NEONATAL SCREENINGS

Prof. ANTONIO ANGELONI

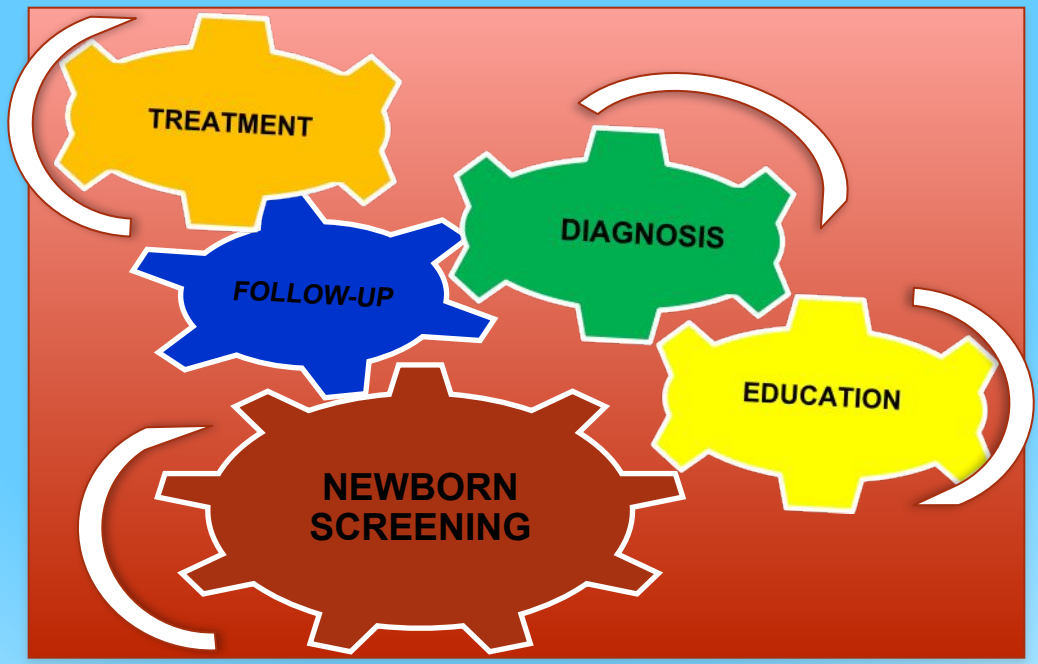
CRITERIA FOR SCREENING PROGRAMS

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: 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



What you should know before using a test (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 (Specificity)

- 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)

Newborn screening: How does it work

A drop of blood is obtained within 48-72 hrs after birth and samples are transferred to a Lab which is capable of highly specialized analyses.

Years of activation of the newborn screenings in regione Lazio.

- **1972 PHENYLKETONURIA**
- **1980 CONGENITAL HYPOTHYROIDISM**
- **1988 CYSTIC FIBROSIS**
- **1999 GALACTOSEMIA**
- **2004 EXTENDED METABOLIC SCREENING**
- **2019 DEFICIT OF BIOTINIDASIS**
- **2021 SPINAL MUSCULAR ATROPHY**

EXTENDED METABOLIC SCREENING

- **Law 167/2016:**

From 2018 is mandatc
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

CONGENITAL HYPOTHYRODISM

- Incidence: 1/3500 newbors

✔ Embryonic alterations in the development of the gland

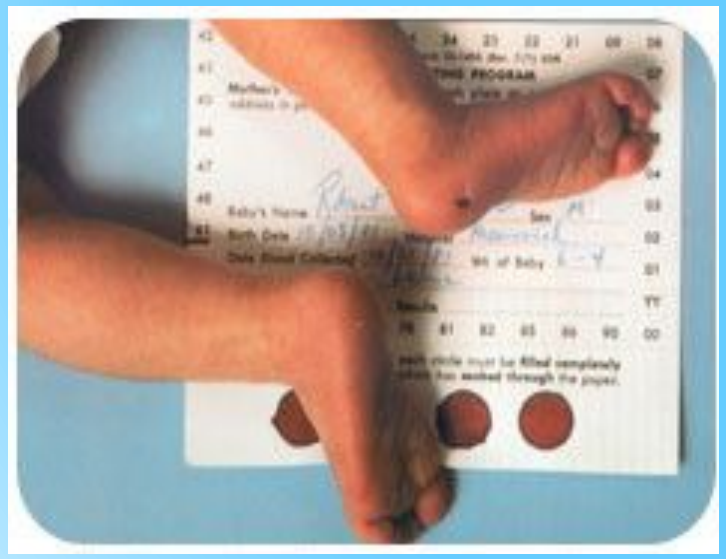
✔ Genetic defects affecting the enzymes involved in thyroid hormones synthesis

✔ Hypothalamic/pituitary axis deficit

❖ **Mental and growth retardation**

NEONATAL DIAGNOSIS

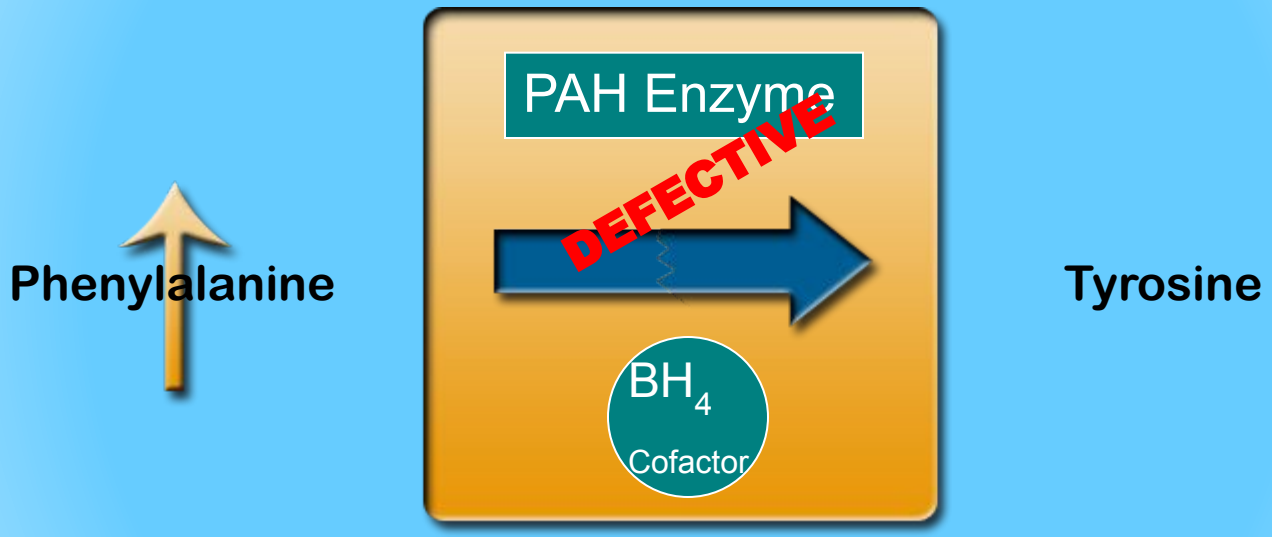
- Determination of TSH within day 3 (PPV 95-98% of the cases).



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

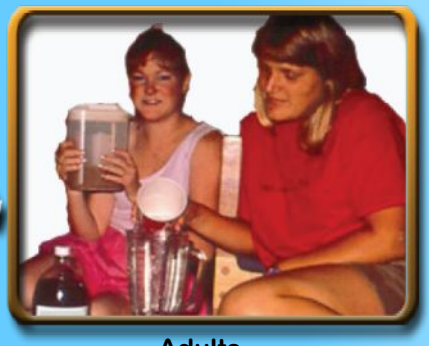
Success of the diet followed newborn screening

- "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 on PKU

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 important.

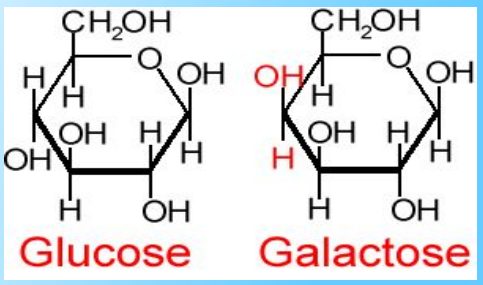
Despite the overall success of the PKU diet, adherence into adulthood continues to be a problem.

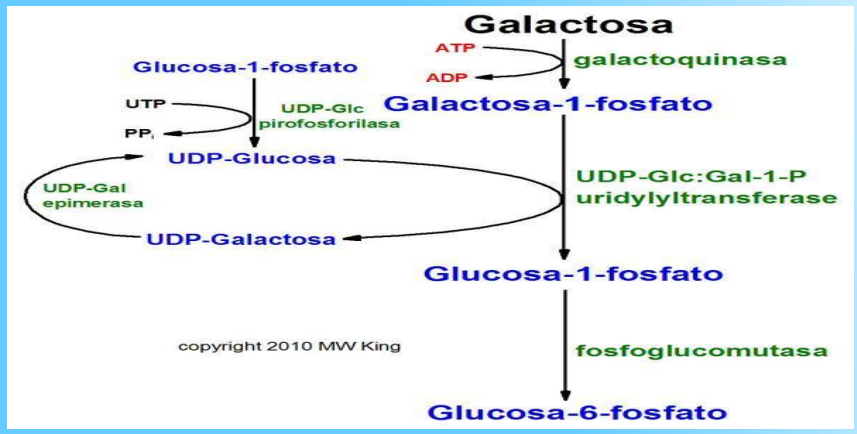
G A L A C T O S E M I A

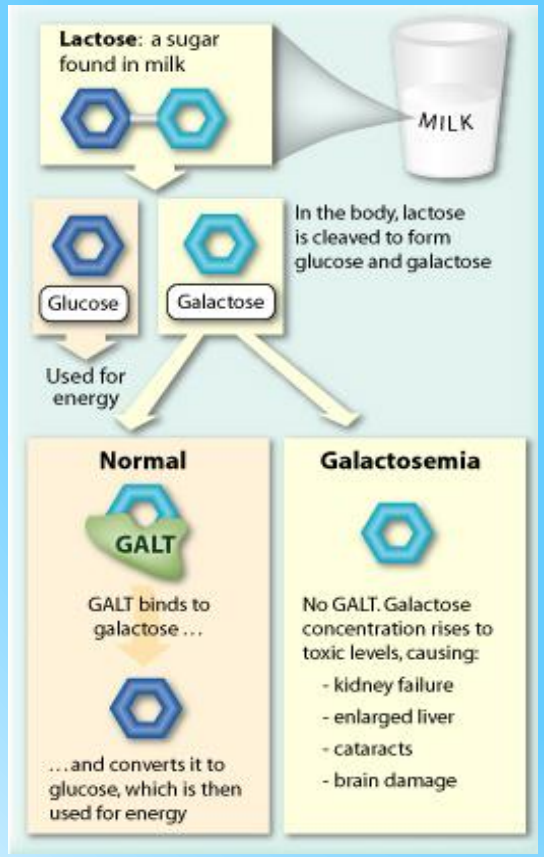
- Galactosemia is an inherited recessive deficiency in enzymes that metabolize galactose

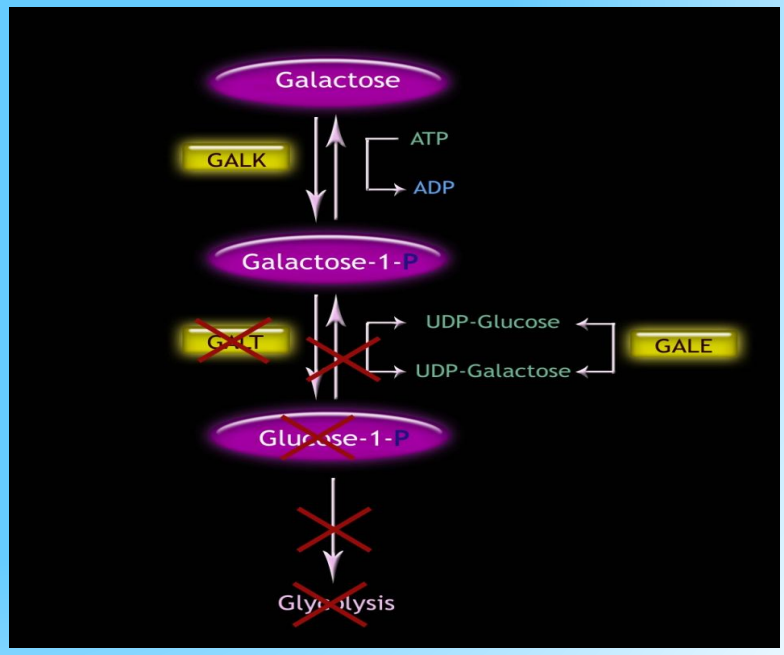
- 1 in 60 000 newborns are diagnosed with Galactosemia every year

Sources and metabolism of galactose









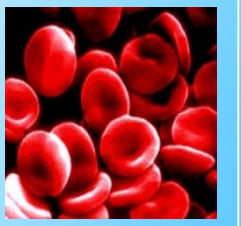
NORMAL GALACTOSE METABOLISM

- 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

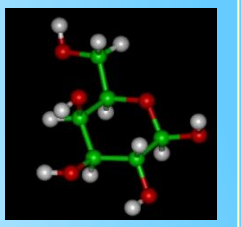
- Glu-1-P proceeds on to glycolysis
- UDP-Galactose is recycled back to UDP-Glucose by *Uridyl Diphosphate Galactose 4-Epimerase* (*GALE*)

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

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)

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

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.

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 affected 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

PANNELLO DM 13 ottobre 2016

15-11-2016 GAZZETTA UFFICIALE DELLA REPUBBLICA ITALIANA *Serie generale* - n. 267

ALLEGATO

Tabella 1. Malattie metaboliche ereditarie oggetto di screening neonatale con metodica di massa tandem

Malattia	Acronimo	Numero MIM	Gruppo (**)	Denominazione del Gruppo Patologia (D.M 279/2001 All. N.1)	Cod. di esenzione (D.M 279/2001 All. N.1)	Marker primari (vedi legenda***)
Fenilchetonuria*	PKU	261600				Phe
Iperfenilalaninemia benigna	HPA	261600				Phe
Deficit della biosintesi del cofattore biopterina	BIOPT (BS)	261640				Phe
Deficit della rigenerazione del cofattore biopterina	BIOPT (REG)	261630				Phe
Tirosine						SUAC
Tirosine						Tyr
Malattia						Val Xleu
Omocist						Met alta
Omocist						Met bassa
Acidemi						C5-DC
Acidemi						C5
Deficit d						C5:1 C5-OH
Acidemia 3-idrossi 3-metilglutarica	HMG	246450				C5-OH C6-DC
Acidemia propionica	PA	606054				C3
Acidemia metilmalonica (Mut)	MUT	251000				C3
Acidemia metilmalonica (Cbl-A)	Cbl A	251100	OA			C3
Acidemia metilmalonica (Cbl-B)	Cbl B	251110				C3
Acidemia metilmalonica con omocistinuria (deficit Cbl C)	Cbl C	277400				C3 alta Met bassa
Acidemia metilmalonica con omocistinuria (deficit Cbl D)	CblD	277410				C3 alta elo Met bassa
Deficit di 2-metilbutirril-CoA deidrogenasi	2MBG	610006				C5
Aciduria malonica	MAL	606761				C3-DC
Deficit multiplo di carbossilasi	MCD	253270				C5-OH
Citrullinemia tipo I	CIT I	215700				Cit
Citrullinemia tipo II (deficit di Citrina)	CIT II	605814				Cit
Acidemia argininosuccinica	ASA	207900	UCD	DISTURBI DEL CICLO DELL'UREA	RCG050	ASA
Argininemia	ARG	207800				Arg
Deficit del trasporto della carnitina	CUD	212140				C0 bassa
Deficit di carnitina palmitoil-trasferasi I	CPT Ia	255120				C0 alta C16 bassa C18 bassa
Deficit carnitina-acilcarnitina traslocasi	CACT	212138				C16 C18:2 C18:1 C18
Deficit di carnitina palmitoil-trasferasi II	CPT II	600650		O DELLE LIPOPROTEINE		C16 C18:2 C18:1 C18

36 condizioni: pannello primario
- 13 Aminoacidopatie
- 13 Acidurie organiche
- 10 Deficit della β-ossidazione degli acidi grassi

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Deficit di acil-CoA deidrogenasi a catena molto lunga	VLCAD	609575	FAO	RCG070	C14:2 C14:1 C14
Deficit della proteina trifunzionale mitocondriale	TFP	609015			C16:1-OH C16-OH C18:1-OH C18-OH
Deficit di 3-idrossi-acil-CoA deidrogenasi a catena lunga	LCHAD	609016			C16:1-OH C16-OH C18:1-OH C18-OH
Deficit di acil-CoA deidrogenasi a catena media	MCAD	201450			C6 C8 C10:1 C10
Deficit di 3-idrossi-acil-CoA deidrogenasi a catena media/corta	MSCHAD	231530			C4-OH
Acidemia glutarica tipo II	GA II/MADD	231680			da C4 a C18 sature e insature

ALTERAZIONI CONGENITE DEL METABOLISM

Si segnala la possibilita', per alcune condizioni e per cause fisiologiche, di normali concentrazioni del biomarcatore in epoca neonatale pur in presenza di patologia (falso negativo).

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Tabella 3. Malattie metaboliche ereditarie che entrano in diagnosi differenziale con le malattie oggetto di screening neonatale con metodica di massa tandem elencate in tabella 1 in quanto condividono i biomarcatori primari

Deficit di S-adenosilomocisteina idrolasi	SAHH	613752	Met
Acidurie 3-metil glutaconiche	3MGCA		C5-OH
Deficit di 3-metilcrotonil-CoA carbossilasi	3MCC	210200	C5-OH
Deficit di 2-metil 3-idrossibutirril-CoA deidrogenasi	2M3HBA	300438	C5:1 C5-OH
Deficit di Isobutirril-CoA deidrogenasi	IBG	271980	C4
Deficit di acil-CoA deidrogenasi a catena corta	SCAD	606885	C4

9 condizioni: pannello secondario
- 4 Aminoacidopatie
- 5 Acidurie organiche

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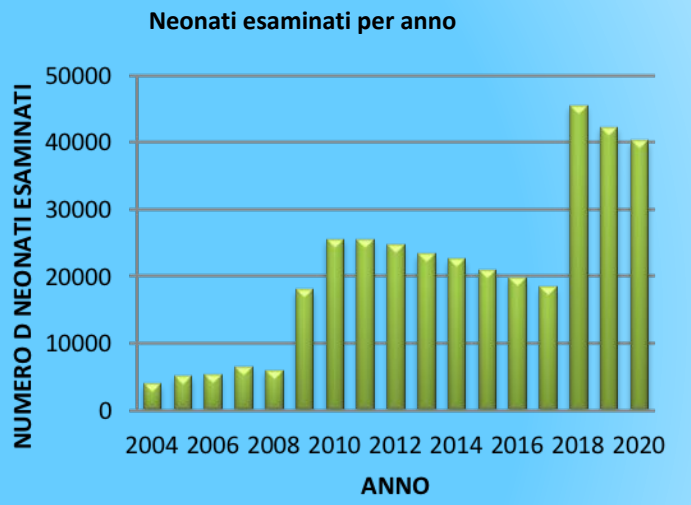
Tabella 2. Malattie metaboliche ereditarie oggetto di screening neonatale con metodiche diverse dalla spettrometria di massa tandem

Malattia	Acronimo	Numero MIM	Denominazione del Gruppo Patologia (D.M 279/2001 All. N.1)	Cod. di esenzione (D.M 279/2001 All. N.1)
Galattosemia	GALT	230400	DISTURBI DEL METABOLISMO E DEL TRASPORTO DEI CARBOIDRATI	RCG060
Difetto di biotinidasi	BTD	253260	DISTURBI DEL METABOLISMO E DEL TRASPORTO DEGLI AMINOACIDI	RCG040

Galattosemia
Deficit di Biotinidasi

SCREENING NEONATALE ESTESO NEL POLICLINICO UMBERTO I: DAL PROGETTO PILOTA AL PROGRAMMA OPERATIVO

- 2004 inizio dello studio pilota: applicazione dello screening esteso a circa il 20 % della nostra popolazione (nati nelle AOU Policlinico Umberto I, S. Eugenio e S .Giovanni)
- da Giugno 2009 screening pilota è stato esteso a tutto il bacino di utenza del centro: 49 % Lazio e 100% Molise
- dal 01/01/2018 avvio del programma di screening neonatale esteso per tutti i nati nel Lazio e Molise

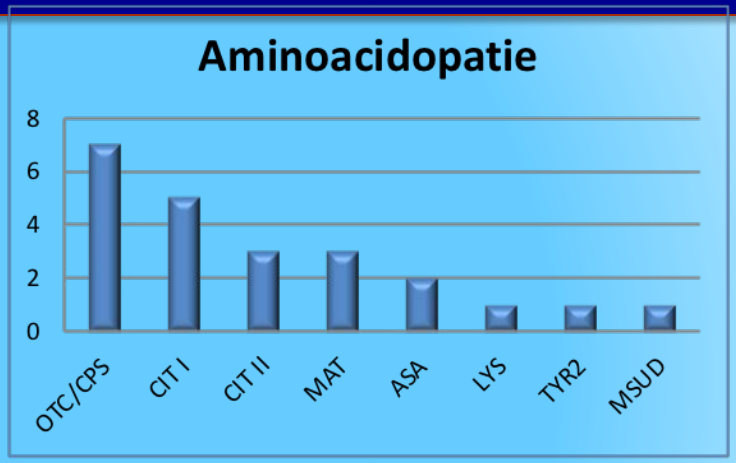


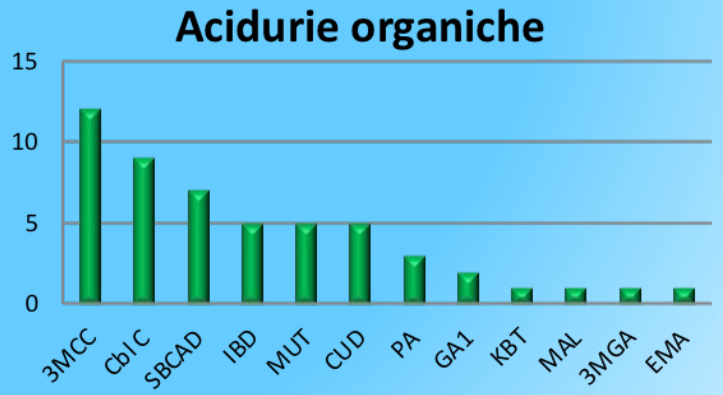
NEONATI SOTTOPOSTI A SCREENING ESTESO: 313776

SCREENING ESTESO NEL LAZIO: DAL PROGETTO PILOTA AL PROGRAMMA OPERATIVO

128 NEONATI POSITIVI CONFERMATI

	2004-2017	2018-2020
Campioni analizzati	226473	128011
Positivi	83	45
INCIDENZA COMPLESSIVA	1:2729	1:2845
SPECIFICITA'	98.5 %	99.1%
SENSIBILITA'	100 %	100%



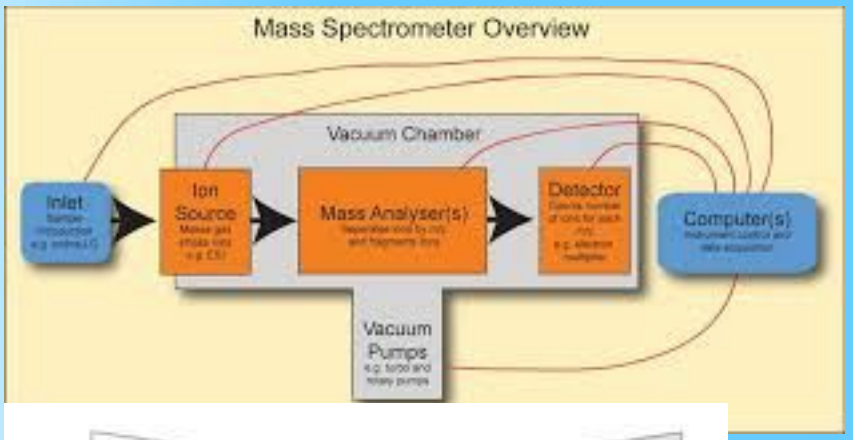


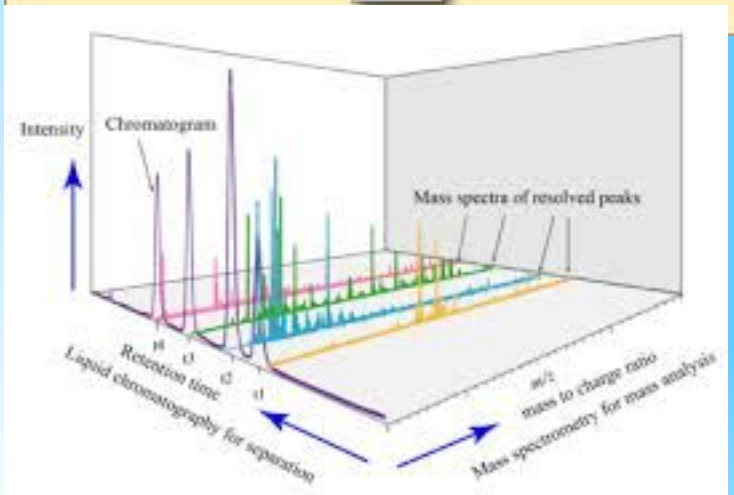
NEWBORN SCREENING (DATA FROM 2010)

ANNO	NEONATI ESAMINATI	DIAGNOSI * SCREENING DI BASE				DIAGNOSI SCREENING BIOTINIDASI	DIAGNOSI * SCREENING METABOLICO
		HPA	IC	FC	GAL		
2010	25550	12	24	3	1		11
2011	25509	9	22	5	1		7
2012	24748	9	24	2	3		7
2013	23330	8	29	4	4		11
2014	22585	9	28	3	1		10
2015	21017	11	26	7	2		9
2016	19756	8	24	8	2		9
2017	18448	9	17	5	1		8
2018	45499 §	19	40	10	7		18
2019	42235	17	61	11	2@	12	14
2020	40277	13	46	8	4	11	13
TOT parziale (dal 2010)	308954	124	341	66	28	23	117
	INCIDENZA COMPLESSIVA 2010-2019 1:442						

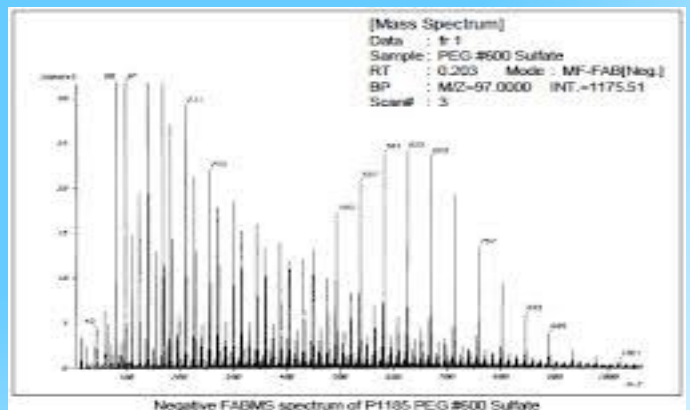
EXTENDED METABOLIC SCREENING

Mass spectrometry for EMS





- How it looks like



Negative FABMS spectrum of P1185 PEG #600 Sulfate

Newborn screening for SMA

- ✔ SMA is a genetic neuromuscular disease.
- ✔ It is caused by a mutation affecting the SMN gene which code a defective protein, thus affecting surviving of motoneurons.
- ✔ In severe SMA (type I) clinical evidences are present at 6 months ffcting all the neuromuscular districts, not only bein responsible for impairment of the movements but also for respiratory activity. If untreated it leads to death within 2 years.

Tecnologia oligonucleotidi antisenso: Nursinen

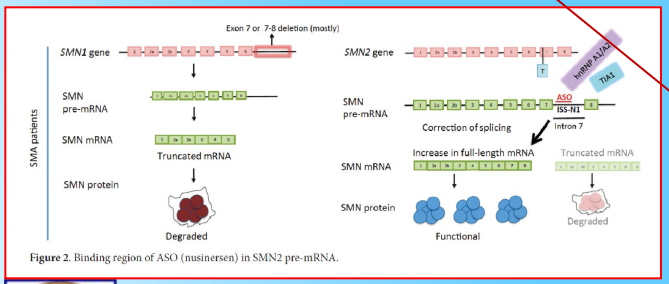
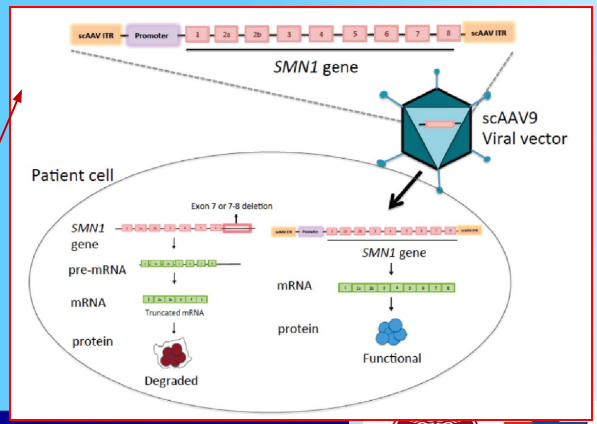


Figure 2. Binding region of ASO (nusinersen) in SMN2 pre-mRNA.

New therapies are now available that are changing the clinical outcome of SMA

Terapia genica : Onasemnogene abeparvovec



Screening for SMA

- ✔ A pilot study has been carried out in Lazio and Tuscany from 2019-2021.
- ✔ **Risultati**: Compliance of the screening has been about 90%. 60.000 newborns have been screened and 8 identified as affected by the disease. All of them have been treated and are currently in good health. Incidence has been calculated about 1/7500