Phenylketonuria (PKU)

- classic “inborn error of metabolism”
- autosomal recessive disease characterized by mutations in the liver enzyme, *phenylalanine hydroxylase*, encoded by the *PAH* gene

  PAH converts phenylalanine to tyrosine  
  (reaction requires O₂ and co-factor BH₄)

- HPA or non-PKU hyperphenylalaninemia are related disorders of phenylalanine hydroxylation involving several enzymes necessary for the synthesis and recycling of co-factor for PAH, tetrahydrobiopterin (BH₄)

- incidence: 1 in 10,000

History of PKU

1934: Asbjorn Folling described an inherited metabolic disorder characterized by severe intellectual impairment, motor problems and skin abnormalities - affected individuals identified by abnormal excretion of phenylpyruvic acid (high frequency of consanguinity in parents of PKU patients; Mendelian disease)

1950s: PKU patients shown to have deficient activity of PAH

1960s: treatment of PKU with low phenylalanine diet shown to be effective

1980s: mapping and cloning of PAH gene

1990s: PKU more than a simple Mendelian trait; also behaves as complex, multifactorial disorder

2000s: Non-dietary treatments for PKU developed
PKU has a multifactorial cause:

- mutation in PAH gene (genetic)
- exposure to dietary phenylalanine (environmental)

Clinical features of PKU

enzyme deficiency is a primarily hepatic phenotype but major clinical presentation is abnormal brain development and function

- reduced higher-brain abilities (executive functions)
- neuropsychological dysfunction (imbalance of neurotransmitters)
- emotional disturbance and behavioral problems (clinical depression)
- severe mental retardation will result in untreated cases (estimated that 1% of patients in mental institutions have PKU)
dopamine (neurotransmitter)

Phenylalanine metabolism

(Klug & Cummings 1992)

Phenylketonuria

(Klug & Cummings 1992)
### Subtypes of PKU, phenylalanine levels & clinical outlook

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Fold increase blood [Phe] (over normal)</th>
<th>Clinical picture (brain dysfunction)</th>
<th>Treatment required?</th>
</tr>
</thead>
<tbody>
<tr>
<td>classic PKU</td>
<td>&gt;20</td>
<td>severe mental retardation</td>
<td>yes</td>
</tr>
<tr>
<td>(untreated)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>mild PKU</td>
<td>10-15</td>
<td>cognitive loss</td>
<td>yes</td>
</tr>
<tr>
<td>(untreated)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-PKU mild HPA</td>
<td>2-8</td>
<td>normal</td>
<td>maybe</td>
</tr>
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</table>

### Newborn screening for PKU

- done with a simple blood test, screening is standard in many developed countries
- resource for sampling of mutant PAH genes
- prenatal diagnosis is possible
- classification of severe and less severe forms as well as non-PKU HPA requires Phe and BH₄ measurements in several body fluids
Maternal PKU

- pregnant mothers with untreated PKU can give birth to children with severe defects
  - congenital malformations
  - microcephaly
  - severe mental retardation
  
- careful treatment with diet is compatible with normal outcome for fetus

Pathogenic PAH alleles

• null alleles or gene deletions (no activity)
• $V_{max}$ alleles (reduced activity)
• kinetic alleles (altered affinity for substrate or cofactor)
• unstable alleles (increased turnover and loss of PAH protein)

majority of mutations
Effects of disease-causing PAH mutations on a patient can be measured at three levels:

- proximal (enzymatic): in vitro assay
- intermediate (metabolic): plasma phenylalanine levels
- distal (cognitive function): IQ tests

genotype-phenotype correlations show good correlations at the proximal levels

at intermediate and distal levels, phenotypes behave as complex traits suggesting the presence of “modifiers”

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**Pathophysiology of PKU**

- metabolites of PKU (i.e. phenylpyruvate) not present in high enough concentrations to be toxic
- is phenylalanine the neurotoxic agent?

  1) brain protein synthesis

  2) transport processes and neurotransmitter biosynthesis
     (tyrosine (Tyr) and tryptophan (Trp) are transported across blood-brain barrier for synthesis of the neurotransmitters, dopamine and serotonin, respectively)
- hypotyrosinemia: low [tyrosine], low neurotransmitters (loss of biogenic amines at critical stages in postnatal brain maturation)
- decreased protein synthesis in brain (weak evidence)
- defective brain myelination (chronic and irreversible)

Potential problems with the low tyrosine theory...

• postnatal tyrosine supplementation without reduction of phenylalanine intake does not prevent mental retardation in PKU
• no consistent or pathological reduction in plasma tyrosine content in untreated PKU patients
• tyrosine supplements during treatment of PKU sufficient to increase plasma tyrosine levels do not improve neurophysiological parameters
Treatment: dietary restrictions

adherence to a phenylalanine-free diet postnatally can prevent mental retardation and improve behavior in children with PKU

Target Your Food Choices

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synthetic dietary supplement needed to avoid malnutrition

**Phenyl-free:** Phe-free amino acid mixture, vitamins, minerals, fat (marketed by Mead Johnson)

- offensive in odor and taste
- must be continued for life
- emotional stress in PKU families
- high cost ("patient years")
Aspartame (Nutrasweet™) is an amino acid sweetener, with two constituent amino acids, aspartic acid and phenylalanine, both commonly found in food.

![Phenylalanine structure](image)

- synthetic diet not perfect...

  - produces several biological side effects due to periodic nutrient deficiencies
  - needs improvement in organoleptic properties (essential fatty acids) and nutrient composition (ratios of amino acids)

**treatment alternatives:**

- gene therapy (not yet applicable)
- enzyme replacement therapy (PAL and PEG-PAL papers)

PAL: non-mammalian enzyme; degrades Phe to ammonia and trans-cinnamic acid (harmless metabolite)
treatment of mild PKU with tetrahydrobiopterin (BH₄) loading

- several recent studies suggest that BH₄ can be a treatment alternative to dietary restriction of phenylalanine

**Tetrahydrobiopterin as an alternative treatment for mild phenylketonuria**


- out of 38 with PAH deficiency, 87% showed responsiveness to BH₄ (i.e. had lower blood phenylalanine levels)
- no response in 7 patients with classic PKU
- long-term treatment with BH₄ in 5 patients increased daily phenylalanine tolerance enough to discontinue Phe-restricted diet
- mutations connected to BH₄ responsiveness predominantly in the catalytic domain of the protein and were not directly involved in cofactor binding

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**Treatment of classical PKU with BH₄**

- recent reports indicate that BH₄ loading was also beneficial to patients with more severe forms of PKU not just mild non-PKU HPA

38 US PKU patients were given single dose of BH₄ and Phe levels were monitored

- 58% responded at 24 h (>30% decrease in Phe levels); some who responded favorable were clinically described with classical PKU

- mutant PAH responds with increase in the residual enzyme activity following BH₄ administration
  - increased stability
  - chaperone effect (better folding)
  - correction of mutant Km

**Kuvan™**: synthetic form of BH4 that is approved in Europe for treatment of non-PKU HPA
Diet and disease: vitamin deficiencies

Beriberi - vitamin B-1 (thiamin) deficiency

whole* grains and lean pork# are a good dietary source

# Japanese navy and Dr. Takaki
* Christiaan Eijkman, chickens

http://nobelprize.org/medicine/educational/vitamin_b1/eijkman.html
- thiamin (vitamin B-1) in its pyrophosphate form acts as a coenzyme in the decarboxylation and transketolation pathways (pentose phosphate pathway) of carbohydrate metabolism, and possibly in nerve conduction (essential for the synthesis of acetylcholine)

\[ \text{TPP} \]

*when proton dissociates a carbanion is formed which readily undergoes nucleophilic addition to carbonyl groups*

- affects the cardiovascular, muscular, gastrointestinal, and nervous systems (weight loss, cardiac abnormalities and neuromuscular disorders such as tremors)
- pathophysiology of clinical manifestations of beriberi not known

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**Rickets** - **vitamin D deficiency**

- caused by insufficiency or inefficient action of activated vitamin D in the body during childhood
- vitamin D is fat-soluble vitamin either absorbed from intestines or produced by the skin when skin is exposed to sunlight; converted to its active form in two steps
- in its active form, vitamin D acts as a hormone to regulate calcium absorption from the intestine and to regulate levels of calcium and phosphate in the bones
- deficiency causes progressive softening and weakening of the bone structure

**hereditary rickets**, an inherited, sex-linked disorder, is a vitamin D-resistant form of rickets caused mutations in the vitamin D receptor (VDR); vitamin D-resistance prevents kidneys from retaining phosphate
**Scurvy:** vitamin C deficiency

- sailors at sea for long periods developed several debilitating symptoms

  1) joint pain and weakness
  2) internal hemorrhaging and bruising
  3) loose and bleeding teeth
  4) mental disturbances

the story of a sailor “who ate grass, like a beast, and survived”
James Lind discovered that citrus fruits could prevent scurvy and developed a method for concentrating and preserving citrus fruit juices for use at sea.

In 1795, the British Royal Navy provided a daily ration of lemon or lime juice ("limeys").

In 1932, vitamin C or ascorbic acid was isolated and synthesized.

**Biological functions of vitamin C**

- A reducing agent (or antioxidant); essential nutrient in humans, apes.

- Necessary to maintain the enzyme prolyl hydroxylase in its active form by keeping its iron atom in a reduced state.

- Enzyme converts the prolines and lysines in procollagen to their hydroxlated forms.

- Many other functions.
Formation of collagen fibril

Formation of collagen fibril

Disease mechanism and pathophysiology of scurvy

- hydroxyl groups form interchain hydrogen bonds that stabilize the triple-stranded collagen helix

- underhydroxylation of proline in scurvy leads to unstable helix and immediate degradation within the cell

- collagen needed for connective tissue, bones, and dentin (major portion of teeth) as well as maintenance of blood capillary structure

- turnover of collagen in blood vessels and teeth is rapid; slow in bone
many steps in collagen processing

many genetic diseases affecting fibril formation

<table>
<thead>
<tr>
<th>mutation</th>
<th>disease</th>
<th>clinical feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>type I collagen</td>
<td>osteogenesis imperfecta</td>
<td>weak bones</td>
</tr>
<tr>
<td>type II collagen</td>
<td>chondrodysplasias</td>
<td>bone &amp; joint deformities</td>
</tr>
<tr>
<td>type III collagen</td>
<td>Ehlers-Danlos syndrome</td>
<td>fragile skin and blood vessels, hypermobile joints</td>
</tr>
</tbody>
</table>

Other examples of the relationship between diet and disease

- high fat diet and type II diabetes
- Equator and spices
- Native American Indians and succotash (corn low in lysine, beans low in methionine)
- malaria and quinine