Classic Phenylketonuria (PKU)
Erin Syverson

National PKU Awareness Month
What is PKU?

Permanent brain damage, cognitive disabilities
Seizures
Reduced melanin
Microcephaly
Behavioral problems
Musty odor
Tyrosine starvation

The enzyme phenylalanine hydroxylase converts the amino acid phenylalanine to tyrosine.

Miller-Keane Encyclopedia and Dictionary of Medicine, Nursing, and Allied Health, Seventh Edition.
**Treatments**

**Kuvan:**
Supplement of BH4
Successful in 10% of classic PKU cases

[Chemical structure of Kuvan](http://images.rxlist.com/images/rxlist/kuvan1.gif)

**LNAA Supplementation:**
Balances Phe levels with other LNAA

[Chemical structure of LNAA](http://upload.wikimedia.org/wikipedia/commons/5/57/L-serine-2D-skeletal.png)
The Diet
The Diet
Regulated by Phe, phosphorylation, and BH4

Most null mutations occur in catalytic or tetramer-forming sites.

Cytosolic - in the liver and minimally in kidneys and pancreas

The PAH protein is very well conserved

Data generated from PFAM
PAH has high similarity across the animal kingdom

- Human: 100% identical, 100% similar
- Chimpanzee: 99% identical, 100% similar
- Mouse: 92% identical, 96% similar
- Chicken: 83% identical, 91% similar
- Zebrafish: 75% identical, 84% similar
- Fruit Fly: 62% identical, 77% similar
- Nematode: 57% identical, 71% similar
How does PAH function in the body?

Tyrosine metabolism

BH4 synthesis

L-amino acid catabolism/ carboxylation/ monooxygenation

Generated from the String Database
What is the state of current research?

Research has mostly focused on finding new mutations.

Most knowledge comes from trial and error/correlation observations.

Not much concrete or whole-body evidence.
How do varying levels of Phe affect gene expression in the liver, brain, and kidneys?

**Hypothesis**: certain levels of Phe are dangerous for some traits, but not for others (i.e. myelin production may be less sensitive than melanin production).
Why is this relevant?

Be able to define causes behind problems in treated patients

Patients will be informed of effects of certain Phe levels at specific ages

Make more informed dietary choices

http://www.tdalfredos.com/Ice-CreamSundae.jpg
When, if ever, is it ok to stop the diet?
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How can we study whole-body effects?

Different aged PKU mice (infancy, adolescence, maturity)
Liver, brain, and kidneys
Perform microarrays
How do varying levels of Phe affect gene expression in the liver, brain, and kidneys?

<table>
<thead>
<tr>
<th>Serum Phe Levels/Diet</th>
<th>App. 0</th>
<th>100</th>
<th>450</th>
<th>750</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain - DOPA</td>
<td>Lowered</td>
<td>Lowered</td>
<td>Lowered</td>
<td>Lowered</td>
<td>Lowered</td>
</tr>
<tr>
<td>Liver - PAH</td>
<td>Lowered</td>
<td>Normal</td>
<td>Raised</td>
<td>Raised</td>
<td>Raised</td>
</tr>
<tr>
<td>Kidney - PAH</td>
<td>Lowered</td>
<td>Normal</td>
<td>Raised</td>
<td>Raised</td>
<td>Raised</td>
</tr>
</tbody>
</table>

Relative expression compared to healthy mice
How does PAH relate to kidney disease?

PAH activity is lowered in rats (and presumably humans) with Chronic Renal Failure (CRF), though hepatic expression remains normal.

**Hypothesis**: PAH in patients with Chronic Renal Failure is being over-phosphorylated at sites in the catalytic domain.
Finding the phosphorylation sites of PAH

Catalytic Domain

NetPhos 2.0: predicted phosphorylation sites in Sequence

Sequence- and structure-based prediction of eukaryotic protein phosphorylation sites.
Blom, N., Gammeltoft, S., and Brunak, S.
Using immunoprecipitation and mass spectrometry to determine phosphorylation status

1. Normal Phosphorylation
2. Increased Phosphorylation

http://www.leinco.com/includes/templates/LeincoCustom/images/immunoprecipitation.gif

Thursday, May 9, 13
Where can we go from there?

Research phosphorylation of other important kidney proteins

BH4 supplementation

Specific role of PAH within the kidney
What are the implications?

PKU is tested for in all newborns.

Phe is an important amino acid and should only be avoided if absolutely necessary.

Lack of biochemical and genetic evidence - how many unnecessary dieters?

http://www.healthy.arkansas.gov/programsServices/familyHealth/ChildAndAdolescentHealth/newBornScreening/PublishingImages/photo1.jpg
Questions?


• http://www.wormbase.org/species/c_elegans/gene/WBGene00000240#0a-9e-3

• http://zfin.org/ZDB-GENE-031006-2

• http://www.informatics.jax.org/marker/MGI:97473

• Timothy L. Bailey and Charles Elkan. "Fitting a mixture model by expectation maximization to discover motifs in biopolymers", Proceedings of the Second
References