Severe Combined Immunodoficiency (SCID)

- The most serious human immunodeficiency disorder.
- 1:50.000 newborns
- Caused by mutations of about **50 genes**
- It is a group of congenital disorders in which both the **humoral and cell-mediated immunity fail to work properly**.
- Children with SCID suffer from recurrent severe infections, retarded growth, and early death.

Most common monogenic SCID

1) X-SCID: Mutation of interleukin receptor γC (*chromosome X*) (SCID X-linked). The most common form (1/58.000)

2) ADA-SCID: Linked to *chromosome 2*0 (mutation of ADA); 25% of all cases. **Autosomal recessive**. 1/100.000 newborns

X-linked SCID (bubble disease)

"bubble boy" disease, named after David Vetter, a Texan born in 1971 who lived out his 12 years in a plastic, germ-free bubble.

More severe than ADA-SCID,
as X-SCIDs have no T-, NK cells

David received bone marrow
from his sister; **she was EBV positive**
David died in 1983 (BL)

Photo: Courtesy of Duke Medical Center News Office

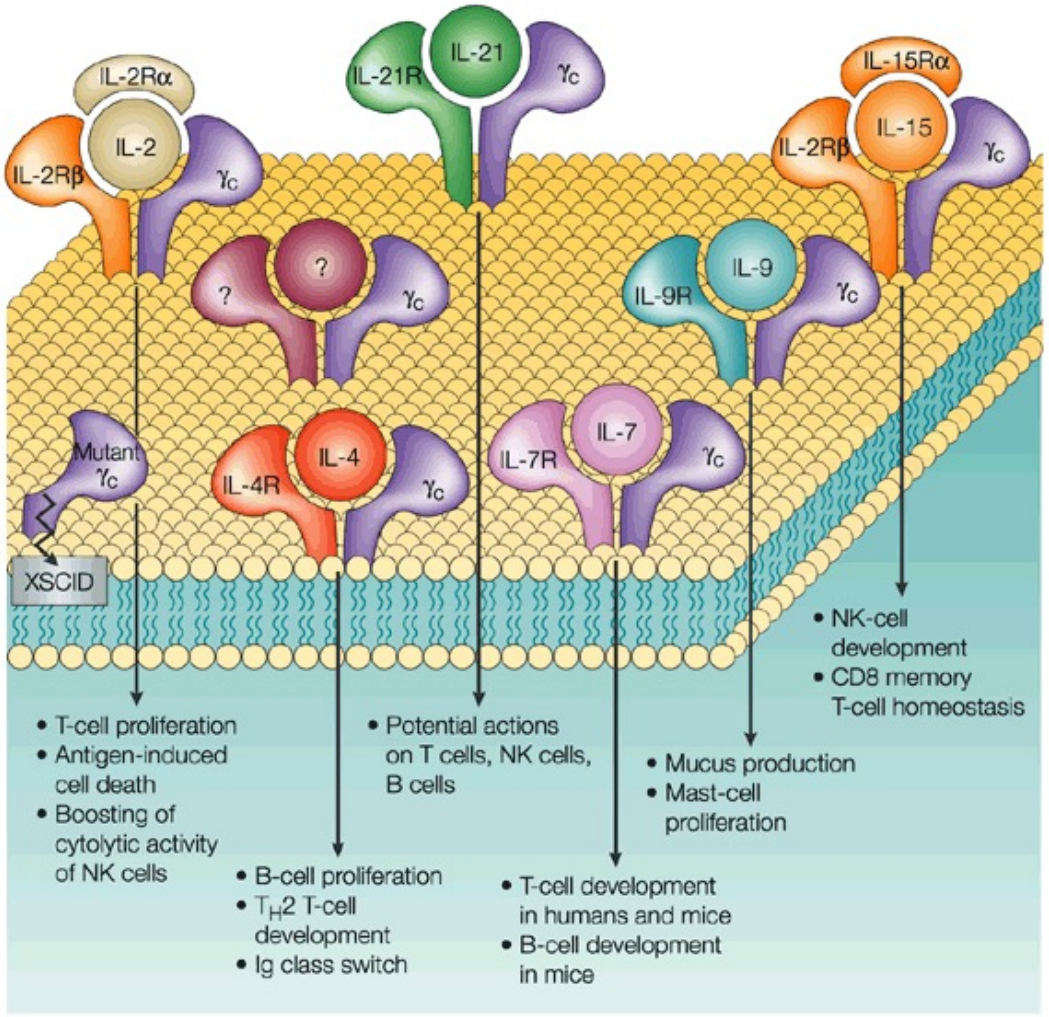
Genetics of X-SCID

- Mutations of ***IL2RG*** **gene encoding the gamma subunit (γ_c)**, common to interleukin receptor 2, 4, 7, 9, 15, 21.

- Cromosome Xq13. 8 exons and 7 introns, the mRNA is 3.6 Kb, the protein 369 aminoacids.

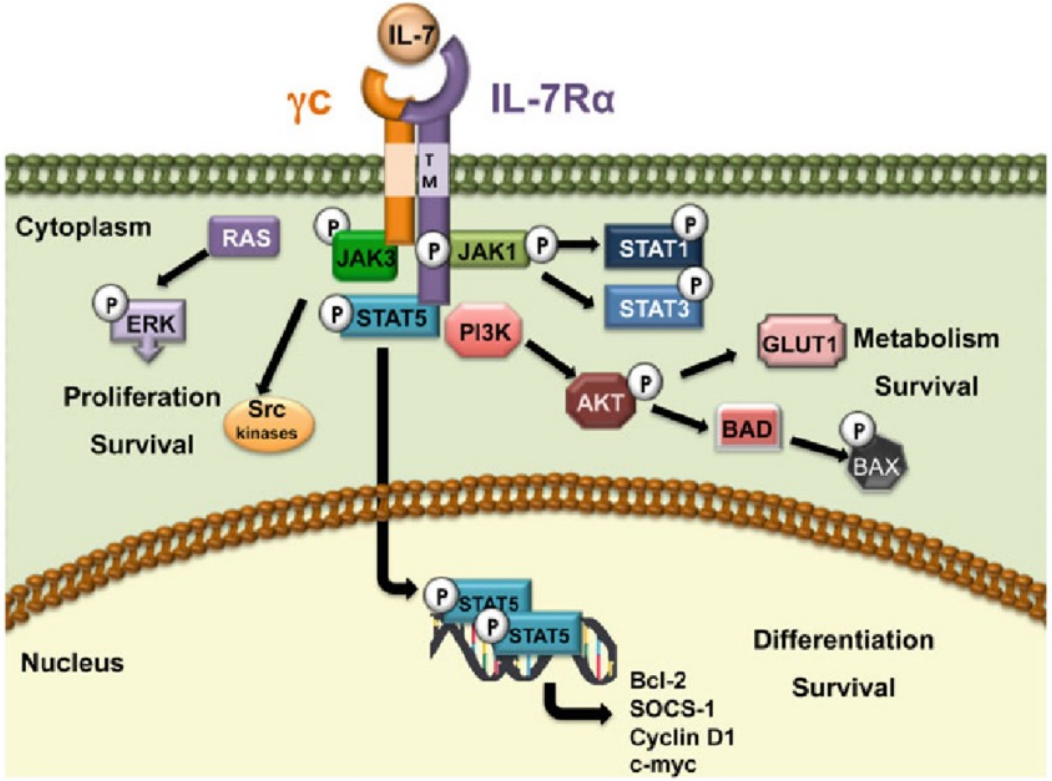
- The activation of these receptors promotes the **proliferation and differentiation of T and NK cells.**

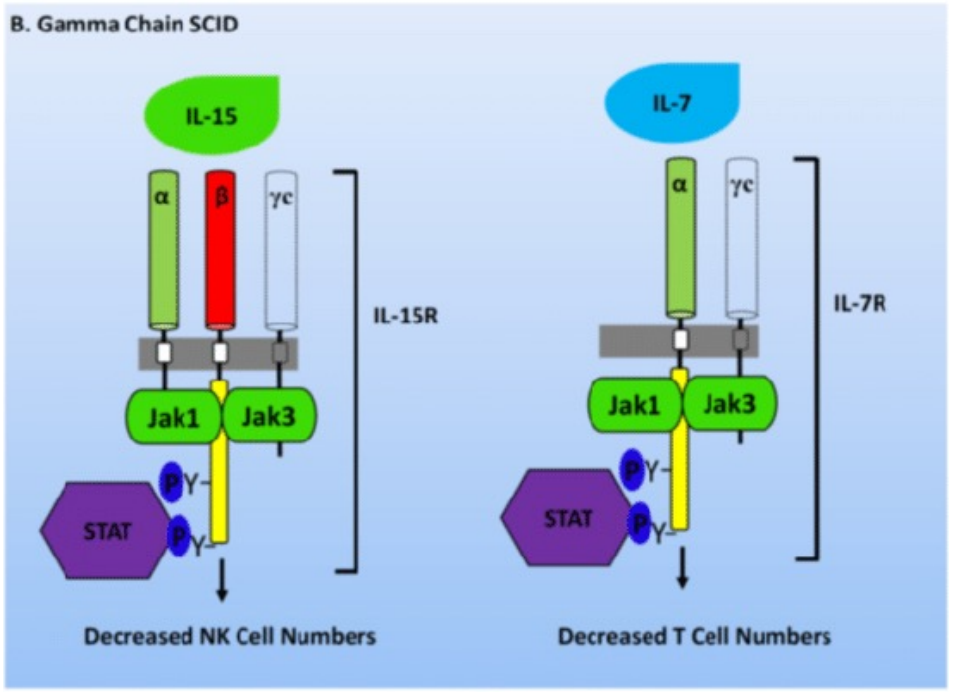
- **Deletions or point mutations:** γ_c chain unable to interact with the subunits of the other receptors.



Nature Reviews | Immunology

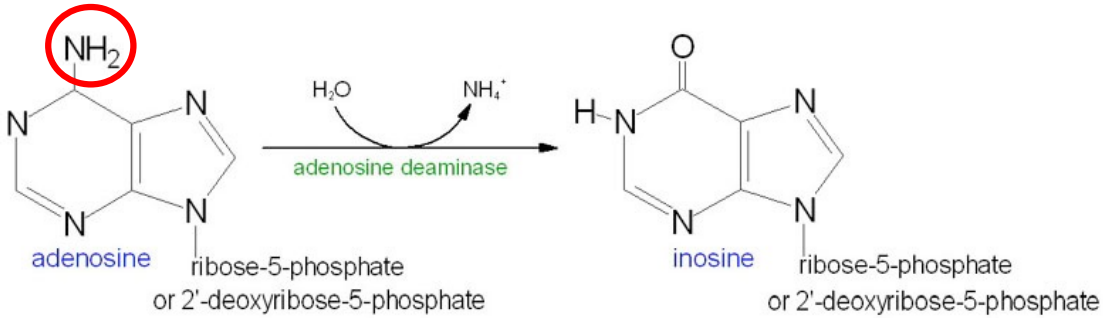
Interleukin signaling





ADA SCID

Adenosine deaminase is a glycoprotein
and acts as a hydrolase,
catalyzing the deamination of adenosine into inosine + ammonia.



Nitrogen base,
One of the four
nucleotides
of the nucleic acids

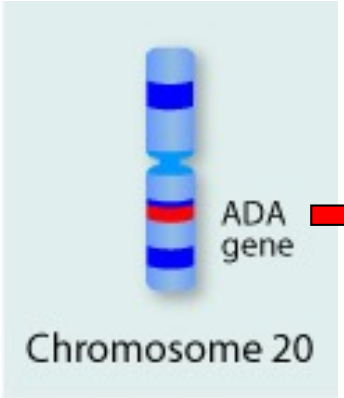
$Adenosine + H_2O = Inosine + NH_4^+$

Adenosine is toxic for B- and T-cells

ADA is essential for the proper growth and function of infection-fighting T and B lymphocytes.

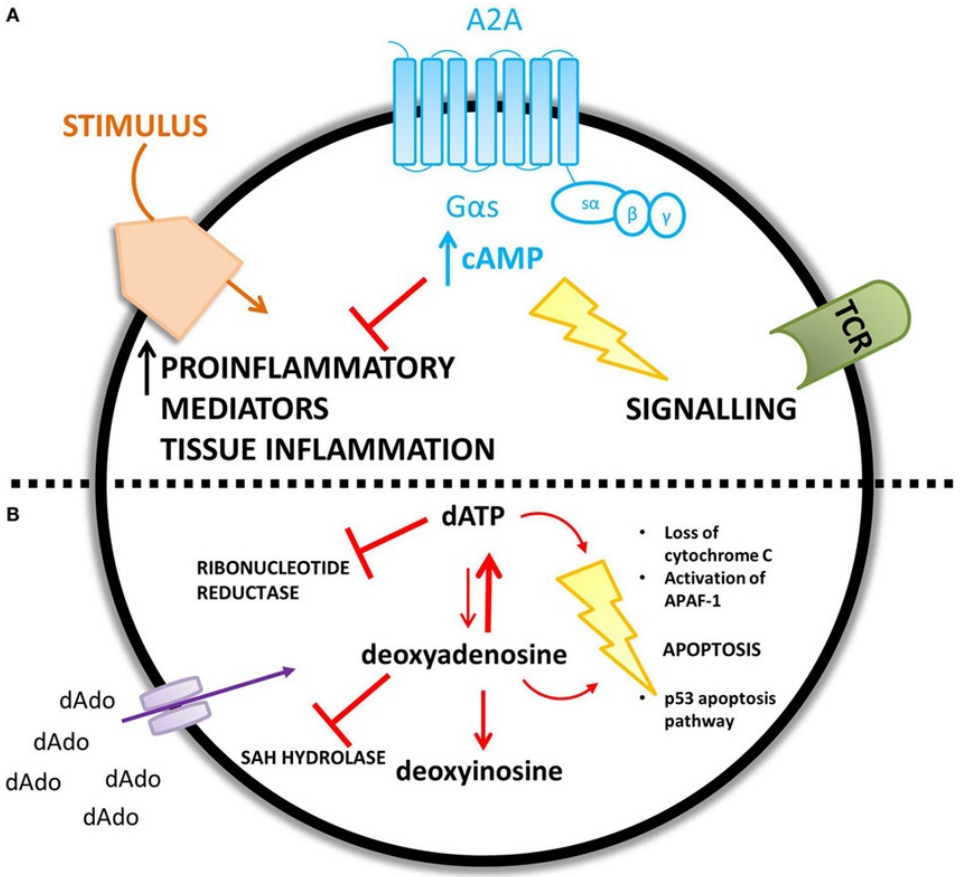
ADA SCID

14 newborns / year in Europe
Very severe: death within the first year of life



-Point mutations in the CDS
-Promoter Deletions

Lymphotoxicity of adenosine



- ADA deficiency leads to an accumulation of adenosine (A) and deoxyadenosine (B) – different mechanisms are proposed for the increased concentration of each metabolic substrate. (A) An increase in extracellular adenosine concentration leads to **an increase in intracellular cyclic AMP (cAMP)** caused by increased A2A receptor activation. cAMP is proposed to mediate lymphotoxic effects by disrupting TCR signaling and inhibiting the immune response to a stimulus. (B) Extracellular **accumulation of 2'deoxyadenosine** increases the intracellular concentration of 2'deoxyadenosine via diffusion down its concentration gradient. 2'deoxyadenosine inhibits SAH hydrolase and plays a role in apoptosis, by activating the p53 pathway. Alternatively, 2'deoxyadenosine can undergo conversion to dATP. dATP inhibits ribonucleotide reductase and also plays a role in apoptosis.

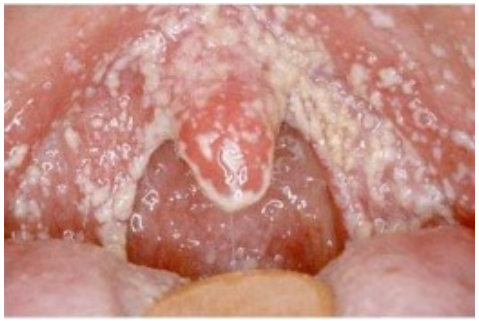
Symptoms of SCID

- Precocious recurrent **opportunistic infections** (viruses, bacteria, fungi, and parasites) within the first three months of life

- Severe **infections of upper and lower airways and GI tract (severe diarrhea), sepsis, high fever**

- **Moniliasis:** common in SCID**.** Fungal infection by *Candida Albicans* affecting skin, mouth, respiratory tract, blood. Difficulties to swallow and lesions of the oral cavity.
- **Pneumonia**: often caused by *pneumocystis jirovecii*

- **Death occurs within the first year of life**.



Diagnosis

- **-Blood count: Lymphopenia**
  - **X.SCID**: T and NK negative; B positive
  - **ADA SCID**: B, T, NK negative
- -Lack of antibody response to vaccination
- -Low circulating levels of immunoglobulins
- -Genetic tests

Cell type	Normal lymphocyte count average (range)	SCID count average (range)
T-cells	3,680 (2,500–5,500)	200 (0-800)
B-cells	730 (300–2,000)	1,300* (44 - >3,000)
NK cells	420 (170–1,100)	<100 (X.SCID)
Total	0–3 months: 5,400 (3,400–7,300)	<2,000

* Non functional

THERAPY

A. **Ordinary treatment** develops on two main fronts:

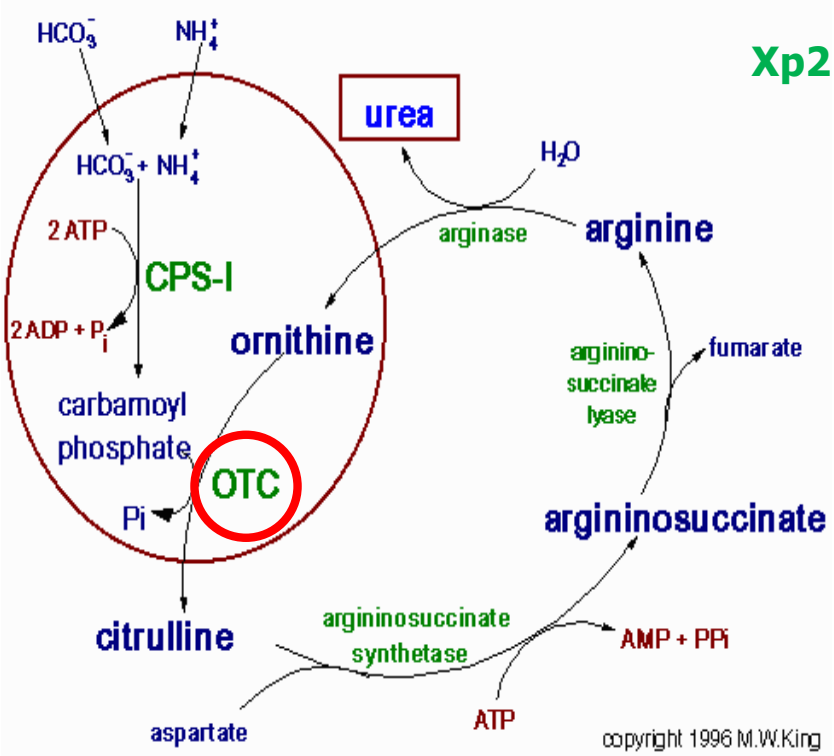
1) prevention of serious infections using prophylaxis measures that include the infusion of **immunoglobulins**, antimicrobial prophylaxis (**antibiotics, antifungals and antivirals**). The infusion of immunoglobulins intravenously or subcutaneously every 2-3 weeks represents a fundamental aid against infections. Vaccinations with live attenuated microbes (measles, chicken pox, rotavirus, Bacillus-Calmette Guérin) are absolutely contraindicated while other vaccinations, although not contraindicated, are often not effective

2) Early and intensive treatment of intercurrent infectious episodes.

B. The **resolutive treatment** of the disease involves **hematopoietic stem cell transplantation from a compatible family member (possible in 20% of cases)** or, more recently, **gene therapy.**

Ornithine Transcarbamylase (OTC) Deficiency
(X-linked disorder 1: 80.000 newborns)

OTC is a key **urea cycle** enzyme (break down and removal of ammonia from the body)



Xp21.1

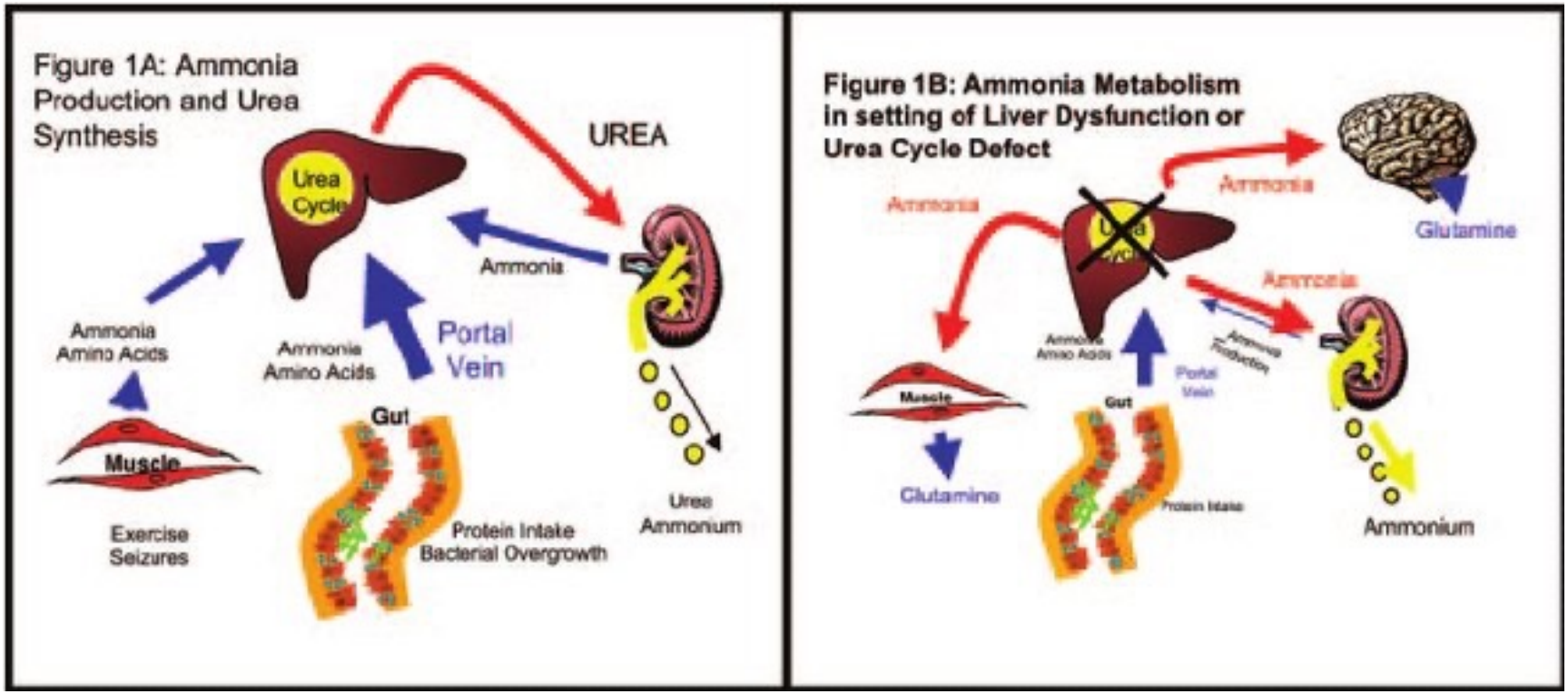
OTC deficiency

hyperammonemia in the blood

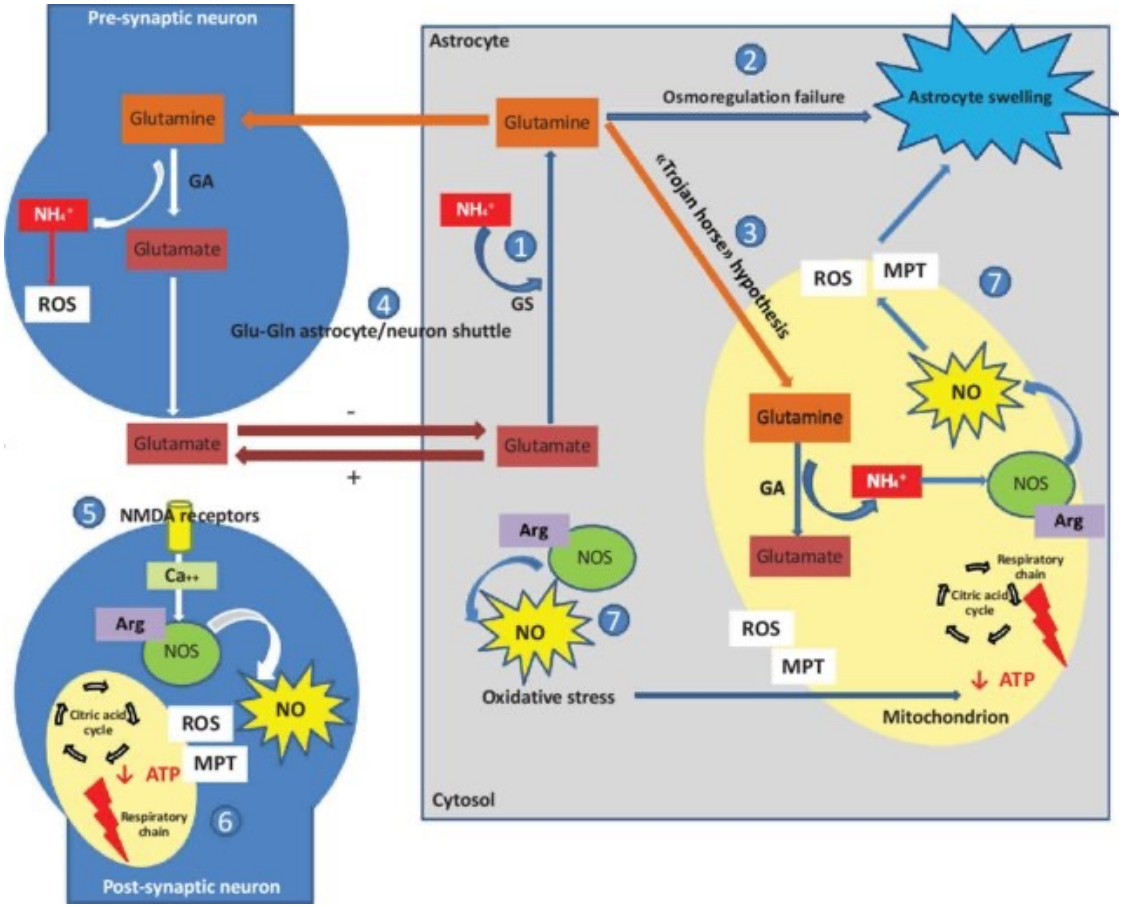
Ammonia is neurotoxic

Neurological abnormalities

AMMONIA METABOLISM



Neurotoxicity of ammonia



MPT: mitochondrial permeability transition

A) Since the brain lacks the urea cycle, detoxification from ammonia relies only on the synthesis of glutamine by the astrocytic enzyme glutamine synthase GS (1). High intracellular concentration of glutamine can lead to **osmotic swelling of astrocytes** (2).

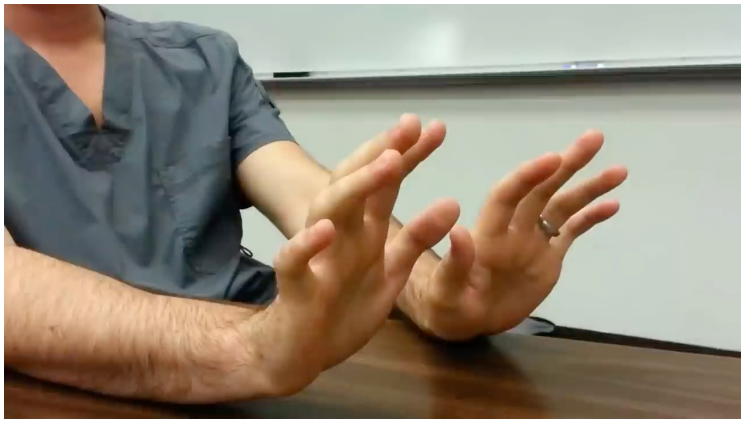
B) The transport of glutamine into the mitochondria and its cleavage by glutaminase (GA) into glutamate and NH4+ (the **'Trojan horse' hypothesis**) seem to play the most important role (3).

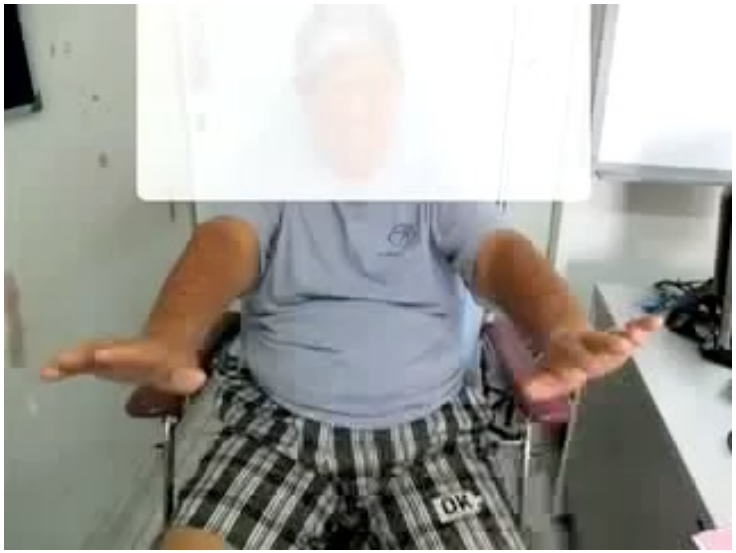
C) Exposure to ammonia can also induce **alterations in the astrocyte-neuron Glu-Gln shuttle** (4) and, consequently, in extracellular glutamate levels (5). The increase in **glutamate** levels leads to hyperstimulation of neurons essentially through the **activation of N-methyl-D-aspartate (NMDA) receptors** (5). This leads to an increase in intracellular calcium, which initiates several calcium-dependent processes, including the formation of NO.

Excessive NO formation is the main cause of mitochondrial dysfunction, **accumulation of free radicals** and impaired energy metabolism in both neurons (6) and astrocytes (7).

Clinical manifestations of OTC

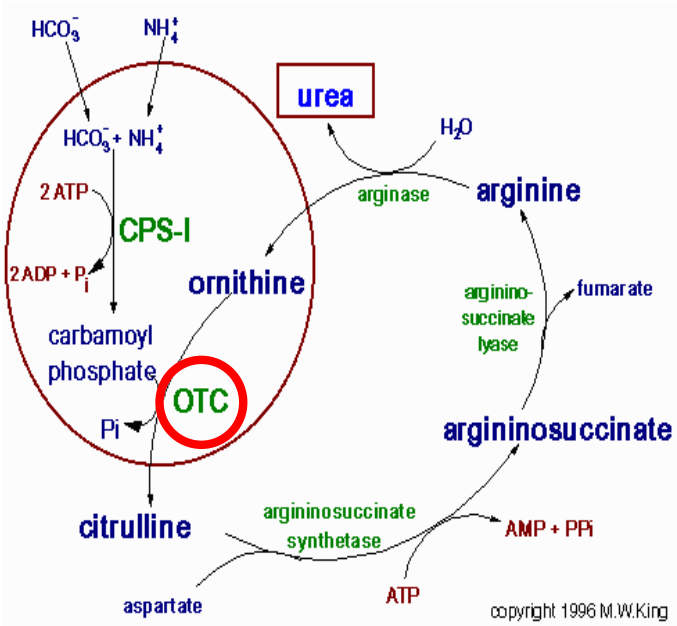
- Hyperammonaemia and increased circulating glutamine
- Refusal to eat
- Tachypnea / apnea
- Lethargy and coma (hyperammonaemic coma, hepatic encephalopathy)
- Convulsions
- Encephalitis
- Flapping tremor (asterixis)
- A large percentage of **deaths** are caused by **white matter edema and demyelination**
- There are also **attenuated forms** with late onset



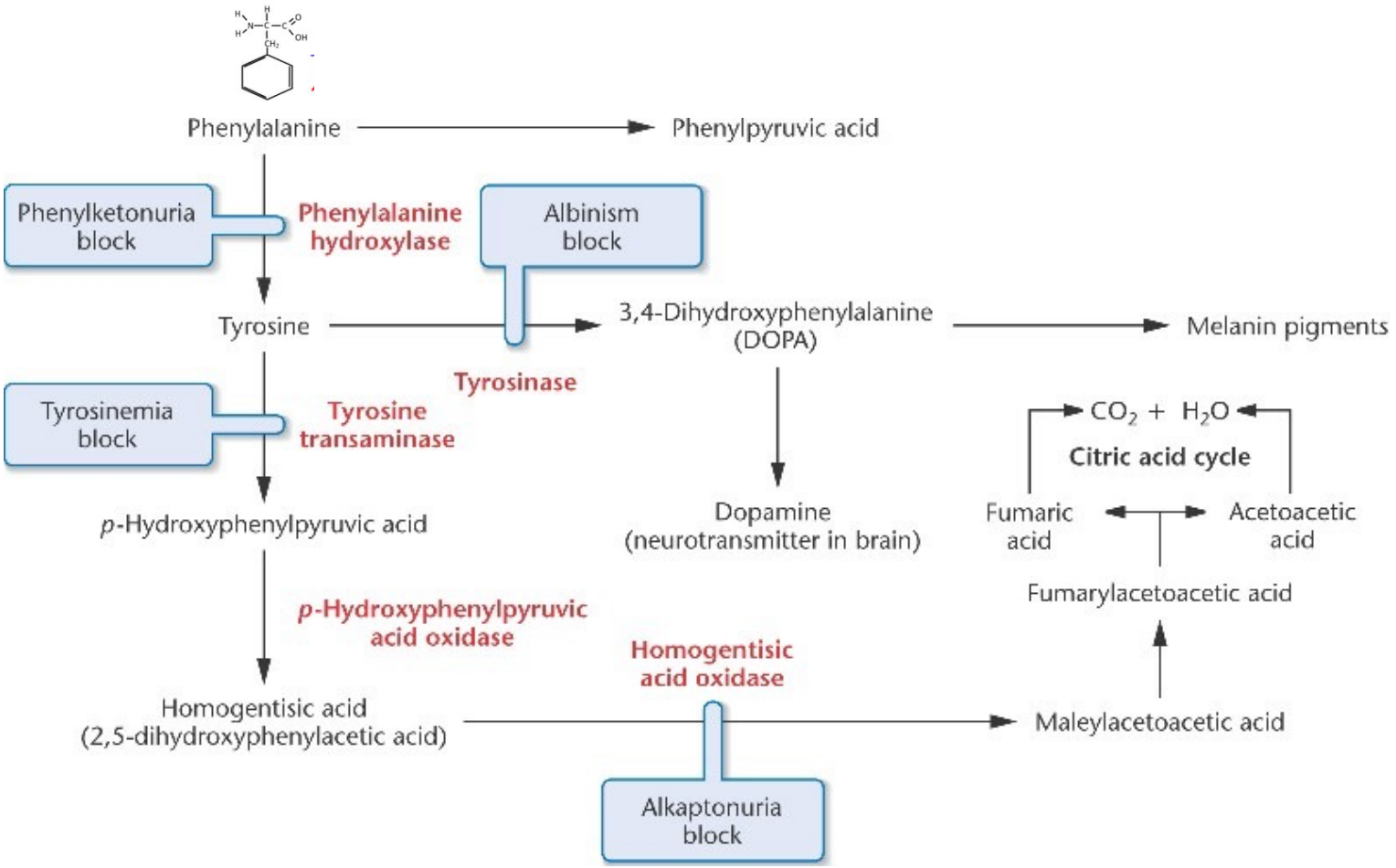


THERAPY

- Strict **low-protein diet** or adapted to tolerance
- Supplementation of **L-citrulline** (substrate for the synthesis of arginine)
- **Hemodialysis** in case of hyperammonemia
- **Glucose** by mouth or intravenously during infections (increased ammonia production)
- Liver transplantation
- **Gene therapy: AAV-OTC**

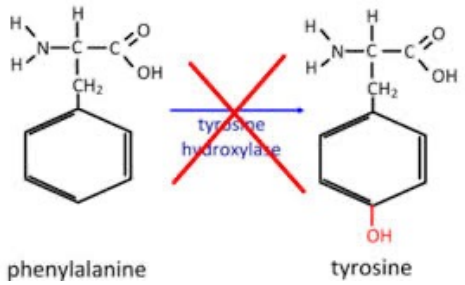


Aromatic AA metabolism



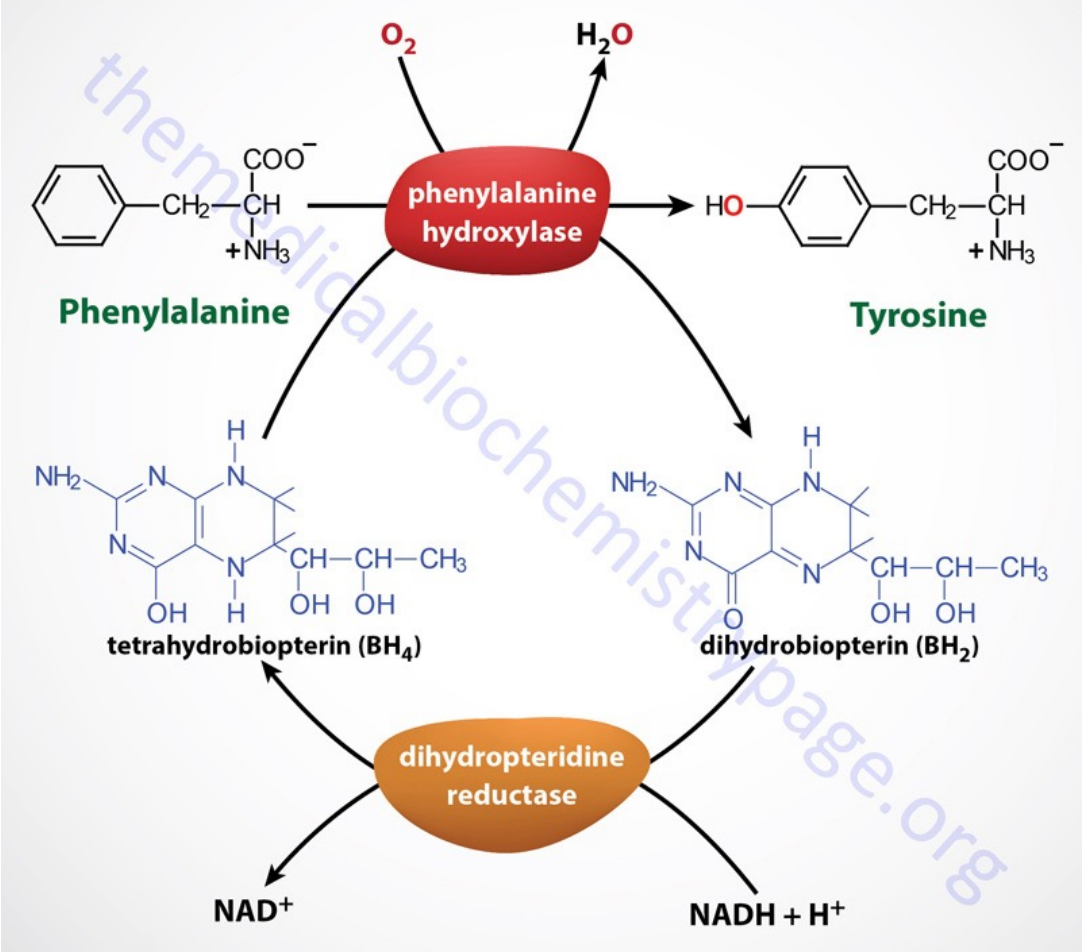
PHENYLKETONURIA (PKU)

- The most common inherited disease of aminoacid metabolism
- **Autosomal recessive**
- **1:10.000 newborns** in Caucasian or oriental population.
- Mutation of **phenylalanine hydroxylase (PAH)** gene on chromosome 12 (400 mutations identified)
- Results in **mental retardation and other neurological problems** when treatment is not started within the first few weeks of life.
- The incidence in Africans is far less.
- Carriers: 1:50 in Caucasians



ENZYMATIC ACTIVITY

- **Phenylalanine hydroxylase (PAH)**, is completely or nearly completely deficient.
- This enzyme is highly expressed in the liver and converts phenylalanine to tyrosine
- PAH uses O_2 and **tetrahydrobiopterin** (BH4) as hydrogen donor.

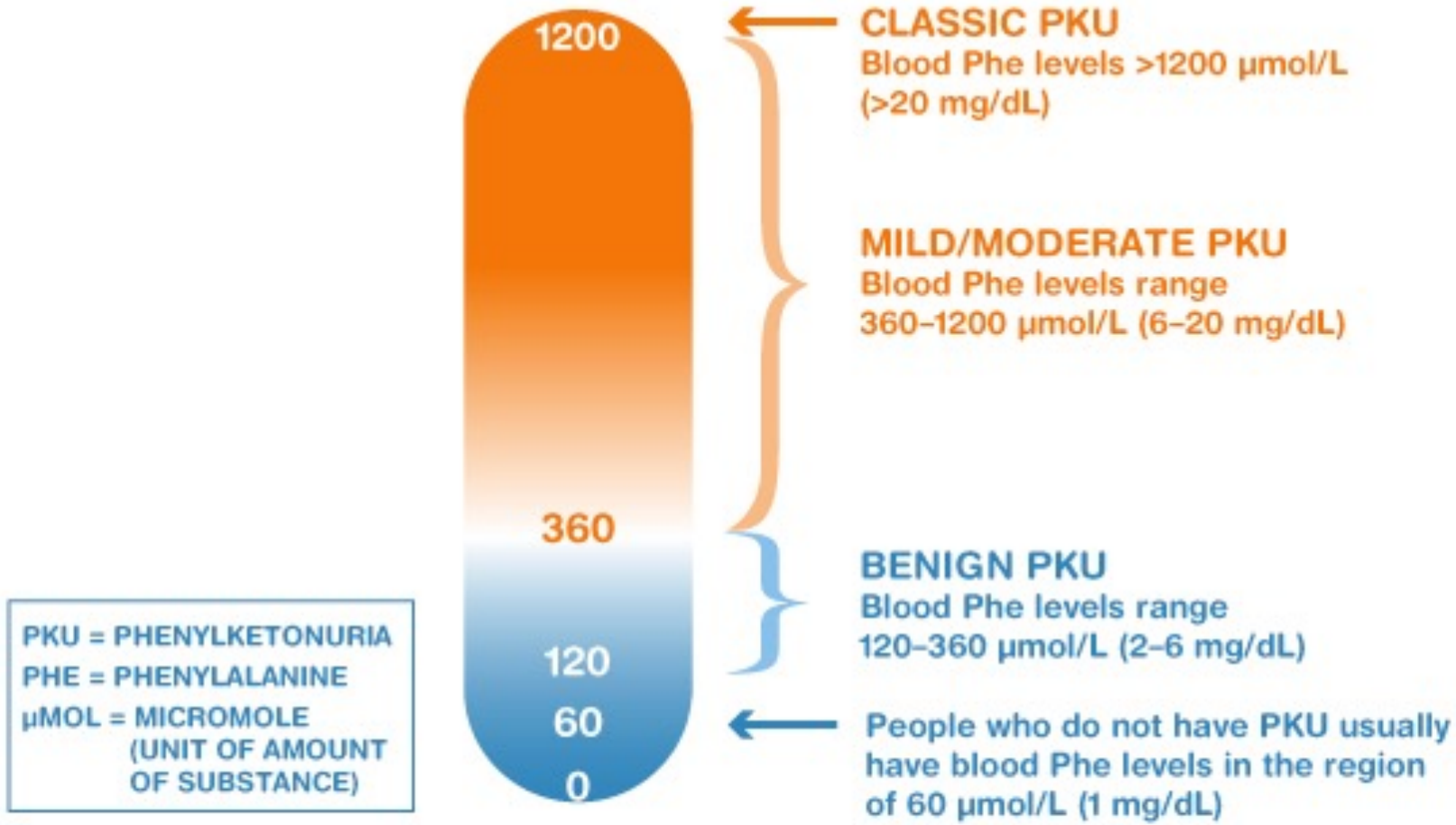


LEVELS OF BLOOD PHENYLALANINE

- A **normal** blood phenylalanine level is about **1mg/dl**.

- In cases of **PKU**, levels may range from **6-80mg/dl**, but are usually greater than 30mg/dl.

Classification of PKU

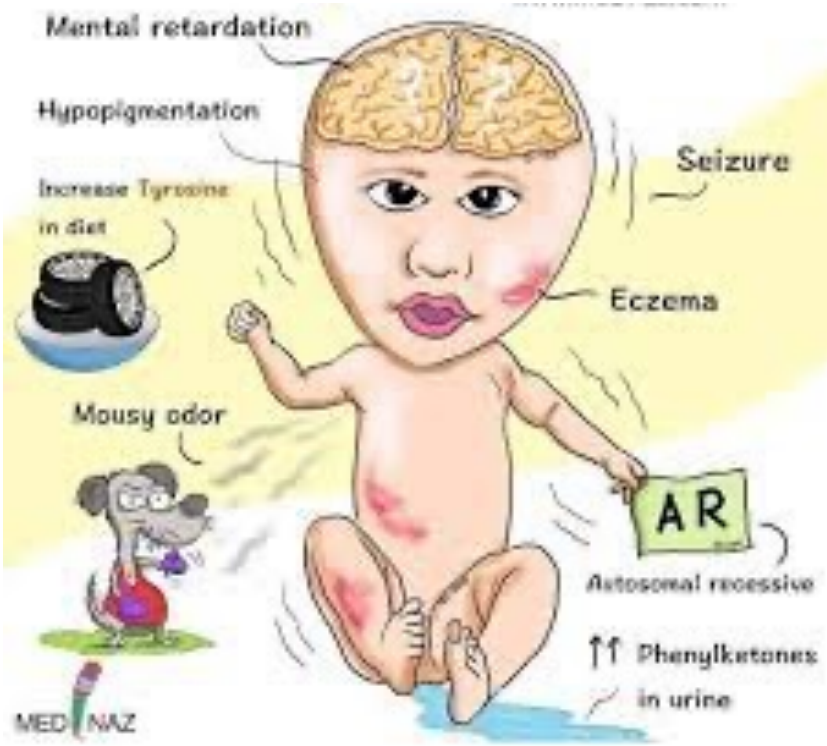


SYMPTOMS OF PKU

in untreated infants

- Normal at birth, then in the first months:

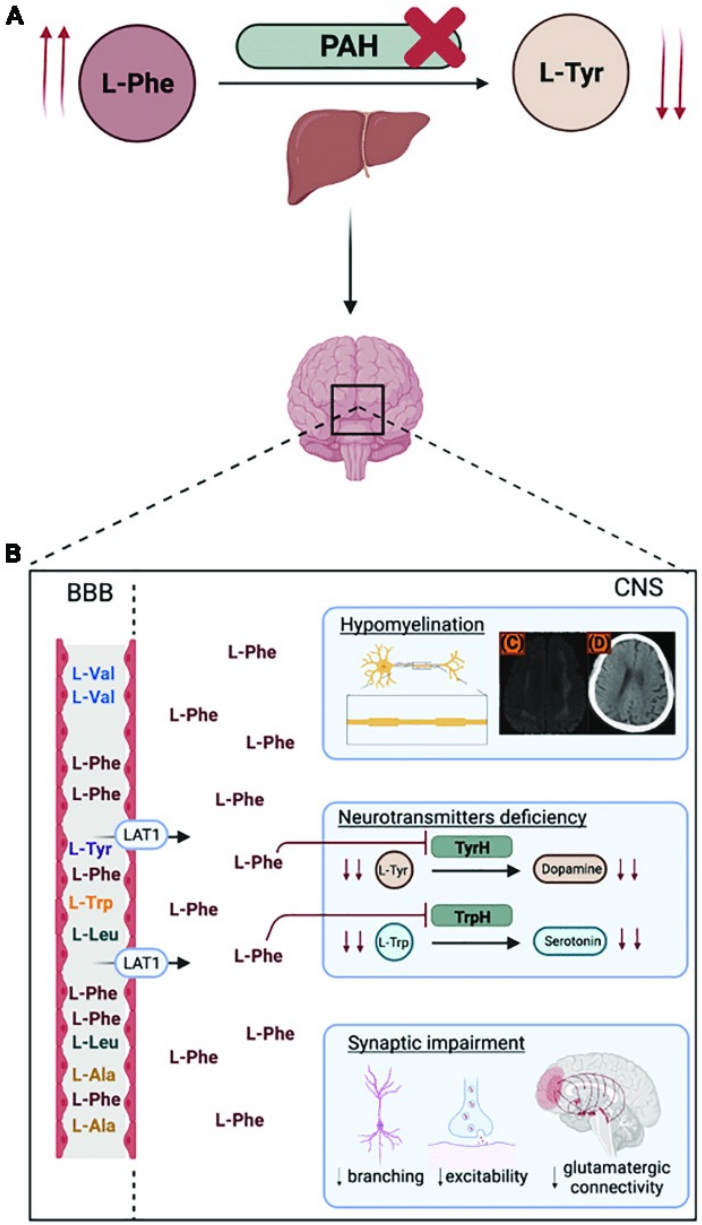
- **Mental retardation:** learning disabilities, behavioral difficulties. Epilepsy, tremors, mood disorders
- **Vomiting**
- **Irritability**
- **Eczema-like rash**
- Increased muscle tone, more active tendon reflexes
- Microcephaly
- **Decreased body growth**
- Clear skin, blue eyes, blond hair (low melanin)
- **Unusual odor to urine (like mouse), sweat, breath**

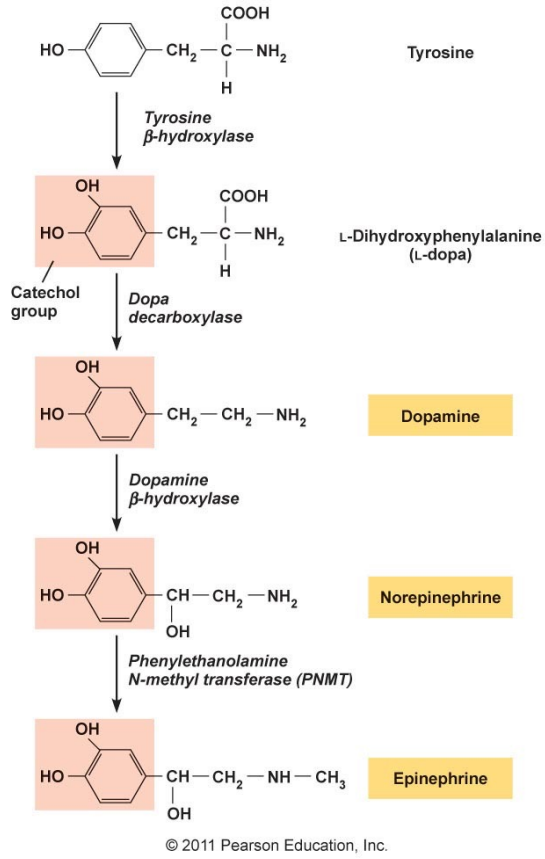


Mechanism of Brain Toxicity

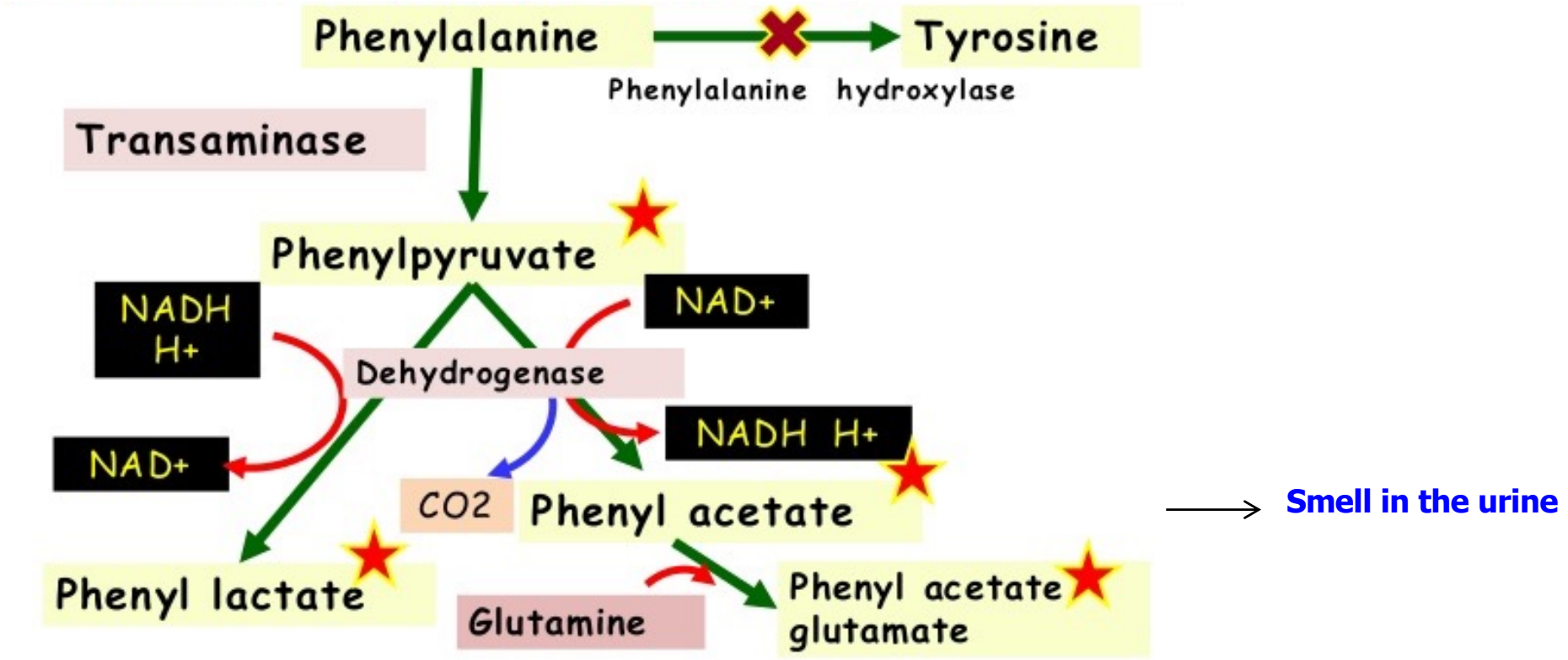
1. Elevated levels of **phenylalanine compete with aminoacid uptake** and block the transport of tyrosine and other aa in the brain. This causes **reduction of protein and myelin synthesis** in the brain

2. The reduction of tyrosine causes a **decrease of dopamine and catecholamines**.



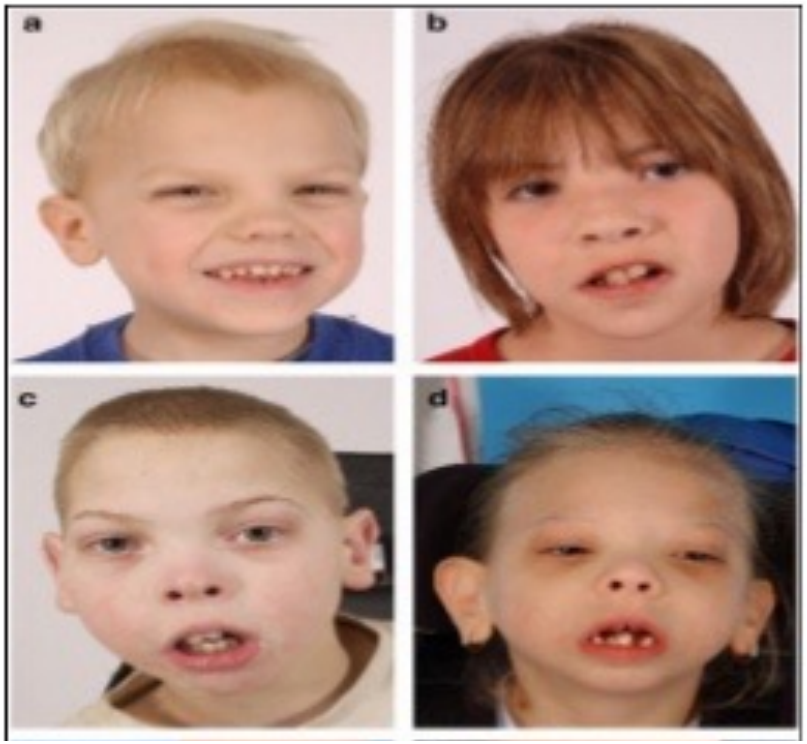


Accumulation of phenyl keto acids

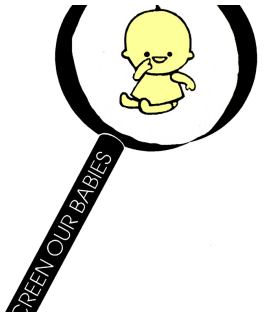


OTHER SYMPTOMS OF PKU

- **Prominent cheek and jaw bones**
- widely **spaced teeth**
- Poor development of tooth enamel.



PKU SCREENINGS

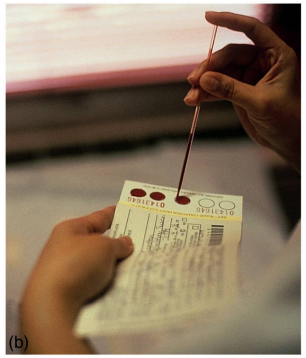


- Screening of the blood phenylalanine level is performed **to all newborns** at about 3 days of age.

- Usually, a few drops of blood are obtained by a **small prick on the heel**, placed on a **card** and then sent for measurement.

- If the screening test is abnormal, other tests are needed to confirm or exclude PKU.

- Newborn screening allows early identification and early implementation of treatment



- Methods:
  - Mass spectrometry or
  - Guthrie Test

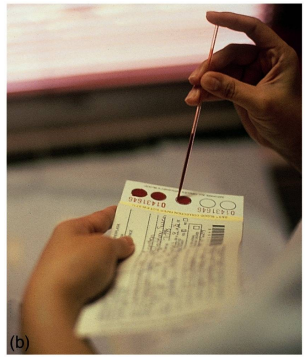
Guthrie Test

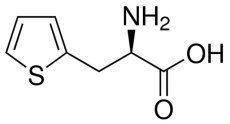
Has been used over the last 30 years. Currently is being replaced by MS

A small drop of blood is taken from the heel of a newborn and applied to a card, in a disc.

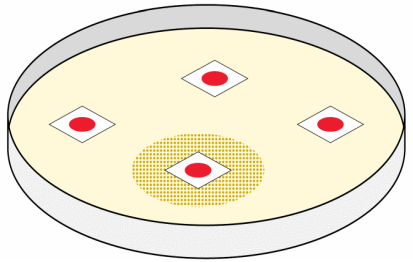
The dried disc is incubated on a petri dish plated with bacteria (*Bacillus subtilis*) in the presence of a growth inhibitor, **B-2-thienyl-alanine**.

High levels of Phe in the blood sample overcome the inhibition and allow the bacteria to grow.

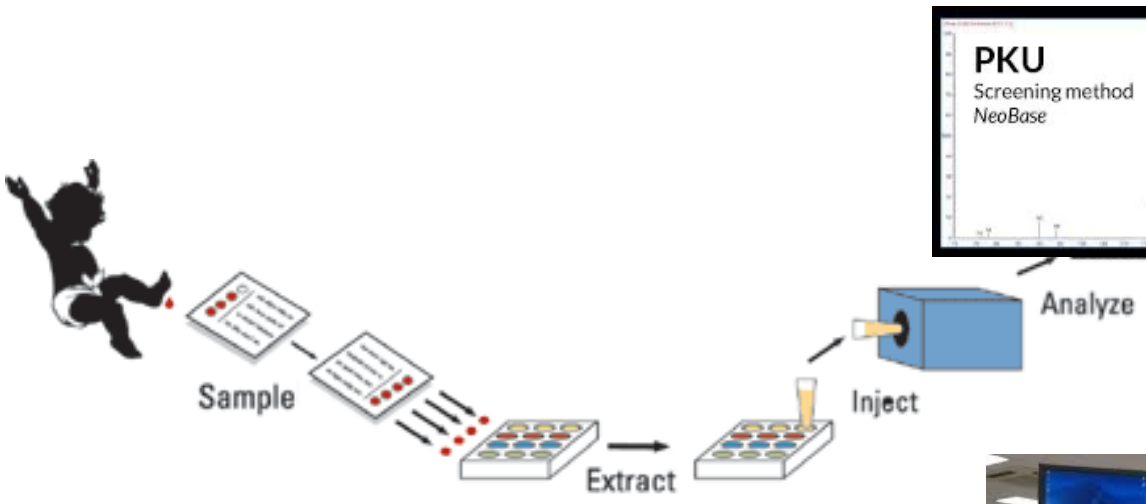


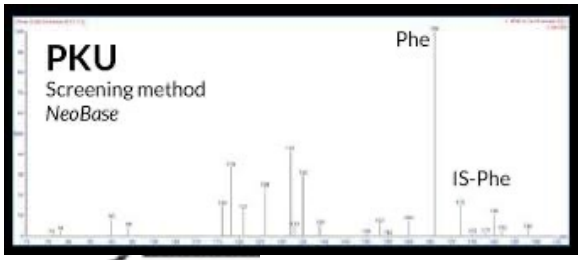


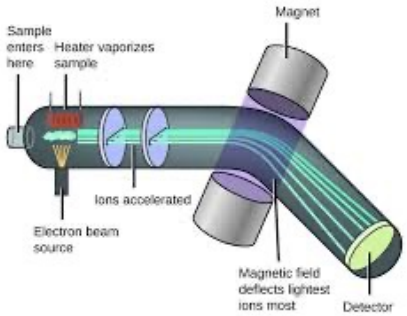
Place patients' blood samples on inoculated agar—bacteria will grow around samples containing excess phenylalanine



Mass spectrometry







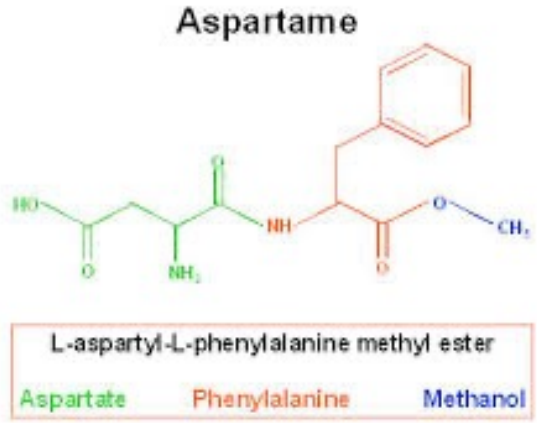
Treatment of PKU

- **DIET:** The goal of PKU treatment is to maintain the blood levels of pheylalanine between 2 and 10mg/dl.

- Treatment for PKU consists of a **diet low in phenylalanine**,
  - **Infants**: with special formulas
  - **Adults**:
    - Eliminate meat
    - Use low protein grain products. Measured amounts of cereals, starches, fruit, and vegetables, along with a milk substitute are recommended instead.

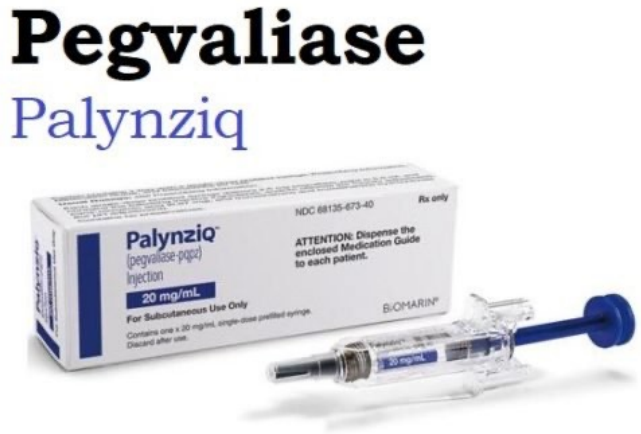
Aspartame

- Individuals with PKU should stay away from food sweetened with **aspartame (aspartic acid and phenyalanine)**



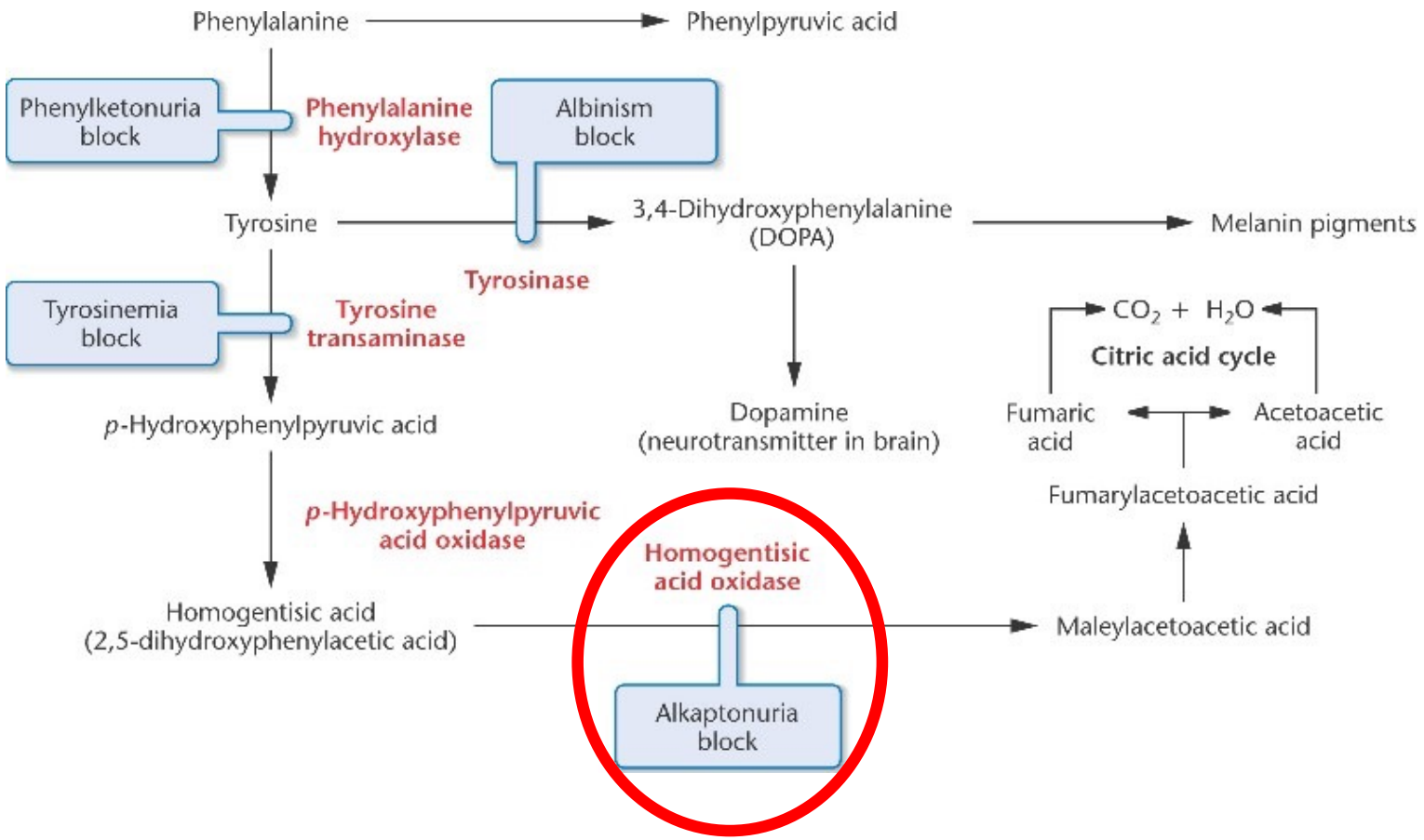
- **Many light foods and drinks contain aspartame**

Palynziq (Pegvaliase)



- New drug approved by FDA and EMA in 2018
- Bacterial enzyme: **phenyalanine ammonia lyase** (brakes down Phe and reduces its blood levels)
- **Conjugated to PEG** to increase its half life
- Given to patients 16 Y/O by SC injections (doses) for the entire life
- Patients who are not treatable with diet (the gold standard)

Alkaptonuria



Alkaptonuria

- Autosomal recessive, mutations of ***Homogentisic acid oxyidase*** (*HGD*) gene

- Rare: 1:250.000 people; more common in Slovakia and Dominican Republic (1:19.000 people)

- **Accumulation of oxidized homogentisic acid (HGA) in the cartilages, connective tissues (*ochronosis*)**

Alkaptonuria

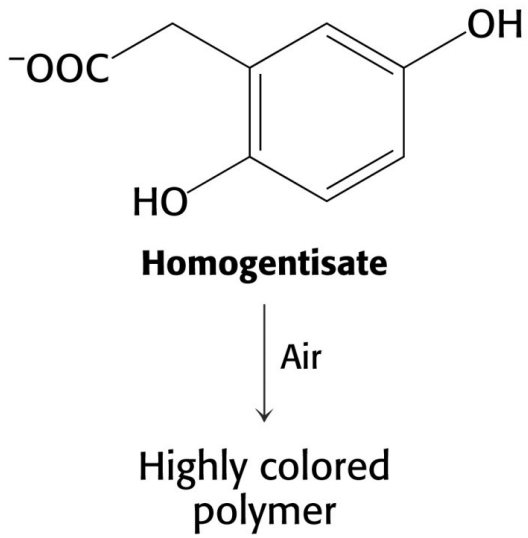
- homogentisic acid (HGA) is accumulated and excreted in the urine and turns a black color upon exposure to air

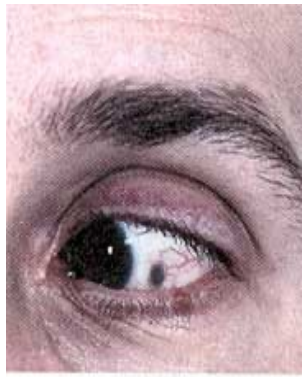
➢ **In children:**

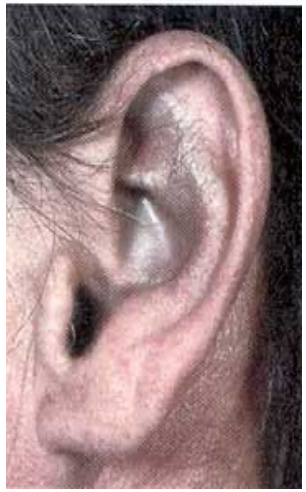
- urine in diaper may darken

➢ **In adults:**

- darkening of the **ear**
- dark **spots on the sclera** and cornea
- arthritis







Clinical manifestations

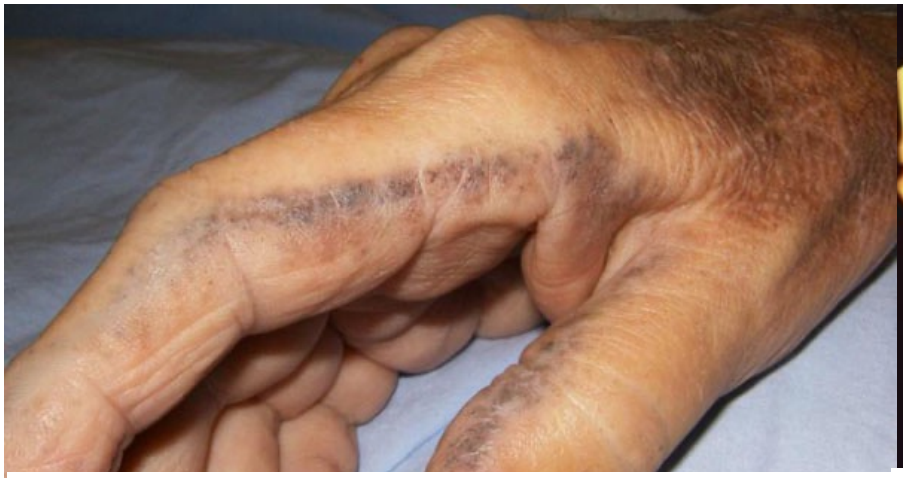
1. **Black urine**, which appears from birth and can lead to early diagnosis of the disease.
2. **Black spots in the eyes**, discolored ears and dark earwax. However, these symptoms do not affect vision or hearing and are often used for diagnostic purposes later in life. This process of ochronosis may also occur **under the nails**, on the **face and hands**.

HGA Build-up causes:

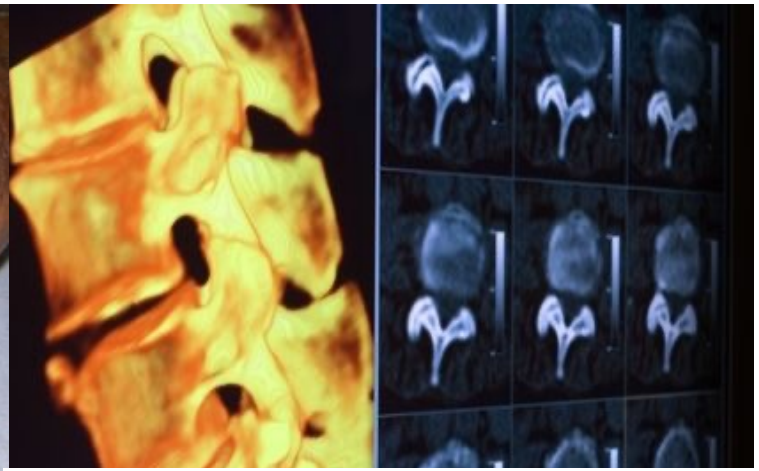
1. **Osteoarthritis**. homogentisic acid (HGA) build up in the connective tissue of patients. This can be extremely painful and gets worse with age.

2. **Kidney, prostate and bladder stones** due to the build-up of HGA in the genito-urinary tract, during the production of urine.

3. **Heart complications**. In severe cases, it may cause heart disease and patients may require heart valve replacements.



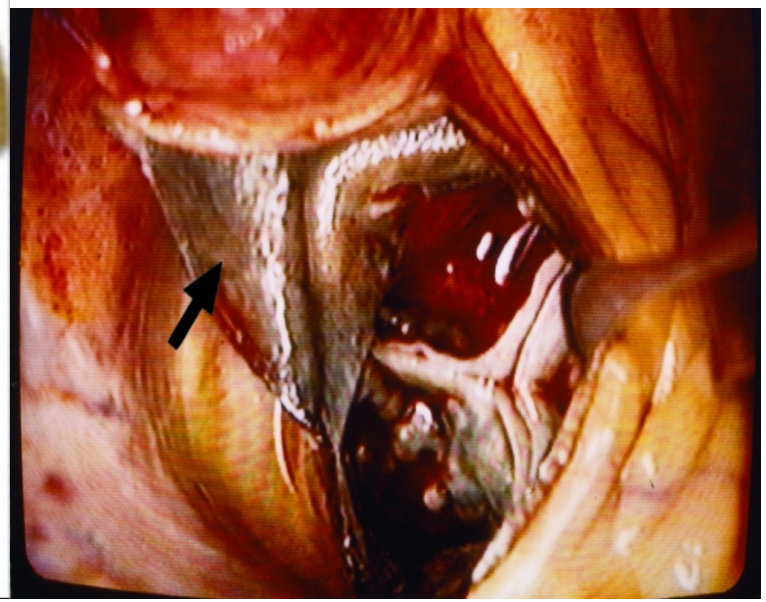
Accumulation of oxidized homogentisic acid pigment in connective tissue (ochronosis)



Arthritis of the spine is a complication of alkaptonuria ochronosis

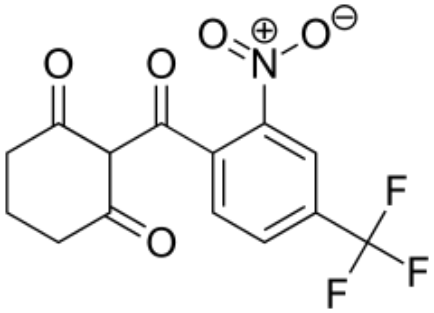
Aortic valve stenosis in alcaptonuria

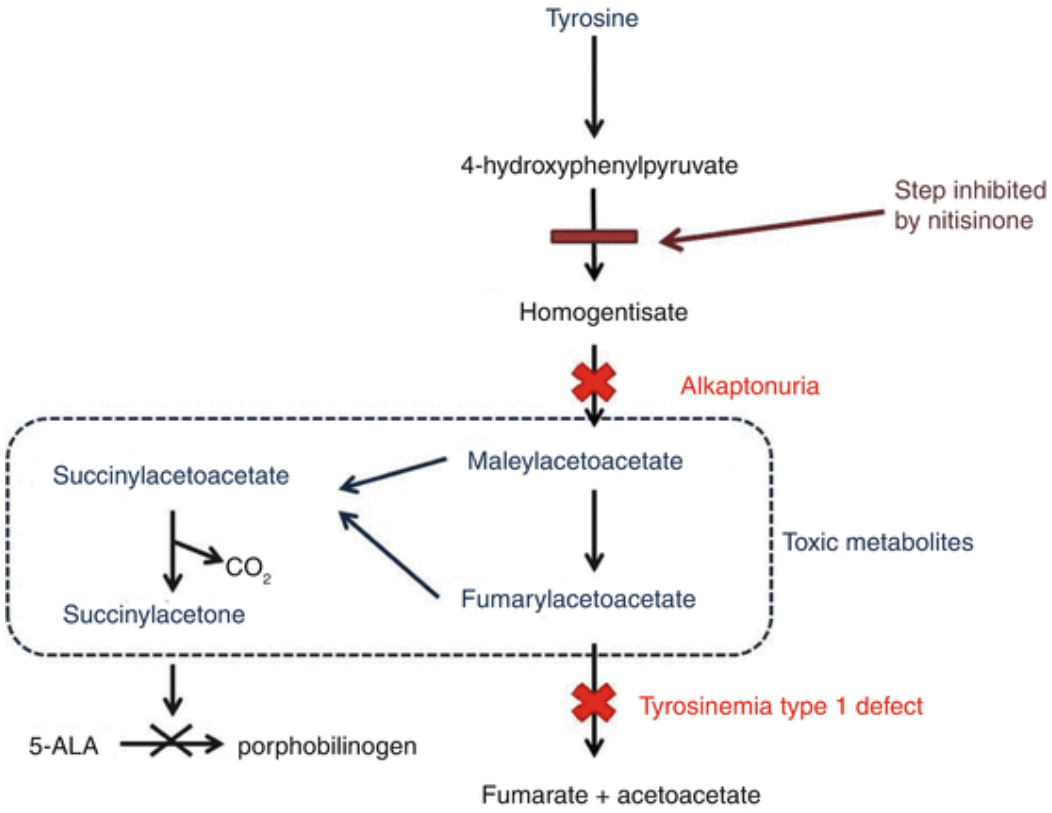
Urine turns a black color upon exposure to air



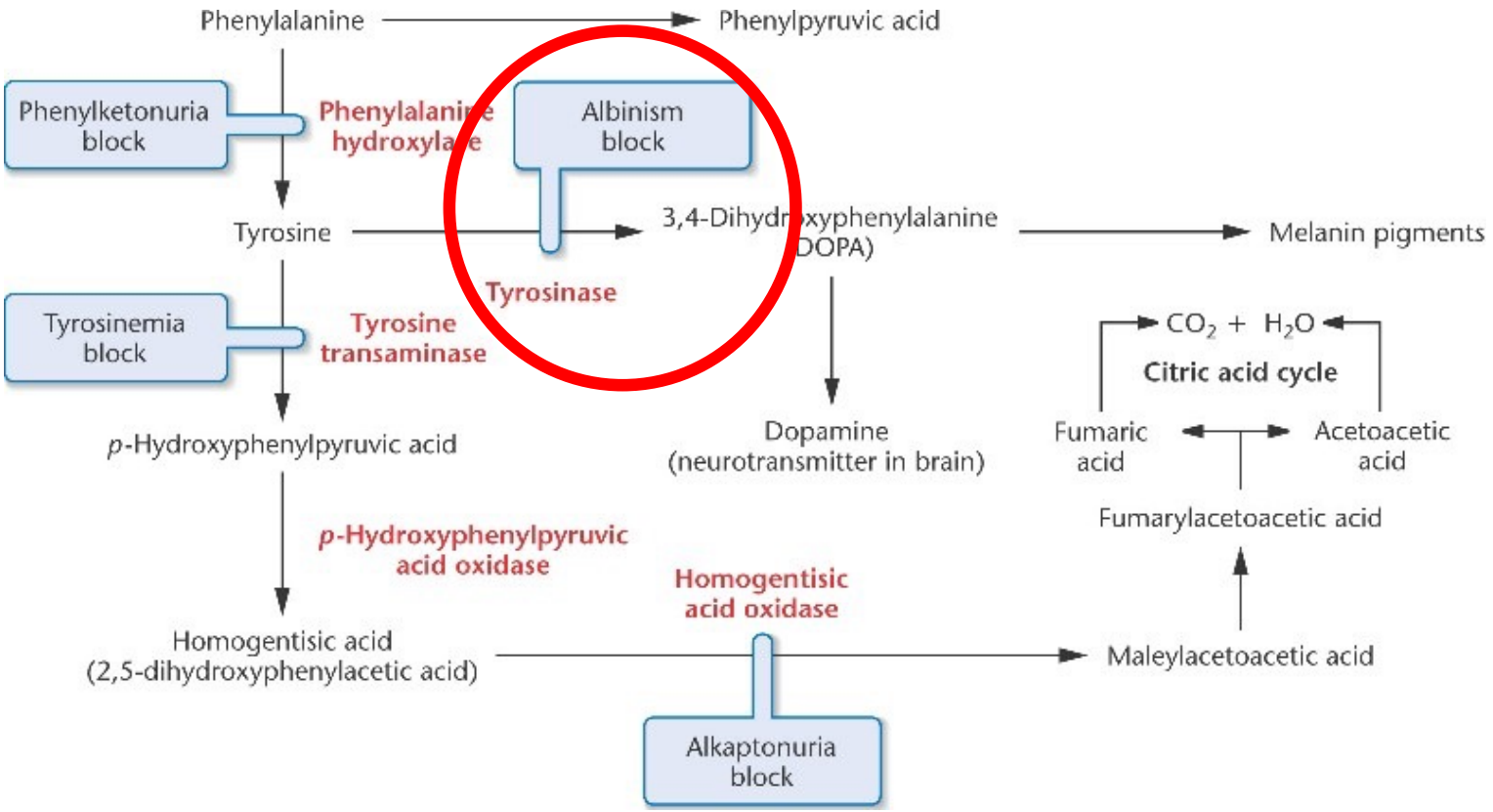
NITISINONE (Orfadin)

- Herbicidal
- New drug to treat AKU
- **Inhibits 4-hydroxyphenylpyruvate oxidase**
- 1 mg/ day *per os* in two doses





Albinism


Phenylalanine
Phenylpyruvic acid
Phenylketonuria block
Phenylalanine hydroxylase
Albinism block
Tyrosine
3,4-Dihydroxyphenylalanine (DOPA)
Melanin pigments
Tyrosinase
Tyrosinemia block
Tyrosine transaminase
CO_2 + H_2O
Citric acid cycle
p-Hydroxyphenylpyruvic acid
Dopamine (neurotransmitter in brain)
Fumaric acid
Acetoacetic acid
Fumarylacetoacetic acid
p-Hydroxyphenylpyruvic acid oxidase
Homogentisic acid oxidase
Homogentisic acid (2,5-dihydroxyphenylacetic acid)
Maleylacetoacetic acid
Alkaptonuria block

Albinism

Characterized by a reduced or lack of pigment that normally gives color to the skin, hair, and eyes.

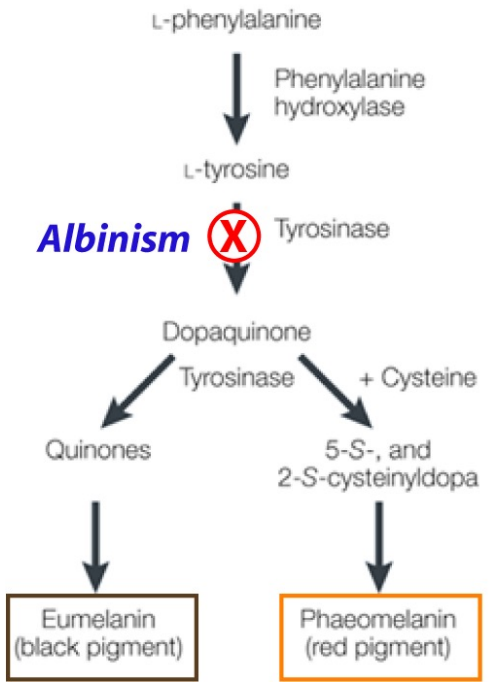
Always associated to **visual defects** (photophobia, nystagmus, amblyopia)

Susceptibility to sunburns and skin cancer

Prevalence: 1/17.000

Autosomal recessive

Mutated gene: **tyrosinase**



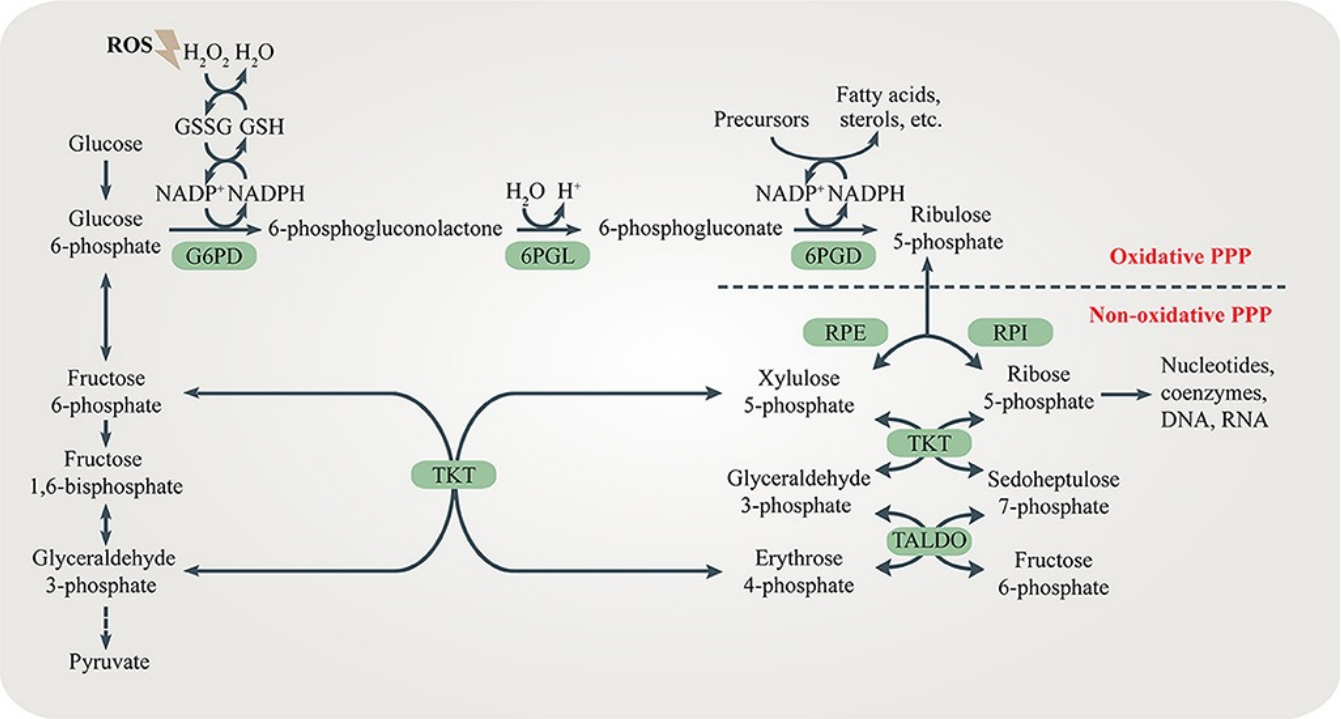
FAVISM

- Recessive, X-linked
- **Mutation of the gene *glucose-6-phopsfate dehydrogenase*** deficiency due to mutation in the G6PD gene, a pentose phosphate shunt enzyme

- The **most common human enzyme deficiency**: 400 million people in the world. Very frequent in **Africa, the Middle East and South Asia**. Related to resistance to **malaria**

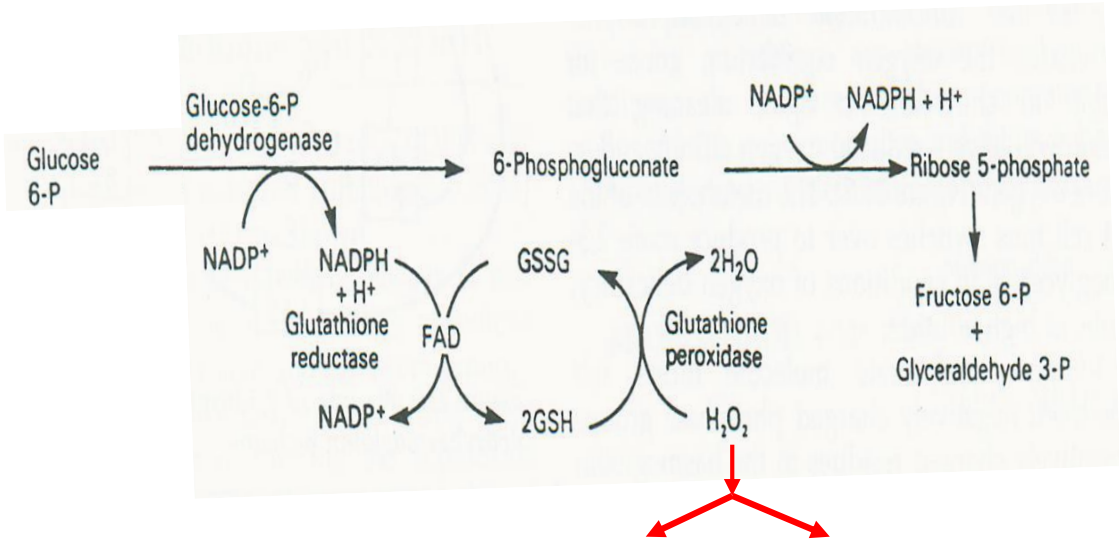
- In **Italy** (continental) the incidence is 0.4%; 1% in Sicily, 14% in Sardinia

Pentose Phosphate Pathway (PPP)



The pentose phosphate shunt is the **only source of GSH for red blood cells**. Their role as oxygen carriers exposes the erythrocytes constantly to the risk of damage by **reactive oxygen species (ROS**), normally prevented by the presence of GSH in adequate quantities.

PPP and GSH



Peroxidation of RBC membrane lipid
→↑↑ membrane fragility → **Hemolysis**

Met-Hb

Meta Hemoglobin

Hb with Fe of the Heme group in the **ferric state** no longer binds O_2

The function G6PD in the red cell is to generate NADPH → reduced glutathione → protect the RBCs from the oxidative damage by H_2O_2

Triggering agents

- People with favism are usually **asymptomatic**
- Hemolysis is caused, with very few exceptions, by specific triggering factors.

TRIGGERING FACTORS:

- ingestion of **fava beans**

- **drugs** with intracellular oxidizing action (antimalalarian, sulfonamides, aspirin, NSAIS, naphthalene)

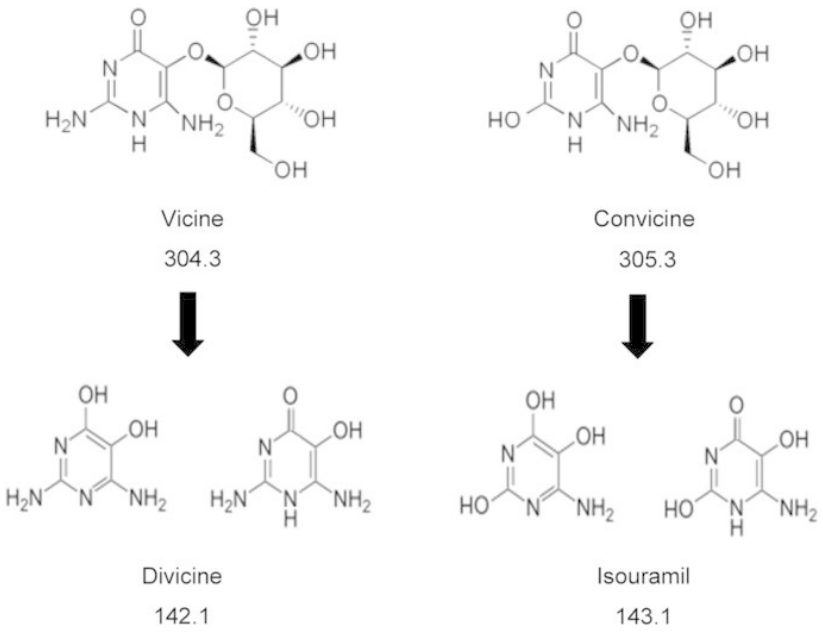
- **Infections** (middle-severe)

- the triggers listed, although apparently heterogeneous, have in common the oxidative action on red blood cells;

- Hemolysis are dose-dependent

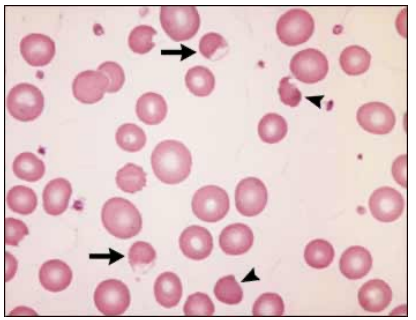
Fava beans: vicine and convicine

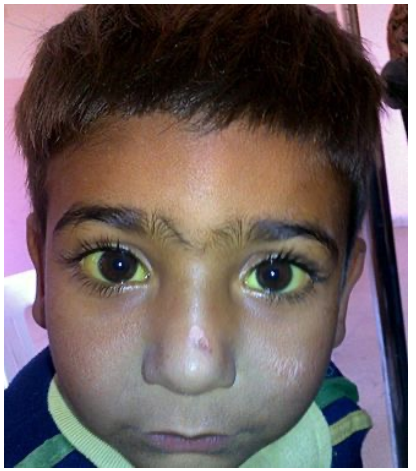
- Fava bean ingestion is the **most common cause of hemolytic crisis**
- Usually raw, but even cooked may cause hemolysis
- Severity depends on the **amount** eaten
- Two molecules responsible: **vicine and convicine**, glycosides contained at high concentrations in fava beans BUT NOT in other legumes (peas, chickpeas, beans, etc)
- Vicine and convicine are glycosides hydrolyzed by the b-glycosidase in divicine and isouramil, which are then further oxidized, with **generation of H_2O_2**



CLINICAL MANIFESTATION (hemolytic crisis)

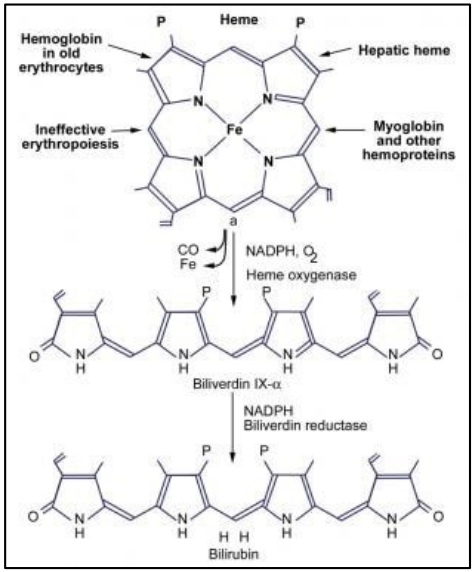
- 12-48 hours after eating **beans or taking triggering drugs**
- Sudden onset of **fever**
- **Jaundice**: skin, sclera, palms of the hands
- Hyper-colored, **yellow-orange urine**.
- **Pallor, weakness**, impaired general condition.
- **Shortness of breath**
- Pulse rapid, weak, not very noticeable.





Jaundice

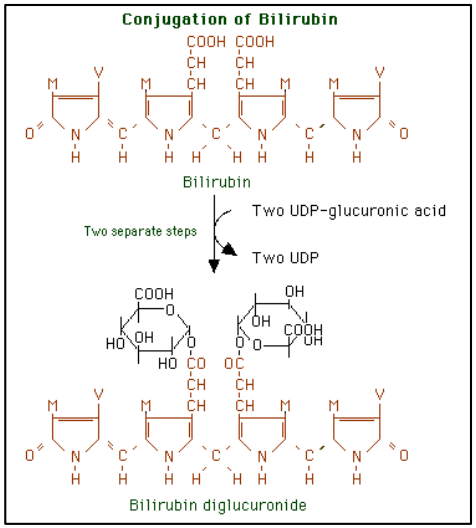
BILIRUBIN METABOLISM



Heme

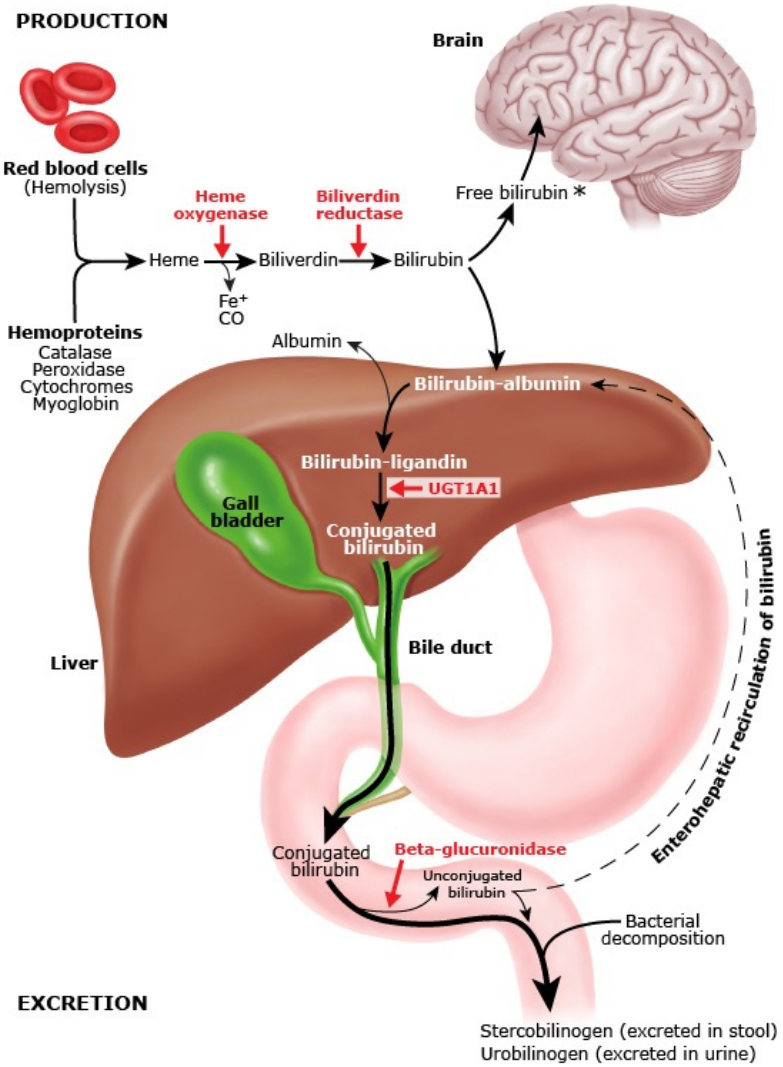
Biliverdin

Bilirubin

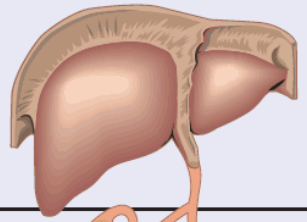
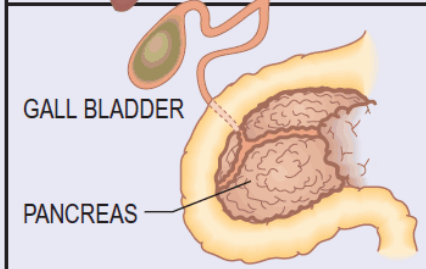


INDIRECT (free)

DIRECT (conjugated)



Types of jaundice

Types	HAEMOGLOBIN ↓ BILIRUBIN	Causes
Prehepatic		Haemolysis
Cholestatic	CONJUGATION	
Intrahepatic		Viral hepatitis Drugs Alcoholic hepatitis Cirrhosis – any type Pregnancy Recurrent idiopathic cholestasis Some congenital disorders Infiltrations
Extrahepatic		Common duct stones Carcinoma – bile duct – head of pancreas – ampulla Biliary stricture Sclerosing cholangitis Pancreatitic pseudocyst

Flavin icterus (pre hepatic)

Straw-yellow flavinic colour in cases of haemolytic anaemia or other massive erythrocyte breakdown

Ruby icterus (intra hepatic)

Intense yellow-red colour in hepatocellular icterus (diffuse hepatitis).

Verdin icterus (extrahepatic)

occlusive icterus: Greenish shade
mechanical obstruction of the bile ducts (stones, cancer etc)

Melas icterus

Dirty dark green color in cachectic liver cirrhosis or liver tumor patients.

Diagnosis of FAVISM: measurement of G6PD enzymatic activity

- The classical method consists in the spectrophotometric measurement of the G6PD activity of a hemolysate, based on the NADPH formation rate (340 nM) , which must be linear over a period of 10 minutes.
- Hemoglobin (Hb) is measured on the same hemolysate and the activity is expressed in IU*/G Hb.
- Normal values at 30 ° C are 7-10 IU / G Hb

*IU= International Unit

Enzymatic assay: interpretation

- **Normal values: 7-10 IU / G Hb**
- **Affected males:** the activity is usually **below 2 IU/G Hb**
- **Females:**
  - if the activity is **below 2** it is likely to be **enzymopenia G6PD homozygous**
  - If the activity is **between 2 and 7** it may be **intermediate (or partial) G6PD** enzymopenia, probably **heterozygous**.

Classification of Favism (WHO)

- **Class I**. Enzyme activity <10%. Patients can have hemolysis even without taking oxidants. **Chronic hemolysis**. It is associated with chronic non-spherocytic hemolytic anemia. It is very rare.

- **Class II** Enzyme activity <10%. Has no baseline haemolysis or chronic non-spherocytic haemolytic anemia. Includes Mediterranean variant (common) and Union variant (454ARG - CYS). **Intermittent hemolysis**

- **Class III** Enzymatic activity 10-60%. More resistant to hemolysis. Includes the African and Seattle variant.

- **Class IV** Enzymatic activity 60-100%.

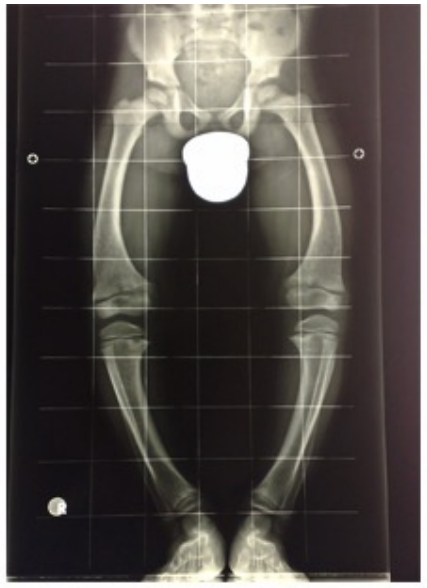
THERAPY

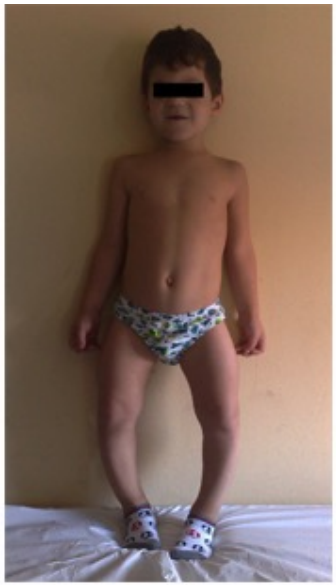
- **Prevention**: avoid taking substances that trigger the haemolytic crisis
- In case of acute haemolysis blood **transfusions** may be necessary
- **Dialysis** if the patient is also suffering from renal insufficiency
- Some patients may benefit from **surgical removal of the spleen** as the spleen is an important site of erythrocyte destruction

X-Linked Dominant Diseases

Vitamin D Resistant Rickets

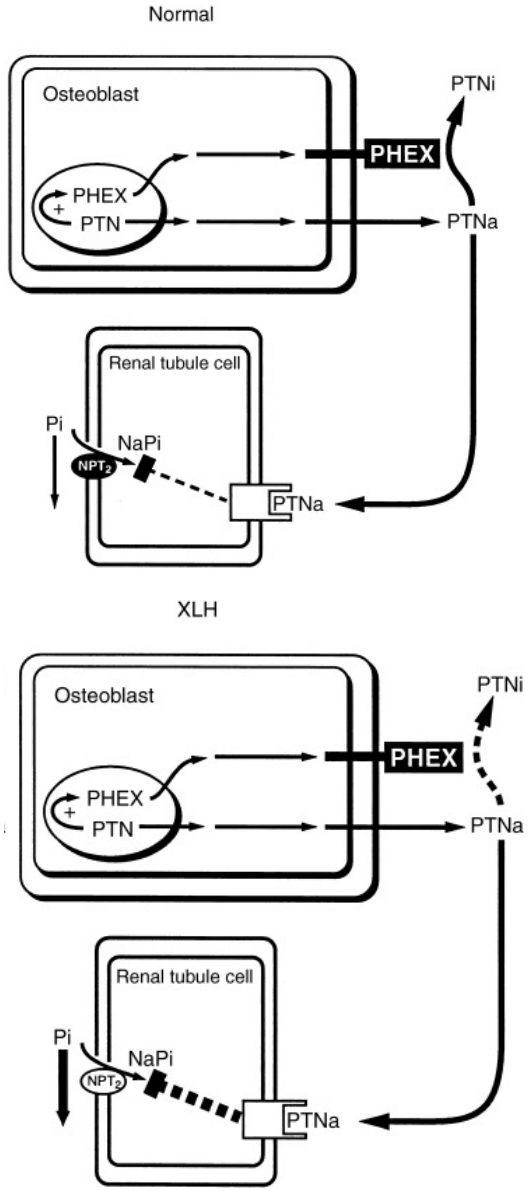
- Hypophosphatemic rickets
- (X-linked Hypophosphatemia, **XLH**)
- Affects equally men and women, more severe in males.
- Prevalence: 1:5.000
- Described by Albright in 1937

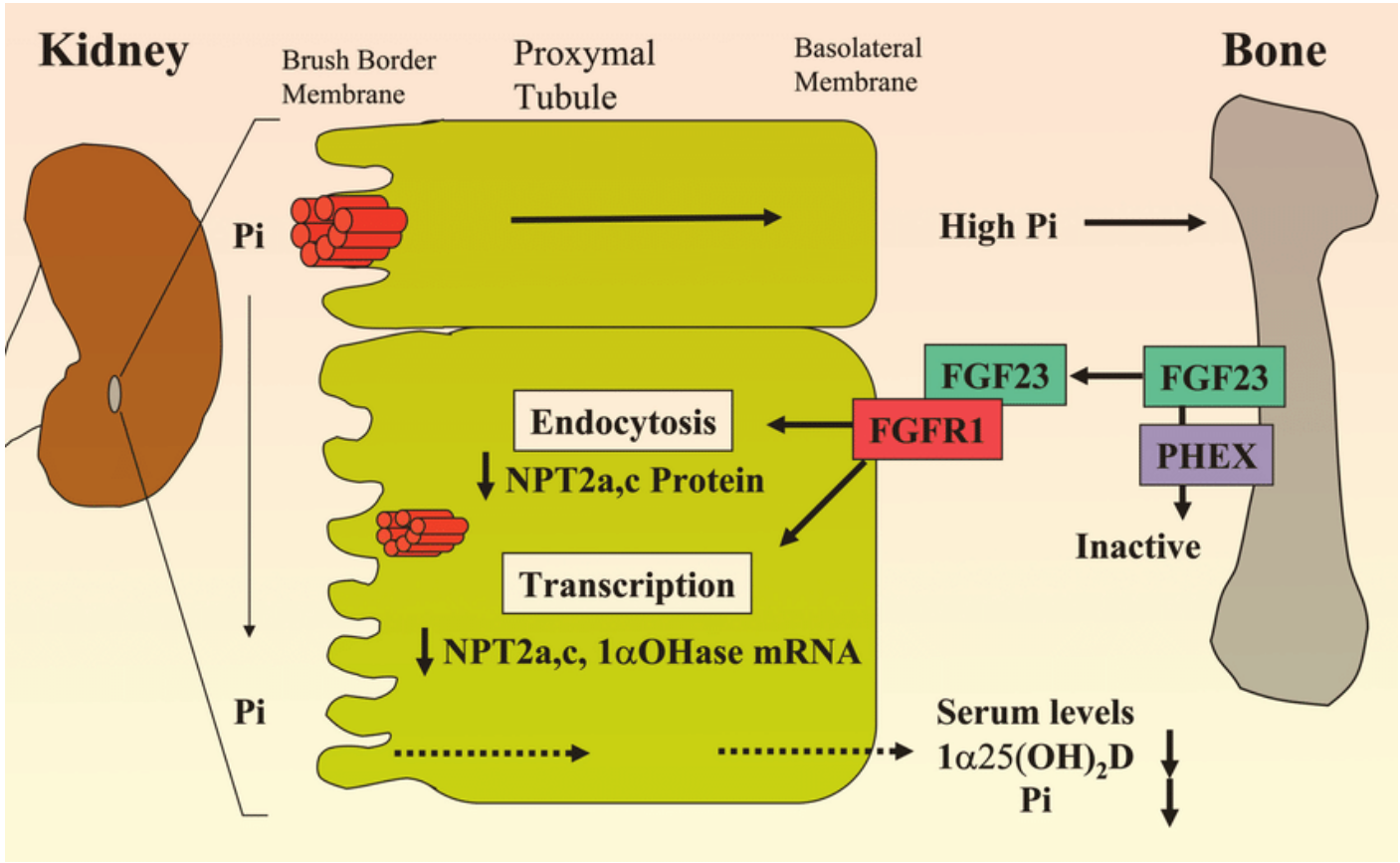




Molecular Pathogenesis

- Mutation of the **PHEX gene**, localized on the chromosome X.
- Membrane **endopeptidase expressed on the** ostoblasts.
- **PHEX degrades phosphatonin PTN/FGF23** a phosphaturic hormone
- **PTN** regulates the phosphate trasporter in the kidney: **promotes the elevated excretion of phosphates in the urine.**
- **A mutation of PHEX causes increased PTN/FGF23 and consequent increased secretion of phosphates in the urine (hyperphosphaturia) and decrease of its plasmatic concentration (hypophosphatemia).**
- The phosphate plays a fundamental role in regulating bone mineralization.





- **FGF23 / PTN acts on the kidneys by inducing endocytosis and reducing the expression of NPT2**, a sodium-phosphate cotransporter located in the proximal tubule.

- **FGF23 reduces the tubular reabsorption of phosphates**, increasing their urinary excretion.

Cinical manifestations

- Onset at 1-2 years with **fragile, subtle and curved bones**.
- Bowed legs
- Spontaneous fractures
- **Deformed** chest
- Reduced height
- **Dental abnormalities**
- Resistance to the therapy with vitamin D

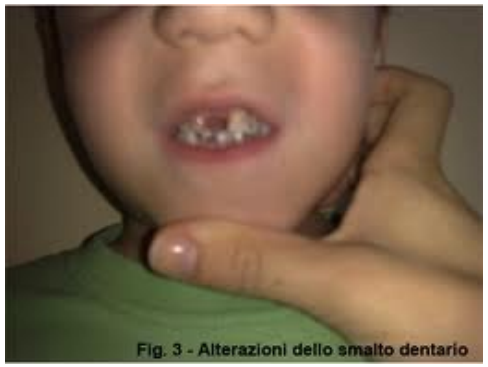
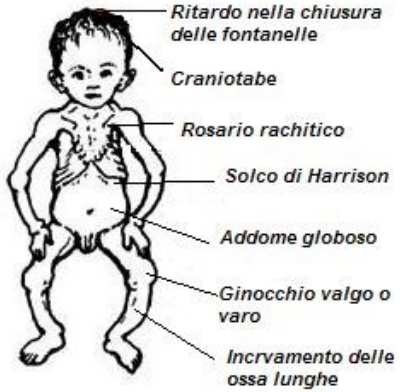


Fig. 3 - Alterazioni dello smalto dentario



- **Diagnosis**: hyposphatemia, elevated alcaline phosphatase, hyperphosphaturia, Vit D levels normal

THERAPY

- **Burosumab (crysvita)** is an <u>anti-FGF-23 monoclonal antibody</u> that has become the treatment of choice for X-linked hypophosphataemia (**approved by AIFA in 2019**).

- Iron deficiency stimulates FGF-23 expression in bone and can exacerbate conditions with high FGF-23 levels. Therefore, **restoration of iron levels** is essential for patients with iron deficiency in the context of elevated FGF-23 hypophosphatemic conditions.