PRENATAL AND NEONATAL SCREENINGS

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(by the way the little one above is not me)

What you should know before using a test (1)

- Sensitivity: property of a test to be altered in patients suffering from a disease. Expresses the capacity to recognize the disease.

- Sensitivity = true positives / true positives + false negatives (ie, all those sick)

What you should know before using a test (2)

- Specificity: the property of a test to be normal in subjects not affected by the disease. Expresses the ability to exclude a disease.

- Specificity = true negatives / true negatives + false positives (i.e. all healthy subjects)

What you should know before using a test (3)

- Positive predictive value: the probability of an individual who has a positive test to be really affected by the disease.

- PPV = true positives / true positives + false positives (ie, all positive)

Prenatal Diagnosis

Prenatal diagnosis indicates the laboratory tests aimed to detect or exclude the presence in the fetus of:

- Chromosomal aberrations (i.e. Down syndrome).
- Genetic diseases (i.e. cystic fibrosis).
- Malformations (i.e. heart defects).

Prenatal Diagnosis

- **Non invasive approaches**:
- Echography (intra/extra uterine; twins; fetus growth, positioning vitality; placenta; sex; amniotic fluid);

- Nuchal translucency (increased as result of fluid accumulation in the neck in malformations; sensitivity 85%, false positive 5%);

- Quadruple screen (AFP and E3 lower in Down syndrome, hCG and Inhibin A higher in Down syndrome).

- **Invasive approaches**:
- Metaphase analysis on chorionic villus sampling or amniotic fluid.

- **Nowdays….DNA analysis on cfDNA!!!**

NEONATAL SCREENING

Neonatal screening is an intervention on the population of apparently healthy infants, with the aim of identifying all infants with specific congenital diseases that, if not treated promptly, will develop serious outcomes for the health and quality of life of the affected subjects.

CRITERIA FOR SCREENING PROGRAMS

In order to undertake a screening program for a disease, this must be:

- relatively frequent;
- serious enough to impose a heavy emotional and financial burden to the family and society;
- the course of the disease must be partly modifiable by treatments;
- there must be available tests with good sensitivity and specificity and relative cost.

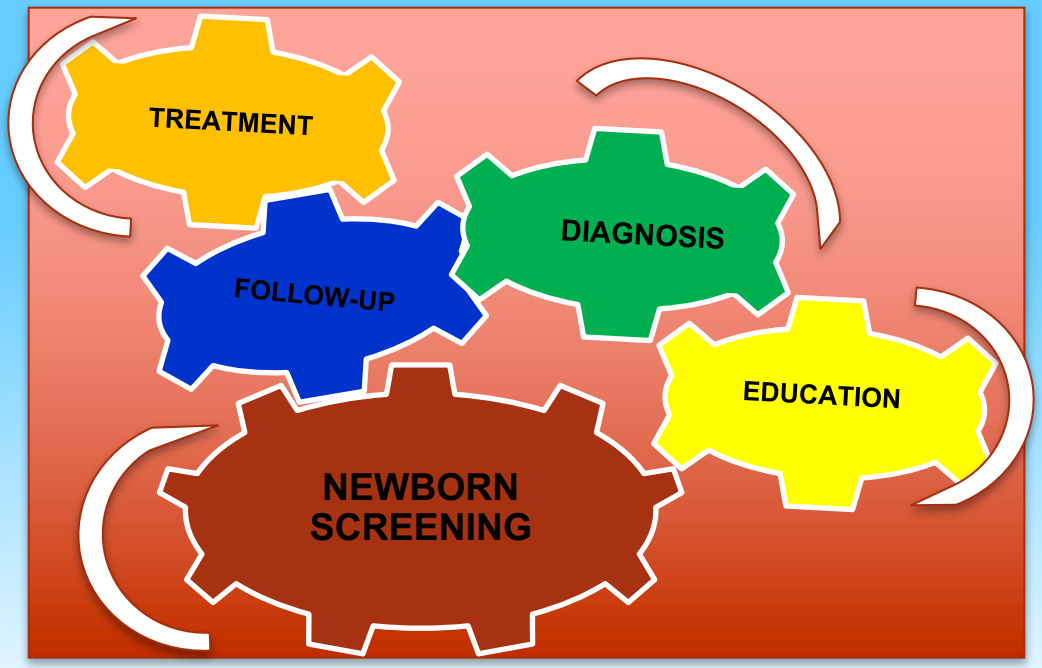
Neonatal screening program in Italy

Starting 1992 it is mandatory for all the Regions to introduce a package of neonatal screening aimed to identify newborns affected by:

- Cystic fibrosis
- Congenital hypothyroidism
- Phenylketonuria
- Galactosemia (in Lazio and two more regions)

Neonatal screening program: a complex network

✓When developing new organization it should be taken into account that Neonatal Screening is not simply the application of a test but it is a complex system



ANALITYCAL ISSUES FOR SCREENING

- PRE-ANALYTICAL ISSUES

1. sample collection (age of collection of blood 48 -72h)
2. sample delivery (arrival to center maximum 24 h after sampling)
3. special procedures (low birth weight, premature, transfused newborns)
4. sample storage (negative sample storage: minimum 8 months at room T; positive sample storage: minimum 12 months at -80° C; confirmed positive sample storage: ∞ at -80° C)

ANALITYCAL ISSUES FOR SCREENING

- ANALYTICAL ISSUES

laboratory organization and certification (personnel and structure)

cut-off evaluation (when possible between the 99%ile of normal distribution and the 5%ile of true positive distribution) and age-related reference range

spectrum of metabolites (acylcarnitines and amino acids)

analytical procedures (mass spectroscopy for acylcarnitines and amino acids)

ANALITYCAL ISSUES FOR SCREENING

- POST-ANALYTICAL ISSUES

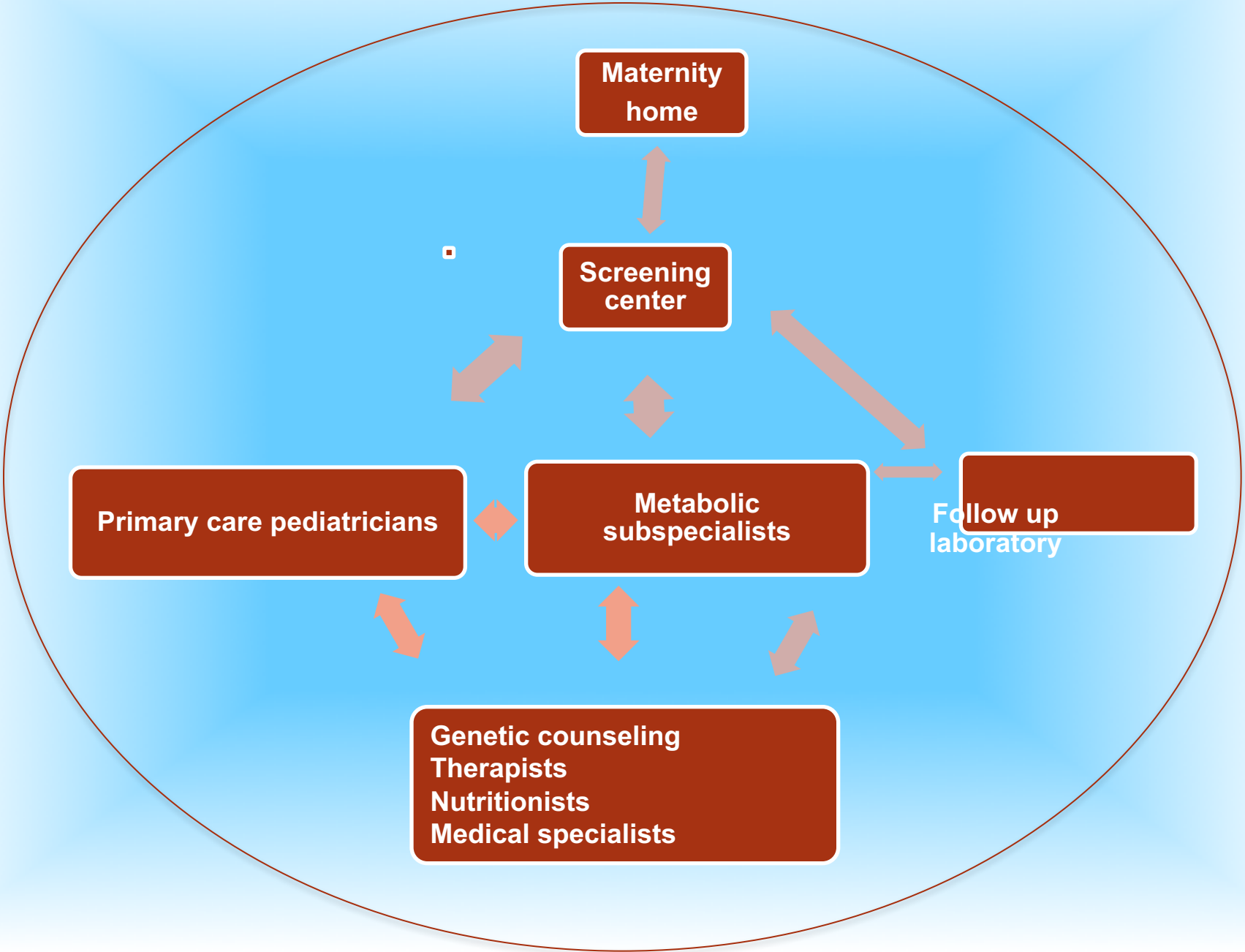
1. profile interpretation and possible interference and artifacts
2. standardization of positive sample procedures (retest – recall – low/high risk)
3. screening performance evaluation (sensitivity, specificity, PPV)
4. proficiency and quality assessment (CDC, ERNDIM, SISN, etc)
5. information system (result communication)

ANALYTICAL ISSUES FOR CONFIRMATION

- METHODS FOR BIOCHEMICAL CONFIRMATION AND FOLLOW-UP

- **Amino acids (plasma, urine) HPLC, LC-MS/MS, GC-MS**
- **Organic acids (urine) GC-MS, LC-MS/MS**
- **Acylcarnitines (plasma, urine) LC-MS/MS**
- Pterines (urine) HPLC
- Homocysteine (plasma) HPLC
- Enzyme activity (DHPR, Biotinidase, Fibroblast fatty acid
- oxidation and carnitine uptake etc..)

GLOBAL ORGANIZATION



Cystic Fibrosis: Clinical Features

Cystic fibrosis is a **heterogeneous recessive genetic** disorder with features that reflect mutations in the cystic fibrosis transmembrane conductance regulator (***CFTR*) gene**.

Classic cystic fibrosis is characterized by chronic bacterial infection of the airways and sinuses, fat maldigestion due to pancreatic exocrine insufficiency, infertility in males due to obstructive azoospermia, and elevated concentrations of chloride in sweat.

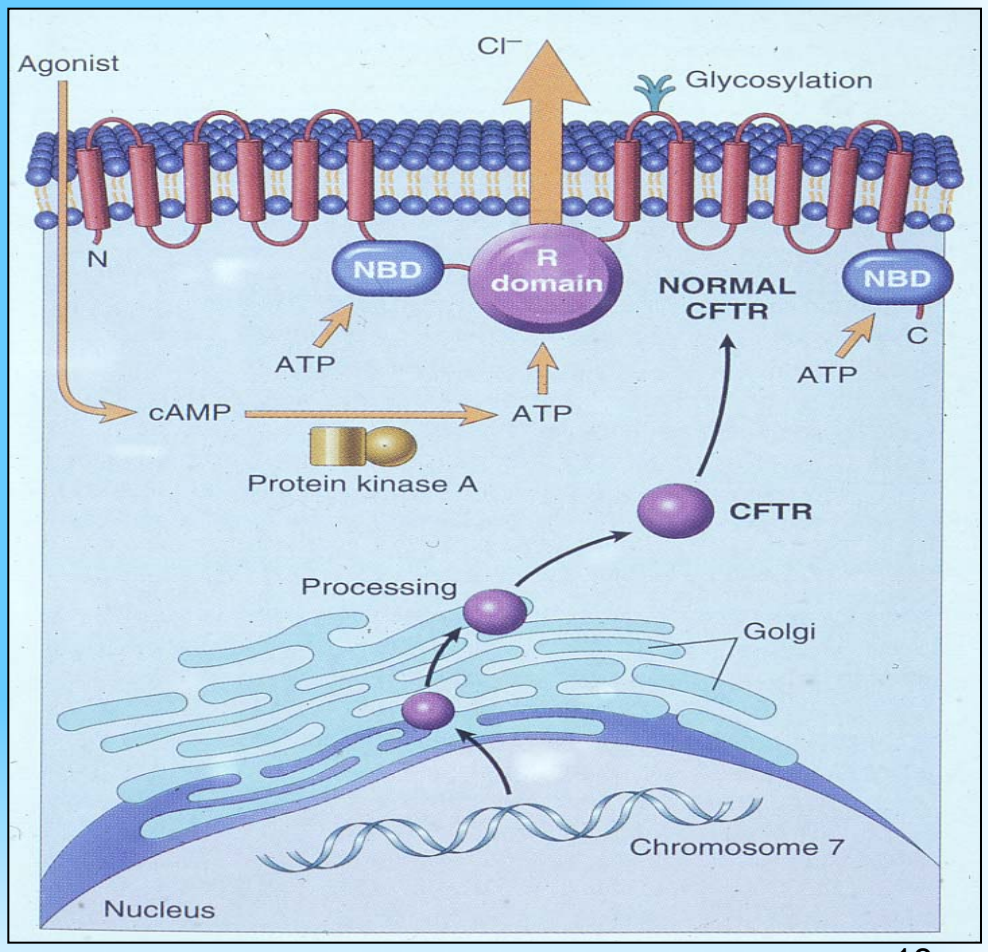
Patients with non-classic cystic fibrosis have at least one copy of a mutant gene that confers partial function of the CFTR protein, and such patients usually have no overt signs of maldigestion because some pancreatic exocrine function is preserved.

Genetics of Cystic Fibrosis

- Autosomal recessive
- Gene located on chromosome 7
- Prevalence- varies with ethnic origin
  - 1 in 3000 live births in Caucasians in North America and Northern Europe
  - 1 in 17,000 live births of African Americans
  - 1 in 90,000 live births in Hawaiian Asians

Protein Function and Biochemistry

- CFTR controls chloride ion movement in and out of the cell.



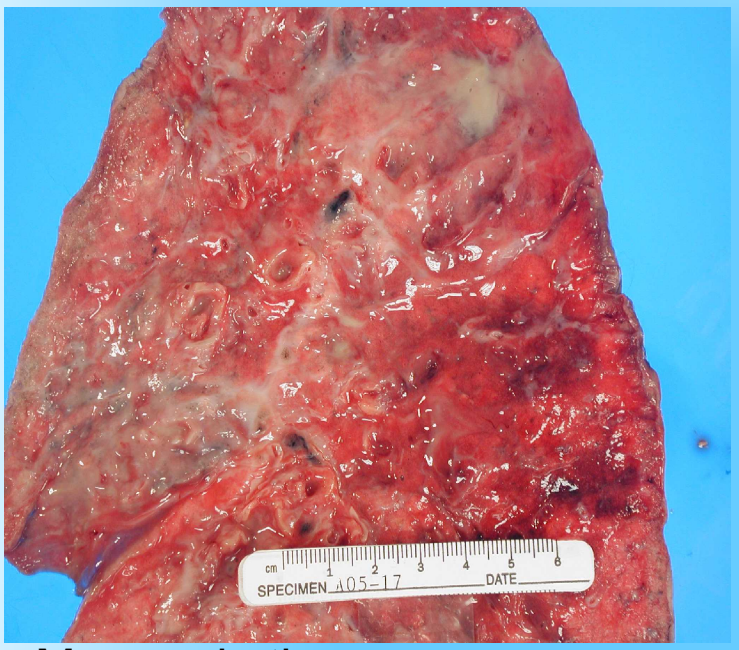
Changes in Protein structure

- CFTR functions principally as a cAMP-induced chloride channel and appears capable of regulating other ion channels.

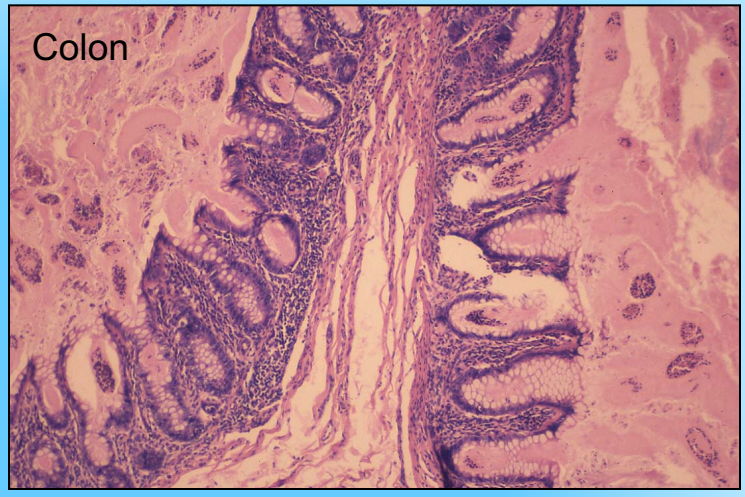
- Besides the most common mutation, ΔF508, accounting for about 70% of CF chromosomes worldwide, more than 850 mutant alleles have been reported to the CF Genetic Analysis Consortium.

- These mutations affect CFTR through a variety of molecular mechanisms which can produce little or no functional CFTR at the apical membrane.

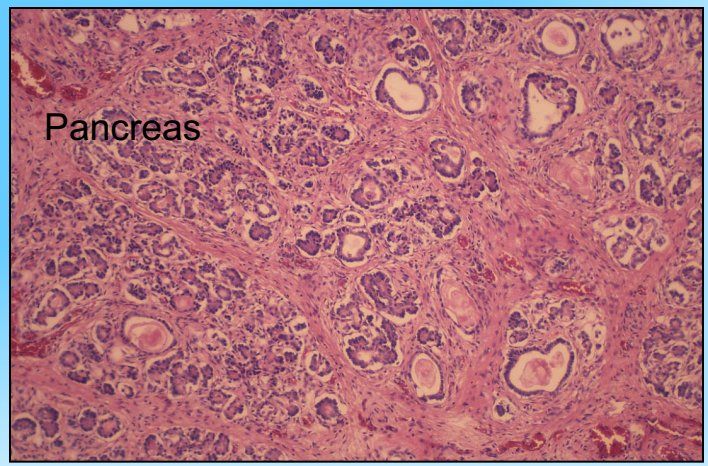
Presentation of Disease



Mucous in the airways cannot be easily cleared from the lungs.

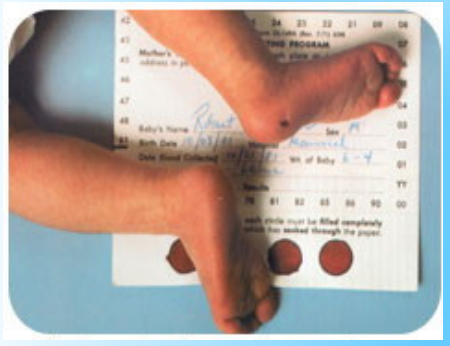


Sticky mucus secretion



Ducts are filled with sticky mucus. Scaring of tissue.

Screening test for cystic fibrosis



Few blood drops from the newborn heel between 48-72 hours are spotted on an absorbent card (Guthrie test)

Trypsin dosage is a test:

High sensitivity

⇩

Among 100 newbors affcted by CF, 98 show high levels of trypsin

Low specificity

⇩

Trypsin levels are increased also in newborns showing:

-respiratory distress syndrome;

-prematurity;

-malformations

Second level tests:

Sweat chloride test

Sweat stimulation with pilocarpine

Sweat collection for 30 min

Cl and Na dosage in the sample

Negative < 40 mEq/l

??? 40 – 70 mEq/l

Positive > 70 mEq/l

GENETIC TESTS FOR CYSTIC FIBROSIS

- FIRST LEVEL (search of the 32 most common mutations that cause cystic fibrosis) through:

  - reverse dot blot

  - amplification refractory mutation systems (ARMS)

  - oligonuclotide specific allele (ASO)

- SECOND LEVEL (identification of 90% of the mutations that cause cystic fibrosis) through:

  -DHPLC

  -full sequence of the gene

CONGENITAL HYPOTHYRODISM

- Incidence: 1/3500 newbors

✓ Embryonic alterations in the development of the gland

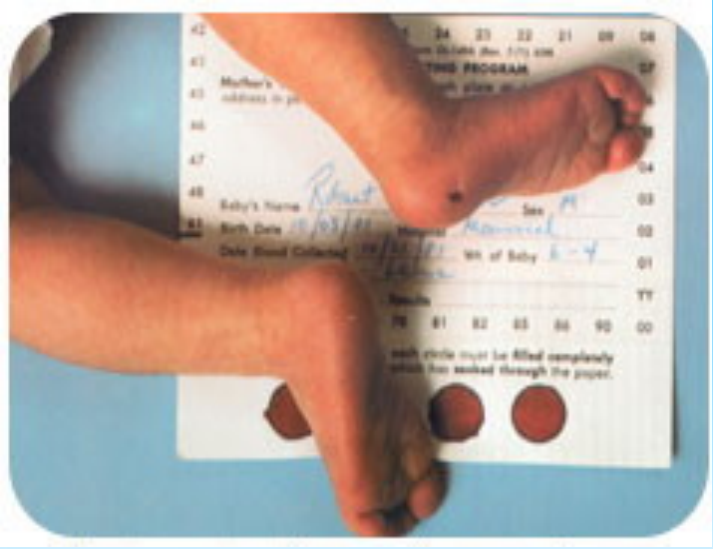
✓ Genetic defects affecting the enzymes involved in thyroid hormones synthesis

✓ Hypothalamic/pituitary axis deficit

- ❖ Mental and growth retardation

NEONATAL DIAGNOSIS

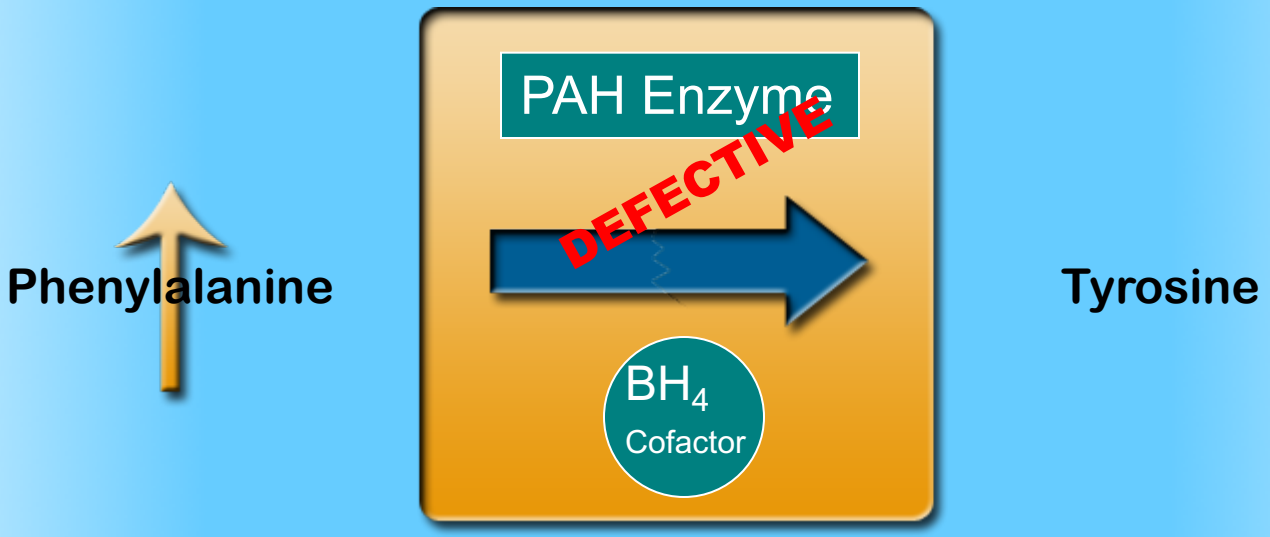
- Determination of TSH within day 5 (PPV 95-98% of the cases).



What is phenylketonuria?

- Persistent elevated blood phenylalanine (Phe) caused by a deficiency of the **phenylalanine hydroxylase (PAH) enzyme**
- The term PKU is reserved for primary dysfunction of the PAH enzyme due to mutations in the PAH gene
- The degree of impairment varies greatly among patients resulting in a broad continuum of phenotypes
- Categories based on blood Phe at diagnosis
  - Classic PKU > 1200 µmol/L (20 mg/dL)
  - Moderate PKU = 900–1200 µmol/L (15–20 mg/dL)
  - Mild PKU = 600–900 µmol/L (10–15 mg/dL)
  - Mild HPA = 300–600 µmol/L (5–10 mg/dL)

Simplified biochemistry of phenylalanine metabolism



PAH = phenylalanine hydroxylase
BH_4 = cofactor tetrahydrobiopterin

Success of the diet followed newborn screening

- "It is reasonable to presume that the best results of dietetic treatment of PKU will be obtained if treatment is started in infancy and particularly in the neonatal period"[1]
- The first method of testing for PKU was the ferric chloride test[2]
  - Detected ketones in urine
  - Limited use in newborns because appearance of ketones can be delayed
- The Guthrie test[3]
  - Developed by Robert Guthrie in the late 1950s
  - Bacteria inhibition assay worked on newborn blood
  - Simplicity (dried blood spot on filter paper) was ideal for mass screening

Consequences of elevated blood phenylalanine levels vary by age

Children

PKU Patients Not on Diet

Adults

When PKU is untreated or treated late, the following may occur

- Mental retardation or reduced IQ
- Seizures and tremors
- Difficulties in executive function
- Psychological and behavioral issues
- Social difficulties
- Impaired growth
- Irritability
- Eczema

When PKU is poorly controlled, the following may occur

- Difficulties in executive function
- Psychological and behavioral issues
- Social difficulties
- Neurological complications
- Irritability
- Eczema

Results of the screening and diet

The combination of newborn screening and Phe-restricted diets has nearly eliminated the severe neurocognitive and motor deficits that occur with untreated PKU

In some studies, difficulty in following the diet and maintaining adequate Phe control resulted in poor outcomes

Nutritional deficiencies have been associated with low-Phe diets, suggesting that increasing natural sources of protein may be of value

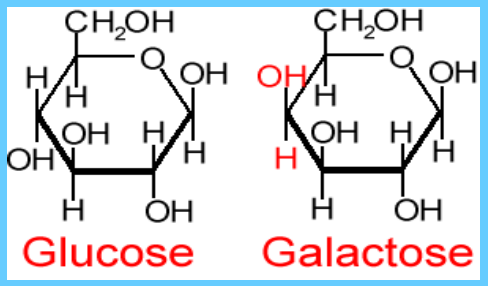
Despite the overall success of the PKU diet, adherence into adulthood continues to be a problem

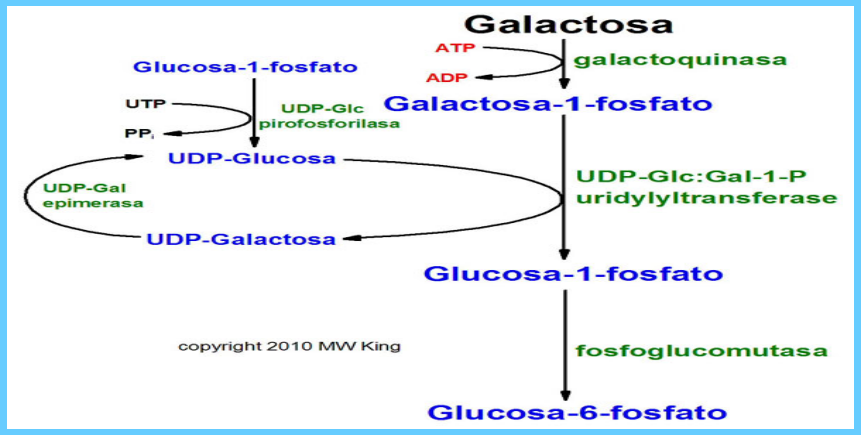
GALACTOSEMIA

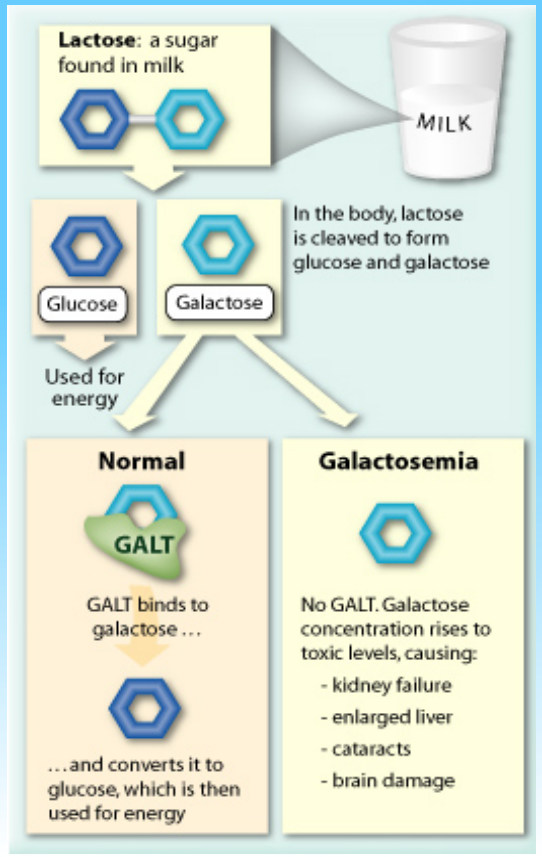
- Galactosemia is an inherited recessive deficiency in enzymes that metabolize galactose

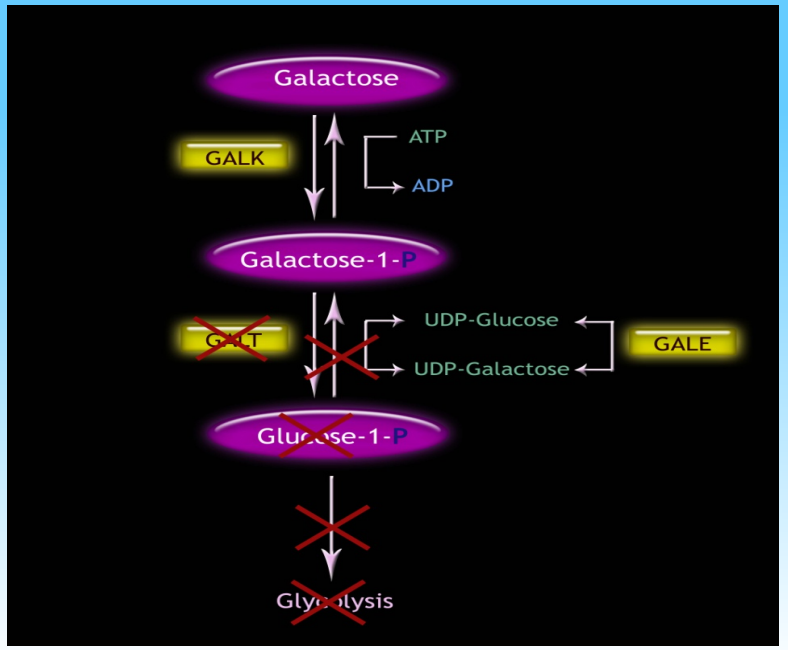
- 1 in 60 000 newborns are diagnosed with Galactosemia every year

Sources and metabolism of galactose









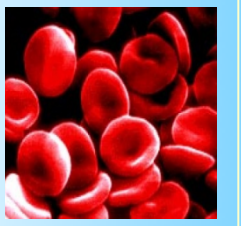
NORMAL GALACTOSE METABOLISM

- Dietary lactose is digested into glucose and galactose, and absorbed through the intestine
- Galactose is taken up by a RBC (carrier-mediated), it is phosphorylated to Galactose-1-Phosphate (Gal-1-P) by *Galactokinase* (*GALK*)
- Gal-1-P is converted to Glucose-1-Phosphate (Glu-1P) using the epimerization of UDP-Glucose to UDP-Galactose by the enzyme *Galactose-1-Phosphate Uridyl Transferase* (*GALT*). That is:

  Gal-1-P + UDP-Glucose $\xrightarrow{\text{GALT}}$ UDP-Galactose + Glu-1-P

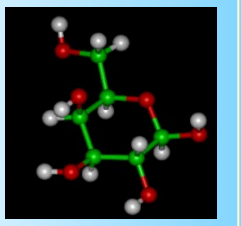
- Glu-1-P proceeds on to glycolysis
- UDP-Galactose is recycled back to UDP-Glucose by *Uridyl Diphosphate Galactose 4-Epimerase* (*GALE*)

THREE TYPES OF GALACTOSEMIA



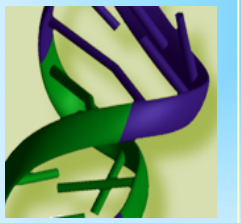
1. *GALT* Deficiency

Most severe form: "classic galactosemia"
Most prevalent: 95% of cases



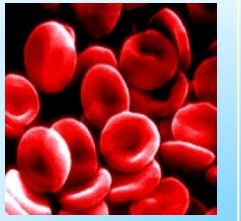
2. *GALK* Deficiency

Milder form
5% of cases



3. *GALE* Deficiency

rare



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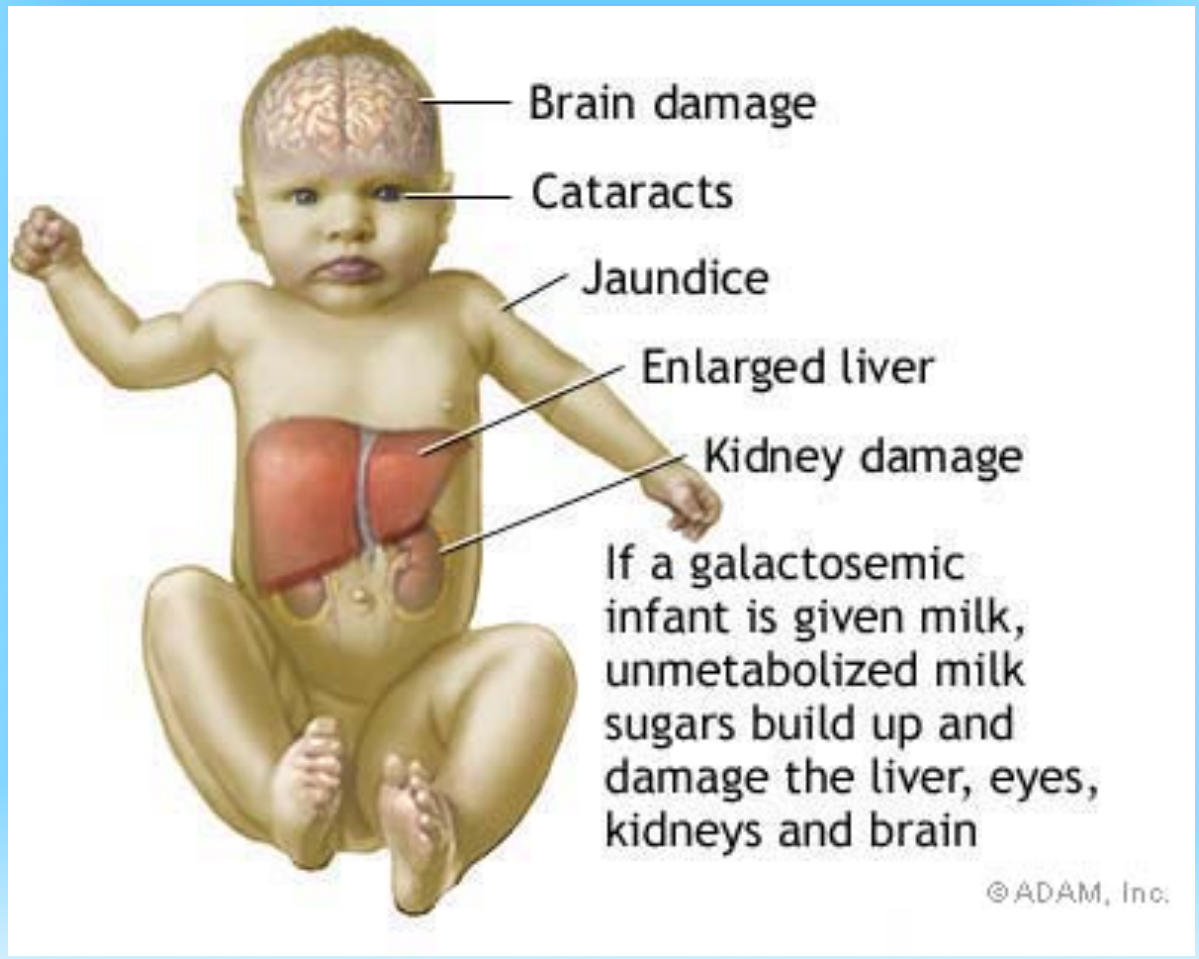


DIAGNOSIS

Tests

- Blood tests
  - Enzyme activity in RBCs

  Normal range for Galactose-1-phosphate uridyl transferase activity is**18.5** to **28.5** U/g Hb.
  - Low blood sugar (hypoglycemia)

- Urine analysis
  - Reducing substances accumulation (i.e. galactose & galactose-1-P)

TREATMENT

- No pharmacological treatment is currently available
- Sources of galactose (especially lactose) must be eliminated from the diet
  - All dairy products (chesses, yogurt, ice cream), breast milk, infant formulas, sweeteners
  - Foods with > 10mg galactose/100g fresh weight must be avoided; dates, papaya, tomatoes, watermelon
- Calcium and vitamin supplementation (vitamin D)

EXTENDED METABOLIC SCREENING

- More than 40 metabolic ereditary diseases
- Tandem Mass Spectrometry to detect anabolites
- Active in USA, Australia, some UE countries. In Italy some regions.
- Highly debated: what's for? Is it worthy?

"Screening everything that can be measured or just what is well known and can be cured?

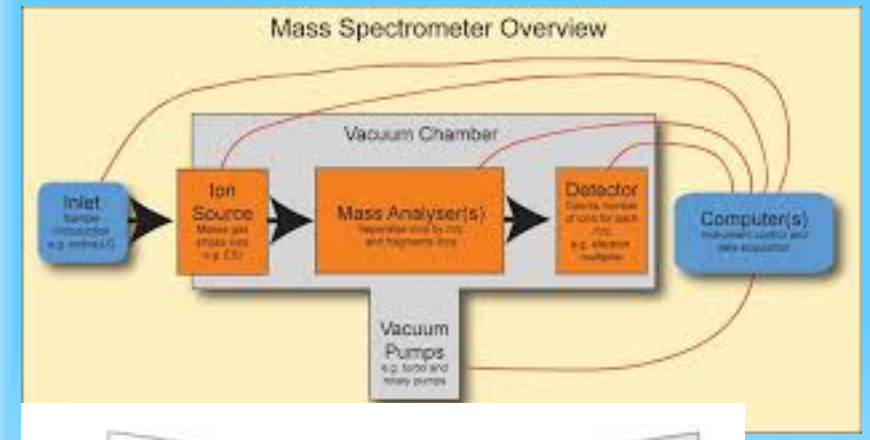
EXTENDED METABOLIC SCREENING

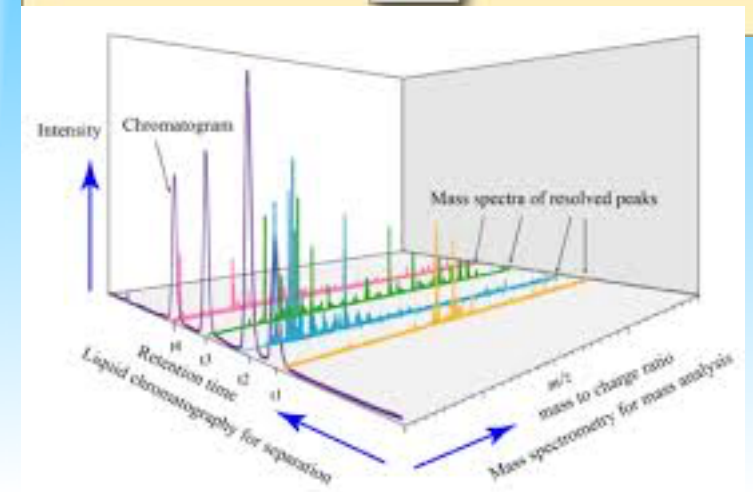
- Now it has become mandatory in Italy.

	GRUPPO	MALATTIA	SIGLA
PANNELLO PRIMARIO	AA	Fenilchetonuria	PKU
	AA	Iperfenilalaninemia benigna	H-PHE
	AA	Deficit biosintesi cofattore tetraidrobiopterina	BIOPT (BS)
	AA	Deficit rigenerazione cofattore tetraidrobiopterina	BIOPT (REG)
	FAO	Deficit dell'acil CoA deidrogenasi a catena media	MCAD
	OA	Acidemia glutarica tipo I	GA I
	OA	Acidemia Isovalerica	IVA
	AA	Malattia delle urine allo sciroppo d'acero	MSUD
	AA	Tirosinemia tipo I	TYR I
	FAO	Deficit del trasporto della carnitina	CUD
	FAO	Deficit dell'idrossiacil CoA deidrogenasi a catena lunga	LCHAD
	FAO	Deficit della proteina trifunzionale	TFP
	FAO	Deficit dell'acil CoA deidrogenasi a catena molto lunga	VLCAD
	OA	Aciduria 3-Idrossi 3-metil glutarica	HMG
	OA	Deficit del Beta-chetotiolasi	BKT
	OA	Acidemia Metilmalonica (CblA)	Cbl A
	OA	Acidemia Metilmalonica (CblB)	Cbl B
	OA	Acidemia Metilmalonica (Mut)	MUT
	OA	Acidemia Propionica	PA
	OA	Acidemia Metilmalonica (CblC)	Cbl C
	AA	Acidemia Argininosuccinica	ASA
	AA	Citrullinemia tipo I	CIT
	AA	Omocistinuria (deficit di CBS)	HCY
	AA	Tirosinemia tipo II	TYR II
	FAO	Deficit di Carnitina palmitoil-transferasi II	CPT II
	OA	Deficit Multiplo delle carbossilasi	MCD
Pannello Secondario	OA	Acidemia Metilmalonica (CblD)	Cbl D
	AA	Argininemia	ARG
	AA	Citrullinemia tipo II	CIT II
	AA	Ipermetioninemia	MET
	AA	Tirosinemia tipo III	TYR III
	FAO	Deficit di Carnitina palmitoil-transferasi (L)	CPT Ia
	FAO	Acidemia glutarica tipo II	GA2
	FAO	Deficit dell'acil CoA deidrogenasi a catena corta	SCAD
	FAO	Deficit Carnitina/acil-carnitina translocasi	CACT
	OA	Deficit del 3-Metil crotonil-CoA carbossilasi	3MCC
	OA	Deficit del 2-Metil butirril-CoA deidrogenasi	2MBG
	OA	Aciduria 3-Metil glutaconica (tipo 1, 2, 3, 4 e 5)	3MGA
	OA	Deficit del Isobutirril-CoA deidrogenasi	IBG
	OA	Aciduria Malonica	MAL
	FAO	Deficit del 3-OH acil-CoA deidrogenasi a catena media/corta	M/SCHAD
	OA	Aciduria 2-Metil 3-idrossi butirrico	2M3HBA
	OA	Encefalopatia Etilmalonica	EE
	OA	Deficit di Ornitina transcarbamilasi	OTC
	AA	Deficit di metilene tetraidrofolato reduttasi	MTHFR

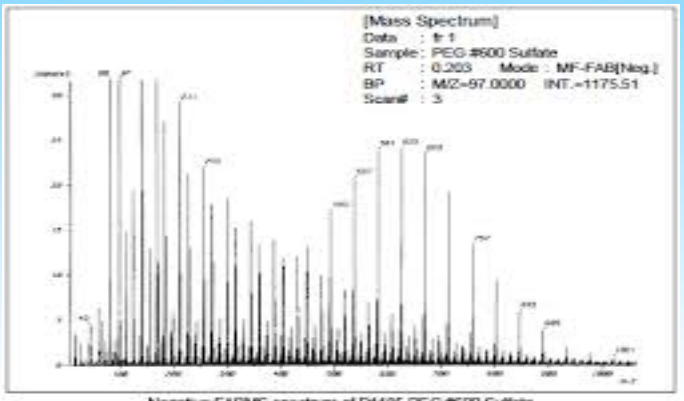
EXTENDED METABOLIC SCREENING

Mass spectrometry for EMS





- How it looks like



Negative FABMS spectrum of P1185 PEG #600 Sulfate