Phenylalanine hydroxylase deficiency

John J. Mitchell, MD[1,2,3], Yannis J. Trakadis, MD[1], and Charles R. Scriver, MD[1,2]

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Abstract: Phenylalanine hydroxylase deficiency is an autosomal recessive disorder that results in intolerance to the dietary intake of the essential amino acid phenylalanine. It occurs in approximately 1:15,000 individuals. Deficiency of this enzyme produces a spectrum of disorders including classic phenylketonuria, mild phenylketonuria, and mild hyperphenylalaninemia. Classic phenylketonuria is caused by a complete or near-complete deficiency of phenylalanine hydroxylase activity and without dietary restriction of phenylalanine most children will develop profound and irreversible intellectual disability. Mild phenylketonuria and mild hyperphenylalaninemia are associated with lower risk of impaired cognitive development in the absence of treatment. Phenylalanine hydroxylase deficiency can be diagnosed by newborn screening based on detection of the presence of hyperphenylalaninemia using the Guthrie microbial inhibition assay or other assays on a blood spot obtained from a heel prick. Since the introduction of newborn screening, the major neurologic consequences of hyperphenylalaninemia have been largely eradicated. Affected individuals can lead normal lives. However, recent data suggest that homeostasis is not fully restored with current therapy. Treated individuals have a higher incidence of neuropsychological problems. The mainstay of treatment for hyperphenylalaninemia involves a low-protein diet and use of a phenylalanine-free medical formula. This treatment must commence as soon as possible after birth and should continue for life. Regular monitoring of plasma phenylalanine and tyrosine concentrations is necessary. Targets of plasma phenylalanine of 120–360 μmol/L (2–6 mg/dL) in the first decade of life are essential for optimal outcome. Phenylalanine targets in adolescence and adulthood are less clear. A significant proportion of patients with phenylketonuria may benefit from adjuvant therapy with 6R-tetrahydrobiopterin stereoisomer. Special consideration must be given to adult women with hyperphenylalaninemia because of the teratogenic effects of phenylalanine. Women with phenylalanine hydroxylase deficiency considering pregnancy should follow special guidelines and assure adequate energy intake with the proper proportion of protein, fat, and carbohydrates to minimize risks to the developing fetus. Molecular genetic testing of the phenylalanine hydroxylase gene is available for genetic counseling purposes to determine carrier status of at-risk relatives and for prenatal testing. ***Genet Med* 2011:13(8):697–707.**

Key Words: *PAH deficiency, hyperphenylalaninemia, phenylketonuria*

Hyperphenylalaninemia (HPA) has been called the epitome of human biochemical genetics.[1,2] In 1934, Folling[3] recognized that a certain type of intellectual disability was associated with elevated levels of phenylpyruvic acid in body fluids. He called the condition "phenylpyruvic oligophrenia," soon to be renamed "phenylketonuria" (PKU).[4] Penrose and Quastel,[4] who had recognized "phenylketonuria" (PKU) to be the first form of

From the [1]Department of Medical Genetics, McGill University Health Center; [2]Department of Human Genetics, McGill University; and [3]Division of Endocrinology and Metabolism, Department of Pediatrics, McGill University Health Center, Montreal, Canada.

John J. Mitchell, MD, Department of Medical Genetics, 2300 Tupper, Montreal Children's Hospital, McGill University Health Center, Montreal, Canada H3H 1P3. E-mail: john.mitchell@muhc.mcgill.ca.

Disclosure: The authors declare no conflict of interest.

Submitted for publication December 8, 2010.

Accepted for publication February 1, 2011.

Published online ahead of print May 6, 2011.

DOI: 10.1097/GIM.0b013e3182141b48

Table 1 Prevalence of PAH deficiency by population

Population	PAH deficiency in live births	Carrier rate
Turks[8]	1/2600	1/26
Irish[9]	1/4500	1/33
White, East Asian[10]	1/10,000	1/50
Japanese[11]	1/143,000	1/200
Finnish, Ashkenazi Jewish[10]	1/200,000	1/225
African (anecdotal)	~1/100,000	?

intellectual disability with a chemical explanation, introduced the idea that PKU was not randomly distributed in human populations and could be treatable. Subsequently, it was shown that affected individuals responded to dietary restriction of the essential nutrient phenylalanine (Phe).[5] A microbial inhibition assay was introduced in the 1960s for mass screening of newborns, providing early diagnosis and access to successful treatment.[6] During the 1980s, the human phenylalanine hydroxylase (*PAH*) gene was mapped and cloned and the first mutations identified.[7] This opened the way for in vitro expression analysis to study the effects of different PAH alleles on enzyme function in the 1990s. It was at the same time that the crystal structure of PAH was elucidated.

Since the appearance of universal newborn screening, symptomatic classic PKU is infrequently seen in the developed world. The prevalence of PAH deficiency in various populations is depicted in Table 1.

CLASSIFICATION SCHEMES

PAH deficiency results in intolerance to the dietary intake of the essential amino acid Phe and produces a spectrum of disorders. Phe in excess can be toxic to brain and cognitive development; thus, any degree of HPA could be called a "phenylketonuric" phenotype and would be a risk factor to be managed accordingly.[12] However, because the risk seems to vary relative to the degree of HPA, various classification schemes have emerged.

The initial classification scheme proposed by Kayaalp et al.[13] was meant to keep the nomenclature simple. This system uses the following terms:

1. PKU is the most severe of the three types and in an untreated state is associated with plasma Phe concentrations >1000 μmol/L and a dietary Phe tolerance of <500 mg/day. The Phe tolerance was defined by the quantity of Phe that could be ingested to maintain a "safe" Phe level in late infancy or before 5 years of age. PKU is associated with a high risk of severely impaired cognitive development.
2. Non-PKU HPA (non-PKU HPA) is associated with plasma Phe concentrations consistently above normal (i.e., >120 μmol/L) but lower than 1000 μmol/L when an individual is on a normal diet. Individuals with non-PKU HPA have a much lower risk of impaired cognitive development in the absence of treatment.
3. Variant PKU includes those individuals who do not fit the description for either PKU or non-PKU HPA.

The classification scheme proposed by Guldberg et al.[14] subdivides PAH deficiency into the following four categories based on Phe tolerance before the age of 5 years:

1. Classic PKU is caused by a complete or near-complete deficiency of PAH activity. Affected individuals tolerate <250–350 mg of dietary Phe per day to keep plasma concentration of Phe at a safe level of no more than 300 μmol/L (5 mg/dL). Without dietary treatment, most individuals develop profound, irreversible intellectual disability.
2. Moderate PKU: affected individuals tolerate 350–400 mg of dietary Phe per day.
3. Mild PKU: affected individuals tolerate 400–600 mg of dietary Phe per day.
4. Mild HPA: affected infants have plasma Phe concentrations lower than 600 μmol/L (10 mg/dL) on a normal diet.

Despite the widespread utilization of these schemes, there may be little value in trying to classify this disorder with our current knowledge as there is no clear clinical application. Current classification schemes do not capture the intricacies of this complex trait. We know that Phe tolerance changes with age.[15] Moreover, the issue may be further clouded by the large variability of treatment targets for Phe around the world; Phe tolerance (and therefore classification) may change depending on which target is being used. The more we are able to understand the allelic (PAH) variation and genomic (modifier loci) variation, the better we will understand genotype-phenotype variation and the more we will aspire to patient-specific therapy.[2]

CLINICAL DESCRIPTION

Untreated PKU

Untreated children with classic PKU show impaired brain development. Signs and symptoms include microcephaly, epilepsy, severe intellectual disability, and behavior problems. The excretion of excessive Phe and its metabolites can create a musty body odor and skin conditions such as eczema. The inhibition of tyrosinase by elevated levels of Phe is believed to be responsible for decreased skin and hair pigmentation.[16] The mechanisms by which elevated blood Phe concentrations disturb cerebral metabolism and cognitive function are not fully understood. The proposed mechanisms include a potential effect of disturbed large neutral amino acid (LNAA) transport from blood to brain on neurotransmitter and protein synthesis, as well as dopamine depletion and white matter pathology.[17,18] Affected individuals have decreased myelin formation and dopamine, norepinephrine, and serotonin production. Further problems can emerge later in life and include exaggerated deep tendon reflexes, tremor, and paraplegia or hemiplegia.[19–21] Individuals treated late or never treated may develop severe behavioral or psychiatric problems (depression, anxiety, and phobias) in the third or fourth decade.[22] In a few case reports, untreated individuals with PKU with normal intelligence were diagnosed in adulthood only as a result of a sudden and severe psychiatric deterioration.[23]

Outcome in treated PKU

Early-dietary treatment in individuals with PKU prevents the severe complications associated with the classical form of the disease. However, a growing body of evidence suggests that individuals with strict adherence to diet may still have some underlying sequelae and suboptimal cognitive outcome.[24,25] A systematic review by Enns et al.[26] concluded that despite the

remarkable success of newborn screening and early dietary treatment for PKU, the neurocognitive, psychosocial, quality of life, growth, nutrition, bone pathology and maternal PKU outcomes are suboptimal. Despite early and continuous therapy, individuals with PKU may develop neurocognitive sequelae, particularly deficits in executive function (difficulties in planning, organization, working memory, and inhibitory control).[27,28] In addition, there is some evidence that childhood metabolic control correlates with development of these problems.[29]

Waisbren et al.[25] reported a significant inverse correlation between Phe level and intelligence quotient (IQ) in patients with PKU. Specifically, from birth to 12 years of age, each 100 micromol/L increase in Phe predicted a 1.3–3.1 point reduction in IQ. There is evidence that less variation around the mean may also affect outcome with individuals with less fluctuation having better outcome.[29] In treated individuals, psychological problems are increased, when compared with siblings or children with other chronic diseases. Moreover, despite normal IQ, early treated children and adolescents have a higher frequency of ADHD, decreased autonomy, and school problems compared with either healthy controls or chronically ill peers.[30–32]

Noncompliance and treatment termination

It is clear that if the diet is not followed closely, especially during childhood and if plasma Phe concentration is allowed to rise frequently above the recommended concentration, some impairment is inevitable. However, as the diet is unpalatable, many individuals are not able to adhere to diet. Walter et al.[33] found as many as 28% of children younger than 10 years and 79% of adolescents were unable to meet their target Phe level. High levels of nonadherence are reflected in many centers around the world. The changes that occur in adolescence and adulthood as a result of high plasma Phe concentrations are both chronic and acute. The important chronic effects of nonadherence are neuropsychological and include a decrease in cognitive function and structural changes visible on magnetic resonance imaging.[17] The acute toxic effects are initially neurophysiologic. They affect neurotransmitter production and can be reflected in electroencephalographic changes. These changes are reversible. However, our understanding of these issues is not complete.[18]

Several studies support that if the diet is relaxed after the age of 12 years, IQ can remain stable but other functions deteriorate. Adolescents and young adults with PKU who had received early treatment but subsequently discontinued diet at the age of 10 years had no reduction of cognitive and motor ability compared with subjects with PKU on diet and normal controls but did have minor deficits in executive function.[34] Adults who have abandoned the Phe-restricted diet tend to have a reduced attention span, slow information-processing abilities, and slow motor reaction time.[35,36] These findings seem to be related to both current and historic Phe levels.[37] Early treated adults who discontinue diet are also at risk for minor neurologic abnormalities such as tremor and brisk reflexes.[38]

A meta-analysis on the neuropsychological symptoms of adolescents and adults with PKU by Moyle et al.[24] concluded that the psychological profile of early treated but off diet at the time of the study, patients with PKU was characterized by reduced information-processing speed. Neuropsychological testing showed a reduction in the Perceptual Organization Index, Processing Speed Index from the Wechsler Adult Intelligence Scale Third Edition, and Part A of the Trail Making Test for the PKU group relative to controls. Moreover, adults who come off diet also develop changes in the frequency distribution of brain electrical activity,[38] increased muscle tone, and tremor,[39] as well as lowered bone mineral content[40] (for guidelines on treatment termination please refer to "Management" section).

DIAGNOSIS

Clinical diagnosis

Neonates with PAH deficiency show no physical signs of HPA except tendency toward lower birth weight and smaller head circumference at birth.[41]

Testing

Plasma Phe concentration

The main route for Phe metabolism is hydroxylation of Phe to tyrosine (Tyr) by PAH. The diagnosis of primary PAH deficiency is based on the detection of an elevated plasma Phe concentration and evidence of normal tetrahydrobiopterin (BH4) cofactor metabolism. Individuals with PAH deficiency show plasma Phe concentrations that are persistently higher than 120 μmol/L (2 mg/dL) in the untreated state.[10,42]

Newborn screening

PAH deficiency is most commonly diagnosed on routine screening of newborns for HPA. PAH deficiency can be detected in virtually 100% of cases by newborn screening using the Guthrie card blood spot obtained from a heel prick. Newborn screening for HPA has been routine throughout North America and the United Kingdom since the mid-1960s and in most other developed countries since the early 1970s.[43,44] The test became routine because of the excellent prognosis for children with PAH deficiency who are treated early and the high risk for severe and irreversible brain damage for children who are not treated. In most countries, a parental right of refusal for this test exists; however, this right is exercised only in rare circumstances.

Infants whose initial test results exceed the threshold concentration of Phe are referred for definitive diagnosis and treatment. The initial screening test yields a significant number of elevated Phe results not related to HPA or biopterin deficiencies, and this may lead to stressful experiences for parents. An elevated Phe concentration (HPA) may result from a blood spot that is too thick, a sample that is improperly prepared, or combinations of the following: liver immaturity, protein overload (in newborns who are fed cow's milk), and possible heterozygosity for PAH deficiency in premature babies.[45] Treatment of babies with elevated Phe level is often initiated before the results of confirmatory testing being available to avoid unnecessary delay in therapy. If the second test confirms HPA, further tests are performed to distinguish those infants with PAH deficiency from the approximately 3% of infants with HPA who have impaired synthesis or recycling of BH4 (see "Differential Diagnosis").[10,42] Molecular genetic testing of the *PAH* gene in these infants can be used to confirm PAH deficiency.[46]

Current newborn screening tests for PKU

A concern is the reliability of current tests to accurately measure Phe concentrations in infants <24 hours old, as HPA manifests itself as a time-dependent increase of Phe concentration in the blood. Three methods of newborn screening are currently in use:

1. Guthrie card bacterial inhibition assay, a time-tested, inexpensive, simple, and reliable test.
2. Fluorometric analysis, a reliable quantitative and automated test, which produces fewer false-positive test results than the bacterial inhibition assay.
3. Tandem mass spectrometry (MS), which has the same benefits as fluorometric analysis, can also measure Tyr concentration and can be useful in interpreting Phe concentration. MS has the capacity to improve both sensitivity and predictive value by allowing for a Phe:Tyr ratio with 2.5 as the discriminator (cutoff) above which persistent HPA is highly likely.[42] Tandem MS can be used to identify numerous other metabolic disorders on the same sample.[47] Cost-effectiveness analysis, with economic modeling, suggests that to replace existing methods of newborn screening for PKU with tandem MS would not be justified, unless the intent is to capture additional metabolic disorders, notably medium-chain acyl-coA dehydrogenase deficiency.[48]

Testing strategy

To establish the diagnosis in a proband: once the initial screen is positive for HPA, diagnostic tests must be performed:

1. Blood for amino acid analysis permits confirmation of HPA and the exclusion of the diagnoses of hepatic insufficiency or other aminoacidopathies.
2. To exclude HPA as a secondary consequence of an inborn error of biopterin metabolism, it is essential to collect a urine sample to measure pterins and a blood sample to assay dihydropteridine reductase (DHPR) activity. A BH4 loading test with pre and postmeasurements of Phe and Tyr can be useful in the rapid diagnosis of biopterin variants[49] (see "Differential Diagnosis"). Measurement of DHPR activity can be performed in blood from the Guthrie card. If these initial tests are positive, further testing such as neurotransmitter metabolites in cerebrospinal fluid may be required to pinpoint the defect in biopterin metabolism.[49]

Once these blood samples have been collected, a low Phe diet is started. Although molecular genetic testing is not necessary for diagnosis or treatment in the immediate newborn period, it is often performed for genotype/phenotype correlation (see "Genotype-Phenotype Correlations").

Differential diagnosis

HPA may also result from the impaired synthesis or recycling of BH4, the cofactor in the Phe, Tyr, and tryptophan hydroxylation reactions. Defects in BH4 synthesis result from guanosine triphosphate cyclohydrolase deficiency or 6-pyruvoyl tetrahydrobiopterin synthase (PTPS) deficiency. Impaired recycling of BH4 is caused by deficient DHPR or deficient pterin-4 acarbinolamine dehydratase. All the HPAs caused by BH4 deficiency are inherited in an autosomal recessive manner. They account for approximately 3% of individuals with HPA.[50] BH4 is also involved in catecholamine, serotonin, and nitric oxide biosynthesis (www.bh4.org). Blau et al.[51] and Scriver and Kaufman[10] emphasize that all neonates with persistent HPA must be screened for the BH4 deficiencies. Prenatal diagnosis is possible for all forms of BH4 deficiencies.

The typical (severe) forms of guanosine triphosphate cyclohydrolase, PTPS, and DHPR deficiencies have the following variable, but common, symptoms: intellectual disability, convulsions, disturbance of tone and posture, drowsiness, irritability, abnormal movements, recurrent hyperthermia without infections, hypersalivation, and swallowing difficulties. Microcephaly is common in PTPS and DHPR deficiencies. Plasma Phe concentrations can vary from slightly above normal (>120 μmol/L) to as high as 2500 μmol/L. Mild forms of BH4 deficiency have no clinical signs and may not need intervention.[49] Pterin-4 acarbinolamine dehydratase deficiency, sometimes referred to as "primapterinuria," is associated with benign transient HPA.

Elevated Phe on newborn screening can also be caused by liver dysfunction. When mild HPA is encountered (120–240 μmol/L), reflexive testing for diseases such as galactosemia should be taken into consideration if newborn screening programs do not already screen for this disease.[52]

MOLECULAR GENETICS

Gene

PAH is the only gene associated with PAH deficiency.[10]

Normal allelic variants

The *PAH* gene contains 13 exons and spans 90 kb[10,42]; the genomic sequence is known to code for a 2.4-kb mature mRNA. Thirty-one different normal allelic variants causing minor changes in the gene sequence have been identified (Table 2, PAH Locus Knowledgebase); all are presumed to be neutral in their effect on protein product.[53]

Pathologic allelic variants

More than 500 different disease-causing mutations have been identified to date in the *PAH* gene (Table 2, PAH Locus Knowledgebase). This database provides information on mutations, associated phenotypes, gene structure, enzyme structure, clinical guidance, and much else.[54] Mutations observed in the *PAH* gene (Table 3) include missense, splice site, and nonsense mutations, small deletions, and insertions.

Normal gene product

The normal product of the *PAH* gene is the protein PAH, containing 452 amino acids (Table 2, PAH Locus Knowledgebase). PAH enzymes can exist as tetramers and dimers in equilibrium.[56] The PAH enzyme hydroxylates Phe to Tyr, this

Table 2 Phenylalanine hydroxylase deficiency: Genes and databases

Gene symbol	Chromosomal locus	Protein name	Locus specific	HGMD
PAH	12q23.2	Phenylalanine-4-hydroxylase	BIOPKU: database of mutations causing BH4-responsive HPA/PKU; phenylalanine hydroxylase locus knowledgebase	PAH

Data are compiled from the following standard references: gene symbol from HGNC; chromosomal locus, locus name, critical region, and complementation group from OMIM# (PKU OMIM# 261600; PAH OMIM# 612349); and protein name from UniProt. For a description of databases (Locus Specific, HGMD) refer to http://www.ncbi.nlm.nih.gov/books/NBK1336/.

Table 3 Classes of mutations observed in the PAH gene

Percent of mutations	Genetic mechanism
62	Missense
13	Deletion (mainly small)[a]
11	Splice
6	Silent
5	Nonsense
2	Insertion
<1	Deletion or duplication of exon(s) or whole gene

From PAHdb Knowledgebase (http://www.pahdb.mcgill.ca/).
[a]Large deletions account for 2–3% of mutations.[55]

reaction being the rate-limiting step in the major pathway by which Phe is catabolized to CO_2 and water.[10]

Abnormal gene product

The mutations that confer the most severe phenotypes are known or predicted to completely abolish PAH activity. These "null" mutations are of various types. Missense mutations usually permit the enzyme to retain some degree of residual activity; however, it is difficult to assess severity in vivo because the in vivo activity is not the simple equivalent of the in vitro enzymatic phenotype.[53,57]

MOLECULAR GENETIC TESTING

Clinical testing

Targeted mutation analysis. A panel of 1–15 common point mutations and very small deletions have a detection rate of approximately 30–50%. Alleles may be population related (Table 4).

Mutation scanning

Mutation scanning detects virtually all point mutations in the *PAH* gene. Mutation scanning by denaturing high performance liquid chromatography, a fast and very efficient method to detect locus-specific point mutations, has shown its relevance for detection of PKU-causing alleles.[58]

Sequence analysis

Sequencing of all 13 exons has a mutation detection rate of approximately 98%.

Duplication/deletion analysis

Comparative multiplex dosage analysis may be useful in detecting large duplications or deletions when no mutations have been identified by mutation scanning or sequence analysis. This technique has been used to detect abnormal dosage in 20% of uncharacterized PKU alleles,[59] and therefore, duplications and deletions may account for up to 3% of mutations.[55]

Linkage analysis

For families in which only one or neither PAH mutation has been identified, linkage analysis may be an option for carrier testing and prenatal diagnosis. Linkage studies are based on accurate clinical diagnosis of PAH deficiency in the affected family member(s) and accurate understanding of the genetic relationships in the family. Samples from multiple family members, including a sample from at least one affected individual, are required to perform linkage analysis. The markers used for linkage are highly informative and are both intragenic and flanking to the PAH locus; thus, they can be used with >99% accuracy in families with PAH deficiency.

Table 4 Summary of molecular genetic testing used in phenylalanine hydroxylase deficiency

Gene symbol	Test method	Mutations detected	Mutation detection frequency by test method[a]
PAH	Targeted mutation analysis	1–15 common mutations (alleles may be population related)	30–50%
	Mutation scanning	Common and private sequence variants	99%
	Sequence analysis	Common and private sequence variants	99%[b]
	Duplication/deletion analysis[c]	Exonic or whole-gene deletions/duplications	Rare

[a]The ability of the test method used to detect a mutation that is present in the indicated gene.
[b]The mutation detection frequency may be lower than 99% if not all the *PAH* exons are included in the sequence analysis.
[c]Testing that identifies deletions/duplications not readily detectable by sequence analysis of genomic DNA; a variety of methods including quantitative PCR, long-range PCR, multiplex ligation-dependent probe amplification (MLPA), or targeted array GH (gene/segment specific) may be used. A full-array GH analysis that detects deletions/duplications across the genome may also include this gene/segment. See array GH.

GENOTYPE-PHENOTYPE CORRELATIONS

PAH deficiency is a "multifactorial disorder" in that both environment (dietary intake of Phe) and genotype (combinations of allelic [PAH] variation and genomic [modifier loci] variation) are necessary causal components of disease.[60] Because each individual has a personal genome, even those with similar mutant *PAH* genotypes may not have similar "PKU" phenotypes. As explained elsewhere,[61–63] *PAH* genotype may not be a robust predictor of phenotype and to evaluate and treat the individual (the actual phenotype), rather than the phenotype predicted from genotype, is the correct approach.

Variability of metabolic phenotypes in PAH deficiency is caused primarily by different mutations within the *PAH* gene.[13,14] DiSilvestre et al.[64] found that genotype does predict biochemical phenotype (i.e., by Phe loading tests) but does not predict clinical phenotype (i.e., occurrence of intellectual disability). PAH deficiency is, therefore, a "complex" disorder at the cognitive and metabolic levels.[60] It is becoming more difficult to assess clinical phenotypes given that most individuals with PAH deficiency in developed countries are treated successfully.

Some untreated individuals with PAH deficiency and *PAH* mutations that usually confer classic PKU have elevated plasma Phe concentration but normal intelligence. Some sibs with the same genotype have different clinical and metabolic phenotypes. The mechanisms that cause dissimilarities in pathogenesis at the level of the brain despite comparable plasma Phe concentrations are still unclear. These atypical individuals have

significantly lower brain Phe concentrations than do individuals with similar blood Phe concentrations. Moller et al.[65] suggest that different brain Phe concentrations in individuals with similar blood Phe concentrations are the result of individual variations in the kinetics of Phe uptake and distribution at the blood-brain barrier. Trefz et al.[66] speculate that these individuals with atypical manifestations may be protected by a second catalytic variant affecting an amino acid transporter. Pietz et al.[67] have demonstrated that loading individuals who have classic PKU with LNAAs decreases Phe uptake into the brain at the transporter and improves neurophysiologic parameters.

A theory that attempts to explain the variations in plasma Phe concentrations in individuals with the same genotypes[60,68,69] suggests that some missense mutations affect protein folding, thus altering the oligomerization of the nascent PAH protein. This process is likely influenced by an individual's genetic background, including, potentially, differences in the quality and quantity of chaperones and proteases. Gersting et al.[70] emphasize the presence of BH4-dependent cooperativity of the activated enzyme that is determined by activating conformational rearrangements.

Despite all the different factors influencing PAH deficiency phenotypes, the specific *PAH* genotype is the main determinant of metabolic phenotype in most cases. In compound heterozygotes with functional hemizygosity (null/missense paired alleles), the less severe of the two *PAH* mutations determines disease severity. When two mutations with similar severity are present, the phenotype may be milder than predicted by either of the mutations.[13,14,53]

Many physicians are now advocating genotyping all newborns identified with HPA to better anticipate dietary needs.[46,47,71] Zschocke and Hoffman[71] even suggest that individuals identified as having mild HPA associated with known "mild" mutations be treated as such with relaxation of their follow-up control analyses at a relatively early stage. Greeves et al.[72] suggest that genotype can help predict the likelihood of intellectual change if or when individuals relax their dietary restrictions later in life.

Genotype-phenotype correlation is also seen with a large number of mutations that are BH4 responsive. Therefore, genotyping may help to predict which individuals will respond to BH4 supplementation and what the response will be.[73] Mutations that are found in the regulatory domain of PAH are more likely to show inconsistency in BH4 responsiveness from person to person[74] (see "BH4 Databases"). Although the genotype/phenotype relationship for BH4 responsiveness is fairly robust, discrepancies have been described (PAHdb Knowledgebase).

MANAGEMENT

Classic PKU

Restriction of dietary Phe

The generally accepted goal of treatment for the HPAs is normalization of the concentrations of Phe and Tyr in the blood and, thus, prevention of the cognitive deficits that are attributable to this disorder.[75] Because the *PAH* genotype may not be a robust predictor of phenotype (see "Genotype-Phenotype Correlations"), the individual's diet should be tailored to calculate Phe tolerance irrespective of genotype. Phe concentrations of 120–360 μmol/L (2–6 mg/dL)[76] or 40–240 μmol/L (1–4 mg/dL) during the first 10 years of life[75] are generally regarded as safe. A diet restricted in Phe should be initiated as soon as possible after birth and continued at least into adolescence,[38] for life.[47] The 2000 NIH Consensus Statement on PKU recommends a target blood Phe range of 120–360 μmol/L (2–6 mg/dL) from infancy until 12 years of age and 120–900 μmol/L (2–15 mg/dL) after 12 years of age. However, controversy remains about the therapeutic blood Phe target concentrations in adolescent and adult life leading to differences in diagnostic and treatment practices across centers.[77] There is some evidence that a high lifetime Phe to Tyr ratio (Phe:Tyr) is more strongly associated with impaired executive function development than high Phe alone.[78] In addition, attempts to restrict wide fluctuations in blood Phe levels and ensuring Phe levels are within the recommended treatment range of PKU may optimize outcome.[29]

The restricted Phe diet is adapted to individual tolerance for Phe and includes appropriate protein and energy for age. Plasma concentrations of Phe within the effective treatment range and normal nutritional status cannot be achieved by a low-protein diet alone but rather requires the use of a Phe-free medical formula. The diet must be carefully monitored, so that growth and nutritional status are unaffected, and deficiency of Phe or Tyr is not created. The diet must be adjusted for growth, illness, and activity.

Treatment in infancy

A Phe-restricted diet and a Phe-free medical formula must be started as soon as possible after birth under the direction of a nutritionist. Breastfeeding is encouraged along with Phe-free formula.[47] Intake of Tyr and total amino acids must be monitored. The UK Medical Research Council[79] recommends that children younger than 2 years should maintain a total amino acid intake of 3 g/kg/day including 25 mg Tyr/kg/day. In practice, there exists a large degree of variability regarding protein intake (dependent on factors such as Phe tolerance or cultural norms), and consensus recommendations require further research.[80] The consumption of Phe-free formula should be spread out evenly over the 24 hours of the day to minimize fluctuations in blood amino acid concentrations. Care must be taken to avoid long periods of low blood Phe concentration, which is also harmful to development,[81] although a safe lower limit has not been well established. Blood Phe concentration should be monitored weekly or biweekly to evaluate control.[75]

Treatment in childhood

Children older than 2 years should maintain a total amino acid consumption of 2 g/kg/day including 25 mg Tyr/kg/day. Monitoring on a biweekly basis is recommended until age 7 years and on a monthly basis thereafter.

Treatment in adolescence and adulthood

Recommendations for treatment of adolescents and adults vary.[82] In general, support for "diet therapy for life" is increasing.[47] Some recommendations are more liberal than others and indicate that relaxation (not elimination) of the strict diet in adolescence does not affect nonexecutive functions when plasma Phe concentration remains below 1200 μmol/L.[83] As previously stated, other studies indicate that if the diet is relaxed after the age of 12 years, IQ can remain stable but other functions deteriorate. Hence, controversy remains over the plasma Phe concentration to be achieved for individuals older than 12 years. The general consensus is that the closer the Phe concentration is to the recommended normal value, the better the individual's general state of well-being.[24,25,38,75,84,85]

Supplementation with BH4

An ever-growing body of evidence indicates that many individuals with primary PAH deficiencies are responsive to the

6R-BH4 stereoisomer in pharmacologic doses (≤20 mg/kg daily in divided oral doses).[86–88] BH4 loading tests determine which persons are BH4 responsive and which are not to relax or discontinue restriction of dietary Phe in those who are responsive. By increasing the Phe tolerance, BH4 supplementation may allow for a more complete diet, thus minimizing risk of nutritional deficiencies associated with a low-protein diet.[89] Moreover, in one case report, pregnancies were successfully managed with BH4 supplementation; however, more data are needed to prove the safety and efficacy of this alternative treatment.[90]

The majority of individuals with mild or moderate PKU may be responsive to BH4, whereas up to 10% of individuals with classic PKU can show a response.[88,91–93] Most responders can be detected with a standard 24-hour load, but some slow responders escape detection unless a more prolonged protocol is used.[92,94,95] Blau et al.[96] discuss some of the controversial issues of how, whom, and when to test for BH4 responsiveness.

Individuality in BH4 pharmacokinetics implies the need for patient-specific dosage schedules.[97,98] Long-term treatment in a small number of BH4-responsive persons has documented the maintenance of the Phe-lowering effect and the absence of major side effects.[99] In the majority of individuals, the BH4 response is likely a result of correction of PAH mutant kinetic effects and/or a chaperone-like effect of BH4.[100] Whatever the mechanism of the therapeutic effect, the 6R-BH4 enhances in vivo Phe hydroxylation and the corresponding oxidative flux and lowers plasma Phe concentration with improved tolerance of dietary Phe.[101]

Psychosocial support

Management in the patients with PKU needs to extend beyond biochemical management and control of blood Phe levels to optimize the outcome. Treatment for affected individuals of all ages is enhanced with the teaching and support offered by an experienced healthcare team consisting of physicians, nutritionists, genetic counselors, social workers, nurses, and psychologists. Given the difficulties with adhering to diet and the evidence that control of blood Phe levels in PKU can still lead to subtle but measurable cognitive function deficits and predisposition to certain psychiatric abnormalities, additional psychosocial support through professional psychologists may be helpful.[102]

NUTRITIONAL DEFICIENCIES

The nutritional management of patients with PKU seeks to optimize patients' growth, development, and dietary compliance. Dietary restriction of Phe raises many nutritional concerns: the quality of the available amino acid substitutes, the neurotrophic and neuroprotective effects of added long-chain polyunsaturated fatty acids (LCPUFA), micronutrient deficiencies, bone disease, and antioxidant status.[102] As a result, the nutritional status of patients with PKU should be regularly monitored.

Decreased levels of essential nutrients such as selenium, carnitine, and LCPUFA have been reported with strict dietary protein restriction.[103] Protein-rich foods are the predominant dietary source of LCPUFA, which are essential for adequate growth, as well as visual and cognitive development.[104] Hence, patients treated for PKU with protein-restricted diet are at risk for LCPUFA deficiency.[105,106] A recent cross-sectional study evaluated the LCPUFA composition in patients with PKU treated with BH4 and concluded that LCPUFA status is within the reference range and significantly greater than patients with PKU treated with diet alone.[89] This may mark an additional advantage of BH4 therapy as it may diminish some of the nutritional deficits by increasing the natural protein intake.

Adequate calcium and vitamin D intake is an important component of care. Numerous studies indicate that individuals with PKU have a high incidence of osteopenia (as measured by DEXA).[107–109] Although adolescence is an important time to accrue bone mass, adolescents with PKU are often not adherent with diets. However, Schwahn et al.[110] found osteopenia in an individual diagnosed late who had not received an artificial diet, suggesting that a component of abnormal bone metabolism may be a result of the disease itself. It is unclear what clinical significance this may have on the aging PKU population. Currently, there are no recommendations for surveillance for osteopenia, but baseline bone scans may be of value for long-term follow-up.

Vitamin B12 deficiency can occur when individuals with PKU relax their diet in adolescence.[111] This vitamin is found in natural animal protein; when patients decrease their amino acid supplement, they often still choose low-protein foods and are, therefore, at risk of vitamin B12 deficiency.[112] Vugteveen et al.[113] stated that measurement of methylmalonic and/or homocysteine may reveal functional B12 deficiencies in patients with PKU even when B12 levels are normal.

Finally, some of the neuropsychological problems exhibited in PKU may be due to a deficiency of the amino acid Tyr. A recent Cochrane review aiming to assess the effects of Tyr supplementation in people with PKU analyzed all randomized or quasi-randomized trials (six studies in total) investigating the use of Tyr supplementation versus placebo in people with PKU. The authors concluded that based on the available evidence, no recommendations can be made regarding Tyr supplementation in routine clinical practice emphasizing that further randomized controlled studies are needed.[114]

AGENTS/CIRCUMSTANCES TO AVOID

Aspartame, an artificial sweetener in widespread use, contains Phe. Persons with PKU should either avoid products containing aspartame or calculate intake of Phe and adapt diet components accordingly.

THERAPIES UNDER INVESTIGATION

Although the treatment of PKU with Phe-restricted diets has been hugely successful, the poor palatability of the diet results in poor compliance in adolescence and adulthood. A number of attempts to find other treatment modalities for PKU are ongoing.

Glycomacropeptide

Glycomacropeptide (GMP) is a protein derived from cheese whey that is rich in specific essential amino acids but is naturally low in Phe.[115] Recent evidence supports improved nutritional management of PKU by using a diet containing GMP, when compared with conventional amino acid formulas.[116] GMP, when supplemented with limiting AAs, may be a safe and an alternative therapy to synthetic amino acids as the primary protein source in the nutritional management of PKU.

LNAA transporters

At the blood-brain barrier, Phe shares a transporter with other LNAAs. Some individuals exclude excess Phe, more or less efficiently, because they show evidence of variation in the high capacity/high kilometer component of Phe transport across the blood-brain barrier.[117] LNAA supplementation has reduced

brain Phe concentration despite consistently high serum concentrations of Phe by competition at this transporter.[67,118] In nonadherent adults, this may help to protect the brain from acute toxic effects of Phe. Significant improvement in ability to concentrate and decreased self-injurious behavior were seen with trial of LNAA supplements in a small number of untreated adults with PKU.[119]

A similar transporter for LNAA also exists in the intestine, and supplementation with a different formulation of amino acids, including LNAA reduced the blood Phe concentration by 40–50% in a small number of individuals with PKU.[120] These supplements will not replace the Phe-restricted diet but may help relax dietary restriction of treated individuals or may aid in management of adults who are not treated. Larger clinical trials are needed before conclusions on the effectiveness of these treatments can be made.

Enzyme substitution

Under investigation is the administration of the enzyme Phe ammonia lyase (PAL) by oral routes to degrade Phe to transcinnamic acid and ammonia.[121,122] The oral route is complicated by proteolytic degradation, whereas injected PAL is complicated by increased immunogenicity.[123,124] PEGylation (conjugation with polyethylene glycol) of PAL has been found to decrease the immune response.[123,124] Clinical trials with this protected form of injectable enzyme are currently underway. Modification of oral PAL to prevent degradation by digestive enzymes is also being investigated.[125]

Somatic gene therapy

Somatic gene therapy is also being explored in animal models and holds some promise for possible future treatment.[47,126–128]

Induced pluripotent stem cells

Another treatment approach under investigation involves therapeutic liver repopulation with *PAH*-expressing cells after hepatocyte or hematopoietic stem-cell transplantation. This treatment has been reported to successfully correct HPA only in an animal model with a selective advantage for the donor cells.[129]

NON-PKU HPA

Individuals with non-PKU HPA who have plasma Phe concentrations consistently below 600 μmol/L (10 mg/dL) may not be at higher risk of developing intellectual, neurologic, and neuropsychological impairment than individuals without PAH deficiency. Although some specialists debate the advisability of nontreatment, others believe that dietary treatment is unnecessary for the individuals in this class. A study by Weglage et al.[130] confirmed the hypothesis of Levy et al.[131] that some individuals with hyper-Phe may not need dietary treatment. Thirty-one individuals with HPA who were never treated and whose plasma Phe concentrations did not exceed 600 μmol/L had normal cognitive neuropsychological development. Larger, well-designed studies are needed.

Care should be taken, so that girls in this group receive proper counseling about the teratogenic effects of elevated maternal plasma Phe concentration (i.e., "maternal HPA/PKU") when they reach childbearing age.[130]

PREGNANT WOMEN WITH PAH DEFICIENCY

Women with PAH deficiency who have been properly treated throughout childhood and adolescence have normal physical and intellectual development. However, if the woman has high plasma Phe concentrations, her intrauterine environment will be hostile to a developing fetus as Phe is a potent teratogen. It is strongly recommended that women with PAH deficiency use reliable methods of contraception to prevent unplanned pregnancies.

Women with PAH deficiency who are off diet and are planning a pregnancy should start a Phe-restricted diet before conception and should maintain plasma Phe concentrations between 120 and 360 μmol/L (2–6 mg/dL), ideally over several months, before attempting conception.[132] During pregnancy, these women should be offered continuous nutritional guidance and weekly or biweekly measurement of plasma Phe concentration because dietary Phe and protein requirements change considerably during pregnancy. It is important that pregnant women with PAH deficiency have an adequate energy intake with the proper proportion of protein, fat, and carbohydrates. They should try to attain normal weight gain patterns to provide the most favorable conditions for fetal growth.[85]

The abnormalities that result from exposure of a fetus to high maternal plasma Phe concentration are the result of "maternal HPA/PKU." The likelihood that the fetus will have congenital heart disease, intrauterine and postnatal growth retardation, microcephaly, and intellectual disability depends on the severity of the maternal HPA and the effectiveness of the mother's dietary management. Although studies have shown that women with non-PKU HPA who have plasma Phe concentrations lower than 400 μmol/L (7 mg/dL) when untreated can give birth to offspring who seem to be normal, the Maternal PKU Collaborative Study reports that even at maternal plasma Phe concentrations of 120–360 μmol/L (2–6 mg/dL), 6% of infants are born with microcephaly and 4% with postnatal growth retardation. If maternal plasma Phe concentrations are >900 μmol/L (15 mg/dL), the risk is 85% for microcephaly, 51% for postnatal growth retardation, and 26% for intrauterine growth retardation. The risk for these abnormalities is both dose dependent and time dependent. Thus, optimal plasma Phe concentrations must be strictly maintained throughout pregnancy to reduce the risk of each individual abnormality; continuing studies corroborate this position.[133]

Unfortunately, many pregnancies are unplanned. Women with HPA who conceive while off the Phe-restricted diet and who manage to bring their plasma Phe concentrations into the recommended range (120–360 μmol/L) as early as possible after conception and no later than 8 weeks of pregnancy can be encouraged by the existing data. Although the risks of congenital abnormalities, especially for congenital heart disease, are greater than if plasma Phe concentration is controlled preconceptually, the possibility for a normal child still exists. If plasma Phe concentrations are not controlled until the second or third trimester, the risks are the same as if the plasma Phe concentration were never brought under control.[85]

Proper prenatal care should include serial ultrasonography to (1) identify nonviable pregnancies in the first trimester; (2) monitor fetal growth; and (3) identify congenital abnormalities (such as congenital heart disease) that are relatively common in babies whose mothers have PAH deficiency. This information can be useful in anticipating the postnatal needs of the infant.

Waisbren et al.[134] contend that suboptimal home environments can have as much of an adverse effect on offspring as a delay in control of maternal plasma Phe concentration. Of concern, the limited intellectual abilities, the reduced social resources, and the emotional difficulties of many women with HPA may complicate adherence to the diet, prenatal care, and the care of their infant.[85] Support for women with HPA/PKU

starting before conception and continuing after delivery is essential for optimal outcome. A successful pilot project involving mothers serving as resources for their daughters with PKU when they reached childbearing age resulted in better dietary control during pregnancy and better outcome for the children.[135]

Too little published data exist on children born to mothers with PKU. One interesting observation of this cohort of children is a decline in the scores achieved on developmental tests at age 4 years from those achieved on similar tests taken at age 2 years.[135] Longitudinal studies of these children are necessary to interpret the significance of these findings.

GENETIC COUNSELING

PAH deficiency is inherited in an autosomal recessive manner. Genetics clinics, staffed by genetics professionals, provide information for individuals and families regarding the natural history, treatment, mode of inheritance, and genetic risks to other family members and information about available consumer-oriented resources. Molecular genetic testing of the *PAH* gene is available for genetic counseling purposes to determine carrier status of at-risk relatives and for prenatal testing. The optimal time for determination of genetic risk, clarification of carrier status, and discussion of the availability of prenatal testing is before pregnancy.

Testing of relatives at risk

Newborn sibs of an individual with PKU who have not been tested prenatally should have blood concentration of Phe measured shortly after birth, in addition to their newborn screen. This will allow earlier detection than by newborn screening alone and, thus, treatment as soon as possible after birth.

CONCLUSION

PAH deficiency is a treatable "multifactorial disorder" leading to HPA. Without treatment, most children with PKU develop profound and irreversible intellectual disability. A diet restricted in Phe should be initiated as soon as possible after birth. Recent findings have stressed the importance of continuing treatment into adulthood to prevent neuropsychological complications. However, more research is needed to standardize the therapeutic blood Phe target concentrations at different age groups and particularly in adolescence and adulthood. Moreover, some individuals with primary PAH deficiencies have been shown to be responsive to the 6R-BH4 stereoisomer in pharmacologic doses. Testing strategies and patient-specific dosage schedules need to be optimized and validated. Continuous efforts are being made to improve current medication (e.g., the dietary supplements) and to develop alternative therapeutic approaches (LNAA, enzyme substitution, and gene therapy). New treatments with better effectiveness and clinical markers to follow-up outcome need to be studied prospectively. Cross-center collaborations will be crucial in providing the evidence allowing for standardization of management. Such collaborations would be fruitful in the effort to better predict the genotype-phenotypes correlation and ultimately provide in a cost-effective way an individualized management plan for patients with PAH deficiency.

ACKNOWLEDGMENTS

This work was supported, in part, by the Canadian Genetic Diseases Network and by the Fonds de la Recherche en Santé du Québec du Réseau de médecine génétique appliquée. The authors thank Shannon Ryan for her contribution to an earlier edition of the Gene Reviews manuscript.

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