Inborn Errors Of Phenylalanine Metabolism
Phenylalanine Metabolism

- Phenylalanine (PHE), an essential aromatic amino acid, is mainly metabolized in the liver by the PHE hydroxylase (PAH) system.
- The first step in the irreversible catabolism of PHE is hydroxylation to tyrosine by PAH.
- PAH enzyme requires the active pterin, tetrahydrobiopterin (BH4), which is formed in three steps from GTP.
- During the hydroxylation reaction BH4 is converted to the inactive pterin, BH2, dihydrobiopterin (quinone).
- Enzyme dihydropteridine reductase (DHPR) regenerate BH4.
Phenylalanine Metabolism

- BH4 is also an obligate co-factor for tyrosine hydroxylase and tryptophan hydroxylase, and thus necessary for the production of dopamine, catecholamines, melanin, serotonin, and for nitric oxide synthase.

- **Defects in either PAH or the production or recycling of BH4 may result in hyperphenylalaninaemia.**

- Also result in deficiency of tyrosine, L-dopa, dopamine, melanin, catecholamines, and 5-hydroxytryptophan.

- When hydroxylation to tyrosine is blocked, PHE is transaminated to phenylpyruvic, phenyllactic and phenylacetic acids.
The enzyme phenylalanine hydroxylase converts phenylalanine to tyrosine together with the co-factor tetrahydrobiopterin (BH4).
Overview of **Biogenic Amines Derived from Aromatic Amino Acids**

- **Phenylalanine** → **Tyrosine**
  - Liver
  - **Skin, brain** → **Melanin pigment**
  - **Adrenal medulla** → **Epinephrine**
  - **Nervous System** → **Norepinephrine**
  - **Thyroid** → **Thyroxine**
- **Liver**
- **Glucose** → **Ketones**
Alternative pathways of phenylalanine catabolism in hyperphenylalaninemia. The reactions also occur in normal liver tissue but are of minor significance.
Hyperphenylalaninemias

- Elevation in blood phenylalanin arise from Defects in either PAH or the production or recycling of BH4.
Classification of Hyperphenylalaninemia

- **Due to PAH defect:**
  - Type I phenylketonuria (PKU)
  - Type II Mild form
  - Type III benign persistent hyperphenylalaninemia

- **Due to defects in the production or recycling of BH4:**
  - Type IV DHPR deficiency
  - Type V BH4 deficiency
Phenylketonuria (PKU)

• PKU is an autosomal recessive disorder.
• It is due an absence or severe deficiency of phenylalanine hydroxylase. (1% enzyme activity).
• Most common form of hyperphenylalaninemia; 97% of cases.
• The clinical features of PKU are absent at birth but develop within a few days if the newborn is untreated.
• Ph. ala. blood levels in newborns with PKU start to rise within 24 hours of feeding breast milk or formula.

• The signs and symptoms of PKU vary from mild to severe, include vomiting, irritability, eczema, increased muscle tone, increased muscle reflex activity, seizures, and severe mental retardation.
Initial symptoms may include:

- a musty or "mousy" odor of the body and urine
- movement disorders
- slower than normal growth rate
- developmental delays in sitting, crawling, and standing

If patients remain untreated they will develop:

- decreased skin and hair pigmentation (due to lack of tyrosine)
- Eczema (Skin Rashes)
- heart defects and other heart problems
- behavior problems
- Seizures
- Microcephaly (small head size)
- Growth retardation
- profound and irreversible mental retardation which develops within three to six months following birth.
Metabolic Derangement

- The consequence of the accumulation of ph.ala. and its metabolized are:
  - **Mousy odor** due to excretion of **phenylacetic acid**.
  - **Hypopigmentation** of hair and skin because of competitive inhibition of tyrosine hydroxylase enzyme by increased concentration of ph.ala. This enzyme is key enzyme for Dopa and subsequent melanine formation from tyrosine.
  - Neurologic symptoms: the pathogenesis of brain damage in PKU is not fully understood but the following
  - 1- Reduced blood levels of tyrosine leading to impaired synthesis of other biogenic amines including melanin, dopamine, and norepinephrine.
• 2- effects on amino acid transport into the brain
• 3- Increased blood PHE levels result in an imbalance of other large neutral amino acids (LNAA) within the brain, resulting in decreased brain concentrations of tyrosine and serotonin. The ratio of PHE levels in blood/brain is about 4:1
• 4- PHE impairs the metabolism of tyrosine hydroxylation to dopamine and tryptophan decarboxylation to serotonin.
PAH Gene:
- Location: chromosome 12q22-24
- Length: 79,278 bp’s (13 exons)
- Over 450 mutations in the gene have been identified in patients with PKU, explaining the wide spectrum of clinical severity.
- Most common is located at position 408 a substitution of an Arginine with a Tryptophan (Arg408Trp).
- PKU affects on average, about every 1 in 15,000 births.
- However the incidence varies between different human populations.
- In the United States, PKU is detected in 1/10,000 (Caucasian population) to 1/50,000 (African American population) newborns.
- The incidence varies greatly in other populations: Turks - 1/2,600; Finnish - 1/200,000
Biochemical Findings

• Hyperphenylalaninemia; blood ph.ala level ranging from 20-80 mg/dl. (N: 1 mg/dl)
• Low blood tyrosine level.
• Phenylpyruvate, phenylacetate and phenyllactate are (not abnormal metabolites) but appear in increased concentration and are excreted in large amounts in urine.
diagnosis

• Screening test: Ferric chloride solution gives dark green color with urine because of the presence of phenylpyruvic acid.
• Determination of phenylalanine and tyrosine blood levels (ph.ala ↑, tyr. ↓) (Guthrie microbiological inhibition test, enzymatic techniques, HPLC, or tandem mass spectrometry)
• PHE/tyrosine ratio > 3.
• If the mutation is known, definitive diagnosis is done by DNA analysis.
• Prenatal diagnosis is possible by PAH DNA analysis on CV or amniotic fibroblasts where the index case has had mutations identified previously.
Treatment

- The goal of PKU treatment is to maintain the blood levels of phenylalanine between 2 and 10mg/dl.
- It involves restricting the dietary intake of phenylalanine.
- In the early stages of life, when the brain is developing rapidly, strict control of phenylalanine concentrations must be imposed to prevent brain damage.
- The concentration of this amino acid is low but cannot be zero since phenylalanine is an essential amino acid and some must be provided in the diet to support protein synthesis.
- Adequate quantities of tyrosine must also be provided in the diet.
- The diet can become somewhat less rigorous after the age of 10, although many clinicians now believe that dietary restriction should be continued throughout life.
- Children who have been diagnosed shortly after birth and properly treated by dietary management develop normally.
- The level of blood phenylalanine should be monitored during treatment.
- Special milk formula low or free phenylalanine are available eg. Lofenalac and Phenyl-free milk.
Newborn Screening for PKU

- Neonatal screening programs for PKU are well established in practically all the countries of the developed world.
- Screening for PKU involves collecting a specimen of capillary blood from the baby at 4 to 10 days after birth, which allows for sufficient time for protein intake to become established. The test involves determining the concentration of phenylalanine in the plasma.
- If the result is indicative of PKU, further definitive tests are performed.
- The plasma phenylalanine concentration used to be determined by the Guthrie test, which involves determining the ability of plasma to support the growth of the bacterium Bacillus subtilis, which can only grow if phenylalanine is present in the medium.
- Nowadays, however, most laboratories use chromatographic, fluorimetric or mass spectrometric methods for the estimation of phenylalanine.
- With immediate diagnosis and treatment by early introduction and maintenance of special diet, normal IQ and development can be expected.
Guthrie bacterial inhibition test

- Bacterial inhibition assay to measure blood phenylalanine. Using a cut-off level of 4 mg/dL, miss 16% under 24 hours, 2% over 48 hours old. Preferable sample at > 72 hr of life.
- The prototype of metabolic screening tests relying on bacterial inhibition.
- Filter paper is saturated with heel-stick blood, allowed to dry.
- Small disks are punched out for use in tests. Bacillus subtilis is spread uniformly on agar.
- Inhibitory amino acid analogs block specific metabolic pathways.
- Bacterial can grow only if exogenous amino acids competitively overcome the block.
- Can test in this manner for phenylalanine, leucine, methionine, galactosemia, histidine, and tyrosine.
Severely retarded brothers with untreated PKU. They were quite fair of hair and skin.
Classical Untreated PKU phenotype
Two cases with PKU were diagnosed early after birth and treated by early introduction and maintenance of special diet. They have normal IQ and development.
Maternal PKU

- Women with PKU and uncontrolled Phe levels also have an increased risk of pregnancy loss.
- Children born to women with PKU are at risk for "maternal PKU" because high levels of Phe interfere with normal embryonic development.
- Keep level <10mg/dl
- Strict dietary control is necessary in pregnant women who have PKU, since maternal hyperphenylalaninemia can affect the fetus in utero, even if the fetus itself does not have PKU. Mental retardation and congenital abnormalities can occur in a large proportion of these infants.
Type II Mild PKU

- Due to incomplete deficiency of PAH enzyme, the residual activity is about 6% of normal.
- Phenylalanine level in blood about 12mg/dl.
- Moderate excretion of phenylpyruvic acid in urine.
- Mild symptoms.
- Diet restriction is necessary but does not need to be severe.
Type III: Benign Persistent Hyperphenylalaninemia

- Residual PAH activity from 10-20% of normal.
- Blood ph.ala. Less than 8mg/dl.
- No excess phenylpyruvate in urine.
- No symptoms.
- No treatment is necessary but follow-up is important to assure that blood ph.ala. will not be elevated to avoid mental retardation.
Type VI: DHPR deficiency

• Autosomal recessive disorder.
• Due to the deficiency of DHPR enzyme.
• The gene locus for DHPR on chr.4p15.3.
• Represent less than 1% of hyperphenylalaninemia cases.
• Blood ph.ala. Level less than in PKU.
• Has the same clinical PKU symptoms but with more severe neurological symptoms because the enzyme involves also in hydroxylation of tyrosine and tryptophane which is an essential step in bisynthesis of neurotransmitters dopamine and serotonine.

• Diagnosis: blood ph.ala. level blood DHPR activty, urine and blood pterin analysis.

• Treatment: ph.ala. restriction diet and replacement therapy by taking 5- hydroxytryptophane and DOPA. (precursors for neurotransmitters)
Type V : BH4 deficiency

- Autosomal recessive disorders.
- Due to the deficiency of BH4 synthesis.
- 4 enzymes are involved in synthesis of BH4, a mutation in the genes of any one of these enzymes results in BH4 deficiency.
- Same biochemical and clinical findings as type IV.
- **Diagnosis**: blood ph.ala. level, urine and blood pterin analysis.
- **Treatment**: ph.ala. restriction diet and replacement therapy by taking 5- hydroxytryptophane and DOPA. (precurssuros for neurotransmitters) and small doses of BH4.