Phenylalanine Hydroxylase Deficiency: *Phenylketonuria and Hyperphenylalaninemia*

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Amino Acids

- **Essential amino acids**
  - Essential amino acids cannot be made by the body. As a result, they must come from food.
  - The nine essential amino acids are: histidine, isoleucine, leucine, lysine, methionine, threonine, tryptophan, valine and **phenylalanine**.

- **Nonessential amino acids**
  - "Nonessential" means that our bodies produce an amino acid, even if we don't get it from the food we eat.
  - They include: alanine, asparagine, aspartic acid, and glutamic acid.

- **Conditional amino acids**
  - Conditional amino acids are usually not essential, except in times of illness and stress.
  - They include: arginine, cysteine, glutamine, tyrosine, glycine, proline, and serine.
Two Pathways of Phenylalanine Metabolism

Dietary Protein → Phenylalanine

1. Phenylalanine hydroxylase (PAH)
   *Requires cofactor/co-substrate tetrahydrobiopterin (BH₄)

Phenylalanine → Tyrosine → Tyrosine Derivatives (e.g. dopamine, epinephrine)

Tyrosine → Proteins

Breakdown

*Requires cofactor/co-substrate tetrahydrobiopterin (BH₄)
Phenylalanine Hydroxylase (PAH) Deficiency

First inborn error of metabolism (IEM) identified by population screening

Traditionally called Phenylketonuria (PKU)
  • Urine accumulation of phenylpyruvic acid

PAH deficiency terminology encompasses
  • PKU
  • Hyperphenylalaninemia
Genetics of PAH Deficiency

- Autosomal recessive IEM

- PAH gene
  - Chromosome 12q23.1
  - 991 variants (as of March 6, 2017)

- Incidence of PAH deficiency
  - 1 in 10,000 to 15,000 live births (United States)
  - 1 in 4,500 (Ireland)
  - 1 in 2,600 (Turkey)
Symptoms due to neurotoxic accumulation of PHE/PHE metabolites

1. Developmental delay
2. Abnormal gait
3. Spastic reflexes
4. Eczema
5. Aberrant behaviors
6. “Mousy Odor” (phenylpyruvic acid)
BH₄ Generation/Regeneration Pathway

*BH₄ deficiency can lead to secondary PAH deficiency
Evolution of Screening Methodology

1960s: Guthrie Test

- Bacterial inhibition assay
- Manual sample analysis; semi-quantitative
- **High** false positive/negative results

1990s: Tandem mass spectrometry (MS/MS)

- Automated sample analysis
- **Low** false positive/negative results
- Mean cutoff for PHE of 130 µmol/L
- PHE:TYR ratio >3 as abnormal
Plasma Amino Acid Analysis

Plasma amino acid analysis (AAA)
- Quantify PHE, TYR, PHE:TYR and complete AAA
- Methods
  - HPLC: UV/derivatization
    - Precolumn
    - Postcolumn
  - Mass Spectrometry

Enzymatic PHE Dehydrogenase
- Rapid PHE determination
- Historic/less common

Additional Diagnostic Testing

Cofactor deficiency testing
• Disorders of BH₄ (PAH enzyme cofactor) synthesis and regeneration

Genotyping
• Mutation analysis for improved therapy planning

PAH activity
• Enzymatic activity in hepatic and renal tissues
• Not recommended for either screening or diagnostic testing
Treatments for PAH Deficiency

Initiation requires prompt NBS, follow-up and diagnostic testing

Treatments

• Dietary
• Pharmaceutical

Treatment of infants within first 2 weeks of life

• PHE >600 µmol/L
• PHE >360 µmol/L (North America)
• PHE between 120 and 360 µmol/L not recommended
Dietary Therapy

- Decrease in natural protein intake
- Supplement with a protein source devoid of PHE
- PHE intake recommendations are based on: residual PAH activity, patient age, growth rate, cofactor responsiveness, etc.

**Phenylketonurics:** Contains phenylalanine

**INGREDIENTS:** Citric acid, malodextrin, aspartame**, natural and artificial flavor, caffeine, magnesium oxide, contains 2% or less of: acesulfame potassium, calcium silicate, gum arabic, niacinamide, calcium pantothenate (vitamin B6), biotin (B vitamin).

**Dietary label**
Pharmacotherapy

Sapropterin dihydrochloride (Kuvan)
- First pharmacologic agent for PAH deficiency (FDA 2007)
- Synthetic BH₄ (tetrahydrobiopterin)
- 25-50% of patients with PAH deficiency respond

Large Neutral Amino Acids (LNAA)
- Block uptake of PHE from intestine and blood-brain barrier
- Limited to older patients (adolescents/adults)
- Not recommended for pregnant patients

Polyethyleneglycol-conjugated PHE ammonia lyase
- Currently in Phase 3 clinical trials (as of 2017)
Phenylalanine Embryopathy (Maternal PKU)

*In utero* exposure to elevated PHE (>360 µmol/L)
- Microcephaly
- Poor fetal growth
- Congenital heart defects
- Intellectual disability (>90% of live births)

Management during pregnancy
- <360 µmol/L prior to conception (<240 µmol/L international)
- LNAAs not recommended
- Sapropterin (benefits/risks)
- Close monitoring of fetal growth
Summary

• PAH deficiency is an autosomal recessive IEM

• Widely considered the dawn of NBS

• Following up testing is needed to confirm PAH deficiency
  • HPLC (pre/post-column derivatization)
  • MS/MS

• Multiple therapies available

• Monitoring/treatment may be for life
References


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