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PEARLS OF LABORATORY MEDICINE

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.

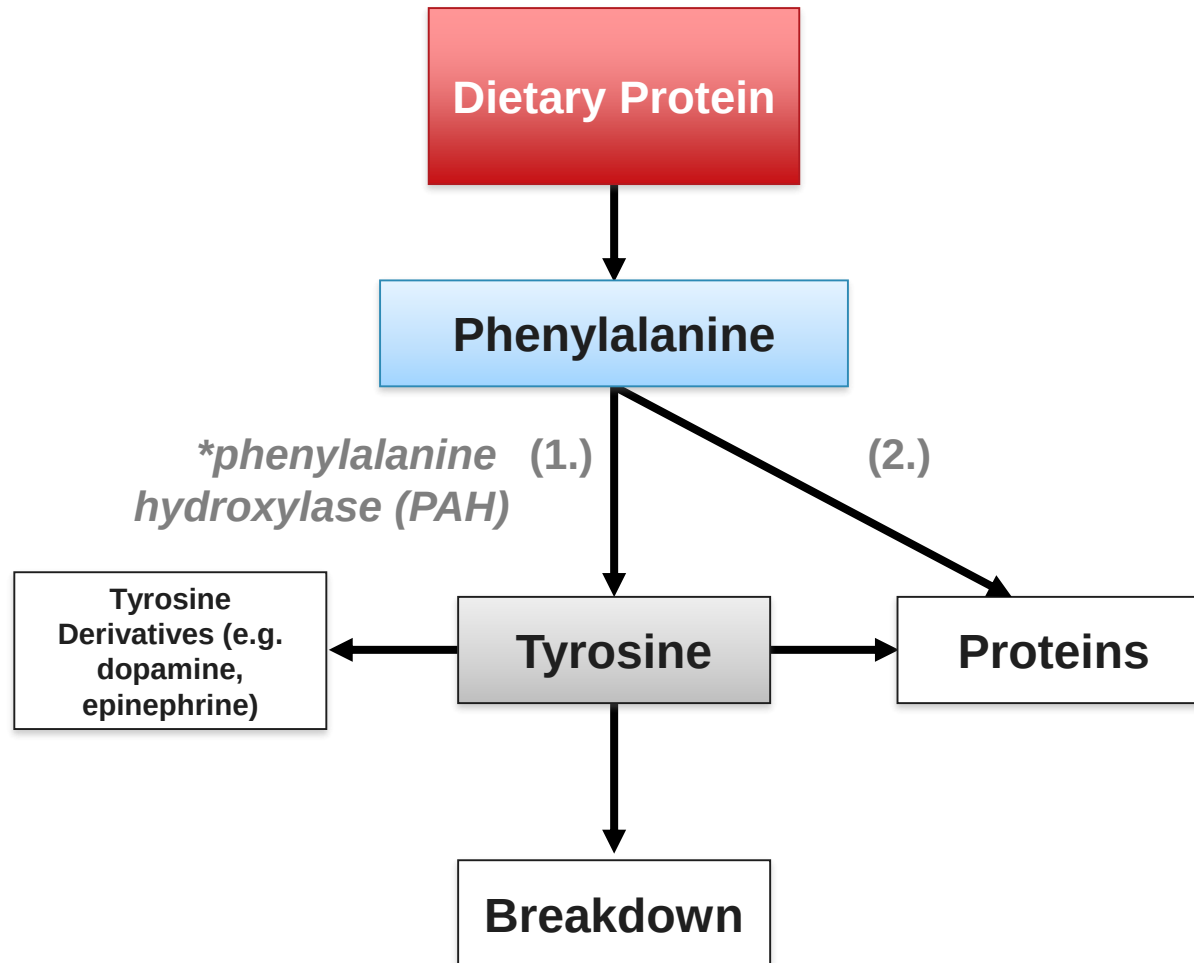
- **Nonessential amino acids**

- "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



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

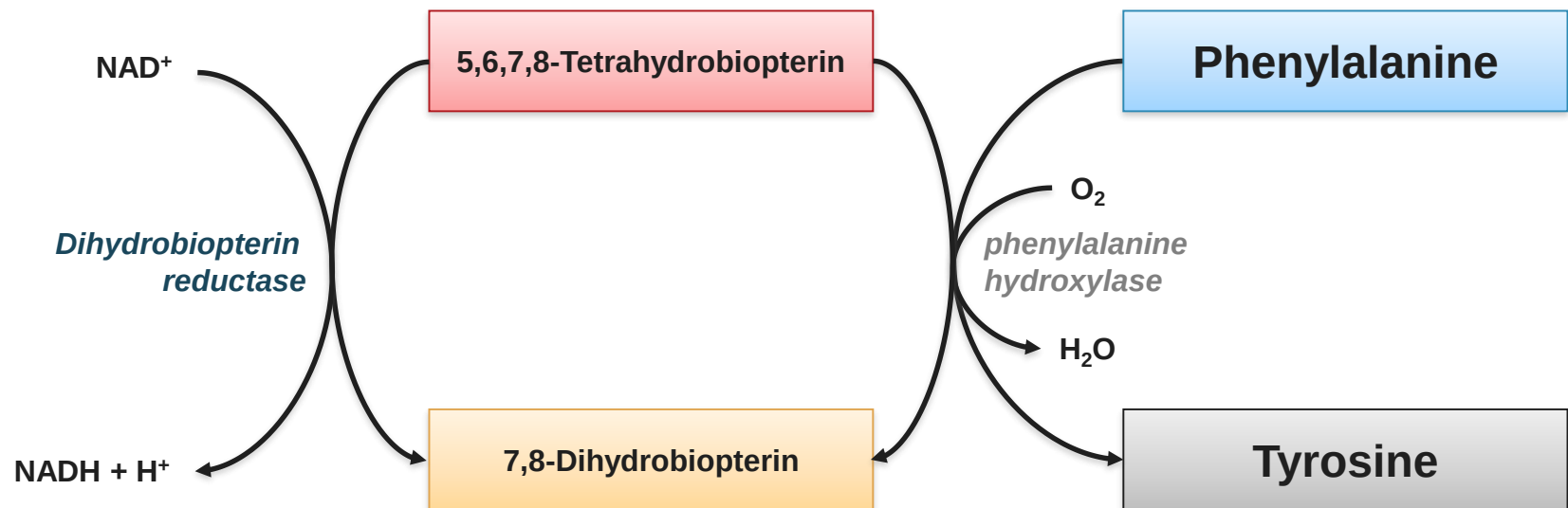
- Autosomal recessive IEM
- PAH gene
 - Chromosome 12q23.1
 - 991 variants (as of March 6, 2017)
- Incidence of PAH deficiency
 - 1 in 10,000 to 15,000 live births (United States)
 - 1 in 4,500 (Ireland)
 - 1 in 2,600 (Turkey)

Pathology of untreated PAH Deficiency

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 $\mu\text{mol/L}$
- PHE:TYR ratio >3 as abnormal

Plasma Amino Acid Analysis

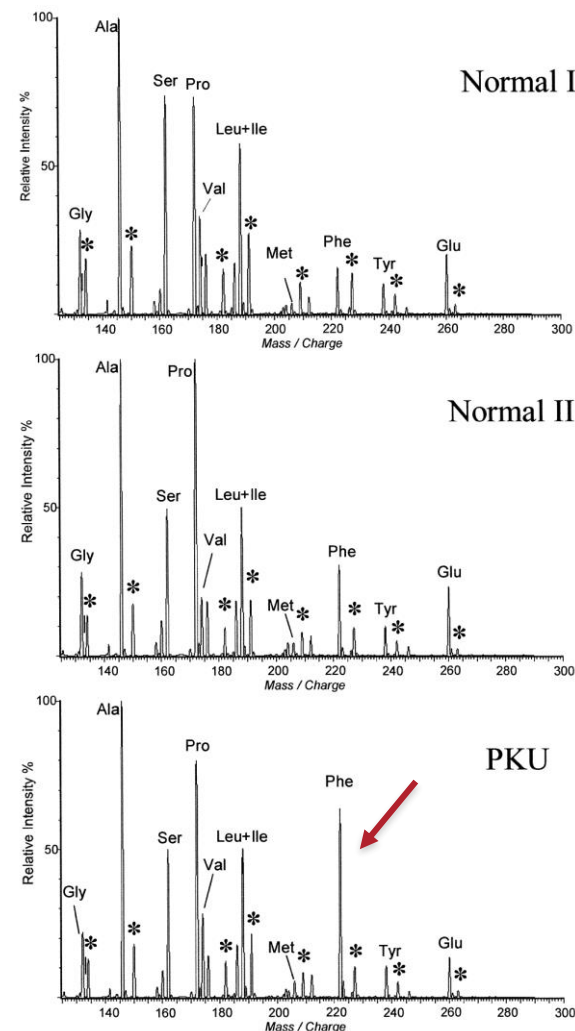
Plasma amino acid analysis (AAA)

- 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

MS/MS Amino Acid Profiles



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 $\mu\text{mol/L}$
- PHE >360 $\mu\text{mol/L}$ (North America)
- PHE between 120 and 360 $\mu\text{mol/L}$ 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.

Dietary label

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).

****PHENYLKETONURICS:
CONTAINS PHENYLALANINE**



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 $\mu\text{mol/L}$)

- Microcephaly
- Poor fetal growth
- Congenital heart defects
- Intellectual disability (>90% of live births)

Management during pregnancy

- <360 $\mu\text{mol/L}$ prior to conception (<240 $\mu\text{mol/L}$ international)
- LNAAAs 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

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