Hyperphenylalaninaemia

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Phenylalanine Metabolism

Phenylalanine (PHE), an essential aromatic amino acid, is mainly metabolised in the liver by the PHE hydroxylase (PAH) system (Fig. 16.1). The first step in the irreversible catabolism of PHE is hydroxylation to tyrosine (TYR) by PAH. This enzyme requires the active pterin, tetrahydrobiopterin (BH_4), which is formed in three steps from guanosine triphosphate (GTP), and DNAJC12 which functions as a co-chaperone with HSP70 for correct folding and stability of the aromatic amino acid hydroxylases. During the hydroxylation reaction BH_4 is converted to the inactive pterin-4a-carbinolamine. Two enzymes regenerate BH_4 via quinoid-dihydrobiopterin (qBH_2). BH_4 is also an obligate co-factor for tyrosine hydroxylase and tryptophan hydroxylases 1 & 2, (and thus necessary for the production of dopamine, catecholamines, melanin and serotonin), and for alkylglycerol monooxygenase (AGMO) and 3 isoforms of nitric oxide synthase [1]. The physiological role of AGMO, which is involved in ether lipid metabolism, is not yet fully characterised.

Defects in either PAH, the production or recycling of BH_4 or DNAJC12 may result in hyperphenylalaninaemia (HPA), as well as in deficiency of TYR, L-dopa, dopamine, melanin, catecholamines and 5-hydroxytryptophan (5HT). When hydroxylation to TYR is impeded, PHE may be transaminated to phenylpyruvic acid (a ketone excreted in increased amounts in the urine, whence the term phenylketonuria or PKU), and further reduced and decarboxylated.

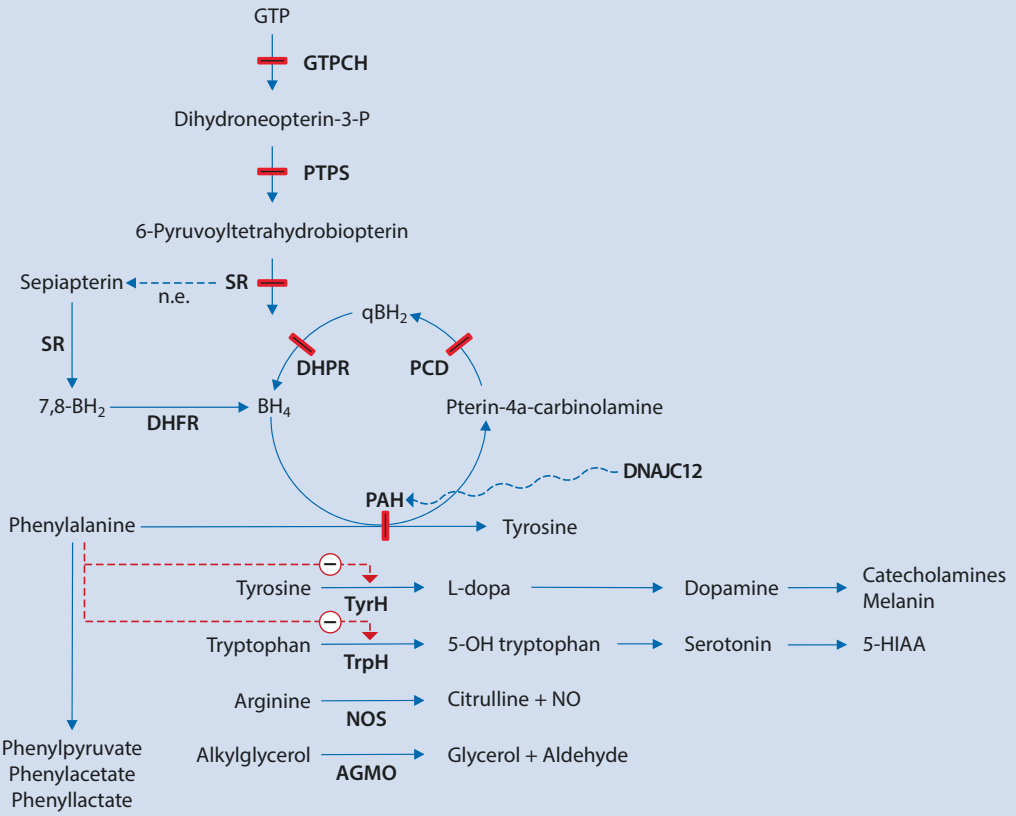

Fig. 16.1 The phenylalanine hydroxylation system, including the synthesis and regeneration of pterins and other pterin-requiring enzymes. AGMO, alkylglycerol monooxygenase; BH_2, dihydrobiopterin (quinone); BH_4, tetrahydrobiopterin; DHFR, dihydrofolate reductase; DHPR, dihydropteridine reductase; DNAJC12, heat shock protein of the HSP40 family; GTP, guanosine triphosphate; GTPCH, guanosine triphosphate cyclohydrolase; 5HIAA, 5-hydroxyindoleacetic acid; NO, nitric oxide; n.e., non-enzymatic; NOS, nitric oxide synthase; P, phosphate; PAH, PHE hydroxylase; PCD, pterin-4a-carbinolamine dehydratase; PTPS, pyruvoyl-tetrahydrobiopterin synthase; SR, sepiapterin reductase; TrpH, tryptophan hydroxylase; TyrH, tyrosine hydroxylase. Encircled minus sign indicates inhibition. The enzyme defects are depicted by *bars* across the *arrows*

Introduction

Mutations within the gene for the hepatic enzyme phenylalanine hydroxylase (PAH) and those involving production or recycling of tetrahydrobiopterin metabolism or DNAJC12 are associated with hyperphenylalaninaemia (HPA). Severe PAH deficiency, which results in a blood phenylalanine (PHE) greater than 1200 μmol/L when individuals are on a normal protein intake, is referred to as classic phenylketonuria or just PKU. Milder defects associated with concentrations between 600 μmol/L and 1200 μmol/L are termed HPA, and those with concentrations less than 600 μmol/L but above 120 μmol/L, mild HPA (MHP). Disorders of biopterin metabolism have in the past been called malignant PKU or malignant HPA. However, such disorders are now best named according to the underlying enzyme deficiency. A comprehensive nomenclature is provided in [2]. Deficiency of DNAJC12, a heat-shock protein (HSP40) which functions as a co-chaperone with HSP70, necessary for correct folding and stability of the PAH protein, also leads to HPA in individuals without mutant PAH genes [3]. PKU if left untreated leads to permanent central nervous system damage. Dietary restriction of PHE along with amino acid, vitamin and mineral supplements, started in the first weeks of life and continued through childhood, is an effective treatment and allows for normal cognitive development. Pharmacologic treatment with BH_4 can reduce blood PHE concentrations in individuals with residual PAH activity. Therapy with PEGylated recombinant *Anabaena variabilis* phenylalanine ammonia lyase (PAL), a non-human enzyme, can also reduce blood PHE concentration. Lifelong treatment is now generally recommended for all patients with PKU, although, as yet, there is insufficient data to know how necessary this is. Less severe forms of PAH deficiency may or may not require treatment, depending on the degree of HPA; however, there is no evidence-based concentration for a raised blood PHE below which treatment is not required. PHE is a potent teratogen and high blood concentrations during pregnancy lead to the maternal PKU syndrome [4]. This can be prevented by strict dietary control of maternal blood PHE throughout pregnancy. Disorders of pterin metabolism lead to both HPA and disturbances in central nervous system amines. Generally, they require treatment with oral BH_4 and neurotransmitters.

16.1 Phenylalanine Hydroxylase Deficiency

16.1.1 Clinical Presentation

The natural history of untreated PKU is progressive, irreversible neurological impairment during infancy with the subsequent development of mental, behavioural, neurological and physical impairments. The most common outcome is moderate to profound intellectual developmental disorder (IQ ≤ 50), often associated with a mousey odour (resulting from the excretion of phenylacetic acid), eczema (20–40%), reduced hair, skin and iris pigmentation (a consequence of impaired melanin synthesis), reduced growth and microcephaly, and neurological impairments (25% epilepsy, 30% tremor, 5% spasticity of the limbs, 80% EEG abnormalities). The brains of patients with PKU untreated in childhood have reduced arborisation of dendrites, impaired synaptogenesis and disturbed myelination. Other neurological features include pyramidal signs with increased muscle tone, hyperreflexia, Parkinsonian signs and abnormalities of gait and tics. Almost all untreated patients show behavioural problems, which include autistic spectrum disorders, hyperactivity, stereotypy, aggressiveness, anxiety and social withdrawal. The clinical phenotype correlates with PHE blood concentrations, reflecting the degree of PAH deficiency.

16.1.2 Metabolic Derangement

Although the pathogenesis of brain damage in PKU is not fully understood, it is causally related to the increased concentrations of blood PHE. Tyrosine (TYR) becomes a semi-essential amino acid, with reduced blood concentrations leading to impaired synthesis of other biogenic amines, including melanin, dopamine and norepinephrine. Increased blood PHE concentrations cause an imbalance of other large neutral amino acids (LNAA) within the brain, resulting in decreased brain concentrations of methionine, TYR and serotonin. The ratio of PHE concentrations in blood/brain is about 4:1 [5]. In addition to the effects on amino acid transport into the brain, elevated PHE inhibits TYR hydroxylation to dopamine and tryptophan decarboxylation to serotonin. The phenylketones phenylpyruvate, phenylacetate, phenylacetylglutamine and phenyllactate are not abnormal metabolites, but appear in increased concentration and are excreted in the urine.

16.1.3 Genetics

PAH deficiency is autosomal-recessively transmitted. At the time of writing >1200 different *PAH* mutations have been described (► http://www.biopku.org/home/pah.asp). Most patients are compound heterozygous. Although there is no single prevalent mutation, certain ones are more common in different ethnic populations: the R408W mutation accounts for approximately 30% of alleles in Europeans with PKU; in East Asians and

South East Asians the R243Q mutation is the most prevalent (13% of alleles). The prevalence of PAH deficiency varies between different populations (e.g. 1 in 1,000,000 in Finland and 1 in 4,200 in Turkey). Overall global prevalence in screened populations is approximately 1 in 12,000, giving an estimated carrier frequency of 1 in 55.

Genotypes correlate well with biochemical phenotypes, pre-treatment PHE concentrations and PHE tolerance [6], which are determined by the milder mutation in compound heterozygotes. However, owing to the many other factors that affect clinical phenotype, correlations between mutations and neurological, intellectual and behavioural outcome are weak. Genetic analysis is of limited practical use in clinical management, but may be of value in determining genotypes associated with BH_4 responsiveness (► http://www.biopku.org/BioPKU_DatabasesBIOPKU.asp) [6] and is essential to diagnose DNAJC12 deficiency [3].

16.1.4 Diagnostic Tests

Blood PHE is normal at birth but rises rapidly within the first days of life. In most Western nations PKU is detected by newborn population screening (NBS). There is variation between different countries and centres in the age at which screening is undertaken (day 1 to day 5), in the methodology used (Guthrie microbiological inhibition test, enzymatic techniques, HPLC, or tandem mass spectrometry) and the concentration of blood PHE that is taken as a positive result requiring further investigation (120–240 μmol/L, but with some laboratories also using a PHE/TYR ratio >3).

Co-factor defects must be excluded by investigation of pterins in blood or urine and dihydropteridine reductase (DHPR) in blood and DNAJC12 deficiency by mutation analysis (► Sect. 16.2). HPA may be found in preterm and sick babies, particularly after parenteral feeding with amino acids and in those with liver disease (where blood concentrations of methionine, TYR, leucine/isoleucine and PHE are usually also raised), and in treatment with chemotherapeutic drugs or trimethoprim.

PAH deficiency may be classified according to the blood PHE concentration when patients are on a normal protein-containing diet, after a standardised protein challenge, or after standardised loading with BH_4 [2].

- Classic PKU (PHE ≥1200 μmol/L; less than 1% residual PAH activity),
- Hyperphenylalaninaemia (HPA) or mild PKU (PHE >600 μmol/L and <1200 μmol/L; 1–5% residual PAH activity), and
- Non-PKU-HPA or mild hyperphenylalaninaemia (MHP) (PHE ≤ 600 μmol/L; >5% residual PAH activity),
- BH_4-Responsive PKU/HPA (blood PHE concentrations decrease substantially after oral administration of BH_4, thus increasing dietary PHE tolerance.

Although the spectrum of severity is continuous, such a classification has some use in terms of indicating the necessity for and type of treatment.

Prenatal diagnosis, rarely requested, is possible by means of *PAH* analysis on chorion villus biopsy (CVB) or amniocentesis where the index case has mutations identified previously.

16.1.5 Treatment and Prognosis

16.1.5.1 Principles of Treatment

Dietary Treatment

Dietary treatment for PKU has proved highly successful and has provided a model for the dietary management of other aminoacidopathies, such as MSUD and classical homocystinuria. The principle of treatment in PAH deficiency is to reduce the blood PHE concentration sufficiently to prevent the neuropathological effects but also to fulfil age-dependent requirements for protein synthesis. Blood PHE is primarily a function of residual PAH activity and PHE intake. For the majority of patients with PKU the former cannot be altered, so that blood PHE must be reduced by restricting dietary PHE intake. The blood PHE concentration while on a normal protein-containing diet, defines whether treatment is indicated. There are some differences in the recommended cut-off above which PHE restriction is required: Germany >600 μmol/L [7], France, USA, Australasia >360 μmol/L [8–10], and 2016 European Society for PKU (ESPKU) guidelines >360 μmol/L [11]. To stay below these, patients with classic PKU have to reduce nutritional PHE intake to 200–400 mg/day or 4–8 g natural protein per day. In all but the USA recommendations, treatment target blood PHE concentrations are age related but show substantial variation. ◘ Table 16.1 shows recommendations for Germany, the USA, France, the Netherlands, Switzerland, Australasia, and the 2016 ESPKU guidelines. With the exception of blood PHE concentrations for the first decade of life and during pregnancy, reported evidence levels are most often low (quasi-experimental designs, non-analytic studies or expert opinion) or not specified and most recommendations are classified as weak. Blood PHE target ranges differ particularly for age groups older than ten years, without clinical evidence that these differences matter. French guidelines accept 900 μmol/L for adults without

Table 16.1 Daily phenylalanine (PHE) tolerances and target blood ranges, showing different targets aimed for in various countries

		Germany [7]	Netherlands [26]	Switzerland [100]	USA [10]	Australasia [7, 71]	Europe [11]	France [8]
Blood PHE concentration indicating treatment (μmol/l)		>600	Not specified	>400	>360	>360	>360	>360
Patient age (years)	PHE tolerance mg/day	Target blood PHE range (lower- upper boundary; μmol/L)						
0	130–400	40–240	120–240	100–300	60/120–360	120–360	120–360	120–360
1	200–400		120–360					
2	200–400			100–400				
3–4	200–400							
5–9	200–400		120–480					
10–11	350–800	40–900		100–600				
12–14	350–800					120–360 (>360[b])	120–600	120–600
15	350–800		120–600					
16–17	450–1000	40–1200						
>17	450–1000							120–600 (900[c])
Pregnancy	120–400[a]	120-360	Not specified	100–300	120–360	70–250	120–360	120–360

[a]tolerance will usually increase in later stages of pregnancy
[b]acceptable after informed decision
[c]acceptable in individuals without clinical signs

clinical signs and Australasian guidelines recommend accepting patients' informed decisions for concentration above 360 μmol/L after childhood.

Since PHE is an essential amino acid, excessive restriction is also harmful and, particularly in infancy, will result in impaired growth and cognitive development. In order to prevent PHE deficiency a lower limit for blood PHE is also defined. The lower limit is formulated ambiguously in the US guideline recommending 120 μmol/L but stating that concentrations 60–120 μmol/L should not be regarded as too low.

The degree of protein restriction required means that in order to provide a nutritionally adequate supply a semi-synthetic diet is necessary. This is composed of the following:

- Unrestricted natural foods with a very low PHE content (<30 mg/100 g; e.g. carbohydrate; fat, some fruit and vegetables).
- Calculated amounts of restricted natural and manufactured foods with medium PHE content (30–100 mg/100 g; e.g. potato, spinach, broccoli; special bread and special pasta). In the United Kingdom a system of 'protein exchanges' is used, with each 1 g of natural protein representing a PHE content of approximately 50 mg.
- Calculated amounts of PHE-free amino acid mixtures (AAMs) supplemented with vitamins, minerals and trace elements. The biological value of AAMs is lower than that of natural protein; the equivalent daily protein from this source needs to be 20% higher than the age-related reference values for natural protein.

Intake of these three components – including the PHE-free amino acid mixture – should be distributed as evenly as possible with meals during the day.

Foods with a higher concentration of PHE (e.g. meat, fish, cheese, egg, milk, yoghurt, cream, rice, corn) are not allowed. Aspartame (L-aspartyl L-phenylalanine methyl ester), a sweetener for foods (e.g. in soft drinks) contains 50% PHE and is therefore inappropriate in the PKU diet.

PHE-free amino acid infant formulas that also contain adequate essential fatty acids, minerals and vita-

mins are available. Human breast milk has relatively low PHE content; in breast-fed infants, PHE-free formulas are given in measured amounts followed by breast feeding to appetite. In the absence of breast feeding a calculated quantity of a normal formula is given to provide the essential daily requirement of PHE. In older patients Glycomacropetide, a 64-amino acid glycophosphopeptide containing 2.0–5.0 mg PHE per gram [12] may partly substitute AAMs thereby improving bioavailability and palatability but there is insufficient evidence to advocate its use as an alternative to traditional treatment [13].

With intercurrent illness, individuals may be unable to take their prescribed diet. During this period high-energy fluids may be given to counteract catabolism of body protein.

Treatment with BH_4

Pharmacological doses of BH_4 can reduce blood phenylalanine concentrations in some patients with PKU [14]; sapropterin dihydrochloride (Kuvan®), a synthetic formulation of the active 6R-isomer of BH_4 is approved in Europe and the USA for the treatment of patients with HPA and PKU, of all ages, who have been shown to be responsive to such treatment. Most frequently BH_4 responsiveness is defined by a reduction of ≥30% in blood PHE concentration after a single dose of 20 mg BH_4/kg body weight, but there are alternative criteria [15]. It has been suggested that a more clinically relevant assessment is to initially determine BH_4-responsiveness with a screening test, measuring the decrease of blood PHE after a single BH_4 dose of 20 mg/kg, followed, if there has been a decrease ≥30%, by a further period of BH_4 treatment to assess the increase in natural protein tolerance setting a goal (e.g. an increase of at least 100%) to define responsiveness in clinical practice [2, 11].

Studies on the PKU Pah^{enu1} mouse, a model of the mild hyperphenylalaninaemia phenotype, and expression studies of mutations found in BH_4-responsive patients have shown that reduced function of PAH can result from misfolding, aggregation and accelerated degradation of the enzyme. BH_4 may act as a chaperone, providing conformational stabilisation and augmenting the effective PAH concentration [16], with different genotypes showing optimal responses at different PHE concentrations [17]. Treatment with BH_4 consists of single daily doses of 5–20 mg/kg body weight, with the aim of decreasing blood PHE concentrations or increasing dietary PHE tolerance. Both effects have been demonstrated in placebo controlled trials [18].

BH_4 responsiveness is most often found in those with mild PKU, who have a higher residual PAH activity. Except for where there are two null-mutations, the association between genotype and BH_4-responsiveness is probabilistic, and BH_4 responsiveness should always be tested clinically. The manufacturer's prescribing information [19] and a US FDA drug review recommend that Kuvan be used in combination with a PHE-reduced diet, leaving open the question of BH_4 monotherapy for those patients who would with treatment have PHE concentrations sufficiently low not to require diet. There are no serious side effects in the short and mid term [20]. Given the different protocols and the limitations of the 30% criterion in determining BH_4 responsiveness, it is impossible to predict the proportion of patients who might benefit significantly from long-term treatment [18]. Limited data suggest that the use of BH_4 in pregnancy is effective and safe in controlling PHE concentrations in responsive patients [20, 21] but diet remains the first-line treatment for pregnant women. Despite increased cost and regimen complexity, treatment with BH_4 can result in improvement in the quality of life in a subgroup of patients with PKU [22].

Sepiapterin, a natural precursor of BH_4 in the salvage pathway of pterins is more stable and crosses cell membranes more efficiently than BH_4. Exploratory studies in adult healthy volunteers showed that after oral doses of CNSA-001, a pharmaceutical preparation of sepiapterin, increases of BH_4 in CSF [23], and plasma BH_4 concentrations were larger than after equivalent doses of sapropterin dihydrochloride [24]. However, trials are required to evaluate possible clinical effects.

Treatment with Pegvaliase

Enzyme substitution therapy with pegvaliase (PEGylated recombinant *Anabaena variabilis* phenylalanine ammonia lyase (PAL) (Palynziq®) is approved for individuals >16 years. The enzyme converts PHE independently from PAH and BH_4 to a harmless compound, transcinnamic acid, and ammonia metabolised in the liver to urea. Covalent attachment of polyethylene glycol polymer chains (PEGylation) 'mask' the agent from the host's immune system, reducing immunogenicity and antigenicity. However, during early treatment (≤6 months) but also later (at year 1) all patients develop antibodies against PEG and PAL and more than 90% experience adverse events like hypersensitivity, arthralgia/arthritis, injection site/generalized skin reactions or lymphadenopathy, and about 9% anaphylaxis episodes [25]. Treatment is initiated by a titration phase when subcutaneous injections must be accompanied by a trained observer (able to recognise signs of acute systemic hypersensitivity/anaphylaxis, to administer an epinephrine autoinjector, and call emergency services if necessary) for at least one hour following each injection. Patients must always carry the epinephrine autoinjector and be able to master its application. Daily subcutaneous injection of 20–60 mg of the enzyme per maintenance dose is effective in reducing PHE concentrations below 120 μmol/L and often allows a normal diet. In clinical trials up to 40% of

patients showed episodes of hypophenylalaninaemia (<30 µmol/L). As the treatment does not increase TYR concentration, blood TYR should also be monitored and if necessary supplemented. Pegvaliase is not recommended for use in women who are currently planning to become pregnant [25].

16.1.5.2 Monitoring of Treatment

A low-protein diet brings the risk of nutritional deficiency. Therefore, treatment is monitored by regular assessment of dietary intake and blood PHE concentrations, as well as neurological, physical, intellectual and behavioural development. Timetables and procedures vary between recommendations [7–10, 26, 27]. Blood PHE should be measured weekly during the first year of life, fortnightly during childhood, and monthly afterwards. Samples ideally should be taken early morning when concentrations are likely to be at a peak or at least 4 hours post-prandially.

16.1.5.3 Alternative Therapies/Experimental Trials

Although dietary treatment is highly successful, it is difficult and compliance is often poor, particularly as individuals reach adolescence. Hence there is a need to develop more acceptable therapies.

- The **large neutral amino acids (LNAA;** phenylalanine, tyrosine, tryptophan, leucine, isoleucine and valine) compete for the same transport mechanism (the L-type amino acid carrier) to cross the blood-brain barrier as well as for the absorption by the intestinal mucosa [28]. Studies in the $PAH^{enu2/2}$ mouse and in patients have shown a reduction in brain PHE concentrations and some positive effect on neuropsychological functions when LNAAs (apart from PHE) have been given enterally [29]. The greatest benefit may be to patients who are unable to comply with conventional dietary management, but it is likely to be of limited efficacy.
- **Gene therapy**. A number of different PAH gene transfer vehicles have been tried in the $PAH^{enu2/2}$ mouse. Vectors based on recombinant adeno-associated viruses (rAAVs) expressed in either liver or muscle are currently the favoured vector system. An rAAV vector with genes for PAH and BH_4 synthesis injected into skeletal muscle or infused into the intraportal vein or naked DNA injected in the tail vein of $PAH^{enu2/2}$ mice, showing a classical PKU phenotype, resulted in correction of PHE for more than 1 year [30]. A phase 1/2 trial of liver delivery with an AAV vector is now underway in adults with PKU (► ClinicalTrials.gov Identifier: NCT04480567).
- **Liver transplantation** fully corrects PAH deficiency [31], but the risks of transplantation surgery and post-transplantation immune suppressive medication are too high for it to be a realistic alternative to dietary treatment. The same is true for liver repopulation with PAH-expressing cells following hepatocyte or haematopoietic stem cell transplantation [32].

16.1.5.4 Compliance with Treatment

Compliance with treatment is best in infancy and childhood. The diet severely interferes with culturally normal eating habits, particularly in older children and adolescents, and this often results in problems with keeping to treatment recommendations. Up to the age of 10 years only 40% of the German Collaborative Study of PKU patients could keep their PHE concentrations in the recommended range [33]. After the age of 10 years 50–80% of blood PHE concentrations measured in a British and Australian sample were above recommendation [34]. In the US, patient, social, and economic factors prevent >70% of adult patients from accessing treatment [35] and two thirds have PHE concentrations above the recommended range [36]. Dietary treatment of PKU is almost impossible without the support of a specialised team, which should include a dietitian, a metabolic paediatrician or physician for adult patients, a biochemist running a metabolic laboratory and a psychologist skilled in the behavioural management of a life-long diet. All professionals, and the families themselves, must fully understand the principles and practice of the diet. The therapeutic team should be trained to work in an interdisciplinary way in a treatment centre, training camps can improve long-term knowledge about the condition and its treatment [2, 11, 37].

16.1.5.5 Outcome

The outcome for PKU mainly depends upon the age at start of treatment, blood PHE concentrations in different age periods, duration of periods of blood PHE deficiency and the individual gradient for PHE transport across the blood-brain barrier. The most important single factor is the blood PHE concentration in infancy and childhood. Dietary treatment started within the first 3 weeks of life with average blood PHE concentrations ≤ 400 µmol/L in infancy and early childhood result in near-normal intellectual development. However, for each 300 µmol/L increase in blood PHE during the first 6 years of life, IQ is reduced by 0.5 of a standard deviation (SD), and during age 5–10 years the reduction is 0.25 SD. Furthermore, IQ at the age of 4 years is reduced by 0.25 SD for each 4 weeks of delay in the start of treatment and for each 5 months of insufficient PHE intake. After the age of 10 years all large studies show stable IQ performance, at least until mid-adulthood irrespective of PHE concentrations [38–41], and a normal school career if compliance during the first 10 years has been accord-

ing to treatment recommendations [42–44]. A Bayesian meta-analysis covering the age range from 2 to 35 years distinguished long-term and concurrent blood PHE concentrations in a critical (<6 years) and non-critical period (≥6 years) as predictors for an IQ <85 [45]. Effects of long-term PHE were larger than for concurrent values and those for the critical period were stronger than for the later period. The associations of PHE with IQ were negative, with PHE measurements <400 µmol/L predicting probabilities of IQ >85, close to the general population. However, correlations between concurrent and long-term PHE values, between the critical and noncritical period as well as age at start of treatment, degree of PAH activity, different IQ tests and socioeconomic status were not controlled. Compared with a matched control group over a 5 year period IQ of early-treated patients with classical PKU aged 10–41 years with blood PHE concentrations between 600 and 900 µmol/L remained stable. Older adults performed worse than younger ones, explained by higher PHE concentrations during childhood or adolescence. IQs of early and well-treated adults with PKU are similar to those of their unaffected family members [44] however, longitudinal studies covering adulthood are still rare [38, 39]. Quality of life (QoL) has become an important outcome. Despite the burden of strict dietary control, early treated patients can have a normal QoL [46, 47]. QoL issues also apply to parents of patients with PKU [48].

16.1.5.6 Complications in Adulthood

The majority of early-treated patients are now adults. Dietary treatment has transformed their prospects: they can expect normal development, professional careers, to start families and to live independently [47, 49]. Nonetheless, when studied in detail, subtle neuropsychological deficits have been found. Neuroimaging abnormalities are common but frank neurological disease has been reported in only in a few. This is concerning and it is important to define the precise phenotype of adults with early-treated PKU and determine which features relate to raised PHE concentrations, either historical or concurrent.

Neurological Abnormalities

Frank neurological disease is rare and may not be related to PHE concentrations [49]. Subacute combined degeneration of the spinal cord has been reported in adults after dietary relaxation. These patients had all developed profound vitamin B_{12} deficiency because they had stopped taking their amino acid supplements, but continued to follow diets low in high quality natural protein [50]. Early and well treated patients as well as those on relaxed diet can show tremor and brisk reflexes [51] the aetiology and clinical significance of which are unclear. Other complications such as cortical visual loss [52, 53] are very infrequent and possibly associated with poor control in childhood or adolescence [54]. Reinstitution of dietary treatment and amino acid supplements can lead to improvement.

Neuropsychological Abnormalities

Subtle changes in executive function, vigilance, working memory, and motor skills have been described in children, adolescents and adults with early-treated PKU, however, findings are inconclusive regarding the nature of executive impairments as well as their specificity, impact on everyday life, persistence over time, association with PHE concentrations, and aetiology [47, 55, 56]. Meta-analytic results suggest that for the prevention of any impairment there is an upper threshold PHE concentrations of 320 µmol/L for children (7–12 years) and 570 µmol/L for adolescents (13–18 years). In adults the negative effect remained stable between PHE concentrations of 750–1500 µmol/L [57]. Information processing was also stable in a 5 year longitudinal controlled study of adult patients with classical PKU [58]. In one study, the latency of visual saccades [59] in adults with PKU with concurrent PHE concentrations greater than 1200 µmol/L was significantly longer than in control patients, whilst no difference was detected with PHE concentrations below 800 µmol/L. Saccadic latencies normalised with improved metabolic control. Others however have failed to find a relationship between saccadic latency and PHE concentrations [60].

Neuroimaging Abnormalities

White matter abnormalities on brain MRI appear after longer periods of increased PHE concentrations, but are reversible after 3–6 months of strict dietary treatment [61]. In all but one study MRI did not correlate with intellectual or neurological abnormalities. In one study, 67% of patients under 10 years old had normal-appearing white matter. This decreased to only 4% in those over 20 [62] although there was no evidence of any clinical neurological deterioration over this period. Alterations were associated with long-term PHE concentrations. Diffusion tensor imaging demonstrated reduced diffusivity of water molecules with intact fractional anisotropy [63, 64], suggesting that the axons in PKU white matter are structurally intact, but that water molecules move more slowly along them. The clinical relevance of any of these imaging findings needs to be demonstrated [49].

Neuropsychiatric Abnormalities

Although no association of early treated PKU with psychiatric disease has been demonstrated [47, 49], increased emotional and behavioural symptoms have been described [65]. Patients with poor dietary control during infancy show hyperactivity, temper tantrums, increased

anxiety and social withdrawal, frequently associated with intellectual deficits. Well-treated subjects show increased risks of depressive symptoms and low self-esteem. However, without correlation to PHE concentrations, causality remains obscure, and such problems are also common in other chronic disease populations [66, 67].

Dietary Deficiencies

Vitamin B_{12} deficiency is well recognised in patients who have stopped their vitamin supplements but continue to restrict their natural protein intake. For patients on a strict diet there have been concerns regarding deficiencies in vitamins and minerals, including selenium, zinc, iron, retinol and long-chain omega-3 polyunsaturated fatty acids (LC-PUFA). Such deficiencies are sometimes found but it is unclear whether they are of any clinical significance. Low calcium, osteopenia and an increased risk of fractures have also been reported, however despite individual studies reporting reduced bone mineral density, pooled data suggest that these reductions are not clinically important [68]. LC-PUFAs, already added in PHE-free infant formulas, have been shown to be low in children aged >4 years. Experimental supplementation has been tolerated well and resulted in increased visual evoked potentials and motor performance, but the optimal type and dose of supply still needs to be determined [69]. In a double-blind randomized six month supplementation study DHA uptakes up to 7 mg/kg and day did not improve neurological functions in children 5–13 years old [70].

Diet for Life

Adults with PKU face different challenges from children, and it is much more difficult to be proscriptive about their dietary management. Although biochemically attractive, the concept of diet for life does pose substantial obstacles. For adolescents with PKU, the low-protein diet is restrictive, imposes a stark differentiation between them and their peers and is enforced by their parents: it is not surprising that many rebel against it. Those who wish to stay on diet when they leave the parental home may lack the skills, financial resources and time required. With suitable support these problems can be overcome, but the majority of adults are poorly compliant with dietetic advice with less than 30% of PHE concentrations falling within target ranges [34, 71]. This is likely to be because most adults who have had a period when their diet has lapsed have noticed no ill effects. A recent large study of adults with PKU shows that patients who were diagnosed on NBS and started on dietary treatment within the first year of life have excellent educational, occupational, and social outcomes regardless of whether they maintain strict dietary control in adulthood [47]. Although there was evidence of some subtle neurocognitive defects, these were mostly related to metabolic control in childhood and did not seem to affect the ability of people with PKU to function in society.

Despite the lack of consistent evidence for any irreversible effects of PHE on the adult brain, recent guidelines are recommending much more stringent PHE control in adults than has historically been the case [10, 72]. To date, no evidence has been published to show that these new targets have had any effect on the number of adult patients following dietary treatment or on the PHE concentrations they obtain. Although experience with maternal PKU (see below) suggests that women can obtain PHE concentrations below 360 μmol/L if they need to, most don't choose to maintain such a strict diet once their children have been born [73], suggesting that for many the strictures involved in maintaining such a diet do not justify any perceived clinical benefits [74].

It has always been difficult to persuade the many patients who are leading a normal life and eating a normal diet of the need to return to the restrictions of their childhood. It is important, however, that these individuals remain under expert care by metabolic physicians and dietitians with a training in behavioural and adult medicine, to ensure that they are following a nutritionally adequate diet, to monitor their long-term outcome, and to keep them informed about new evidence and treatments. A pragmatic approach is likely to be most productive, recommending broader corridors for PHE target concentrations, and giving adults with PKU the support, training and resources they need to follow their own choices [75]. The policy of personalised treatment is supported by a growing evidence of individual vulnerability to the neurotoxic effect of PHE [76].

16.1.5.7 Management of Late-Diagnosed PKU

Children with classic PKU who have not been screened in the newborn period and who are diagnosed later in infancy or childhood will have suffered permanent brain injury. However, the initiation of dietary treatment to control blood PHE concentrations will often lead to improvement in their neurodevelopmental state, albeit limited, and by preventing further damage allow for some developmental progress. The outcome for such children will depend on a number of factors but most importantly on the age at diagnosis and start of treatment.

Caring for adults with late-diagnosed PKU poses a unique set of problems. Although there is a wide spectrum, most of these patients will not be able to live independently. These older patients were either never treated, or treated late, when brain damage was already established, and often came off a low-protein diet at a young age. Although returning to diet does not affect established neurological disease, it can improve difficult

behaviour. In a randomised double-blind cross-over trial of the reintroduction of diet in patients with late-diagnosed PKU, carers rated behaviour as significantly better when subjects were on a low-PHE diet [77]. A 6-month trial of dietary treatment is warranted in late-diagnosed patients with challenging behaviour.

For late-diagnosed patients who remain at home into adulthood, the major challenge is planning for when their parents are no longer able to care for them. Eventually, these individuals will need alternative arrangements for their long-term care. This is best done in good time and with parental participation. If arrangements have to be made in an emergency, because of ill health or death of a carer, the results can be disastrous.

16.2 DNAJC12 Deficiency

Over 45 patients have now been described with HPA due to deficiency of DNAJC12, a 40 kDa heat-shock protein (HSP40) which functions as a co-chaperone with HSP70 required for the correct protein folding of the aromatic amino acid hydroxylases, PAH, tyrosine hydroxylase and tryptophan hydroxylases 1 and 2. For review see [3].

16.2.1 Clinical Presentation

Blood PHE concentrations at diagnosis have been <600 μmol/L. The clinical phenotype is heterogeneous, ranging from severe intellectual disability, dystonia and early-onset dopa–responsive parkinsonism to mild autistic features or hyperactivity and to individuals who are asymptomatic.

16.2.2 Metabolic Derangement

Deficiency of DNAJC12 is associated with a reduction in the activity of the aromatic amino acid hydroxylases leading to HPA and a reduction in biogenic CSF amines. CSF 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) concentrations are markedly decreased and the HVA/HIAA ratio is elevated. Neopterin and biopterin in CSF, urine and blood are normal. Additionally, DNAJ co-chaperones are thought to be involved in protein folding and degradation, and there is an association of DNAJC-family members with Parkinson's disease, parkinsonism and neurodegenerative diseases. In particular, they are involved in endocytosis of the synaptic vesicle (► Chap. 30).

16.2.3 Genetics

DNAJC12 deficiency is an autosomal recessive disorder caused by biallelic mutations in *DNAJC12*.

16.2.4 Diagnostic and Confirmatory Tests

The diagnosis should be suspected in any patient with HPA in whom PAH deficiency and biopterin synthesis disorders have been excluded. A BH_4 challenge results in a reduction in blood PHE. DHPR activity and urinary and blood pterin levels are normal. The diagnosis is confirmed by finding pathogenic mutations in *DNAJC12* [3].

16.2.5 Treatment and Prognosis

Treatment consists of BH_4 and/or L-dopa/carbidopa and 5-hydroxytryptophan. Normal development should follow early diagnosis and treatment. Late diagnosis can be associated with permanent neurological disease, however, treatment started in older symptomatic individuals may still be of benefit [78].

16.3 Maternal PKU

16.3.1 Clinical Presentation

Before the introduction of NBS and early treatment, it was unusual for women with PKU to have children of their own: as with other women with learning difficulties, positive steps were often taken to control their fertility. Initial observations that some children of mothers with PKU also had learning difficulties and behavioural problems were interpreted as genetic transmission rather than an environmental problem but the first description of maternal PKU syndrome (MPKUS) recognised the teratogenic effects of high maternal PHE concentrations [79]. Offspring of women with untreated classical PKU suffer developmental delay (92%), microcephaly (73%), cardiac defects (12%), low birth weight (40%) and dysmorphic features [4]. Blood PHE should be measured in a mother who delivers a baby with such symptoms, particularly in areas where NBS for PKU is not practiced or has been introduced relatively recently.

Although the pathogenesis of this condition is still poorly understood, much progress has been made and the MPKUS is now a preventable disease.

16.3.2 Metabolic Derangement

Teratogenic Effects of Phenylalanine

The Maternal PKU Collaborative Study (MPKUCS) showed that for maternal PHE concentrations below about 360 μmol/L there was no evidence of any deleterious effect on the foetus [80]. Above 360 μmol/L, developmental indices decreased by about three points for every 60 μmol/L rise in average concentration. Congenital heart disease (CHD) was only seen with much higher PHE concentrations (≥900 μmol/L) [81]. The risk of CHD increased with increasing PHE exposure; 50% of mothers who had children with CHD had average PHE concentrations ≥1500 μmol/L.

16.3.3 Treatment and Prognosis

16.3.3.1 Prevention of the Maternal PKU Syndrome

Strict dietary control for pregnant women with PKU and, preferably, those who are planning pregnancy, is necessary: the institution of strict metabolic control before conception and throughout pregnancy is associated with normal outcomes [80].

The plasma PHE targets used in maternal PKU have changed over time. Initially target concentrations of below 600 μmol/L led to improved outcomes and reduced incidence of CHD to background frequency. The policy to aim for even lower concentrations was based on the fact that, in infancy, PHE concentrations below 360 μmol/L minimised the risk of brain damage and that active placental transport led to an enrichment of PHE in the foetal circulation [82].

16.3.3.2 Current Practice

Prevention of the MPKUS requires time and resources, and the best outcomes are obtained by the centres with the most experience [83]. In 109 pregnancies cared for in a single centre over a 30-year period, preconception diet was established in 69.5% [84]. This centre looks after 15–20 PKU pregnancies annually. Prospective mothers are offered dietary education with partners or families. PHE is monitored twice a week preconceptionally and three times a week in pregnancy, with next day reporting. This level of service requires clinicians trained in metabolic medicine, specialist dietitians, specialised laboratory services, foetal medicine services, and close cooperation with child neuropsychologists to monitor outcomes. These resources are only available in units caring for adults with PKU, and any woman with PKU already pregnant or considering pregnancy should be referred to the nearest such centre.

16.3.3.3 Outcome

All women who plan their pregnancies and start diet before conception can maintain excellent metabolic control throughout pregnancy irrespective of their baseline PHE concentrations [84]. Concentrations may rise transiently during morning sickness or intercurrent illness, but these episodes can be controlled by reducing natural protein intake and increasing amino acid supplements. With morning sickness it is important to maintain calorie and supplement intake, in order to prevent catabolism and early use of anti-emetics is recommended.

After the first trimester protein tolerance increases markedly as the baby grows. For women who remain on a low-protein diet after delivery, greater protein restriction is often required postpartum.

Although PHE concentrations can usually be quickly brought under control, women who start diet after conception have significantly higher PHE concentrations throughout pregnancy [84]. A small subgroup of women, unable to fully comply with a low-protein diet, never obtain satisfactory metabolic control. Admission for full supervision of their diet will bring PHE concentrations down, but prolonged in-patient stays are neither practicable nor acceptable to the patients. Outcomes of such pregnancies remain poor. Often successive pregnancies are affected in the same way. In such pregnancies monitoring of PHE concentrations is infrequent, but often the absolute concentrations remain below 1000 μmol/l. For these women, new interventions are desperately required. BH_4, which is licensed for use in pregnancy, may have a role to play in responsive patients [21, 85]; any significant improvements in IQ for the offspring would justify the cost.

Any effects of the low protein diet on the foetus are much less severe than those of PHE but may still be significant. Maternal PHE below 120 μmol/L is associated with intrauterine growth retardation [86]. Low essential fatty acid intakes have led to the use of amino acid supplements fortified with DHA. Some centres use tyrosine supplements to maintain maternal TYR within the normal range.

The key to preventing MPKUS is planning, with dietary treatment being established prior to conception. This requires all women with HPA to be educated from an early age with the information repeated regularly thereafter.

16.4 HPA and Disorders of Biopterin Metabolism

Disorders of tetrahydrobiopterin (BH_4) associated with HPA and biogenic amine deficiency include deficiencies of GTP cyclohydrolase I (GTPCH), 6-pyruvoyl-tetrahydropterin synthase (PTPS), dihydropteridine reductase (DHPR) and pterin-4a-carbinolamine dehydratase (PCD) (primapterinuria). Dopa-responsive dystonia (DRD), which is due to a dominant form of GTPCH deficiency, and sepiapterin reductase (SR) deficiency, also lead to CNS amine deficiency but are associated with normal blood PHE (although HPA may occur in DRD after a PHE load); these conditions are not considered further here (► Chap. 30). Consensus guidelines for the diagnosis and treatment of BH_4 deficiencies have recently been published [87].

16.4.1 Clinical Presentation

These conditions can present in one of three ways:

- Asymptomatic, but with raised PHE found following NBS; as part of the standard screening protocol the infant is then investigated further for biopterin defects.
- Symptomatic, with neurological deterioration in infancy despite a low-PHE diet. This will occur where no further investigations are routinely undertaken after a finding of HPA in NBS which is wrongly assumed to be PAH deficiency.
- Symptomatic, with neurological deterioration in infancy on a normal diet. This will occur either where there has been no NBS for HPA or if the PHE concentration is not sufficiently raised to have resulted in a positive screen or to require dietary treatment.

Symptoms may be subtle in the newborn period and not readily apparent until several months of age. Birth weight and birth head circumference may be low in some infants, suggesting intrauterine involvement. All conditions apart from PCD deficiency are associated with abnormal and variable tone, abnormal movements, irritability and lethargy, seizures, poor temperature control, progressive developmental delay and microcephaly. An abnormal EEG and cerebral atrophy can occur in PTPS and in DHPR deficiency and basal ganglia calcification is reported in the latter [88]. There is a mild (peripheral) form of PTPS associated with HPA but without neurotransmitter deficiency, where there are usually no neurological symptoms [89]. In PCD deficiency symptoms are mild and transient.

16.4.2 Metabolic Derangement

Disorders of pterin synthesis or recycling are associated with decreased activity of PAH, tyrosine hydroxylase, tryptophan hydroxylase and nitric oxide synthase (◘ Fig. 16.1). The degree of HPA is highly variable, with blood PHE concentrations ranging from normal to >2000 µmol/L. Central nervous system (CNS) amine deficiency is most often profound and responsible for the clinical abnormalities. Decreased concentration of HVA in cerebrospinal fluid (CSF) is a measure of reduced dopamine turnover, and similarly 5-HIAA deficiency is a measure of reduced serotonin metabolism (see also ► Chap. 30).

16.4.3 Genetics

All disorders are autosomal recessive. Descriptions of the relevant genes and a database of mutations are available at ► http://www.biopku.org/pnddb/home.asp. In most series biopterin disorders account for 1–3% of infants found to have a raised PHE on newborn screening; PTPS deficiency is the most common disorder, followed by DHPR deficiency [88]. PTPS deficiency has a higher frequency in Chinese populations, and a genotype phenotype correlation has been reported [90].

16.4.4 Diagnostic and Confirmatory Tests

Diagnostic protocols and interpretation of results are as follows.

16.4.4.1 Urine or Blood Pterin Analysis and Blood DHPR Assay

All infants found to have HPA on NBS should have blood DHPR and urine or blood pterin analysis. The interpretation of results is shown in ◘ Table 16.2.

16.4.4.2 BH_4 Loading Test

If dietary PHE restriction is in place this is stopped 2–3 days before the test. Blood PHE concentrations should be at least 400 µmol/L at the start. An oral dose of 20 mg BH_4/kg is given approximately 30 min before a feed. Blood samples are collected for PHE and TYR at 0, 4, 8 and 24 h. The test is positive if plasma PHE falls to normal (usually by 8 h) with a concomitant increase in TYR. The rate of fall of PHE may be slower in DHPR deficiency. Blood for pterin analysis at 4 h will confirm that the BH_4 has been taken and absorbed.

A combined PHE (100 mg/kg) and BH_4 (20 mg/kg) loading test may be used as an alternative. This combined

Table 16.2 Interpretation of results of investigations in disorders of biopterin metabolism

Deficiency	Blood PHE µmol/L	Blood or urine biopterin	Blood or urine neopterin	Blood or urine primapterin	CSF 5-HIAA and HVA	Blood DHPR activity	Gene
PAH	>120	↑	↑	–	↓[a]	N	*PAH*
GTPCH	50–1200	↓↓	↓↓	–	↓	N	*GTCH1*
PTPS	240–2500	↓↓	↑↑	–	↓	N	*PTS*
DHPR	180–2500	↓↓	N or ↑	–	↓	↓	*QDPR*
PCD	180–1200	↓	↑	↑↑	N	N	*PCBD1*
DNAJC12	>120	**N**	**N**	–	↓	N	*DNAJC12*

CSF, cerebrospinal fluid; DHPR, dihydropterin reductase; GTPCH, guanosine triphosphate cyclohydrolase I; 5-HIAA, 5-hydroxyindole acetic acids; HVA, homovanillic acid; N, normal; PAH, phenylalanine hydroxylase; PCD, pterin-4a-carbinolamine dehydratase; PHE, phenylalanine; PTPS, 6-pyruvoyl-tetrahydropterin synthase

[a]In PAH deficiency, as long as PHE concentrations remain elevated, there is a secondary inhibition of tyrosine and tryptophan hydroxylases causing depletion in CSF amines

loading test is reported to identify BH_4-responsive PAH deficiency and discriminate between co-factor synthesis or regeneration defects and is useful if pterin analysis is not available [91, 92].

16.4.4.3 CSF Neurotransmitters

The measurement of HVA and 5-HIAA is an essential part of the diagnostic investigation and is also subsequently required to monitor amine replacement therapy with L-dopa and 5HT. CSF must be frozen in liquid nitrogen immediately after collection and stored at –70 °C prior to analysis. If blood stained, the sample should be centrifuged immediately and the supernatant then frozen. The reference ranges for HVA and 5-HIAA are age related [93] (see also ► Chap. 30).

16.4.4.4 Confirmatory Tests

Apart from DHPR measurement in erythrocytes, measurement of enzyme activity is not necessary for the initial diagnosis. Molecular analysis is available for all conditions and is now likely to be the method of choice for confirmation of the diagnosis. Where results can be obtained in an acceptable time frame gene panels or next generation sequencing may be used as an alternative to pterin analysis as a first line investigation in infants with HPA on NBS. Where necessary, for further confirmation DHPR activity can be measured in fibroblasts, PTPS activity in erythrocytes and fibroblasts and GTPCH activity in liver, cytokine-stimulated fibroblasts and stimulated lymphocytes. If PAH deficiency and disorders of biopterin metabolism cannot be confirmed as a cause of HPA, molecular analysis should be undertaken for *DNAJC12* mutations [3].

16.4.4.5 Prenatal Diagnosis

If the mutation of the index case is already known prenatal diagnosis can be undertaken in the first trimester by mutation analysis following chorionic villus biopsy. Analysis of amniotic fluid neopterin and biopterin in the second trimester is available for all conditions. Enzyme analysis can be undertaken in foetal erythrocytes or in amniocytes in both DHPR deficiency and PTPS deficiency. GTPCH is only expressed in foetal liver tissue.

16.4.5 Treatment and Prognosis

For GTPCH deficiency, PTPS deficiency and DHPR deficiency the aim of treatment is to control the HPA and to correct CNS amine deficiency. In DHPR deficiency treatment with folinic acid is necessary to prevent CNS folate deficiency [58], and it may also be required in GTPCH and PTPS deficiency, where a reduction in CSF folate can be a consequence of long-term treatment with L-dopa. PCD deficiency does not usually require treatment, although BH_4 may be used initially if the child is symptomatic.

In PTPS and GPCH deficiency, blood PHE responds to treatment with oral BH_4 (available as sapropterin dihydrochloride). In DHPR deficiency, BH_4 may also be effective in reducing blood PHE, but higher doses may be required than in GTPCH and PTPS deficiency. This, in theory, might lead to an accumulation of BH_2 and inhibition of BH_4 dependent enzymes [94]. Consequently, it has been recommended that in DHPR deficiency HPA should be corrected by dietary means and BH_4 should

not be given. However, a number of patients with DHPR deficiency have been successfully treated with BH_4 and in a single case report, BH_4 up to a dose of 40 mg/kg/day did not cause a further increase in CSF BH_2 [95].

CNS amine replacement therapy is given as oral L-dopa with carbidopa (usually in 1:10 ratio, but also available in 1:4 ratio) and 5HT. Carbidopa is a dopa-decarboxylase inhibitor that reduces the peripheral conversion of L-dopa to dopamine, thus limiting side effects and allowing a reduced dose of L-dopa to be effective. Side effects (nausea, vomiting, diarrhoea, irritability) may also be seen at the start of treatment. For this reason L-dopa and 5HT should initially each be started in a low dose (Table 16.3), which is increased gradually to the recommended maintenance dose. Further dose adjustment depends on the results of CSF HVA and 5-HIAA concentrations.

Additional medications, developed primarily for treatment of Parkinson's disease, have been used as an adjunct to therapy, with the aim of reducing the dose and frequency of amine replacement medication and improving residual symptoms and preventing diurnal variation. These include selegiline (L-deprenyl), a monoamine oxidase-B inhibitor [96], entacapone, a catechol-O-methyltransferase (COMT) inhibitor and pramipexole, a dopamine agonist. Pramipexole, in higher doses, has been reported to cause adverse psychiatric effects [97]. For further discussion on the use of medication see [87].

16.4.5.1 Monitoring of Treatment

CSF amine concentrations should be monitored 3-monthly in the 1st year, 6-monthly in early childhood and yearly thereafter. Where possible, CSF should be collected before a dose of medication is given. CSF folate should also be measured.

Hyperprolactinaemia occurs as a consequence of dopamine deficiency and measurement of serum prolactin can be used as a method to monitor treatment, with normal values indicating adequate L-dopa replacement. It has been suggested that 3 blood prolactin measurements over a 6 hour period may be a more sensitive and less invasive marker than the CSF HVA concentration in deciding on dose adjustment [98].

Blood PHE must also be monitored, but this only needs to be undertaken frequently in DHPR deficiency where a low-PHE diet is used.

Table 16.3 Medication used in the treatment of disorders of biopterin metabolism

Drug	Dose (oral)	Frequency	GTPCH	PTPS	PCD	DHPR
BH_4	2–5 mg/kg/day, increasing to 5–10 mg/day according to blood PHE response	Once daily	+	+	±	±[a]
5HT	1–2 mg/kg/day, increasing by 1–2 mg/kg/day every 4–5 days up to maintenance dose of 8–10 mg/kg/day	Give in four divided doses; final maintenance dose dependent on results of CNS neurotransmitters	+	+	–	+
L-Dopa (as combined preparation with carbidopa)	1–2 mg/kg/day, increasing by 1–2 mg/kg/day every 4–5 days up to maintenance dose of 10–12 mg/kg/day	Give in four divided doses; final maintenance dose dependent on results of CNS neurotransmitters	+	+	–	+
Selegiline (L-deprenyl)	0.1–0.25 mg/day	In three or four divided doses (as adjunct to 5HT and L-dopa; see text)	±	±	–	±
Entacapone	15 mg/kg/day	In two or three divided doses	±	±	–	±
Pramipexole	0.006 mg/kg/day increasing to 0.010 mg/kg/day[b]	In two to three divided doses	±	±	–	±
Calcium folinate (folinic acid)	15 mg/day	Once daily	±	±	–	+

BH_4, tetrahydrobiopterin; CNS, central nervous system; DHPR, dihydropterin reductase; GTPCH, guanosine triphosphate cyclohydrolase I; 5HT, 5-hydroxytrytophan; PCD, pterin-4a-carbinolamine dehydratase; PTPS, 6-pyruvoyl-tetrahydropterin synthase

[a]See text

[b]Higher doses (0.030–0.033 mg/kg/day) have been used but may cause psychiatric adverse effects [97]

16.4.5.2 Outcome

Without treatment the natural history of GTPCH, PTPS and DHPR deficiency is poor, with progressive neurological disease and early death. The outcome with treatment depends upon the age at diagnosis and initiation of therapy and the phenotypic severity [88]. Most children with GTPCH deficiency have some degree of learning difficulties despite adequate control. Patients with PTPS deficiency may have a satisfactory cognitive outcome if detected early. Those with DHPR deficiency, if started on diet, amine replacement therapy and folinic acid within the first months of life, can show normal development and growth. Late diagnosis in all these conditions is associated with a much poorer outcome. The outcome of pregnancies in women with biopterin synthesis disorders on treatment appears to be good without worsening of symptoms or other disease specific complications. Foetal outcome was also satisfactory [99].

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