Phenylketonuria
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Abstract
Phenylketonuria remains one of the most common inborn errors in the United Kingdom. It is detected on the newborn heel-prick screening sample allowing early treatment with a strict low phenylalanine diet supplemented with artificial amino acids, and appropriate vitamin and minerals. Although the long-term prognosis is good, there is an increasing body of evidence highlighting subtle problems in neuropsychological function with slower reaction times and poorer executive function than peers. White matter changes clearly seen on brain magnetic resonance imaging may have some relationship to these neuropsychological difficulties but their significance is not clearly understood. The diet, although successful, is difficult to follow lifelong and with its attendant risks of nutritional deficiencies needs careful specialist management. In view of these challenges new treatments such as sapropterin (a tetrahydrobiopterin analogue) and large neutral amino acids are currently being used in phenylketonuria and a human trial has started using ammonia lyase as enzyme replacement therapy. Maternal phenylketonuria syndrome remains a risk for those who conceive whilst blood phenylalanine is elevated and females must be counselled early in childhood to avoid this risk.

Keywords hyperphenylalaninaemia; phenylketonuria; PKU; sapropterin therapy

Phenylketonuria (PKU) can claim at least three ‘firsts’: the first metabolic disorder to have a successful treatment; the first to be controlled by diet; and the first to be detected by newborn screening. This review describes the current management and outcome of PKU and summarizes developments of new therapies.

Terminology
PKU was first described in 1934 by Folling as ‘imbecillitas phenylpyruvic’ following the finding of phenylpyruvic acid (a phenylketone) in the urine of two siblings with mental retardation. The term phenylketonuria was later used by Penrose and has remained the most widely used name for hyperphenylalaninaemia (HPA) due to phenylalanine hydroxylase deficiency. It is now generally applied to the more severe end of the spectrum in which phenylalanine is greater than 1200 μmol/l whilst consuming a normal protein intake and this type is also referred to as classical PKU. HPA is a frequently used term in the United Kingdom (UK) and is used in the remainder of this article.

Natural history
PKU causes severe intellectual impairment. In classical PKU developmental delay is apparent within the first year of life and progresses to severe mental retardation (IQ < 50). Examination shows limb spasticity, tremor and microcephaly. A seizure disorder is frequently present and EEG abnormalities are common. Other findings may include hypopigmentation of the hair, skin and iris due to reduced melanin synthesis. Parkinsonian features and gait abnormalities are also often observed in the untreated individual. Abnormalities of behaviour are very common including hyperactivity, aggression, anxiety and social withdrawal. The natural phenotype is rarely seen now due to widespread newborn screening for this condition. However, PKU should be considered as a possible diagnosis particularly in an individual born in a country where newborn screening may not be available.

Detection
In most first-world countries the diagnosis of PKU is made through newborn screening. PKU can be readily detected by a raised phenylalanine on the newborn heel-prick blood test. Blood phe and tyrosine (tyr) are measured. In PKU the ratio between these two metabolites is greater than three. The cut-off value for a presumed positive screen varies between countries depending on the infant’s age at screening. The UK practice is to sample between days 5–8, using a cut-off phe of 240 μmol/l. Other causes of elevated phe, aside from PKU, include a disorder of biotin production or recycling, liver dysfunction or premature babies receiving amino acid containing parenteral feeds.

Disorders of biotin production or recycling can cause raised phe since tetrahydrobiopterin (BH4) is the co-factor for the phenylalanine hydroxylase enzyme (see Figure 1). These disorders, previously called ‘malignant PKU’, are best named by their respective enzyme deficiency. In all positive screening cases a raised phe level is caused by a defect in the production or recycling of tetrahydrobiopterin (BH4). PKU is still the most commonly used term in the United Kingdom (UK) and is used in the remainder of this article.
Phenylalanine is an essential amino acid which is metabolized in the liver by the enzyme phenylalanine hydroxylase (PAH). The first step of catabolism of phenylalanine is irreversible conversion to tyrosine. The PAH enzyme requires tetrahydrobiopterin as its co-factor. PKU develops due to deficiency in, or absent activity, of the PAH enzyme and results in elevated phe and reduced levels of tyrosine. When the pathway to tyrosine is blocked, excess phe is transaminated to phenylpyruvic acid and excreted in urine. The enzyme is coded by the PAH gene located on the long arm of chromosome 12. More than 400 pathological mutations are recognized and most affected subjects are compound heterozygotes in that they carry two different mutations. There is a good correlation between pre-treatment phe levels, phe tolerance and genotype. However, outcome is affected by many factors and genotype knowledge is of limited value in predicting clinical management. But mutation analysis has some value in predicting BH4 responsiveness (see below).

PKU is inherited as an autosomal recessive condition. Prenatal diagnosis, although rarely requested, is possible by mutation analysis if the mutations are already identified in the index case.

Treatment
The aim of PKU treatment is the reduction of blood phe to a level allowing normal brain development. An individual’s blood phe depends upon dietary intake of phe and the residual activity of phe hydroxylase. Although in some cases it is possible to augment phe hydroxylase activity (see new treatments), in most cases treatment relies upon reducing phe intake by a restriction of natural protein. In most cases meat, cheese, bread, fish and milk must be avoided. A semi-synthetic diet is used which comprises:

- foods of low phe content in unlimited amounts such as many fruits and vegetables;
- weighed amounts of foods containing medium amounts of phe (e.g. broccoli, potato). The amount of phe ingested is often calculated using an exchange system. In the UK system 1 ‘exchange’ = 50 mg phe which is approximately 1 g protein;
- phe-free amino acid mixtures to provide normal or supranormal total protein intake;
- vitamins, minerals and trace elements.

The diet should be strictly followed with these food groups evenly distributed throughout the day. Aspartame should be avoided as it contains large amounts of phe. Infant formulae feeds which are phe-free are available; many contain added essential fatty acids. These are used in conjunction with a small amount of standard infant formulae. It is possible to continue breast feeding even in severe PKU by giving a measured amount of phe-free formula prior to a breast feed. All PKU diets should be administered with the advice of a specialist dietician.

Monitoring of treatment
It is vital to monitor phenylalanine levels, usually through frequent blood spot analysis. Guidelines vary between countries regarding frequency and acceptable phe levels. In the UK, infants and young children should have weekly samples aiming at levels 120–360 μmol/l; school-age children fortnightly samples with a range of 120–480 μmol/l; and in adolescents and adults monthly samples with an upper limit of 700 μmol/l. These guidelines were reviewed in 2010 but despite intense debate on ‘safe’ levels of phe particularly at the milder end of the spectrum, the guidelines were unchanged. Details of these discussions are available through the UK newborn bloodspot screening programme website (see below).

Treatment target debate outside UK
The US PKU guidelines have recently been updated. They recommend lifelong dietary treatment aiming for the therapeutic target used in young children in UK i.e. 120–360 μmol/l rather than allowing any relaxation with age. Recent evidence supports a lowering of the target range for a longer period of time, that is, at least through adolescence. The brain is still undergoing important organization during adolescence and relaxing diet at this age may result in subtle deficits in neuropsychological function as an adult. A new European Guideline is currently underway and is due to be published in 2015. There is an appetite to develop a uniform treatment policy throughout Europe.

In addition to monitoring phe levels, other nutritional indices such as vitamin B12, folate, iron, calcium, phosphate and essential fatty acids should be measured in those with poor dietary adherence. Growth parameters are also monitored. Some clinics advocate regular neuropsychological testing whereas others only refer for such assessment where difficulties are suspected. It is likely that the new European guideline will be more offer more specific recommendations on such
monitoring; the recent US guideline defines timelines for these assessments.

**Nutritional issues in PKU**

The nutritional sufficiency of the PKU diet must be regularly monitored by a specialist dietician. Vitamin B12 deficiency is a particular risk in adolescents and adults who have stopped taking their supplements but are still restricting their protein intake by habit. Other vitamin or mineral deficiencies have occasionally been noted in PKU such as iron, selenium and calcium. Bone mineral density may be lower than normal in this group of patients although the reasons for this are unclear. Polyunsaturated fatty acids levels are frequently low in the plasma and red cells of PKU children on diet. This is probably due to its low animal protein content. Although it seems prudent to supplement PUFAs, there is not yet clear evidence on requirements in this group nor on the long-term impact on neurodevelopment.

**Dietary adherence**

Adherence to treatment in PKU is particularly challenging for several reasons: the strict diet creates awkward social occasions; the diet itself is unpalatable; frequent blood tests leads to needlephobia in some children; and the diet is time consuming and may be costly in some countries. Dietary non-adherence increases with age. It is important to provide education programmes to help compliance; such as toddler groups and teenage camps.

**Duration of diet**

Despite the knowledge that has accumulated on PKU, the risk of stopping diet in adulthood is not yet known. The oldest early-treated patients are now entering middle age. The vast majority of these remain neurologically healthy but the possibility of late neurological decline cannot be excluded. Current recommendation is diet for life. This is based upon the evidence of poorer neuropsychological performance when phe levels are elevated and the knowledge that MRI of the brain shows abnormalities of myelin of uncertain significance. Where diet for life is refused then at least monitoring for life by regular clinic attendance is encouraged.

**New treatments for PKU**

As long-term dietary compliance is difficult there is a need for alternative modes of treatments:

- **Enzyme replacement therapy**

  The non-mammalian enzyme phenylalanine ammonia lyase converts phe to a non-toxic substance called transcinnamic acid. It has been tested using enteral, intraperitoneal and subcutaneous routes. More recently enzyme stability has been achieved by pegylation with polyethylene glycol. Results of the first human Phase I trial of single dose subcutaneous injection have been recently published using this preparation (‘PEG-PAL’). A reduction in blood phe was observed when the highest tested dose was used. Immune reactions to both the PAH enzyme and the polyethylene glycol conjugate were common and further work is underway to test repeated dosing.

- **Large neutral amino acids**

  The large neutral amino acids (LNAA) including phe compete at the blood brain barrier for entry to the brain through the same transporter (LAT1). Increasing the concentration of LNAA in the blood therefore reduces phe entry to the brain. There is a similar mechanism in the gut, and absorbed phe is lower if LNAA are supplemented in generous amounts. It is not yet understood precisely how much advantage LNAA has over current amino acid preparations and it appears unlikely that LNAA would be prescribed as a sole treatment without phe restriction at least in childhood or in pregnant women with PKU. However, it may be a useful approach for adults. The optimal method of administration of LNAA along with phe restricted diet remains under discussion.

- **Glycomacropeptide**

  Glycomacropeptide (GMP) is an intact whey protein which is naturally low in phenylalanine (also in tyrosine, histidine, leucine, tryptophan and arginine). It is being investigated as a useful adjunct to dietary treatment for PKU by allowing a greater intake of ‘natural’ protein in the diet thus allowing a decrease in artificial amino acid products. It has been shown in the mouse model to improve bone density. It is not yet readily available in the UK.

- **Sapropterin therapy**

  BH4 therapy has been used for some time to treat defects in the pterin pathway. However several studies have shown that administration of BH4 can result in a reduction of phe levels even in phenylalanine hydroxylase deficiency. The mechanism is not completely understood but most likely is due to stabilization of a misfolded protein thus suggesting that those with mild PKU are most likely to benefit. However, some patients with classical PKU have also shown a response. It is estimated that 80% of those with mild PKU and 40% of those with classical PKU will benefit from this treatment. Genotype can help in predicting response but it cannot be assumed, and a short therapeutic trial is required to judge BH4 responsiveness. Sapropterin dihydrochloride is a synthetic formulation of the active 6R-isomer of BH4 which is licensed in Europe and US for the treatment of PKU. In Europe the license is granted for individuals over 4 years of age with phenylalanine hydroxylase deficiency that has shown a response to the drug. Two randomized controlled trials and two open label extension Phase III studies have proven the ability of Sapropterin to reduce blood phe or improve phe tolerance in those with PKU. The drug has a good safety profile. Sapropterin is currently only commissioned in England for poorly controlled maternal PKU. However, a commissioning policy for its use in children is under currently under discussion. The drug is used extensively throughout the US and parts of Europe. Longer term reports of its effect on quality of life are awaited.

- **Gene therapy**

  The most promising results come from experiments using recombinant adeno-associated virus vector in which long-term correction without adverse effects has been reported in the mouse model (PKUenu2). There are no human gene therapy studies yet.

- **Liver transplantation**

  This procedure effectively provided phe hydroxylase activity in a child with PKU who required liver transplantation for an
unrelated problem. The risks and complications of transplant render it an unrealistic option.

Transplanted hepatocytes have no proliferative advantage over host PKU hepatocytes. It is postulated that liver progenitor cells could be a potential therapy for PKU. Although hepatocyte and/or hepatic stem cell studies are underway in humans with other metabolic disorders, there are no human trials currently in PKU.

Outcome of PKU
The outcome for PKU is good. If dietary treatment is started early (before 3 weeks of age) and blood phe levels remain satisfactory, then ultimate IQ should be in the average range although slightly reduced in comparison with peers or siblings. Outcome is dependent upon the quality of blood phenylalanine control. The small numbers of adolescents and adults who have developed overt neurological disease have had poor metabolic control in childhood. However recent research does identify some problems in the treated PKU population.

Magnetic resonance imaging (MRI) of the brain
Brain MRI in children and adults commonly shows abnormalities in the cerebral white matter even in treated PKU. These signal changes are likely to be intramyelinic oedema which usually affects the periventricular white matter. Milder changes affect only the occipital lobe but more severe involvement progresses rostrally to the frontal lobe. The degree of white matter change is associated with recent metabolic control (average phe level in the preceding year and current phe levels) but not to early phe levels. Despite years of investigation the functional consequences of these findings is unclear. There is some recent evidence suggesting a correlation between neuropsychological performance and more widespread white matter changes. The MRI changes are reversible upon lowering blood phe within about 2 months. The lesions appear static at least over a 5 year period in adulthood if phe levels remain stable.

Neuropsychological studies
Despite many neuropsychological studies in treated PKU it remains difficult to draw clear conclusions: the numbers studied are often small; the types of neuropsychological test vary between studies; the ages are different (children or adults); the background phe control and phe level at the time of testing vary. The tentative conclusion is that some neuropsychological damage occurs even in treated PKU.

Reaction times are delayed in PKU and this relates to concurrent elevated phe levels. Executive function i.e. higher level processes requiring interactions between several areas of the brain, has been extensively studied as it is governed by the prefrontal cortex. This is a dopamine sensitive area of the brain which may be especially vulnerable in PKU. Of the various subsets of executive function studies, inhibitory control is impaired in early treated PKU. Tests of working memory may have an age-related effect as children show largely normal results but a decline in function is observed in adolescents and adults.

There are other behavioural and psychiatric symptoms attributed to PKU. Poor dietary control early in life results in anxiety, hyperactivity and social withdrawal, and those with satisfactory early treatment still appear to have a higher risk of low self-esteem and possibly depression. Further research is required in this field: larger longitudinal studies with more uniformity are required.

Maternal PKU
Infants born to mothers with blood phe above 1200 µmol/l show fetal damage including low birth weight, microcephaly, dysmorphic facies, slow postnatal growth and development and intellectual impairment. The facial features are similar to fetal alcohol syndrome: small palpebral fissures, epicantthic folds, long philtrum and thin upper lip. Although congenital heart disease is the most common, other organ malformation can occur. The risk to mothers with milder PKU is smaller and appears to correlate with phe level. In view of these risks all females with PKU must be monitored for the duration of their lives, being counselled early in their childhood and having a longstanding trusting relationship with their PKU team. The aim for managing maternal PKU is for women to be on a strictly controlled diet preconception with regular phe monitoring showing levels between 100 and 250 µmol/l. There is also evidence that if diet is started by 10–12 weeks of pregnancy a satisfactory outcome can be achieved. Sapropterin is used in pregnancy. The evidence for its safety is based on case reports and it is unlikely that a clinical trial will ever take place. However, a sub-registry of the US PKUDOS registry called PKU MOMS contains results of its safe use in over 20 pregnancies. This registry will continue to monitor and report safety and efficacy.

Summary
PKU is a success story. It can be detected early in life allowing early instigation of dietary therapy. The treatment is effective and children grow and develop normally. Within this framework of success however there are still unanswered questions about long-term neuropsychological outcome and the necessity of diet for life. Dietary treatment remains challenging for many patients hence the importance of the alternative approaches now on the horizon.

FURTHER READING

Phenylketonuria (PKU) Demographic, Outcomes, and Safety Registry: PKUDOS; clinicalTrials.gov NCT00778206 PKU-MOMS sub-registry.


www.newbornbloodspot.screening.nhs.uk.


www.nspku.org.

**Practice points**

- PKU is one of the most common IEM in the UK with an incidence 1 in 10 000
- Dietary therapy remains the mainstay of treatment
- Long-term monitoring requires a specialist team to avoid nutritional deficiencies
- Prognosis is good as long as phe levels are kept within treatment guidelines
- There may be mild deficits in neuropsychological function even in treated patients
- Treatment with Sapropterin benefits some, usually those with milder disease