Phenylketonuria

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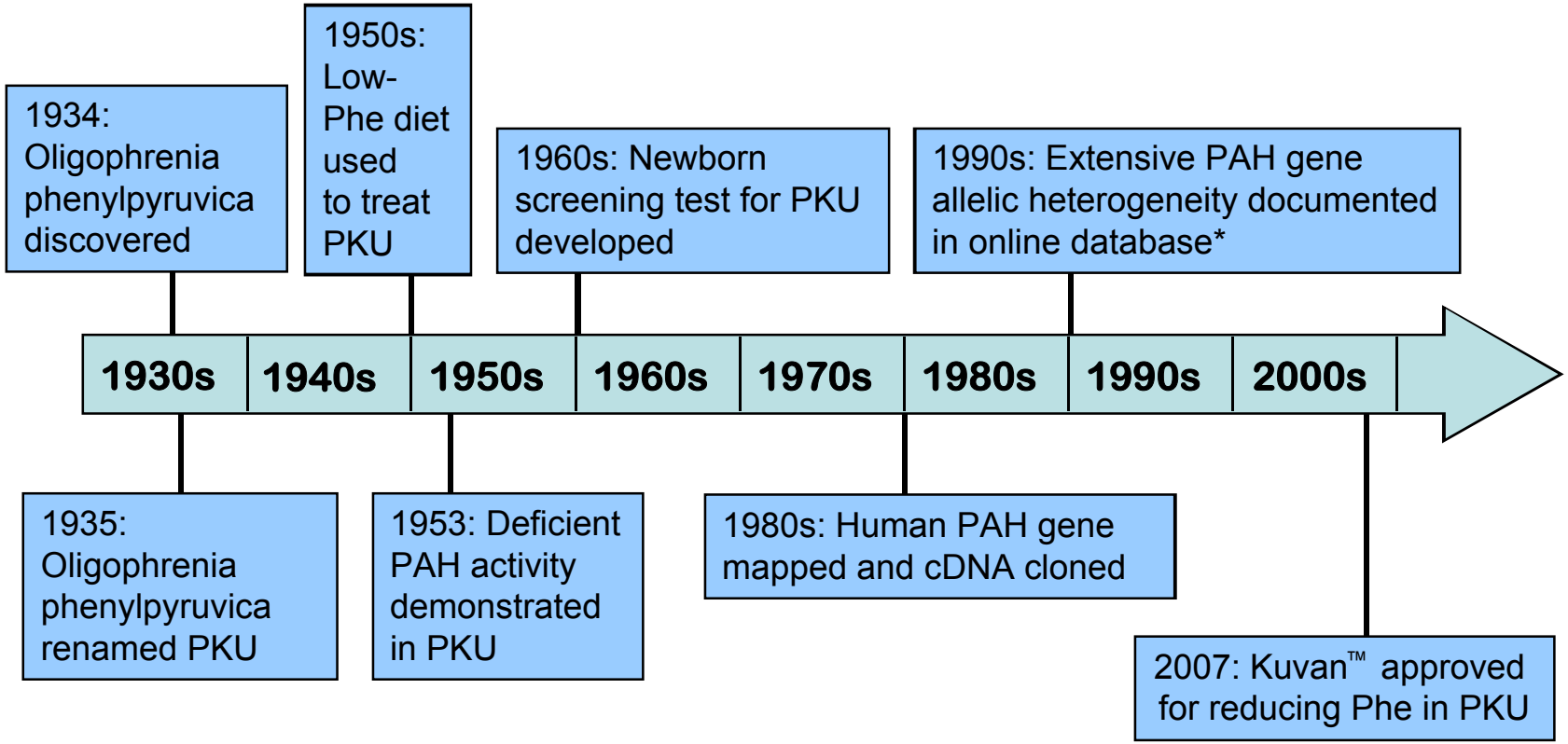
History of PKU Timeline



*http://www.pahdb.mcgill.ca

Scriver, CR. PKU: The Journey; not the Arrival…yet.In: Blau N.
PKU and BH4–Advances in Phenylketonuria and Tetrahydrobiopterin. 1st ed. SPS Publications;2006.

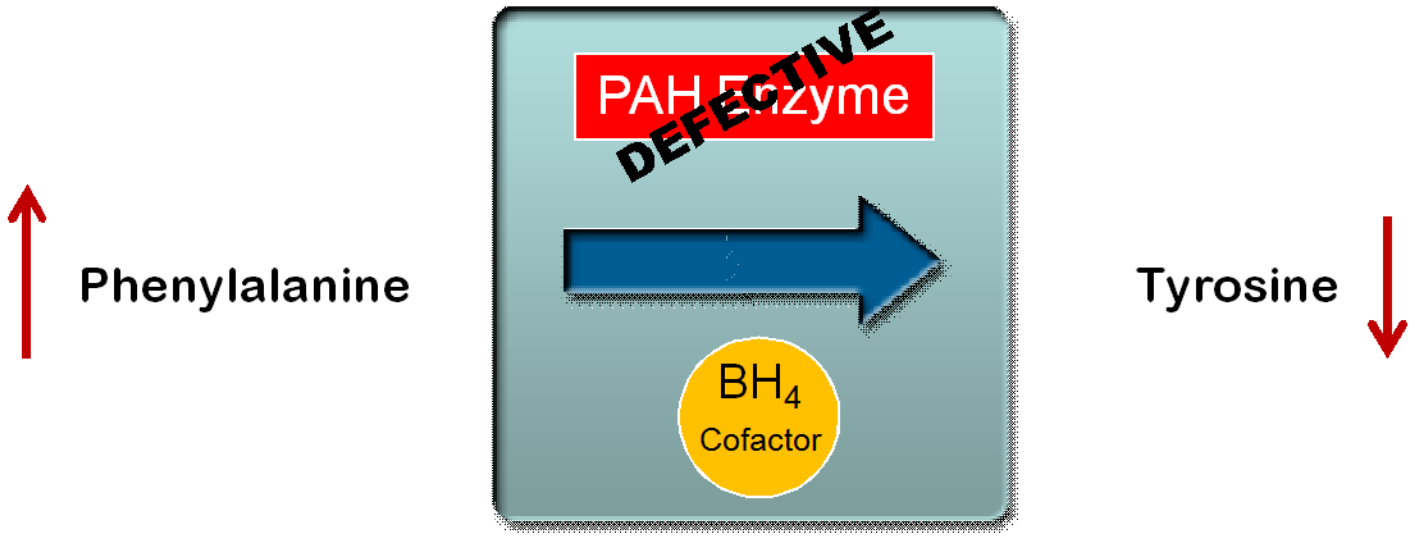
Phenylalanine

- **Phenylalanine** is a primary amino acid that is abundant in dietary protein.
- It's main metabolic pathway yields the amino acid **Tyrosine**, which is involved in the production of **Melanin** pigments.
- Defects of enzymes responsbile for interconversion of metabolites in the pathway are the cause of three well-studied, single-gene **Inborn Errors of Metabolism**:
- **Phenylketonuria** (PKU), **Albinism** (Melanin deficiency), and **Alkaptonuria** (excess HA).

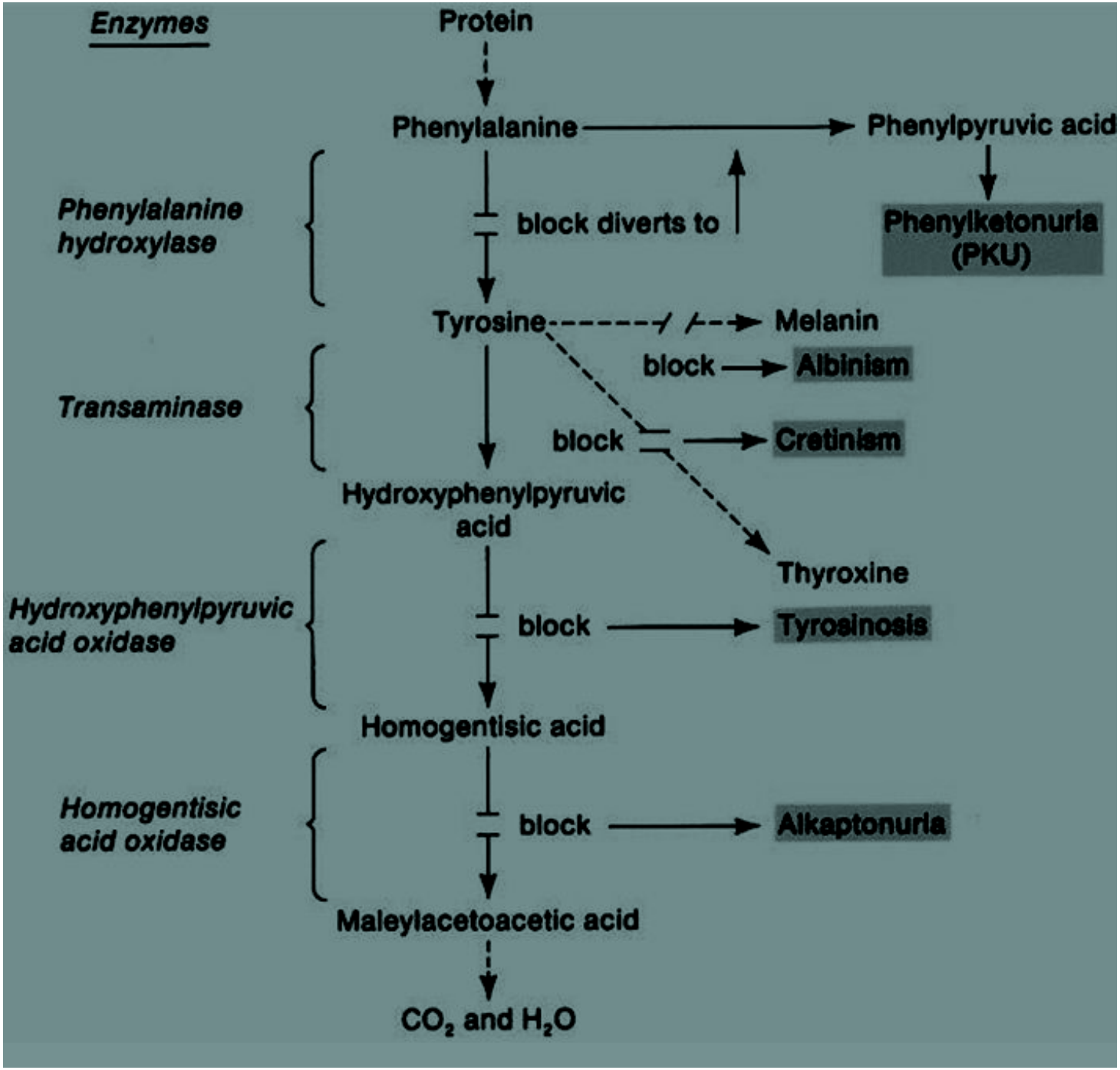
- Archibald Garrod in 1902 written a book named-- In born error of metabolism and proposed One gene one enzyme hypothesis.
- Reported disease-

1. PKU
2. Albinism
3. Alkaptonuria
4. Tyrosinemia

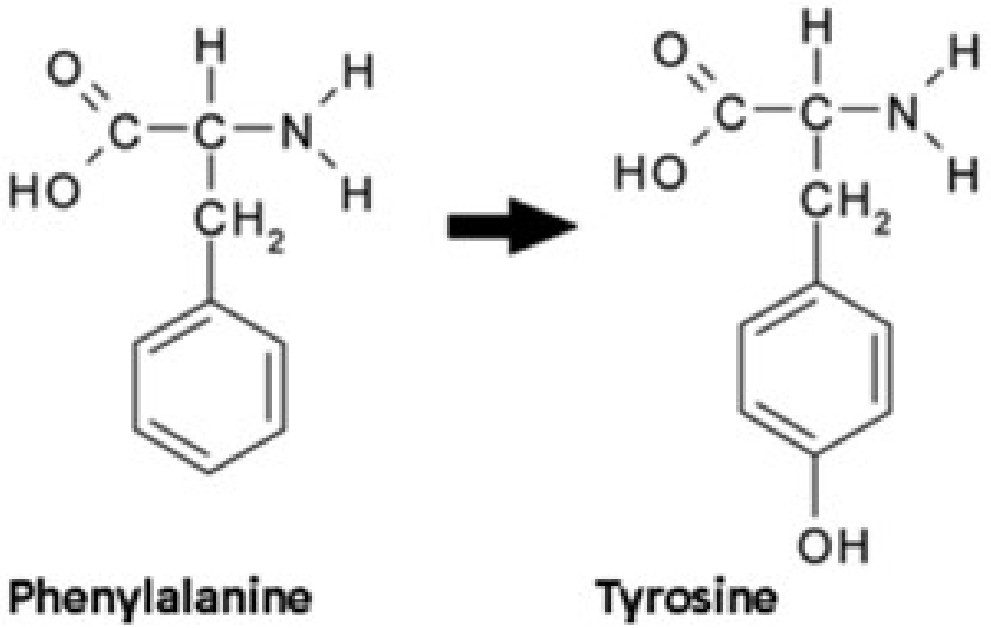
Simplified biochemistry of phenylalanine metabolism



PAH = phenylalanine hydroxylase
BH_4 = cofactor tetrahydrobiopterin

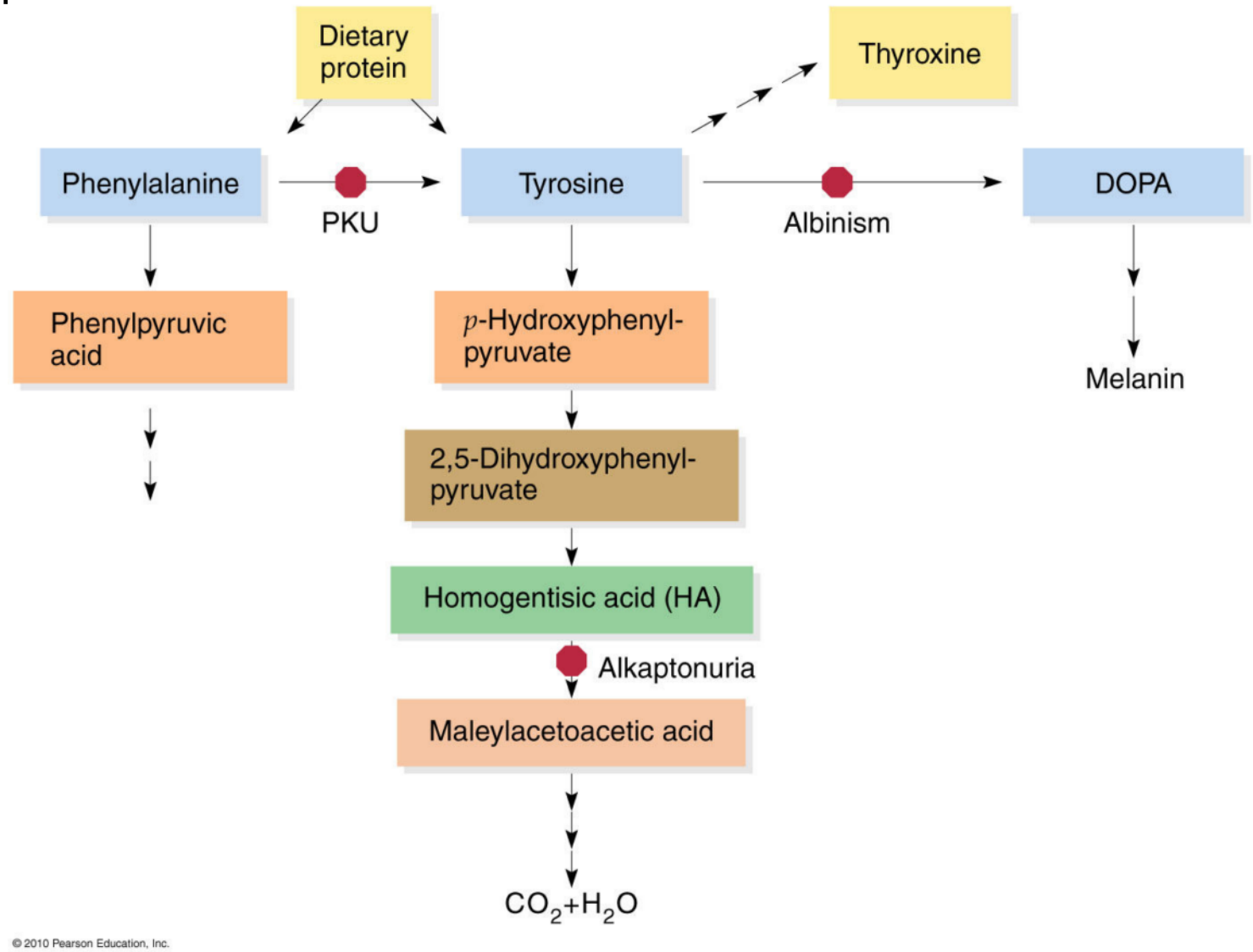

Enzymes
Protein
Phenylalanine
Phenylpyruvic acid
Phenylalanine hydroxylase
block diverts to
Phenylketonuria (PKU)
Tyrosine
Melanin
block
Albinism
Transaminase
block
Cretinism
Hydroxyphenylpyruvic acid
Thyroxine
Hydroxyphenylpyruvic acid oxidase
block
Tyrosinosis
Homogentisic acid
Homogentisic acid oxidase
block
Alkaptonuria
Maleylacetoacetic acid
CO_2 and H_2O

Enzyme phenylalanine hydroxyase breaks down excess phenylalanine in to tyrosine



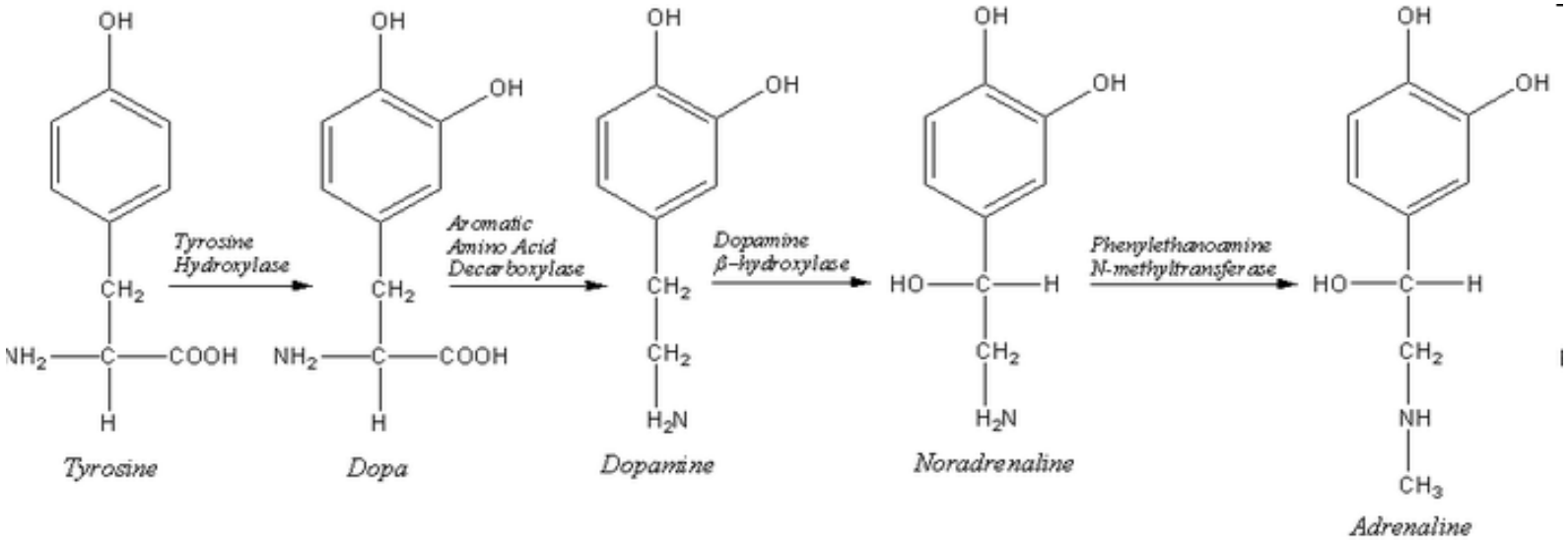
Phenylalanine Tyrosine

Phenylalanine metabolic



https://www.mun.ca/biology/scarr/iGen3_04-01.html

- Low levels of tyrosine also leads to lowered production of the pigment melanin, so children with this condition tend have fairer hair and greener eyes than other members of their family.
- The excess phenylalanine is converted instead into phenylketones, which are excreted in the urine - hence the name for this condition. The sweat and urine of an affected child has a musty odour due to these ketones.



Phenylketonuria (PKU)

- An enzyme related disorder
- Uria=something in urine
- Autosomal recessive disorder.
- The normal blood level of PHE is 30-120 µM (0.5 to 2.0 mg/dL), but it is >1200 µM (20 mg/dL) in phenylketonurics.
- Leads to excessive urinary excretion of phenylpyruvate and phenyllactate, and, if untreated leads to severe mental retardation.
- Newborn screening for PKU is mandatory in the United States. Prenatal diagnosis is now possible using DNA probes

Excess phenylalanine, a common amino acid accumulate.,

Phenylalanine accumulation, causing rashes, seizures, hyperactivity, and mental retardation, if untreated.

No cure.

Without this enzyme, phenylalanine (an essential amino acid) is converted to phenylpyruvic acid, which affects cells of the central nervous system causing mental retardation, slow growth, and early death

Symptoms

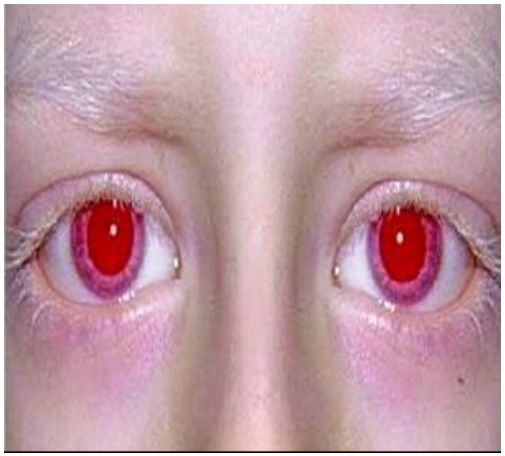
- Mental retardation
- Microcephaly
- Neurological problems –seizures
- Hyperactivity
- Skin rashes/eczema
- A Mousy Odor in the breath, skin or urine, caused by too much phenylalanine in the body
- Delayed development
- Behavioral, emotional and social problems

Fair skin and blue/red eyes, due to absence of melanin — the pigment responsible for hair and skin tone.

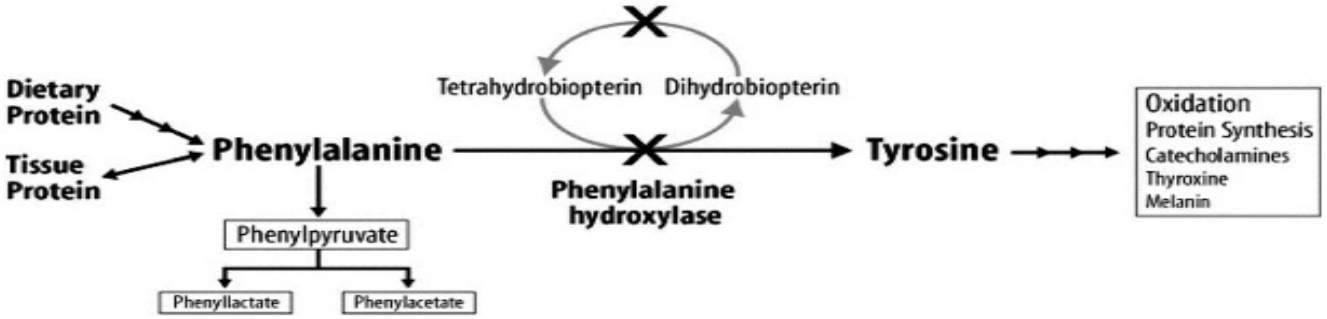
Vision defects and photo phobia

A person with PKU can live normally if phenylalanine is restricted from the diet

Disease is managed by avoiding foods high in proteins containing phenylalanine.



Phenylketonuria (PKU)



PKU is a relatively common metabolic disorder

- Most frequent disorder of amino acid metabolism
- The incidence of occurrence of PKU is about 1 in 15,000 births,
- The incidence varies widely in different human populations from 1 in 4,500 births among the Irish to fewer than one in 100,000 births among the population of Finland.
- Incidence of PKU in the USA
  - 1 per 13,500 to 1 per 19,000 newborns
  - Higher in Whites and Native Americans
  - Lower in Blacks, Hispanics, and Asians

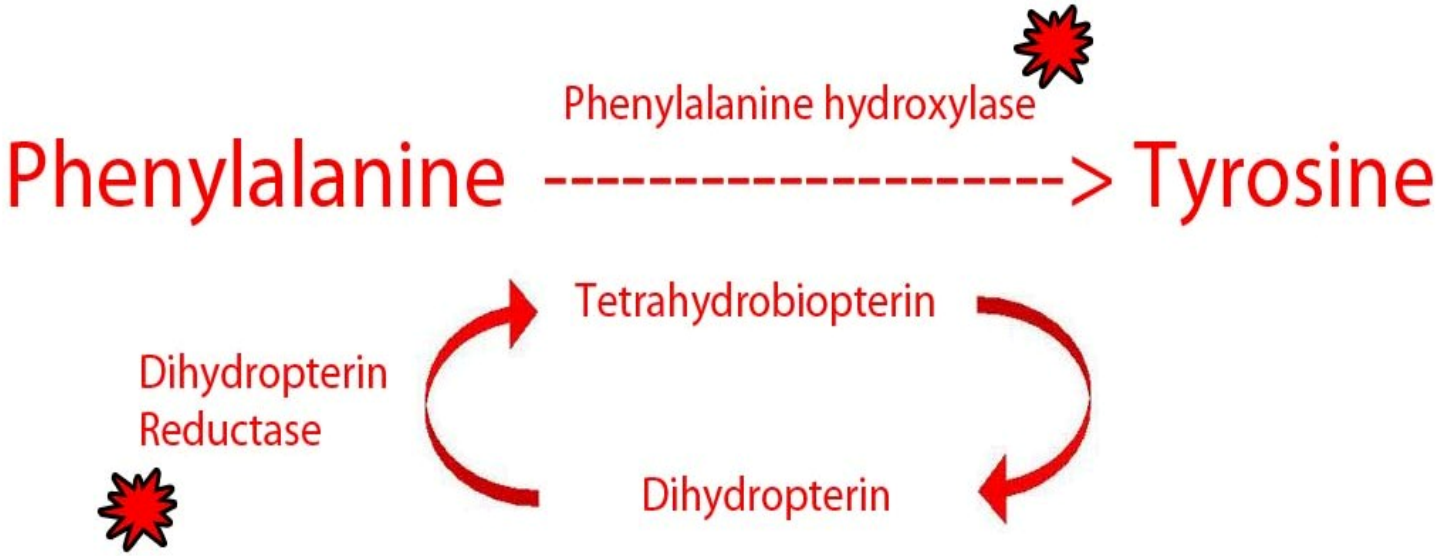
[1]NIH Consensus Development Panel. National Institutes of Health consensus development conference statement: Phenylketonuria: screening and management, October 16–18, 2000. *Pediatrics*. 2000;108:972–982.

Types of Phenylketonuria

- Classical PKU/Type I
  - “severe”
  - <1% residual enzyme activity
  - very high levels PHE-strict diet for life
- Type II/Atypical PKU
  - milder
  - tolerate more liberal protein diet
- Type III/Mild/Benign persistent Hyperphe
  - 5% residual activity
  - levels <600uM
  - no diet needed
- Type IV/Malignant PKU 2%

Two genes

- Phenylalanine hydroxylase (PAH gene)
- Dihydropterin reductase (DHPR gene)



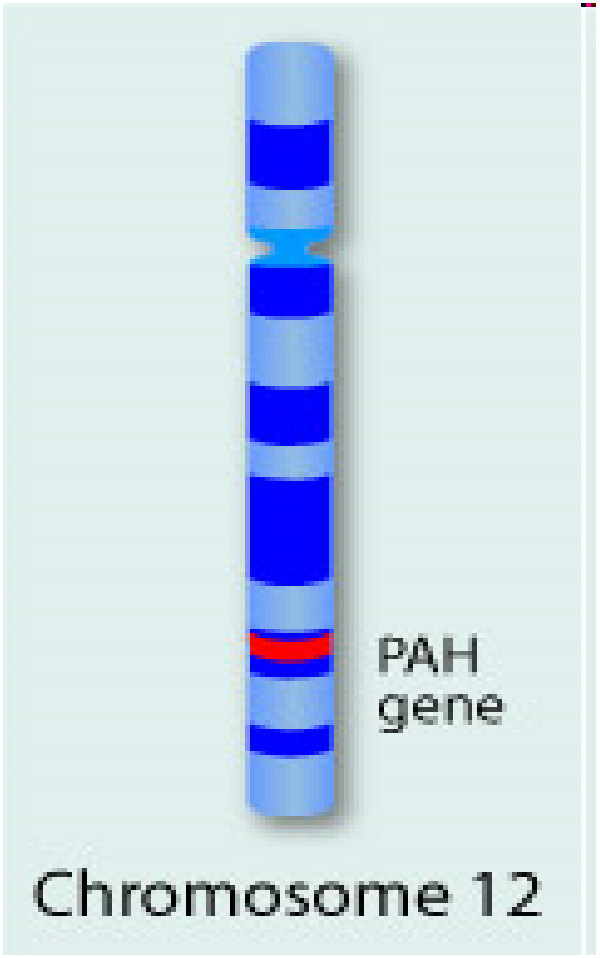
http://www.virtualmedstudent.com/links/metabolism/phenylketonuria.html

Phenylketonuria

PAH Gene-

- Chromosome 12q24.1
- Gene cloned 1983,
- Length: 79,278 bp's (13 exons)

- >450 mutations described-some common
- Little genotype- phenotype correlation -
  - combined/compound heterozygosity
  - mutations in modifying genes

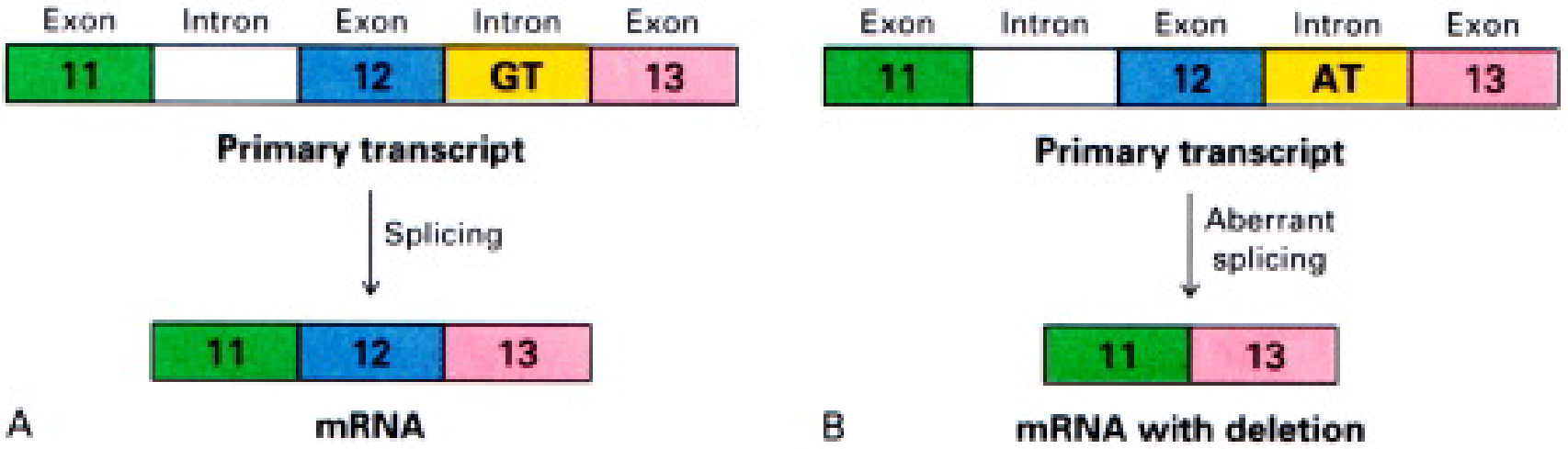


PAH Common mutations

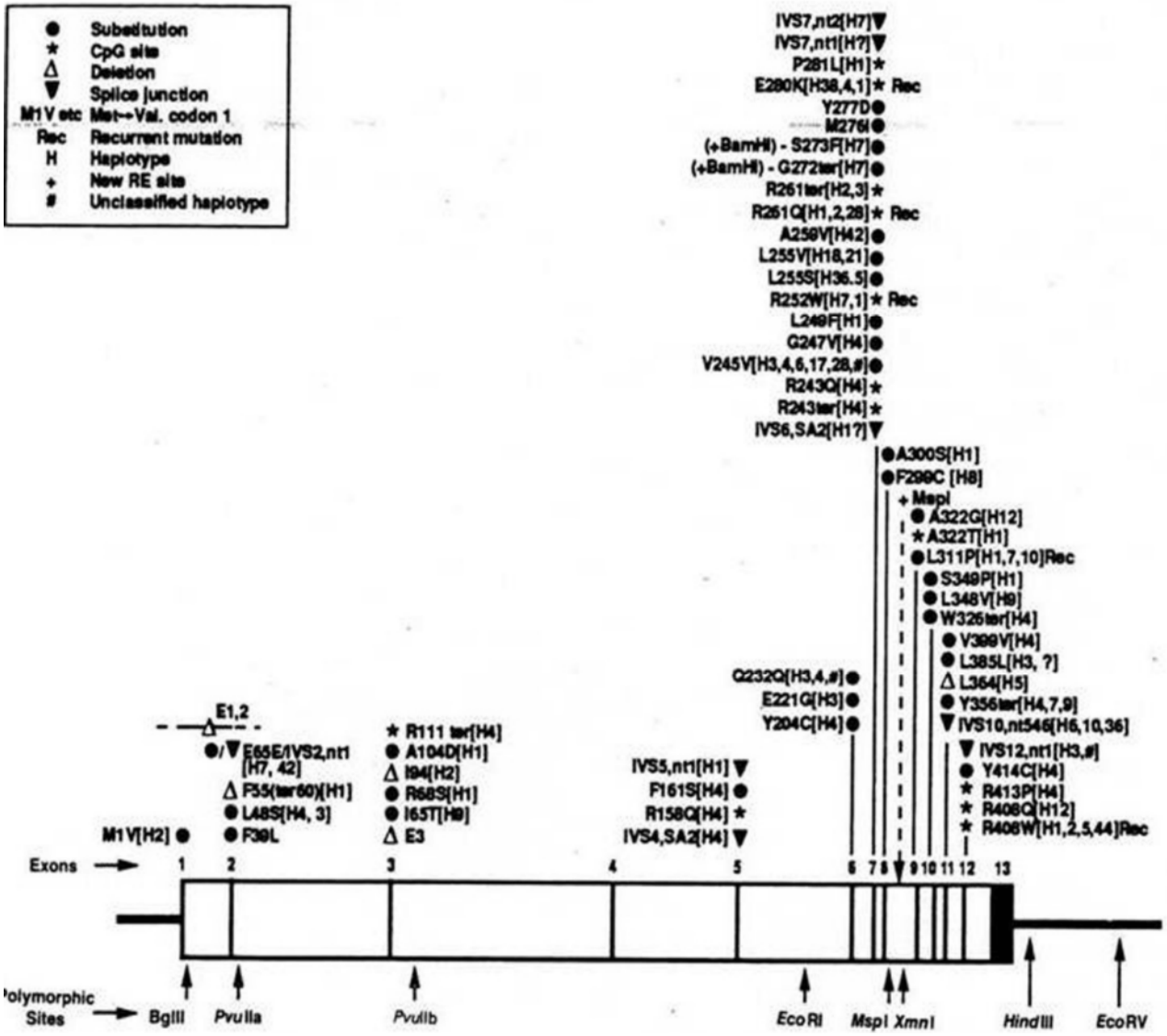
- Most common is located at position 408
- A substitution of an Arginine with a Tryptophan(Arg408Trp).
- transcription- promotor
- RNA splicing/cleavage
- Point mutation: nonsense, frameshift, missense- null
- Large mutations: frameshift deletion, insertions, duplications- all null

% of mutations	genetic mechanism
62	missense
13	deletion
11	splice
6	silent
5	nonsense
2	insertion
<1	deletion or duplication of exon or even gene

Phenylketonuria 2012 - Bioinformatikpedia



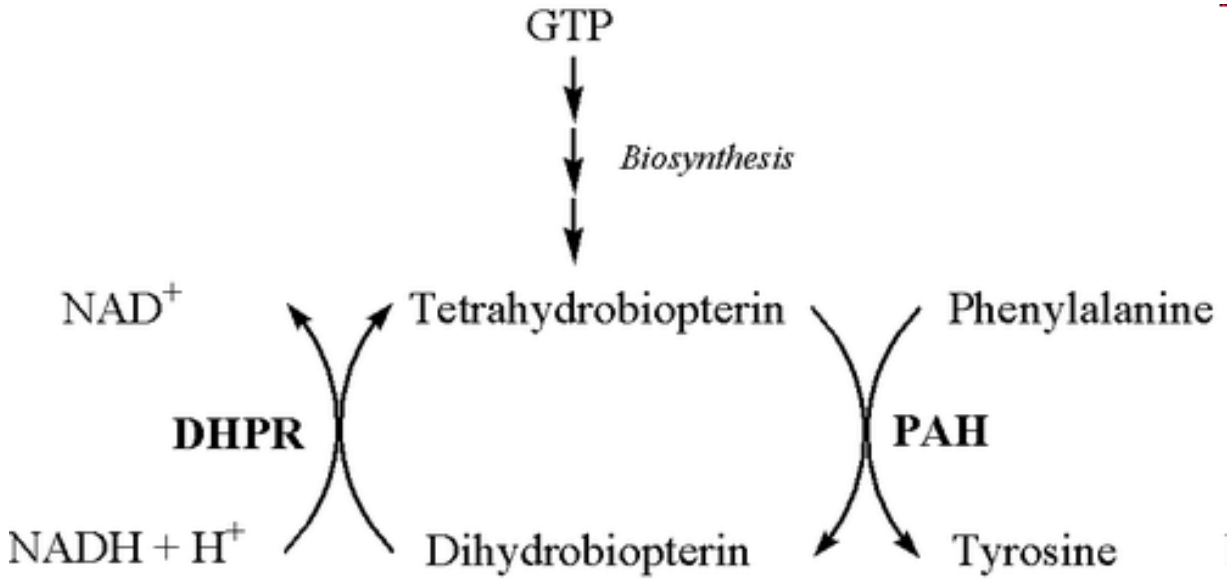
PKU can be caused by an intron mutation that leads to aberrant splicing. (A) Normal primary transcript and mRNA. (B) Mutation of G to A in intron 12 results in the skipping of exon 12. Most mutations causing PKU occur in coding regions.



(From Scriver CR, John SMW, Rozen R, Eisensmith R, Woo SLC, with permission.)

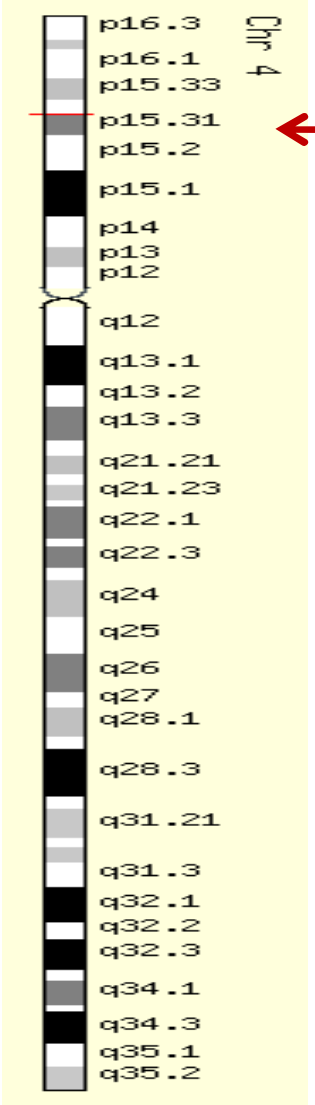
Rare form of PKU

- A rarer form of the disease occurs when PAH is normal but there is a defect in the biosynthesis or recycling of the cofactor tetrahydrobiopterin (BH4) by the patient.

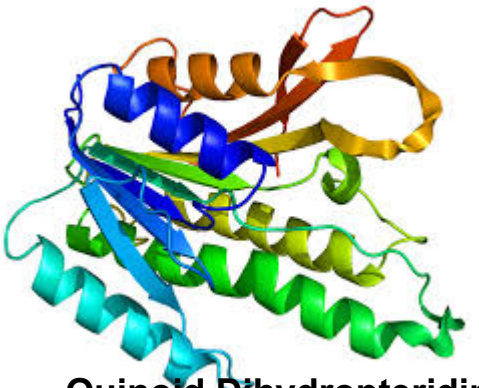


BH_4 - tetrahydrobiopterin (cofactor)
DHPR - dihydropteridine reductase (recycles BH_4)

qdpr protein



Qdpr gene



Quinoid Dihydropteridir Reductase(qdpr) /DHFR protein

The *QDPR* gene provides instructions for making an enzyme called quinoid dihydropteridine reductase.

This enzyme helps carry out one step in the chemical pathway that recycles a molecule called tetrahydrobiopterin (BH4).

Tetrahydrobiopterin plays a critical role in processing several protein building blocks (amino acids) in the body. For example, it works with the enzyme phenylalanine hydroxylase to convert an amino acid called phenylalanine into another amino acid, tyrosine. Tetrahydrobiopterinis also involved in reactions that produce chemicals called neurotransmitters, which transmit signals between nerve cells in the brain. Because it helps enzymes carry out chemical reactions, tetrahydrobiopterinis known as a cofactor.

When tetrahydrobiopterin interacts with enzymes during chemical reactions, the cofactor is altered and must be recycled to a usable form. Quinoid dihydropteridine reductase is one of two enzymes that help recycle tetrahydrobiopterinin the body.

PKU Neonatal screening

Guthrie test for PKU

Horst Bickel and Robert Guthrie

- Guthrie 1961 developed a technique for the diagnosis of phenylketonuria.
- Ontario Started Guthrie test in 1965
- Blood spot (filter paper) samples using the Guthrie bacterial inhibition assay
- Prevention maternal PKU syndrome

Blood spot (filter paper) samples using the Guthrie bacterial inhibition assay

Normal plasma Phe<0.24 mM.

Cost effective: 2.5-6.6 cost benefit ratio

Prevention maternal PKU syndrome

Normal plasma Phe<0.24 mM.

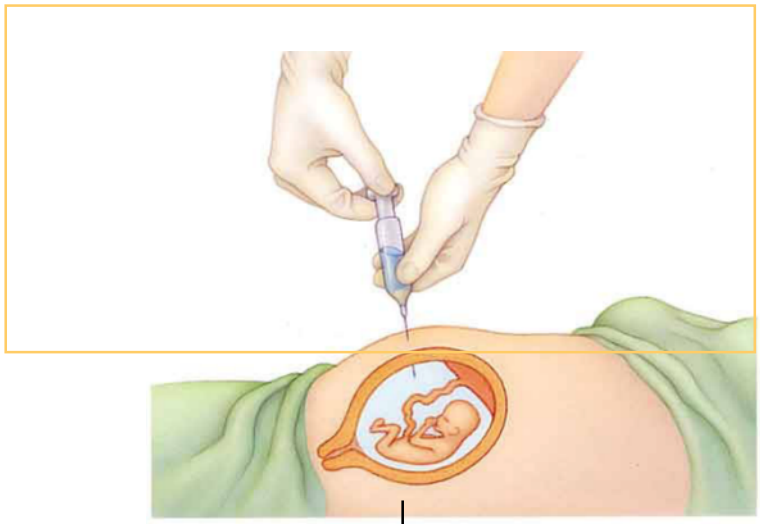
Cost effective: 2.5-6.6 cost benefit ratio

PKU Prognosis

- If the condition was not diagnosed early and a special diet not started, the individual will suffer severe and irreversible brain damage.
- If detected early, the individual will develop normally but will have to follow the special diet at least until adolescence, if not throughout their entire life.
- Women with PKU who wish to become pregnant must also eat the special diet, since children in the womb affected with PKU will not be able to metabolize the phenylalanine the mother ingests.

Test of PKU in fetus

Removal of about 20 ml of amniotic fluid containing suspended cells that were sloughed off from the fetus



A few biochemical analyses with some of the amniotic fluid

Centrifugation

Fetal cells

Quick determination of fetal sex and analysis of purified DNA

Growth for weeks in culture medium

Biochemical analysis for the presence of alleles that cause many different metabolic disorders

Karyotype analysis

PKU Treatment

- The only treatment available for PKU is a diet where phenylalanine levels are strictly limited.
- Artificial protein substitutes are given which contain amino acids without phenylalanine
- Meat, fish, eggs, cheese, milk products, legumes, and bread are all foods that have high levels of phenylalanine should be avoided.

  Phenylalanine restriction in the patient's diet.
- **Aspartame** (artificial sweetener) must be avoided in PKU patients because it contains phenylalanine.

BH4 supplements

- BH4 (tetrahydrobiopterin) is a substance made by the body. It works to help the PAH enzyme change Phe into Tyr.
- Some children with PKU will benefit from taking BH4 supplements in pill form. This treatment is helpful in reducing blood Phe levels in some children with PKU. About 10% of children with classic PKU respond to BH4 pills. Most children with mild PKU are helped by BH4 pills.
- Drug therapy - PAL
- Liver transplant
- Gene therapy- delivery of normal copy of PAH in the hepatocytes of the probands

PKU Diet: low protein & low phenylalanine

- Lofenalac special infant formula, low in phenylalanine
- Phenylalanine-free nutrient mixes for adults.
- Little to no milk, cheese, eggs, meat, fish, beans, nuts, or other high protein foods.
- Fruits and vegetables are the most safe foods.
- Certain “diet” or “light” foods must be avoided completely.

Suggested Reading

1. Human Molecular Genetics – Tom Stratchen & Andrew P. Read. Pub: John Wiley & Sons.
2. An introduction to Genetic Analysis – Griffith, Miller, Suzuki, Lewontin, Gelbard. Pub: W.H. Freeman & Co.
3. Genomes 2 – T.A. Brown, Pub: Wiley-Liss. John W. & Sons.
4. Emery's Elements of Medical Genetics – R.F. Mueller, I.D. Young, Pub: Churchill
5. An Introduction to Human Molecular Genetics – J.J. Pasternak, Pub: Fitzgerald Science