

Phenylketonuria

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Phenylketonuria is the most prevalent disorder caused by an inborn error in aminoacid metabolism. It results from mutations in the phenylalanine hydroxylase gene. Phenotypes can vary from a very mild increase in blood phenylalanine concentrations to a severe classic phenotype with pronounced hyperphenylalaninaemia, which, if untreated, results in profound and irreversible mental disability. Neonatal screening programmes identify individuals with phenylketonuria. The initiation of a phenylalanine-restricted diet very soon after birth prevents most of the neuropsychological complications. However, the diet is difficult to maintain and compliance is often poor, especially in adolescents, young adults, and pregnant women. Tetrahydrobiopterin stimulates phenylalanine hydroxylase activity in about 20% of patients, and in those patients serves as a useful adjunct to the phenylalanine-restricted diet because it increases phenylalanine tolerance and allows some dietary freedom. Possible future treatments include enzyme substitution with phenylalanine ammonia lyase, which degrades phenylalanine, and gene therapy to restore phenylalanine hydroxylase activity.

Introduction

Phenylketonuria is an inborn error of metabolism, characterised by mutations of the phenylalanine hydroxylase (PAH) gene.¹ PAH converts phenylalanine into tyrosine and requires the cofactor tetrahydrobiopterin (BH4), molecular oxygen, and iron to do so (figure 1). Loss of PAH activity results in increased concentrations of phenylalanine in the blood and toxic concentrations in the brain. Untreated phenylketonuria is associated with progressive intellectual impairment, accompanied by a constellation of additional symptoms, which can include eczematous rash, autism, seizures, and motor deficits. Developmental problems, aberrant behaviour, and psychiatric symptoms often become apparent as the child grows.

Until the 1960s, most children born with phenylketonuria became profoundly mentally disabled, often spending their lifetime in institutional care. The foundations for the early detection and modern management of phenylketonuria were laid by three key findings: in the 1930s, Asbjørn Følling² identified raised levels of phenylalanine in the blood (hyperphenylalaninaemia) as the underlying cause of the neuropsychological deficits; in the 1950s, Horst Bickel³ introduced a low-phenylalanine diet to treat phenylketonuria; and in the 1960s, Robert Guthrie⁴ introduced a diagnostic test suitable for mass screening for hyperphenylalaninaemia (the Guthrie test). Nowadays, many countries around the world include a test for hyperphenylalaninaemia in neonatal screening programmes—ie, the Guthrie test or more modern systems based on tandem mass spectrometry.

Early diagnosis and prompt intervention has undoubtedly allowed most individuals with phenylketonuria to avoid severe mental disability. Dietary restriction of phenylalanine remains the mainstay of treatment but phenylketonuria is an active area of research and new treatment options are emerging that might reduce the burden of the difficult and restrictive diet on patients and their families.⁵

Since its detection in 1934, there has been much research into various aspects of this disease. In this

Seminar, we summarise the history, differential diagnosis, pathophysiology, genetics, outcome, and current and future management of phenylketonuria.

Epidemiology

The prevalence of phenylketonuria varies widely around the world. In Europe the prevalence is about one case per 10 000 livebirths,⁶ but for some areas of Europe it is higher. Persistent hyperphenylalaninaemia is detected in about one in every 4000 births in Turkey because of high consanguinity within the population, and in Northern Ireland.^{7,8} Finland has the lowest prevalence in Europe with one case per 100 000.⁹ In the USA the prevalence is one case per 15 000.¹⁰ In Latin America it varies from about one case per 50 000 to one per 25 000 births; prevalence is generally higher in southern Latin America than elsewhere in that region.¹¹ Estimates of prevalence rates in Asia vary from about one per 15 000 to one per 100 500 births in regions of China,^{12,13} less than one per 200 000 in Thailand,¹⁴ and about one per 70 000 in Japan.¹⁵ Africa seems to have a very low prevalence of phenylketonuria¹⁶ and Spain has an especially high prevalence of mild hyperphenylalaninaemia.¹⁷

Search strategy and selection criteria

We searched PubMed with the terms: "phenylketonuria", "hyperphenylalaninemia", and "PKU" in combination with "diagnosis", "treatment", "diet", "tetrahydrobiopterin", "sapropterin", "pharmacotherapy", "gene therapy", "enzyme replacement therapy", "large neutral amino acids", "glycomacropeptide", "nutritional status", "vitamins", "IQ", "growth", "neurocognitive function", "neuropsychology", "psychosociology", "quality of life", "executive function", "attention deficit", "psychiatric symptoms", "brain", "genetics", "outcome", "follow-up", "management", "adult", and "maternal" from January, 1966 until February, 2010. We gave preference to papers published within the past 5 years, but did not exclude some important less recent publications. No restriction was applied on the language of publications.

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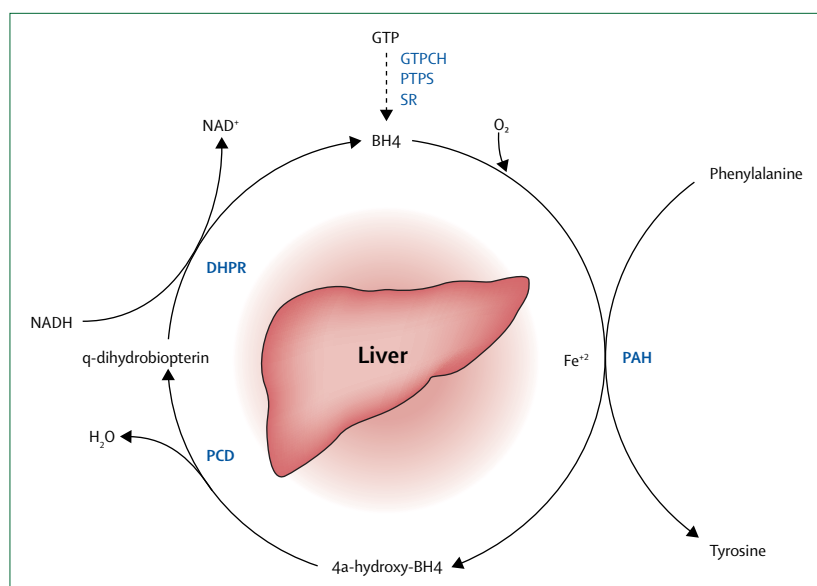


Figure 1: Phenylalanine hydroxylating system

During the hydroxylation of phenylalanine by phenylalanine hydroxylase (PAH), and when molecular oxygen (O_2) and iron (Fe^{2+}) are present, tetrahydrobiopterin (BH4) is oxidised to a 4a-hydroxy-BH4 intermediate, which is subsequently regenerated back to BH4 via quinoid (q) dihydrobiopterin by the enzymes carbinolamine-4a-dehydratase (PCD) and by the NADH-dependent dihydropteridine reductase (DHPR). BH4 is synthesised from guanosine triphosphate (GTP) by three additional enzymes GTP cyclohydrolase I (GTPCH), 6-pyruvoyl-tetrahydropterin synthase (PTPS), and sepiapterin reductase (SR). Mutations in genes coding for PCD, DHPR, GTPCH, PTPS, and SR result in BH4 deficiency.

Molecular genetics and classification

The PAH gene consists of 13 exons and their requisite introns.¹⁸ Phenylketonuria arises when both alleles are mutated. The two mutations can occur in any of the exons, in the splice junctions of the intervening introns, or perhaps in other as yet unidentified areas of the gene, such as in the promoter region. Phenylketonuria is inherited as an autosomal recessive condition. Those who have only one PAH mutation (eg, parents of a child with phenylketonuria) are carriers and have none of the biochemical or clinical characteristics of phenylketonuria.

The Human PAH Mutation Knowledgebase (hPAHdb)—a database of naturally occurring mutations in the human PAH gene—was summarised in 2007 and includes a total of 548 separate mutations.¹⁹ About 50% of these mutations are mis-sense mutations, making them the most frequently occurring type of mutation in the human PAH gene (the next most frequently occurring type, splice junction mutations, account for about 10%). The position and nature of the mutation dictates its effect on the activity of the PAH enzyme, which determines the hyperphenylalaninaemia phenotype of the patient. Little or no enzyme activity results in the classic phenylketonuria phenotype. Other mutations only partly inhibit enzyme activity, giving rise to mild phenylketonuria or mild hyperphenylalaninaemia. PAH alleles are distributed differently in European countries.²⁰ Roughly 5% of mutations do not affect PAH activity.

Phenylketonuria is classified by the severity of hyperphenylalaninaemia. The normal range of blood phenylalanine concentrations is 50–110 $\mu\text{mol/L}$. Individuals with blood phenylalanine concentrations of 120–600 $\mu\text{mol/L}$ before starting treatment are classified as having mild hyperphenylalaninaemia; those with concentrations of 600–1200 $\mu\text{mol/L}$ are classified as mild phenylketonuria (sometimes a moderate classification is included for concentrations of 900–1200 $\mu\text{mol/L}$); and concentrations above 1200 $\mu\text{mol/L}$ denote classic phenylketonuria.²¹ Classification is not always straightforward because phenylalanine concentrations are measured in newborn babies when blood phenylalanine might not have had time to reach its highest value. Classification can also be made on the basis of tolerance for dietary phenylalanine while on diet, which is not always easily and accurately measured. This tolerance is usually not greater than 250 mg per day in classic phenylketonuria, whereas in mild or even moderate phenylketonuria, phenylalanine tolerance can range from 250 to 400 mg per day.²²

PAH requires BH4 as a cofactor. About 1–2% of cases of hyperphenylalaninaemia are due to mutations in genes coding for enzymes involved in BH4 biosynthesis or regeneration.^{23,24} However, some patients with defects in BH4 biosynthesis, such as those with Segawa disease and sepiapterin reductase deficiency, present without hyperphenylalaninaemia.^{25,26}

Pathophysiology and outcomes

Phenylalanine's entry into the brain is mediated by the large neutral aminoacid carrier L-aminoacid transporter 1 (LAT1). Raised phenylalanine concentrations in the brain can impair neuropsychological function through several mechanisms.²⁷ Imaging studies have described white-matter lesions associated with reduced formation of myelin in brain white matter, although no definite causative link between dysmyelination and neuropsychological impairment has been established.²⁸ However, Anderson and colleagues²⁹ did show a relation between white-matter abnormalities and neuropsychological impairment.

Two other large neutral aminoacids—tyrosine, a precursor of dopamine and norepinephrine, and tryptophan, a precursor of serotonin—also enter the brain via the LAT1 carrier.³⁰ High concentrations of phenylalanine in the blood can inhibit LAT1 and other large neutral aminoacids from entering the brain, increasing the potential for neurotransmitter dysfunction and their availability for protein synthesis.³¹ Other possible mechanisms for hyperphenylalaninaemia-induced damage to the brain include reduced activity of pyruvate kinase,³² disturbed glutamatergic neurotransmission,³³ reduced activity of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase,³⁴ and the function of monoamine oxidase B as a modifying gene.³⁵

Adequate control of blood phenylalanine is effective in prevention of most of the CNS deficits associated with phenylketonuria. Most individuals show normal overall development, attain expected educational standards, lead independent lives as adults, form normal relationships, and obtain employment. Their disabilities are usually subtle. Nevertheless, optimum outcome is highly dependent on metabolic control with diet, and this control varies during a patient's lifetime. A meta-analysis³⁶ of five studies of 113 patients with phenylketonuria and 107 controls found a substantially lower average intelligence quotient (IQ) in the phenylketonuria group than in the control group. Another meta-analysis³⁷ showed a 1.9–4.1 point reduction in IQ for every 100 $\mu\text{mol/L}$ increase in mean lifetime phenylalanine concentrations in individuals with phenylketonuria whose treatment was initiated in the neonatal period. This study³⁷ substantiates an older study³⁸ of the UK registry for phenylketonuria, which showed that both early treatment and a decrease of the phenylalanine concentration during treatment resulted in improved outcome.

The subtle abnormalities in phenylketonuria can impair executive function—a broad term encompassing cerebral processes in high-level functions such as planning, problem solving, information processing, bringing previous experience to bear on activities, and sustained attention.³⁹ A study of 14 children with phenylketonuria and matched healthy controls showed that poor metabolic control (blood phenylalanine $>400 \mu\text{mol/L}$) was associated with reduced executive functioning.⁴⁰ However, as with IQ, some degree of deficit in executive functioning was present in all individuals with phenylketonuria irrespective of the degree of blood phenylalanine control. Another study⁴¹ reported children with phenylketonuria to have reduced cognitive function compared with non-phenylketonuric siblings, matched healthy controls, and children from the general population, despite having received early and continuous treatment.

Phenylketonuria also impairs other aspects of neuropsychological functioning. A meta-analysis⁴² reported that phenylketonuria affects a range of neuropsychological speed tests, with higher phenylalanine concentrations associated with a more pronounced deficit; responses in children to a choice reaction time test were especially sensitive to the adverse effects of hyperphenylalaninaemia. In a study of 57 patients with phenylketonuria age 7–14 years whose treatment had been initiated early and had been continuous, those with poor phenylalanine control (blood concentrations $>360 \mu\text{mol/L}$) showed slower information processing, greater susceptibility to task-induced cognitive interference, less consistent performance, and less well maintained performance during a test of sustained attention than did patients with good phenylalanine control and matched healthy controls.⁴³ In the same study

the performance of individuals with good phenylalanine control was not worse than the healthy controls.

Children with phenylketonuria also have behavioural abnormalities and motor dysfunction.⁴⁴ Impairment of memory has been described,⁴⁵ although a meta-analysis showed no difference between well-controlled patients with phenylketonuria and controls for this index.³⁶ Emotional and psychiatric illnesses can also occur. Untreated children with phenylketonuria often have autism. In poorly treated children attention deficit hyperactivity is frequent. In adults agoraphobia, reduced emotional wellbeing, feelings of alienation, depression, social isolation, an impaired ability to communicate, low self-esteem, and poor functioning in social situations have all been reported.^{46–51}

Patients with non-phenylketonuric hyperphenylalaninaemia have a lower risk of neuropsychological dysfunction than do those with phenylketonuria, although compared with healthy controls, some might have decreased executive functioning.⁵² Despite most development of the brain occurring in the early years of life, it seems that discontinuation of dietary management of phenylketonuria during adolescence leads to subtle but measurable deficits in neuropsychological functioning during adult life.⁵³

The universal experience of those caring for individuals with phenylketonuria is that the dietary treatment results in a pronounced improvement in cognitive outcome but imposes a social burden. However, quality of life of those with phenylketonuria has only recently been studied. In the past 3 years two research projects have come to somewhat differing conclusions. On the one hand, Bosch and colleagues⁵⁴ reported no clear abnormalities, concluding that patients with phenylketonuria generally see themselves as healthy, normal people. On the other hand, Simon and co-workers⁵⁵ reported a decreased and delayed autonomy, a lower number of relationships, and having fewer children than seen in healthy individuals. Such conflicting reports perhaps explain the opinion of most health-care professionals that patients with phenylketonuria can vary quite widely in their social capabilities.

Blood phenylalanine concentrations inevitably vary over time in all individuals with phenylketonuria, but some display pronounced variations that might be of clinical importance. Information about the prognostic value of blood phenylalanine variability is sparse. A retrospective study analysed data from 45 children with phenylketonuria who had had their blood phenylalanine managed by a low-phenylalanine diet since infancy (see Management section for a description of dietary management).⁵⁶ The mean blood phenylalanine concentration was 312 $\mu\text{mol/L}$, indicating that overall control of blood phenylalanine was adequate for most patients. The SD of serial blood phenylalanine concentrations from each individual over time was used as a measure of variability. There was no correlation

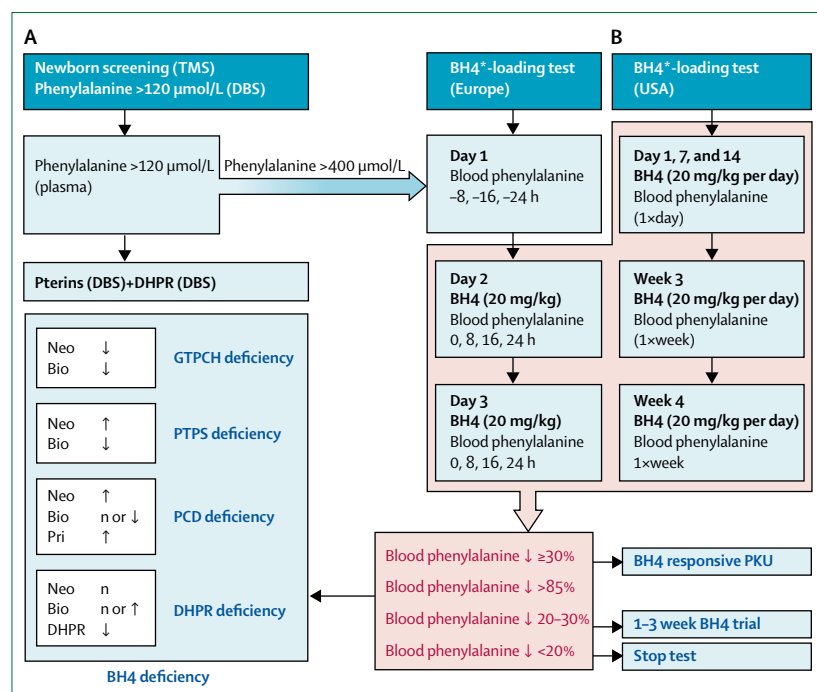


Figure 2: Differential diagnosis of hyperphenylalaninaemia

(A) Flowchart of the differential diagnosis of hyperphenylalaninaemia with relevance to phenylalanine hydroxylase and tetrahydrobiopterin deficiencies. (B) Tetrahydrobiopterin-loading test in Europe⁶⁸ and the USA.⁶⁷ TMS=tandem mass-spectrometry. Phe=phenylalanine. DBS=dried blood spots. DHPR=dihydropteridine reductase. Neo=neopterin. Bio=bipterin. Pri=primapterin. GTPCH=GTP-cyclohydrazine. PTPS=6-pyruvoyl-tetrahydropterin synthase. PCD=pterin-4a-carbinolamine dehydratase. BH4=tetrahydrobiopterin. PKU=phenylketonuria. n=normal. *Tetrahydrobiopterin or sapropterin.

between mean lifetime blood phenylalanine concentrations and most recent IQ measurement (correlation coefficient 0.17, $p=0.38$). By contrast, the correlation between the SD (variability) of blood phenylalanine concentrations and most recent IQ was stronger (correlation coefficient 0.36, $p=0.06$). After stratification for age, the strongest correlation between variability of blood phenylalanine and IQ was recorded in 32 children aged less than 9 years (correlation coefficient 0.36, $p=0.05$). Regression analysis suggested that each one-point increase in the SD of blood phenylalanine reduced IQ by four points.

These data accord with an earlier finding, that increased variability of blood phenylalanine might affect cognitive performance.⁴⁴ They suggest that in addition to maintaining average phenylalanine values, improvement of the stability of blood phenylalanine over time could be an important treatment strategy.

Examination of adults with untreated phenylketonuria in institutional care revealed microcephaly and impaired growth (reduced height and head circumference).⁵⁷ Although phenylalanine restriction in previously untreated adults does not improve cognitive performance, it can reduce aberrant behaviours. In one study, indices of wellbeing were measured before and after treatment in 15 patients who either received dietary management late in life or resumed this treatment after a period on a

normal diet.⁵⁸ 60% ($n=9$) of patients reported an improvement in quality of life after restarting the diet, with 53% ($n=8$) reporting feeling calmer and less upset and 40% ($n=6$) reporting improved general health or alertness.

Another prospective double-blind randomised placebo-controlled crossover trial of phenylalanine-restricted diet was done in a group of 34 adults with late-diagnosed phenylketonuria and severe challenging behaviour.⁵⁹ The results showed that there are substantial difficulties in instituting a phenylalanine-restricted diet in this population, but if attempted, there are potential benefits to quality of life (only 17 of 34 participants completed the 60-week study, although most of the caregivers' comments were positive during the treatment phase).

Mental health outcomes in untreated patients with phenylketonuria are highly heterogeneous, but these patients do not all develop pronounced mental disability. Such disabilities might be affected by variable concentrations of phenylalanine in the brain as shown by magnetic resonance spectroscopy studies.⁶⁰

Untreated or poorly treated phenylketonuria in women during pregnancy is a particular health concern because the fetus is exposed to teratogenic concentrations of phenylalanine. Maternal blood phenylalanine concentrations of less than 900 µmol/L almost always result in mental disability and microcephaly and often result in congenital heart disease and intrauterine growth retardation in the offspring.⁶¹ Maternal blood concentrations less than 360 µmol/L might reduce cognitive ability in the offspring.⁶² These offspring also usually display facial dysmorphisms similar to those seen in individuals with fetal alcohol syndrome. Adequate phenylalanine control before conception and continually throughout pregnancy is important, as cognitive outcome in these offspring is better than in children whose mothers began or resumed dietary phenylalanine restriction after conception.⁶³ However, many pregnancies are unplanned, and cognitive deficits in some mothers with phenylketonuria mitigate against maintaining adequate metabolic control.⁶⁴

Offspring born to women who are fed with a normal diet, unless, as in rare cases, the infant also has phenylketonuria. Mothers with maternal phenylketonuria can breastfeed their non-phenylketonuria infants without restriction. These infants carry a mutant gene for phenylketonuria but their residual PAH activity is sufficient to adequately metabolise phenylalanine, even the additional amount they receive from their mother's breast milk.

Assessment

Every infant identified in newborn screening with hyperphenylalaninaemia should be assessed at a metabolic centre as early as possible. Initial assessment is by blood aminoacid analysis. If the baby has hyperphenylalaninaemia, blood aminoacid analysis will

reveal increased phenylalanine concentrations (>120 µmol/L), normal or reduced tyrosine concentrations (phenylalanine to tyrosine ratio >2), and normal concentrations of the remaining aminoacids. This is the pattern of all forms of hyperphenylalaninaemia/phenylketonuria. In these patients two differential diagnostic assessments should be done. First, whether or not the patient has a defect in BH4 synthesis or recycling should be clarified. If they do not have such defects, whether the patient with hyperphenylalaninaemia/phenylketonuria can be treated with diet restrictions only, or can at least be helped in part with BH4 alone or together with a restricted diet should be assessed.

In European countries,⁶⁵ the infant is treated in hospital and given a BH4 load of 20 mg/kg bodyweight orally. Their blood phenylalanine concentration is measured before and 8, 16, and 24 h after the BH4 load is given. A striking normalisation (within 8 h) in phenylalanine concentrations indicates BH4 deficiency whereas very little or no reduction in phenylalanine indicates BH4-non-responsive phenylketonuria. In the USA BH4 is not given to newborn babies. Instead, urine and a filter-paper-dried blood specimen are obtained for measurements of urinary pterins (neopterin and biopterin) and red blood cell dihydropteridine reductase to assess the possibility of a defect associated with BH4 deficiency.⁶⁶ The following abnormal pterin patterns in blood or urine are diagnostic for different types of BH4 deficiency: in GTP-cyclohydrolase I deficiency, both neopterin and biopterin are very low or not detectable; in 6-pyruvoyl-tetra hydropterin synthase deficiency, neopterin is very high and biopterin is very low or not detectable; in pterin-4a-carbinolamine dehydratase deficiency, neopterin is high, biopterin is low or borderline, and primapterin is high; and in dihydropteridine reductase deficiency, dihydropteridine reductase activity is low, neopterin is normal, and biopterin is high (figure 2). In some patients with dihydropteridine reductase deficiency, pterin patterns can be normal and only dihydropteridine reductase activity in a filter-paper-dried blood specimen is diagnostic.

The BH4-loading test can also identify BH4-responsive phenylketonuria. Various BH4-loading tests have been proposed for the diagnosis of BH4-responsive phenylketonuria after the newborn period. These tests usually involve giving BH4 at doses of 10 or 20 mg/kg, as a single dose or as repeated doses and with or without giving concomitant phenylalanine.^{67–69} A commercial form of BH4 (sapropterin dihydrochloride [Kuvan, BioMarin Pharmaceutical Inc, CA, USA]) is available, but the best test has yet to be established.

The widely quoted definition of BH4-responsiveness is a reduction in blood phenylalanine of at least 30%,⁷⁰ but different goals can be set for individual patients and no cutoff value for the BH4-loading test has been provided (figure 2). Policies for testing BH4-responsiveness differ

between Europe and the USA. In Europe the previous 24-h test was extended to a 3-day test in 2008.⁶⁸ The test consists of phenylalanine monitoring on the first day; challenge with BH4 at a dose of 20 mg/kg per day and measurement of blood phenylalanine concentrations before and 8, 16, and 24 h after giving BH4 on the second day; and a second challenge with the same dose of BH4 and the same blood sampling regimen on the third day. A reduction of blood phenylalanine concentrations greater than 85% is indicative of BH4 deficiency, while a reduction of less than 20% implies that the patient is a non-responder. If a satisfactory treatment response is noted (blood phenylalanine concentrations reduced by at least 30%), the dose is adjusted (recommended range 5–20 mg/kg) according to the phenylalanine response. If an adequate response is not noted (blood phenylalanine reduced by 20–30%), treatment at a dose of 20 mg/kg per day is continued for a further 1–3 weeks with daily blood phenylalanine monitoring, at which time the patient is declared to be responsive (titrate treatment as above) or non-responsive (discontinue treatment). In patients with BH4 deficiency, blood phenylalanine concentrations return to normal 4–8 h after BH4 is given. In the USA, BH4 is given daily (20 mg/kg bodyweight), and patients provide blood samples at home on day 1, 7, and 14.⁶⁷ If necessary, BH4 is given for 2 more weeks and blood phenylalanine concentrations are monitored once per week (figure 2). A reduction of blood phenylalanine concentrations greater than 85% is indicative of BH4 deficiency, while a reduction of less than 20% implies that the patient is a non-responder.

	<2 years	2–6 years	7–9 years	10–12 years	13–15 years	>16 years
Australia	100–350	100–350	100–350	100–450*	100–450*	100–450*
Austria	40–240	40–240	40–240	40–900	40–900	40–1200
Croatia	130–240	130–360	130–360	130–600	130–600	130–960
Denmark	120–300 (<4 years)	120–400 (4–8 years)	120–600 (8–10 years)	120–700	120–900	120–900
France	120–300	120–300	120–300	120–600	120–900	120–1200
Germany	40–240	40–240	40–240	40–900	40–900	40–1200
Hungary	120–360	120–360	120–480	120–480	120–480 (7–14 years)	120–600 (>14 years)
Italy	120–360	120–360	120–360	120–360	120–600	120–600
Japan	120–240	120–360	180–360	180–480	180–600	180–900
Netherlands	120–360	120–360	120–360	120–360	120–600	120–600
Poland	120–360	120–360	120–360	120–720	120–720	120–720
Portugal	120–360	120–360	120–360	120–360	120–480	120–480
Spain	<360	<360	<480	<480	<720	<720
Switzerland	100–300	100–400	100–400	100–600	100–600	100–600
Turkey	60–240	60–240	60–240	60–240	60–240	60–240
UK	120–360	120–360	120–480	120–480	120–700	120–700
USA	120–360	120–360	120–360	120–360	120–600	120–900

*Some phenylketonuria centres accept a concentration of less than 700 µmol/L.

Table: Target blood phenylalanine concentrations (µmol/L) as recommended for treatment of phenylketonuria in different countries, by age group

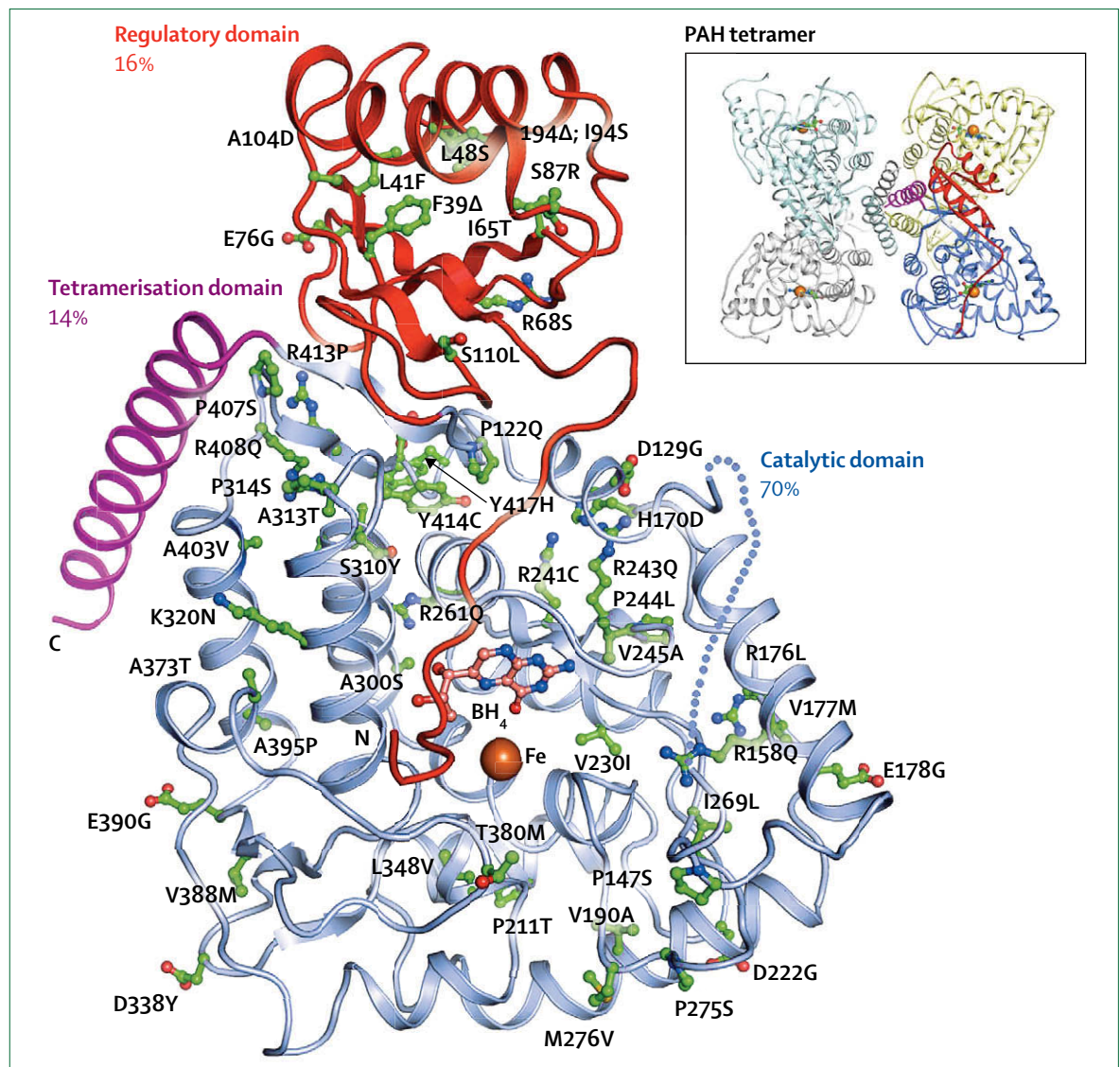


Figure 3: Three-dimensional crystal structure of the human phenylalanine hydroxylase monomer with most common BH₄-responsive mutations. The most mutations (70%) were positioned in the catalytic domain (blue), 16% of mutations are in the regulatory domain (red), and 14% are in the tetramerisation domain (lilac). Inset: phenylalanine hydroxylase tetramer. Modified with permission from Zurflüh and colleagues.⁶¹ PAH=phenylalanine hydroxylase.

Management

Diet therapy

By contrast with the USA,⁷¹ there is great inconsistency between European countries and between clinical centres with respect to target therapeutic blood phenylalanine concentrations, even during the first and most important decade of life (table).^{65,72} After the first decade of life the inconsistency increases in both Europe and the USA.^{65,71,72} There is also a lack of consensus about the severity of hyperphenylalaninaemia—defined as blood phenylalanine concentrations less than 120 $\mu\text{mol/L}$ —at which treatment should be initiated. Most clinical centres use one of three phenylalanine concentration threshold:

greater than 360 $\mu\text{mol/L}$, greater than 400 $\mu\text{mol/L}$, or greater than 600 $\mu\text{mol/L}$.

The restriction of dietary phenylalanine remains the mainstay of phenylketonuria management, and usually begins immediately after confirmation of hyperphenylalaninaemia in a neonate. Patients with phenylketonuria have to accept the phenylalanine-free formula and avoid foods rich in protein (eg, meats, fish, eggs, standard bread, most cheeses, nuts, and seeds) and foods and drinks containing aspartame, flour, soya, beer, or cream liqueurs. Low-protein natural foods such as potatoes, some vegetables, and most cereals can be eaten but only in severely restricted amounts. Low-protein variants of some foods exist, such as low-protein bread and low-

protein pasta. The required amount of daily protein is largely obtained from manufactured, commercially available phenylalanine-free protein substitutes.

During infancy, adherence to the diet is straightforward because the child's parents control nutritional intake. Most centres allow breastfeeding to provide the natural protein.⁶⁵ As children get older, adherence to the diet becomes increasingly difficult because meals have to be planned rigorously and children cannot choose the food routinely consumed by their peers. Consequently, compliance with the diet is often poor, especially when the patient reaches adolescence, as evidenced by poor control of blood phenylalanine concentrations in this age group.^{5,73,74} Long-term maintenance of the diet is important, because patients find it difficult to return to adequate dietary compliance after a period of eating an unrestricted diet. The difficulty of the dietary regimen, psychosocial and emotional factors, issues relating to family cohesion, commitment of parents to maintaining the diet, ethnic background of the mother, knowledge of the disease, attitudes to health-care professionals, and lack of reimbursement for supplements (in some health-care systems) have been cited as reasons for suboptimum dietary compliance.^{10,74–76}

The diet is especially difficult to maintain in adults. Many adults discontinue it or refuse to return to it, which is a continuous challenge in the follow-up of phenylketonuria. Treatment with BH4 cofactor in selected cases or large neutral aminoacid supplements can be helpful. Dietary therapy recommendations vary according to the target blood phenylalanine concentrations in different countries.

Glycomacropeptide

Glycomacropeptide is a protein derived from cheese whey that is rich in specific essential aminoacids but contains no tyrosine, tryptophan, or phenylalanine.⁷⁷ This protein can be a useful adjunct to the phenylalanine-restricted diet, when manufactured to sufficient purity to ensure it is free from phenylalanine that could be contained in traces of other whey proteins, and with supplementation of missing aromatic aminoacids. Studies of patients with phenylketonuria suggest that foods containing glycomacropeptide are palatable;^{78,79} one study showed that ten of 11 patients preferred a diet supplemented with glycomacropeptide to their usual aminoacid formula.⁸⁰

BH4

Some mutations are associated with a BH4-sensitive phenotype of phenylketonuria, in which giving pharmacological doses of exogenous BH4 results in an increase in the activity of PAH that is sufficient to reduce circulating phenylalanine to a therapeutically relevant extent.^{81,82} These mutations usually present with substantial residual activity when expressed re-

combinantly in eukaryotic cell systems and are located in all regions of PAH (figure 3). However, the relation between genotype and BH4-responsiveness is complex.⁸³ A study in Croatian patients with phenylketonuria showed that the prevalence of BH4-responsive phenylketonuria was lower than would have been expected from genotyping alone.⁸⁴ Thus, although genotyping is useful in excluding non-responders (classic phenylketonuria), it is insufficient for a reliable prediction of those who are BH4 responsive. Mechanisms of BH4-responsiveness are multifactorial,⁸⁵ but the main mechanism seems to be stabilisation of the PAH tetramer by preventing misfolding, subunit aggregation, proteolytic degradation, and thermal inactivation.^{86,87}

Two formulations of BH4 have been studied clinically—6R-BH4 dihydrochloride (Schircks Laboratories, Jona, Switzerland) and sapropterin dihydrochloride (BioMarin Pharmaceutical Inc)—but only sapropterin dihydrochloride is approved by the US Food and Drug Administration, the European Medicines Agency, and in Japan for therapeutic use. In some patients, a single daily dose (10–20 mg/kg bodyweight) of this compound is sufficient to maintain a stable blood phenylalanine concentration over 24 hours.⁸⁸ Clinical studies using either formulation^{89,90} have shown BH4-sensitive phenylketonuria in a subset of patients. The proportion of BH4-sensitive patients increases as the severity of the phenotype decreases (figure 4).⁷⁰ In one clinical study,⁹¹ the prevalence of BH4 responsiveness in patients with phenylketonuria (n=557) for blood phenylalanine reductions of 20%, 30%, 40%, and 50% was 55%, 46%, 41%, and 33%, respectively, with the 24-h loading test with 20 mg/kg BH4. Using the 30% threshold, BH4 responsiveness was similar irrespective of the test (8-h vs 24-h) in patients with mild hyperphenylalaninaemia (79–83% responders), mild phenylketonuria (49–60% responders), and classic phenylketonuria (7–10% responders).⁹¹

For more on mutations associated with a BH4-sensitive phenotype of phenylketonuria see BIOPKU database; <http://www.biopku.org>

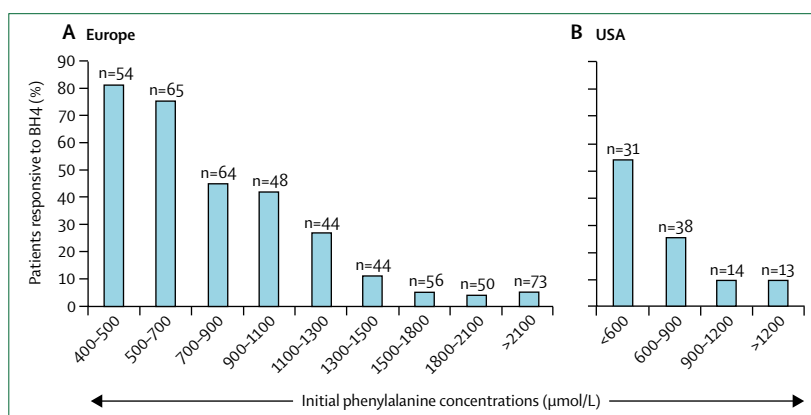


Figure 4: Responsiveness to tetrahydrobiopterin in patients with phenylketonuria according to initial blood phenylalanine concentrations

Outcome of the 24-hour loading test in European phenylketonuria population with 20 mg/kg tetrahydrobiopterin (BH4) and a 30% responsiveness cutoff (A).⁸⁹ Patients in the USA who responded to sapropterin (10 mg/kg per day) on day 8 by at least a 30% reduction of blood phenylalanine concentrations (B).⁹⁰

A screening study using sapropterin dihydrochloride, the registered formulation of BH4, found that about one patient in five responded to the treatment, with greatest response rates in subgroups with less severe hyperphenylalaninaemia.⁹² A group of patients from this study who responded to treatment were enrolled in a subsequent trial in which patients were randomly allocated to double-blind treatment with sapropterin dihydrochloride or placebo.⁸⁸ A reduction in blood phenylalanine of at least 30% was recorded in 44% (n=18) of the active treatment group and 9% (n=4) of the placebo group (p=0·0002), although about two-thirds of patients responding to treatment in the screening study did not respond in the randomised trial. Control of blood phenylalanine in responders was well maintained during a 22-week extension to this trial.⁹³

Data from controlled clinical trials with sapropterin dihydrochloride indicate a similar occurrence of all-cause adverse events with this treatment (64%) and placebo (71%).^{88,93} The most frequent adverse events (all mild or moderate in severity) reported for sapropterin dihydrochloride were headache (20%), pharyngolaryngeal pain (15%), nasopharyngitis (14%), vomiting (13%), and diarrhoea (10%).^{88,93} Thus far, only data for the effect on phenylalanine metabolism (as either a decrease of plasma phenylalanine concentrations or an increase of phenylalanine intake resulting in comparable phenylalanine concentrations) are available. Data for neurocognitive outcomes in these patients are scarce.⁹⁴ The number of patients who, for various reasons, stop BH4 treatment is also unknown.

Large neutral aminoacids

Because phenylalanine competes with other large neutral aminoacids for transport across the blood–brain barrier, supplementation with these aminoacids other than phenylalanine could provide another potential treatment approach. Giving these aminoacids after an oral phenylalanine load abolishes the rise in phenylalanine in the brain of patients with phenylketonuria.³⁰ A double-blind, placebo-controlled study also showed a reduction in blood phenylalanine from baseline of 39% during short-term treatment with large neutral aminoacids.⁹⁵ Additionally, large neutral aminoacid treatment seems to have a beneficial effect on executive functioning.⁹⁶ However, we do not have many clinical data for this treatment strategy.

Phenylalanine ammonia lyase

Phenylalanine ammonia lyase is a bacteria-derived enzyme that catalyses the conversion of L-phenylalanine to transcinnamic acid and ammonia without a cofactor requirement.⁹⁷ In the mouse model of phenylketonuria, blood and brain concentrations of phenylalanine were reduced during 90 days of treatment with injections of phenylalanine ammonia lyase.⁹⁸ A clinical trial with a pegylated formulation of this enzyme for subcutaneous

management of patients is underway. In a study to develop an oral form of this enzyme, stability of phenylalanine ammonia lyase was slightly improved against intestinal proteolytic digestion by site-directed mutagenesis of a chymotrypsin cleavage site, making it potentially useful for oral application.⁹⁹

Gene therapy

In principle, restoration of the function of the affected enzyme could be accomplished with a gene therapy approach. For example, a study has reported restoration of hepatic PAH activity after intramuscular injection of PAH gene vectors in PAH-deficient mice.¹⁰⁰ A similar approach protected offspring of PAH-deficient mice from the teratogenic effects of hyperphenylalaninaemia.¹⁰¹ Another study in animals showed beneficial effects of simultaneous expression of PAH along with two other enzymes in the biosynthetic pathway for BH4.¹⁰² Transplantation of cells with fully functioning PAH/BH4 metabolism (or even liver transplantation)¹⁰³ or gene therapy with phenylalanine ammonia lyase are alternative approaches.

Conclusions

Our understanding of the genetic basis, pathophysiology, and management of phenylketonuria has increased substantially in recent years. In particular, we now understand the importance of maintaining control of blood phenylalanine concentrations during and after childhood to achieve the best long-term neuropsychological outcomes. Long-term compliance with treatment remains a key challenge for the future, especially with respect to adolescents and young adults, those trying to become pregnant, and during pregnancy. The recent introduction of sapropterin dihydrochloride into therapeutic use is likely to contribute to the management of phenylketonuria for a subset of patients who respond well to this treatment. The therapeutic use of large neutral aminoacids should be studied in more detail. In the longer term, novel therapies such as phenylalanine ammonia lyase and, further ahead, gene therapy could ease the considerable burden of the dietary phenylalanine restriction for phenylketonuria patients and their families.

Contributors

All authors contributed equally to the writing of this Seminar.

Conflicts of interest

We declare that we have no conflicts of interest.

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