

Phenylketonuria (PKU)

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Outcome without screening:

PKU is an inherited group of disorders in which the body's normal processing of the essential amino acid, phenylalanine, is disrupted. The clinical effects of untreated classical PKU are developmental delay and mental retardation, usually noticeable within the first 6 months. Seizures, microcephaly, behavioral disturbances, a noxious odor (urine/sweat) and eczema-like rashes may be seen after infancy. First described in Europe in the 1930s, it was not until the 1960s that knowledge of its inheritance, biochemical defect, and potential treatment became known. Patients with the untreated classical form who were born prior to newborn screening have severe disabilities, and several remain in group homes or institutions at significant expense.

Incidence:

The incidence in Caucasians is about 1/10,000 newborns. Ethnic incidence varies, but PKU has been reported in almost all racial groups. It is inherited as an autosomal recessive disorder, with each parent being a carrier, and 25% recurrence risk in each subsequent pregnancy for another similarly affected child to the same biologic parents.

Outcome with screening:

An efficient specific, sensitive, and inexpensive newborn screening test was developed, and PKU became the first human genetic disorder screened in the neonate to detect at risk infants before symptoms developed, and to initiate treatment in confirmed cases to prevent the clinical effects.

Causes of Phenylketonuria(PKU):

The clinical effects of untreated PKU are due primarily to elevations of the amino acid phenylalanine(phe) and its abnormal products, the phenylketones (phenyllactic, phenylpyruvic, and phenylacetic acids, the last responsible for the unusual odor). Additionally, tyrosine, the natural product of phenylalanine, is deficient, resulting in neurotransmission defects in the central nervous system. Many other metabolic processes are involved, but these two areas are the primary substances altered in the genetic defect.

These abnormalities occur because the enzyme, phenylalanine hydroxylase (PH), which normally hydroxylates phenylalanine to tyrosine, is deficient or absent. The direct assay of this enzyme is complicated by its expression primarily in the liver. Therefore, indirect assessment of the level of blockage is achieved usually by measuring the plasma phenylalanine along with known protein intake.

Currently there are >400 reported mutations in the gene coding for PH (PAH). Mutation panels of 4-15 'common' mutations have detected as high as 30-50% of disease causing alleles. Mutation analysis, scanning, and sequencing are clinically available for those cases in whom molecular testing is desired. Mutation type is affected by ethnic background. However, once

the mutations are described in a patient, genetic testing can be performed in other family members.

Screening test and confirmation:

In Tennessee, the newborn screening test is performed on the diluted plasma/serum from the dried filter paper spot. Phenylalanine (Phe), the accumulated metabolite, is quantitatively measured by tandem mass spectrometry (MS/MS). A persistent elevation of plasma phenylalanine above the laboratory's cut-off value is termed hyperphenylalaninemia (HPA).

There are both inherited and non-inherited causes of high plasma phenylalanine.

Inherited forms include:

- 1) classical, severe PKU - forms with persistent levels above 4-5X the cut-off value (usually 1000 $\mu\text{mol/l}$, and spill urinary phenylketones (post-neonatal) when not on restrictive diet)
- 2) variant PKU - forms with persistent levels between PKU and non-PKU HPA
- 3) non-PKU HPA – persistent phe levels above the cutoff but below about 2-3X above the cut-off,.
- 4) cofactor deficiencies – defects in the synthesis and regeneration of the cofactor (BH4) for the enzyme, phenylalanine hydroxylase

These patients are considered for potential treatment, and individual determinations are made.

Non-inherited cases may be due to:

- 1) prematurity (liver enzymes not mature)
- 2) intravenous/oral amino acid solutions/formula, particularly when coupled with #1
- 3) liver disease

These patients are usually monitored until phe levels are in the normal range, and rarely require treatment.

Confirmation by direct assay of the enzyme is impractical due to available laboratory methods (requires a liver biopsy). Therefore, indirect methods are generally used to measure its deficiency in cases of HPA. Classification of PKU/HPA was previously done by giving the 6mo child a 3-day natural protein challenge diet, and measuring plasma phenylalanine/tyrosine, and urinary phenylketones. Because the challenge is currently impractical, most clinics rely on the dietary intake as given by parents and the calculated intake of mg phe/kg to determine the degree of restriction required. Different genetic mutations give rise to variability in expression. Therefore, the level of dietary restriction, if any, varies with the mutations in the PH gene.

Newborn Screening Considerations

There is adequate natural protein present in standard formulas (including soy) and breast milk to cause elevation of plasma phe due to classical PKU in the 24hr old term neonate. However, rarely milder variant forms may be missed if the neonate is less than 24 hr of age and is 1) breast fed (lower Phe content than standard formulas); or 2) on IV glucose. This information, taken with the reasons for non-inherited forms of HPA, is helpful when the genetic center interprets the Phe values, and in deciding to recommend a repeat or to see the baby immediately.

Newborn screening should be done as close to time of discharge as possible in a normal infant. However, there are several issues to consider when deciding on the time of screening of the critically ill neonate, i.e., diet, transfusion status, pre-maturity, other newborn screening tests, etc. Phone consultation with the appropriate state-designated genetic center may be helpful in determining the best time to screen a baby.

The following information is necessary for the state newborn screening follow-up and state-designated genetic center personnel to have when notifying a hospital or primary care provider of presumptive positive results, and recommendations for further evaluation:

- 1) Discharge status of baby
- 2) Diet/IV status within 24 hours of time specimen obtained
- 3) Current primary care provider (hospital or outpatient), and
- 4) Current maternal phone number and address.

Follow the recommendations of state newborn screening personnel if a repeat newborn screen is requested. If directed, notify the state-designated genetic center personnel when necessary. The state newborn screening follow-up program automatically notifies the state-designated genetic center of all presumed positives; personnel at the designated genetic center will help with follow-up.

Treatment is begun only by the state-designated center. Genetic Centers are designated by the state health department to cooperate with the Newborn Screening Program in case detection, follow-up of suspected positive cases, interact with the families for further clinical and biochemical evaluation, determine the type of HPA, and provide treatment prescriptions if necessary. There are medical geneticists, metabolic nutritionists, laboratory services, and other services, such as psychology, social work/genetic counselors, etc. to carry out implementation of the diet and genetic counseling with the families, who in turn communicate with the primary care providers and health departments. These centers are available to see patients on short notice as needed to immediately evaluate an at risk patient.

Treatment:

Phenylketonuria is one of the first genetic disorders for which early treatment intervention has prevented clinical symptoms, increased productivity of affected individuals, and decreased societal burden. This is due to efficient newborn screening programs.

Treatment has focused on restriction of dietary Phe and the replacement of tyrosine. The diet becomes a medical prescription, including all natural protein (breast milk, formulas, solid food). Special synthetic formulas have been created just for patients with PKU/HPA. Most current products contain minimal or no phe, replacement tyrosine, and adequate calories and nutrients required for growth. Variation occurs among the few companies world-wide which supply these products. Therefore, they are not interchangeable with changing a dietary prescription.

Since Phe is essential for body growth, natural protein (in the form of breast milk, formulas and at later ages, solid food) is added in restricted specified amounts to maintain the plasma Phe levels in treatment range. Monitoring of plasma levels of Phe, and tyrosine is done frequently, varying from bi-weekly in the neonatal period during rapid body growth to about every few months in later life depending on the proximity to the target control range. Exchange systems for foods with particular Phe allowances are provided to the parents and eventually to the children. Three day food records are required for calculation of total Phe, tyrosine, calories and total protein intake prior to plasma monitoring, as interpretation of blood levels depends on diet intake, as well as health status. Starvation (either calorie or protein) may result in increases in Phe because the body is breaking down its own natural body protein, resulting in a high Phe, even though the intake is low. Thus careful monitoring is related to a carefully obtained history.

Development of a PKU patient approximates the IQ of his unaffected siblings, if the blood Phe level of a newly treated PKU patient is in target range by before 1 month of age, and if subsequent median phe levels are kept near target range. A national collaborative study in the 1970s-80s revealed that a yearly index of median dietary control of a plasma Phe level below 2-3X the cut-off was adequate to prevent clinical symptoms based on follow-up of comparison study groups. However, recent data from Europe and studies of effects on the pre-frontal cortex have suggested that levels close to 1.5X cut-off afford even better results, and most clinics have adopted that value as a target for most types of HPA. While it was originally believed that PKU treatment could be terminated when myelination was thought to be complete (in early childhood)

collaborative data have supported continuing diet past childhood. Published series of articles support treatment into the adult years, especially in classical cases, and particularly females.

Special concerns and issues:

Cofactor defects include alterations in other enzyme pathways (other than phenylalanine hydroxylase) known to affect the processing of Phe. While these defects constitute less than 5% of the persistent hyperphenylalaninemia (HPA), they are evaluated in all cases, and if confirmed, require different treatment.

Prematurity and infusion of IV amino acid hydrolysates in hyperalimentation are the more common reasons for transient HPA not resulting from genetic defects in Phe processing. Therefore, because not all HPA is genetic PKU, not all HPA requires treatment, nor are all HPA treated alike. Referral to state designated Genetic Centers is recommended for the further evaluation of suspected cases..

PKU is inherited as an autosomal recessive disorder. Each biologic parent is an obligate carrier for at least one HPA gene mutation. While the mutation contributed from each parent is likely to be different (unless the parents are related), both mutations affect the function of the phenylalanine hydroxylase enzyme. Each carrier parent has one normal version and one mutant version of the PKU/HPA gene. When transmitting their genes to their offspring, the parents have a 1 in 4 chance (with each pregnancy) that they will both transmit their mutant HPA versions. Therefore, when an inherited PKU/HPA is detected, genetic counseling is performed by the designated genetic treatment center for the immediate family, and offered to other first-degree relatives.

One of the clinical complications of treated females with PKU is their risk for fetal damage during their own pregnancies. Damage to the fetus from the mother's high levels of Phe may cause irreversible microcephaly and mental retardation, increased risk for congenital heart defects and dysmorphic facial features. Since the great majority of these babies are only carriers, they cannot be treated after birth. The only treatment available if pregnancy is continued is to prevent the defects by modifying the mother's diet. It has been recommended that these women maintain their plasma levels of Phe between even lower than PKU patients on diet prior to conception and throughout the pregnancy to prevent developmental defects of the brain and internal organs. This maternal embryopathy argues for continued treatment of all PKU/HPA females through their childbearing years.