The Molecular Basis of Phenylketonuria

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Background

• What is it?
  – Phenylalanine
  – Untreated …
  – Restricted Diet

• Autosomal Recessive
  – Carriers: 1/50
  – Frequency: 1/10,000
  – Screening: 1960’s
Phenylalanine Hydroxylase Activity
Figure 8.29  Catabolic products of phenylalanine that accumulate in phenylketonuria.

- Phenylalanine
- Phenylpyruvate
- Phenylacetate
- Phenylacetyl glutamine

Reactions:
- Phenylalanine → Phenylpyruvate → Phenylacetate → Phenylacetyl glutamine
- Phenylalanine → Phenylpyruvate → Phenylacetate → Phenylacetyl glutamine
Phenylalanine Toxicity

- Unknown
- Serotonin and Catecholamines
  - Inhibits tyrosine and tryptophan transport into neurons
- Phenylpyruvic acid
  - Pyruvate decarboxylase inhibitor
- Myelin Synthesis
- Alterations in brain architecture
Phenylalanine hydroxylase

- 75% phenylalanine’s disposal
- Converts phe to tyr
- 12q24.1
- 1% map to genes for BH$_4$
Phenylalanine hydroxylase

- **Domains**
  - N-terminus (1-142)
  - Catalytic (143-410)
  - Tetramerization (411-452)

- **Active Site**
  - Non heme iron
    - Iron III resting state
Active site
Phenylalanine hydroxylase

- pH dependent equilibrium
  - Homodimers
  - Homotetramers
  - Antiparallel coiled-coil core
Mutations

- Catalytic
  - 209
- Regulatory
  - 49
- Tetramerization
  - 10
Active-site Mutations

- **T278I, T278A, and T278N**
  - Thr278 location
  - H-bond to Glu280
- **E280K**
  - Electrostatic potential altered
- **F254I**
  - pi-stacks with pterin ring
  - Interfere with pterin binding
Active-site Mutations (cont…)

- **P281L**
  - Defines shape near iron
- **F331C and F331L**
  - Abolish pi-stacking interactions that stabilize the active site wall
- **S349P**
  - Located near active site
  - Total alteration of active site shape
Normal PAH Mechanism

1. Fe$^{3+}$ + Sub. → BH$_4$
2. Fe$^{3+}$ + e$^{-}$ → Fe$^{2+}$
3. Fe$^{2+}$ + O$_2$ → Fe$^{3+}$
4. Fe$^{3+}$ + BH$_4$ → BH$_2$
5. BH$_2$ + Fe$^{3+}$ → Product
S349P Mutation Uncoupling
Regulatory Domain

- Regulation of PAH
- G46S Mutation
  - Distorts secondary structure
  - Inactive aggregates formed
Tetramerization Domain Mutations

- **IVS12 + 1g → a** in intron 12
  - Most prevalent mutation among Caucasians
  - Truncated form of PAH
    - Lacks last 52 amino acids
    - Unstable protein
- **pro407-arg408-pro409**
  - Hinge region
The Heterozygote Advantage

• **Ochratoxin A**
  – *Aspergillus* and *Penicillium*
  – N-acyl derivative of phenylalanine
  – Stops Protein Synthesis
    • Competes for phenylalanyl-tRNA synthetase

• **Celtic Origin**
  – Mild, wet climate
  – Famine, economic hardships
Treatment

• Diet
  – Non compliance
• Gene Therapy
  – Far Future
• Enzyme Replacement Therapy
  – PAL
    • Phenylalanine $\rightarrow$ trans-cinnamic acid
  – Oral
An Interesting Tangent

- Phenylacetate
  - Damage to immature brain
  - Inhibits protein prenylation
  - Other mechanisms?
- Primary brain tumors are very similar to immature CNS
- Significant tumor suppression with no apparent toxicity to the host
Conclusions

• PAH deficiency
• Autosomal recessive
  – Heterozygote advantage
• Almost 400 known mutations
• Future
  – Most promising: Enzyme replacement therapy
References


References (cont…)


References (cont...)  

