation of BH_4 therapy is indicated. The shaded area indicates the normal range

6-Pyruvoyl-tetrahydropterin synthase (PTPS) deficiency (OMIM 261640)

Pyruvoyl-tetrahydropterin synthase (PTPS) deficiency is the most frequent of the disorders of pterin metabolism. Patients can have the typical/severe form (hyperphenylalaninaemia and abnormal CSF neurotransmitters), or the atypical/peripheral form (minor or no changes in neurotransmitter levels and less significant or transient hyperphenylalaninaemia). With the severe form there is an increased risk of prematurity and low birth weight. In most cases, however, children appear normal at birth and present with abnormal movements and delayed developmental milestones in the first few months of life. Patients with the peripheral form usually have an excellent prognosis for normal neurological development, as long as the hyperphenylalaninaemia is corrected by diet or administration of BH_4. The *PTS* gene is composed of 6 exons on 11q22.3-23.3 and more than 46 mutations have been identified in patients with PTPS deficiency (Thöny and Blau 2006). There are no prevalent mutations, although N52S and P87S, appear to be relatively frequent in the Asian population (Thöny and Blau 2006). Two-thirds of these mutations are associated with the severe form and one-third with the peripheral/milder form of PTPS deficiency (Thöny and Blau 2006).

Pterin-4α-carbinolamine dehydratase (PCD) deficiency (OMIM 264070)

Pterin-4α-carbinolamine dehydratase (PCD) is required for the regeneration of tetrahydrobiopterin after phenylalanine hydroxylation (Fig. 2). Deficiency of this activity causes in newborns a mild form of hyperphenylalaninaemia (HPA) with persistent high urinary levels of primapterin (7-biopterin) (Thöny et al 1998). Affected patients appear completely normal, but have elevated phenylalanine levels at birth. Most patients develop no symptoms, although transient hypotonia has been reported in some (Thöny et al 1998). In most patients, phenylalanine levels normalize after few months of life and remain normal or just above the normal range with an unrestricted diet. The reason for this normalization is not completely clear, although it is likely that other enzymes with the same enzymatic activity (such as the recently proposed pterin-4α-carbinolamine dehydratase/DCoHalpha) become able to compensate later in life (Hevel et al 2006). Neurotransmitter levels are not altered in this condition and the outcome of these patients is usually excellent. Pterin-4α-carbinolamine dehydratase can dimerize with HNF-1a and work as a transcription factor. However, there are no known abnormalities related to this function, probably because there are other genes encoding very similar proteins. The *PCDB* gene is composed of 4 exons on 10q22 and 9 different mutations have been identified in affected patients (Thöny and Blau 2006).

Dihydropteridine reductase (DHPR) deficiency (OMIM 261630)

Dihydropteridine reductase deficiency is a defect in the regeneration of tetrahydrobiopterin after hydroxylation of substrates and the action of carbinolamine dehydratase. It is usually detected via newborn screening due to elevated phenylalanine levels and direct measurement of enzyme activity in dried blood spots. Clinically, it is more severe than other forms of pterin deficiency. Many patients have significant developmental delays despite therapy, develop brain abnormalities, and are prone to sudden death. The reason is not completely clear, but might be related to the accumulation of q-dihydrobiopterin (BH_2) and abnormal metabolism of folic acid. Accumulated BH_2 inhibits all enzymes using tetrahydrobiopterin (BH_4) as cofactor. Patients have low CSF folate in addition to abnormal pterins because DHPR helps maintaining folate in the active (tetrahydro) form (Smith et al 1985). There is correlation between genotype and phenotype, with some patients with DHPR deficiency requiring no therapy, others responding to tetrahydrobiopterin alone, others requiring neurotransmitters and diet because of poor response to tetrahydrobiopterin (de Sanctis et al 2000). There can be MRI changes with white-matter abnormalities and basal ganglia calcifications (Longhi et al 1985; Woody et al 1989). The *QDPR* (quinoid dihydropteridine reductase) gene is composed of 7 exons on 4p15.3 and more than 34 different mutations have been identified in affected patients (Thöny and Blau 2006). Two mutations (G151 S and F212C) are associated with a mild form of DHPR deficiency and affect only serotonin metabolism in the brain (Thöny and Blau 2006).

Sepiapterin reductase (SR) deficiency (OMIM 182 125)

Sepiapterin reductase catalyses the NADPH-dependent reduction of carbonyl derivatives, including pteridines, and plays an important role in BH_4 biosynthesis. Unlike other defects of biopterin synthesis, this defect is not

associated with increased phenylalanine levels and is not usually identified by newborn screening. Urine pterin levels can also be normal in these patients (Bonafe et al 2001). It is hypothesized that peripheral tissues can use alternative enzymes such as carbonyl, aldose, and dihydrofolate reductase to perform the last two steps in BH_4 biosynthesis, resulting in selective brain BH_4 deficiency (Fig. 1). SR catalyses a two step reaction. The first step can also be catalysed by the enzyme aldose reductase. In physiological conditions, SR catalyses both reactions to produce BH_4. However, in SR deficiency, aldose reductase catalyses the first step of the reaction and then the product enters the salvage pathway where finally dihydrofolate reductase catalyses the formation of BH_4. This happens in the liver, kidneys and all other peripheral tissues where BH_4 is synthesized. In the brain dihydrofolate reductase activity is low (<10% of the liver) and there is accumulation of sepiapterin—a CSF marker for this disease. Defective synthesis of BH_4 in the brain results in the imbalanced production of neurotransmitters.

In mice in which the sepiapterin reductase gene was knocked out (Spr^{-}/Spr^{-}), dopamine, noradrenaline (norepinephrine), and serotonin were markedly reduced. BH_4 levels were decreased more in the liver than in the brain suggesting that higher levels of the cofactor are required for brain functioning (Yang et al 2006). In addition to neurological abnormalities, these mice also had hyperphenylalaninaemia, dwarfism, and early death, not seen in humans (Yang et al 2006). Patients with this condition present as others with defective metabolism of pterin metabolism. They have psychomotor retardation, inconsolable crying, neurological abnormalities (hypotonia, dystonic posturing, oculogyric crises, spasticity, tremor, ataxia, gait disorder, chorea, Parkinsonism, seizure-like movements, cerebral palsy) with diurnal variation (symptoms are worse in the evening), psychiatric symptoms (depressed affect, aggressive behaviour, hypersomnolence), and occasional physical findings (microcephaly, growth deficiency) (Abeling et al 2006; Neville et al 2005). This disease is hard to diagnose due to lack of peripheral markers of the disease. High levels of prolactin can be present (2 patients) and one patient developed galactorrhoea due to a prolactinoma (Friedman et al 2006; Neville et al 2005). Brain MRI is normal in most cases. Diagnosis requires measurement of CSF neurotransmitter metabolites and pterin analysis: low HVA and 5-HIAA, and high levels of biopterin and dihydrobiopterin with the presence of sepiapterin (Zorzi et al 2002). Diagnosis can be confirmed by documenting low SR activity in skin fibroblast cultures. The *SPR* (sepiapterin reductase) gene is composed of 3 exons on 2p13 and more than 19 different mutations have been identified in affected patients (Thöny and Blau 2006).

Diagnosis

These disorders can be diagnosed in most cases by examining the urine pterin profile (collected on filter paper and protected from light) and measuring the activity of dihydropteridine reductase (on blood spots collected on filter paper) in all patients with hyperphenylalaninaemia. There are patients, however, in whom phenylalanine levels can be either normal or minimally elevated at birth and clinical suspicion should remain for patients presenting with characteristic neurological symptoms (Horvath et al 2008). Patients with the dominant form of GTP cyclohydrolase I deficiency have normal plasma phenylalanine levels. Screening of urinary pterins should be followed with measurement of pterins and neurotransmitters in the CSF. Table 1 indicates the characteristic profiles obtained in these disorders. Phenylalanine levels at

Table 1 Changes in plasma phenylalanine, urine neopterin and biopterin (CSF only when indicated), and CSF neurotransmitter metabolites in disorders of pterin metabolism

	Phe (plasma)	Biopterin (urine)	Neopterin (urine)	DHPR (blood)	HVA (CSF)	5-HIAA (CSF)
GTPCH1 (recessive)	⇧	⇩	⇩	N	⇩	⇩
GTPCH1 (dominant)	N	N (⇩CSF)	N (⇩CSF)	N	⇩	±⇩
PTPS	⇧	⇩	⇧	N	⇩	⇩
PCD	⇧	⇩	Normal ⇩primapterin	N	N	N
DHPR	⇧	⇩	N	⇩	⇩	⇩
SR	N	N (⇩CSF)	N (⇧CSF sepiapterin)	N	⇩	⇩

N, normal; Phe, phenylalanine; DHPR, dihydropteridine reductase activity; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid; GTPCH1, GTP-cyclohydrolase I deficiency; PTPS, 6-pyruvoyl-tetrahydropterin synthase deficiency; PCD, pterin-4α-carbinolamine dehydratase deficiency; DHPR, dihydropteridine reductase (DHPR) deficiency; SR, sepiapterin reductase deficiency

birth are usually elevated in all of these conditions, except for the dominant form of GTP-cyclohydrolase I deficiency, in milder recessive forms of this same condition (Horvath et al 2008), and in patients with sepiapterin reductase deficiency. All patients with elevated phenylalanine levels in the newborn screening should have urine sent for pterin profile and blood spots sent for dihydropteridine reductase activity. The urine pterin profile indicates low levels of both biopterin and neopterin (a by-product of the GTP-cyclohydrolase I reaction) in GTP-cyclohydrolase I deficiency, while biopterin is reduced in pyruvoyl-tetrahydropterin synthase deficiency, with a decreased biopterin/neopterin ratio. Urine biopterin can be reduced in carbinolamine dehydratase and dihydropteridine reductase deficiency. An abnormal pterin (primapterin) can be identified in patients with carbinolamine dehydratase deficiency, while patients with dihydropteridine reductase deficiency have decreased activity of this enzyme in blood spots.

Evaluation of CSF neurotransmitters and pterin/folate levels is an essential component of the confirmation of the diagnosis for these disorders (Hyland 2007, 2008). CSF homovanillic and 5-hydroxyindoleacetic acid, metabolites derived from dopamine and serotonin (respectively), are reduced in all disorders of pterin synthesis and recycling, with the exception of carbinolamine dehydratase deficiency. Lesser variations are seen in the dominant form of GTP-cyclohydrolase I deficiency, with borderline reduction of homovanillic acid and levels of 5-hydroxyindoleacetic acid that can be normal. An abnormal pterin, sepiapterin, can be identified in the CSF of patients with sepiapterin reductase deficiency (Zorzi et al 2002).

Serial measurements of CSF neurotransmitters can help in individualizing the dose of neurotransmitter precursors to be given to each patient. In addition, measurements of prolactin levels (dopamine inhibits prolactin secretion) can also help in screening for these disorders and for adjusting therapy (Concolino et al 2008; Spada et al 1996). In some cases, measurement of prolactin levels seems superior of the actual measurements of CSF metabolites for individualization of therapy (Ogawa et al 2008).

The diagnosis of specific disorders of pterin synthesis or recycling needs to be confirmed by enzyme assay or DNA sequencing of the putative gene.

Differential diagnosis

A large number of disorders can present symptoms similar to those of patients with defects in the synthesis or recycling of pterins. Many of the dystonia syndromes can have similar presentation. In some of them, MRI changes can point to the correct diagnosis. Measurement of CSF neurotransmitters and pterins remains essential for the correct diagnosis. Alternating hemiplegia of childhood can have the same identical symptoms, but neurotransmitters are normal in this condition. Tyrosine hydroxylase and aromatic L-amino acid decarboxylase deficiency can present with a phenotype similar to if not identical to that of patients with disorders of pterin metabolism and recycling. They can also have abnormal neurotransmitters, but pterin measurement in the CSF is normal.

Management

The treatment of disorders of pterin metabolism is aimed at normalizing phenylalanine levels and brain neurotransmitters and correcting deficiency of other chemicals. A diet restricted in phenylalanine is effective in normalizing plasma phenylalanine levels as in patients with classic phenylketonuria (phenylalanine hydroxylase deficiency). However, in most cases this is not needed since administration of tetrahydrobiopterin (BH_4) can improve phenylalanine hydroxylase activity in the liver and normalize phenylalanine levels (Fig. 3). The dose used to normalize plasma phenylalanine levels in these conditions (1–10 mg/kg per day) is usually lower of that necessary in patients with classic phenylketonuria who respond to this therapy (10–20 mg/kg per day). The effect of BH_4 on phenylalanine levels is immediate (when phenylalanine levels start at above 400 μmol/L) with normalization within 6 h and persisting for 20 h or more. There are patients with DHPR deficiency who do not respond to BH_4 cofactor therapy and need dietary treatment in addition to neurotransmitter precursors.

In theory, BH_4 could normalize neurotransmitter levels in the brain. Initial measurement indicated entry of tetrahydrobiopterin into the brain (Kaufman et al 1982). Low doses of tetrahydrobiopterin (1 mg/kg per day) do not enter significantly into the CSF (Komori et al 1995) and a minimum dose of 20 mg/kg per day is required to see the appearance of tetrahydrobiopterin in the CSF (al Aqeel et al 1992). In addition, as seen from the clinical course of the patient presented in Figs. 3–5, the amount of tetrahydrobiopterin that enters the brain is not sufficient to sustain appropriate synthesis of neurotransmitters in patients with disorders of biopterin synthesis. For this reason, normalization or improvement of brain neurotransmitters can be obtained with the administration of neurotransmitter

precursors or inhibitors of their degradation such as dopa/carbidopa, 5-hydroxytryptophan, and selegiline (a monoamino oxidase inhibitor used mostly in DHPR deficiency). Therapy should be initiated at low doses and the dose gradually increased as needed. Motor benefits usually occur within a few days of starting levodopa. The dose of these drugs should be carefully titrated to avoid dyskinesias or other side-effects. Dosage can be monitored by measuring CSF levels of neurotransmitter metabolites (Hyland 2007, 2008) or verifying normalization of serum prolactin levels (Concolino et al 2008; Ogawa et al 2008; Spada et al 1996).

Patients with DHPR deficiency develop cerebral folate deficiency (Smith et al 1985) and therapy with folinic acid has been effective in improving neurological outcome in these patients (Irons et al 1987).

Outcome

In general, the outcome of patients with disorders of biopterin synthesis can be good, with normal or near normal mentality (Jaggi et al 2008; Liu et al 2008). There are reports of significantly delayed development in patients treated later, although significant improvements of the developmental quotient can be observed with treatment (Lee et al 2006). Many patients have residual neurological symptoms that usually have diurnal variation, being worst when the patients get tired or when the dosage or interval for medications is not respected. There is correlation between severity of the mutation, age at which therapy is initiated, type of disease, degree of correction of CSF neurotransmitters, and residual functional deficits (Dudešek et al 2001; Echenne et al 2006; Jaggi et al 2008; Liu et al 2008).

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