Museo Storico della Città
Martedì 2 Giugno 2015
Lushnja, Ore 10

CONFERENZA SCIENTIFICA SULLA TALASSEMIA

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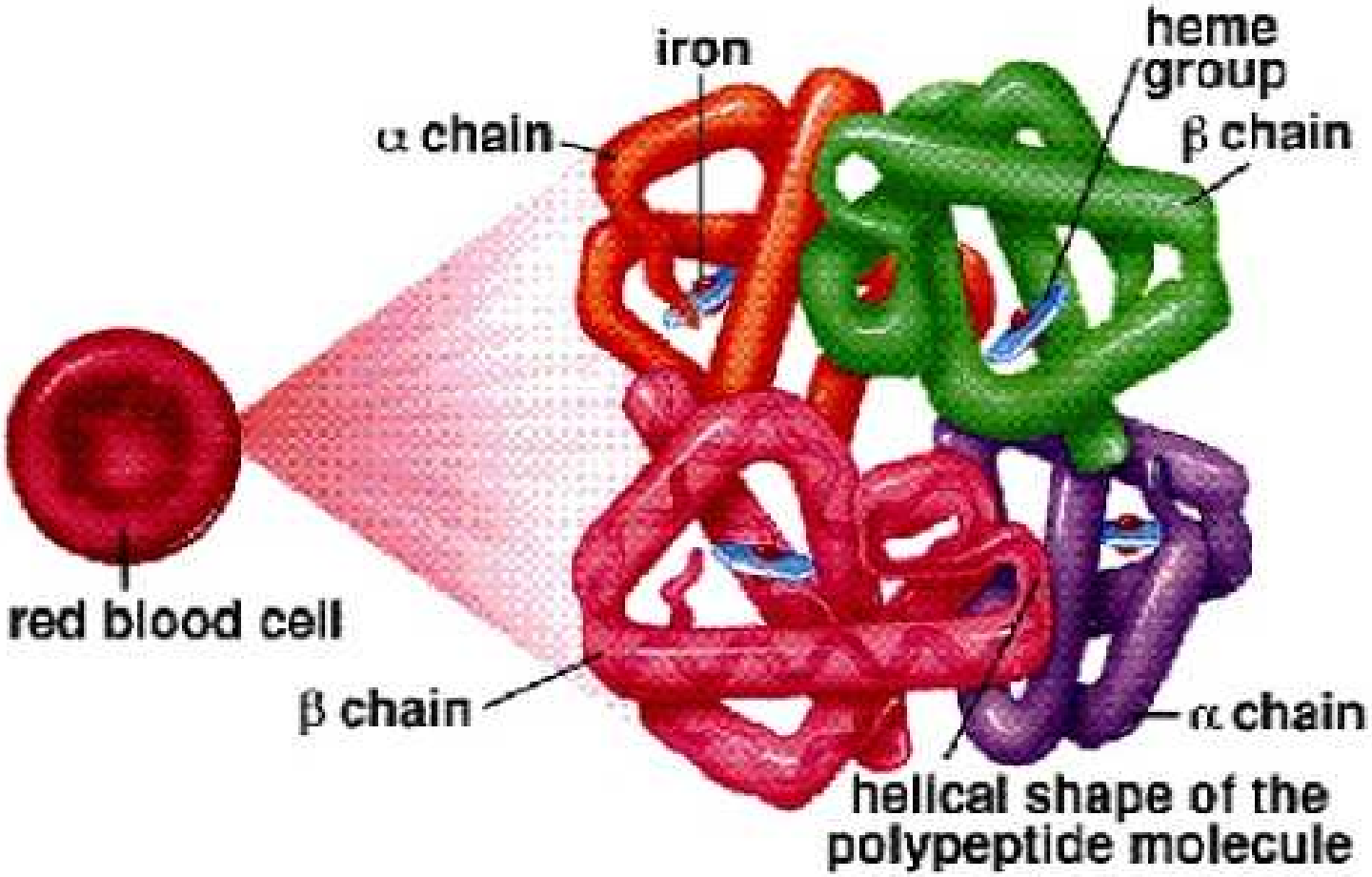
Genetista Medico

Ricercatore Universitario

Hemoglobin Structure

- Four subunits
  - two α
  - two β
- Iron
- Heme
- Binds 4 O_2

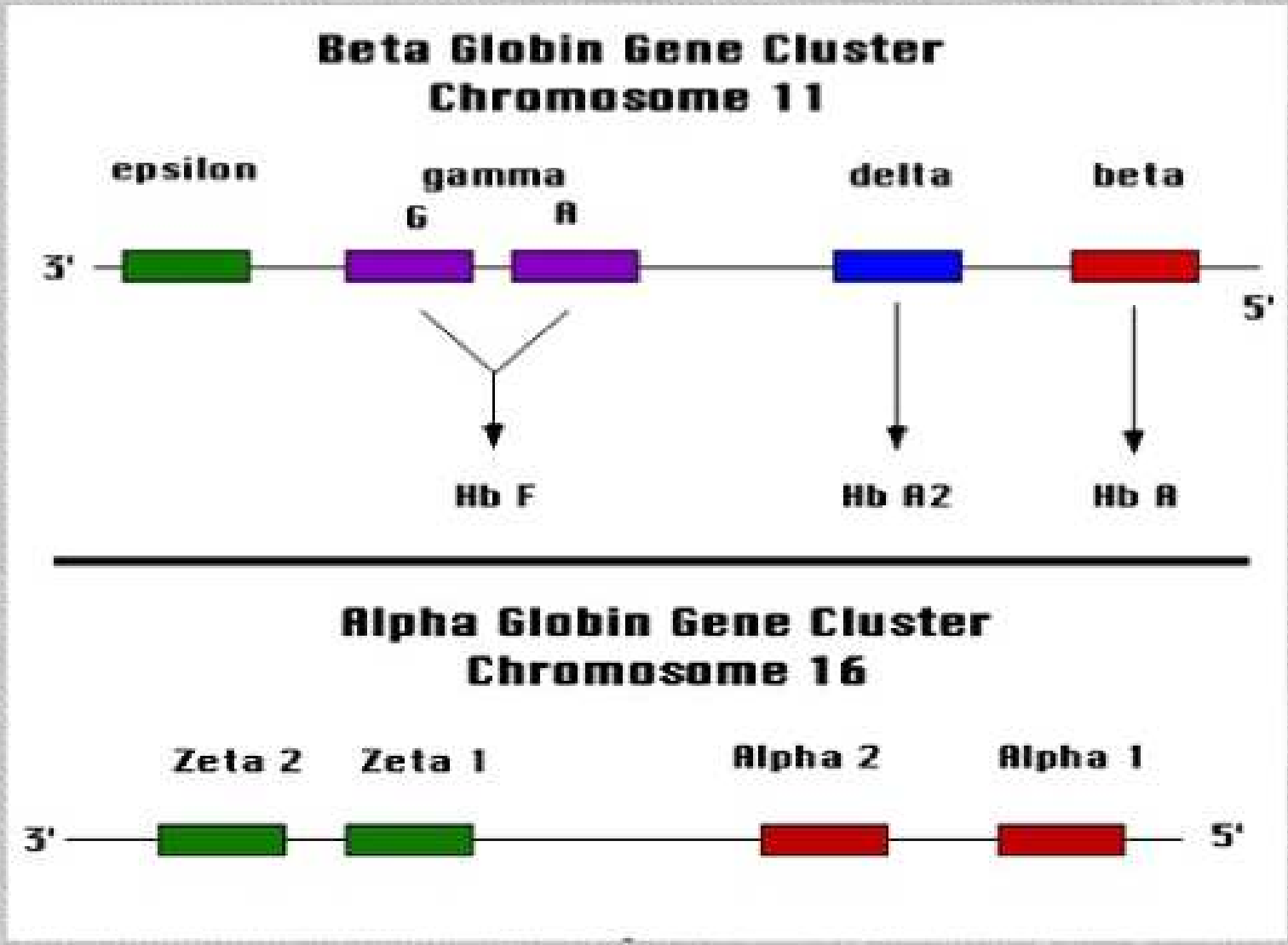


Emoglobine fisiologiche nell'uomo

Haemoglobin	Globin chains	Period of life when present
Gower 1	$\zeta_2\varepsilon_2$	Embryo
Gower 2	$\alpha_2\varepsilon_2$	Embryo
Portland 1	$\zeta_2\gamma_2$	Embryo
Haemoglobin F	$\alpha_2\gamma_2$	Embryo, fetus and neonate; minor component during adult life
Haemoglobin A	$\alpha_2\beta_2$	Minor component in fetus, increasing late in gestation and in the neonatal period to become the major haemoglobin during infancy, childhood and adult life
Haemoglobin A_2	$\alpha_2\delta_2$	Very low levels in infancy; minor component in childhood and adult life

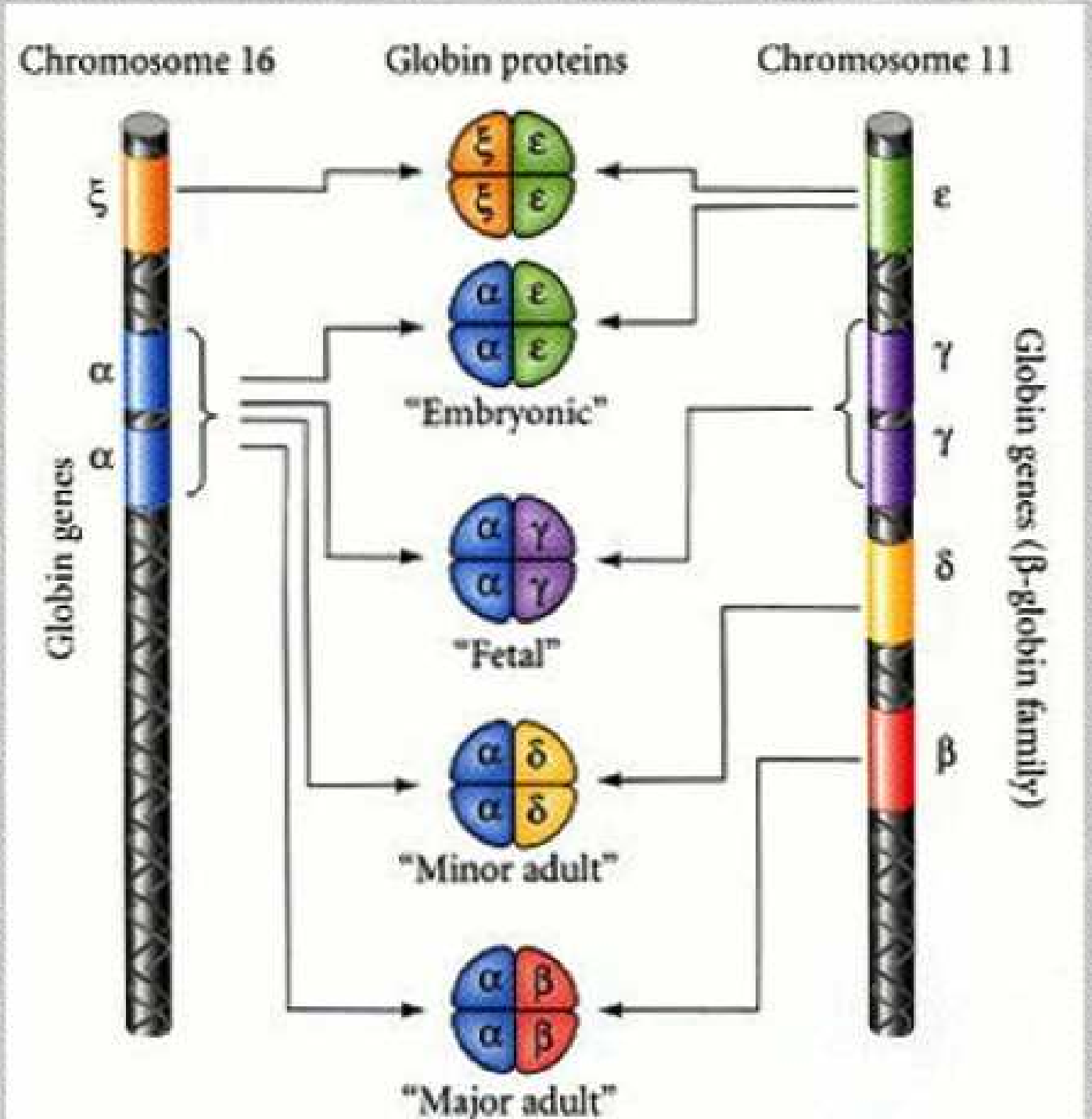
Geni delle catene globiniche

Chr. 11

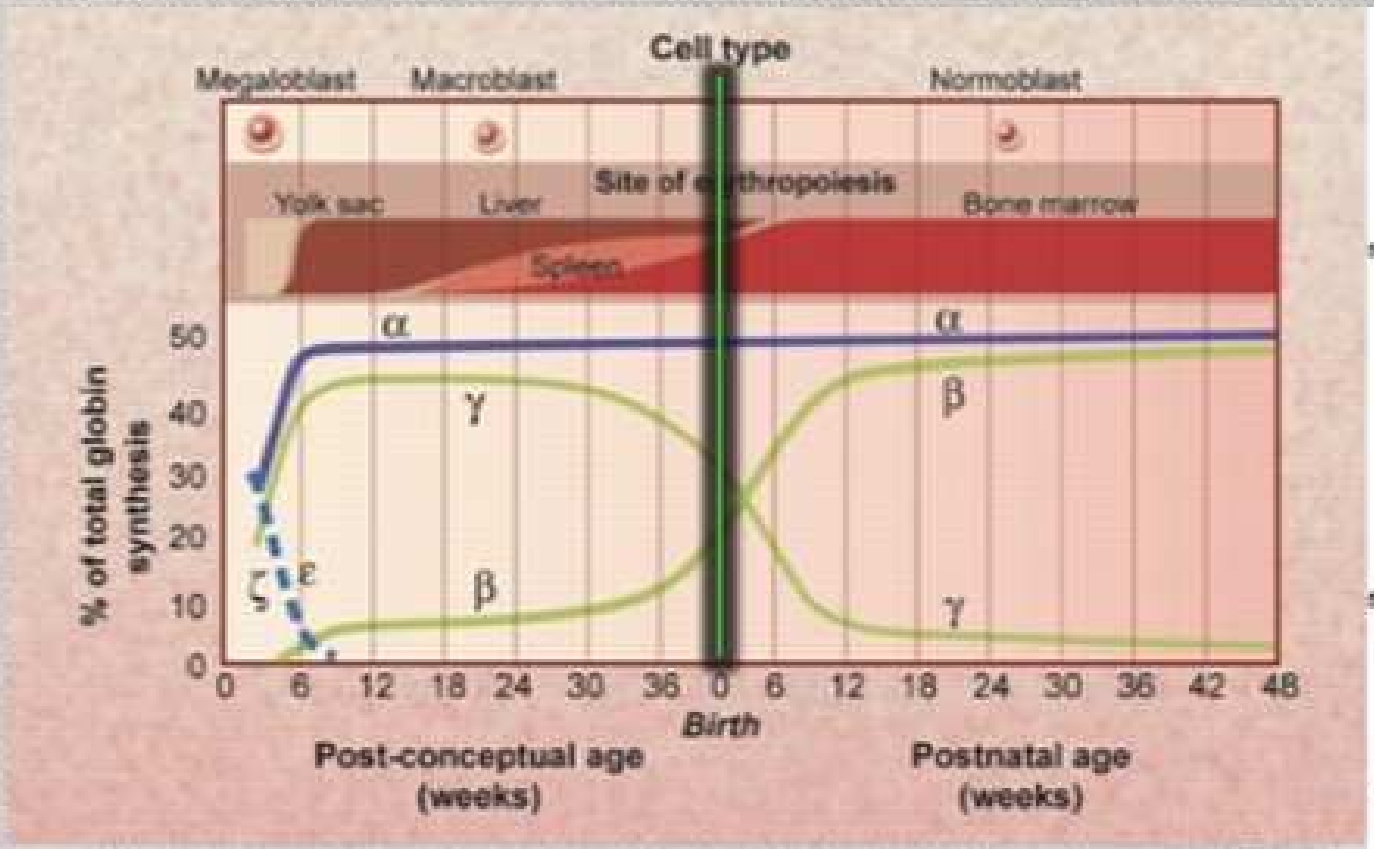


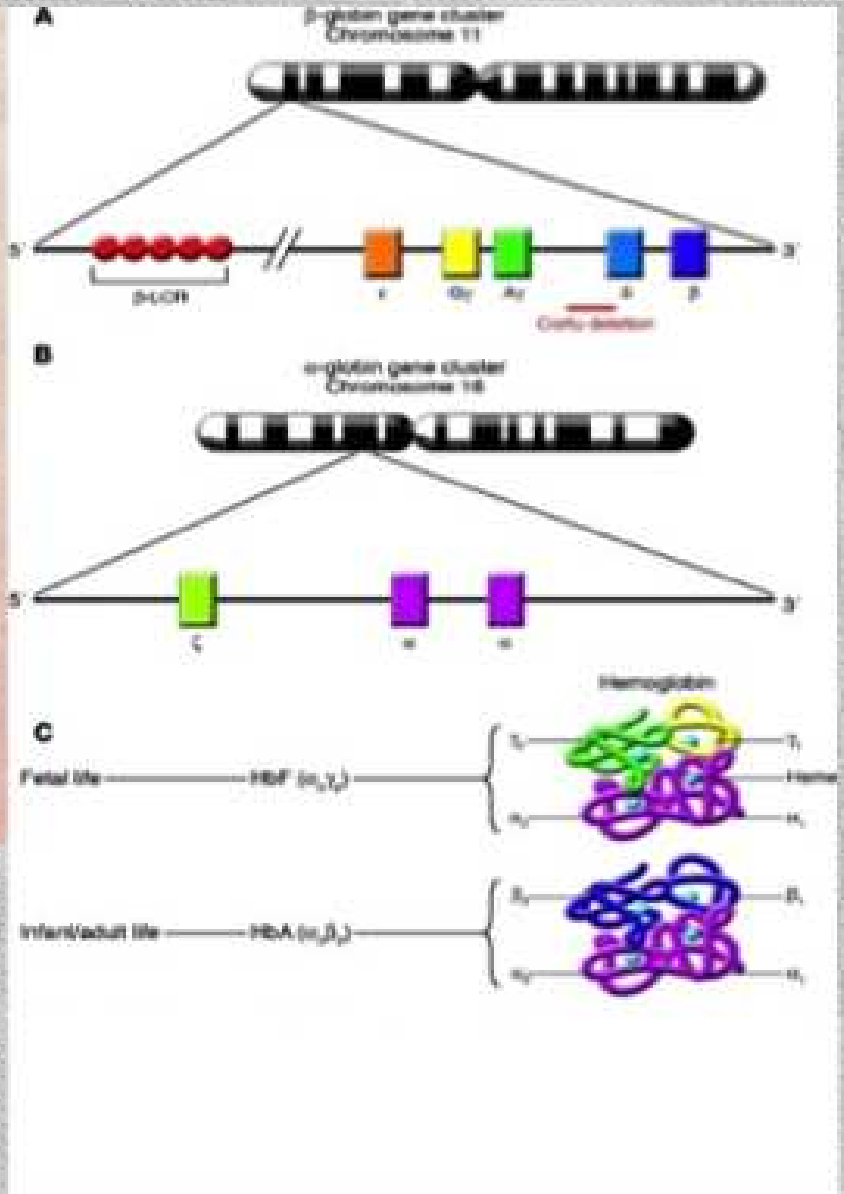
Chr. 16

Dai Geni alle Globine

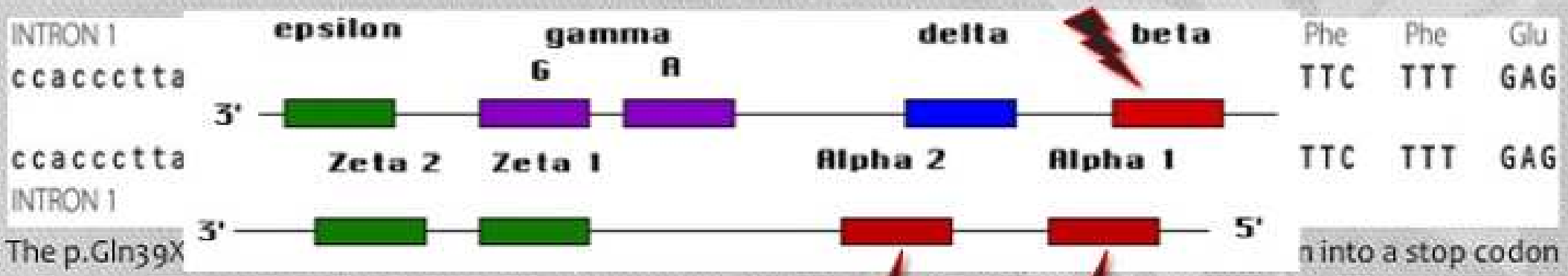


Un emoglobina per ogni età...





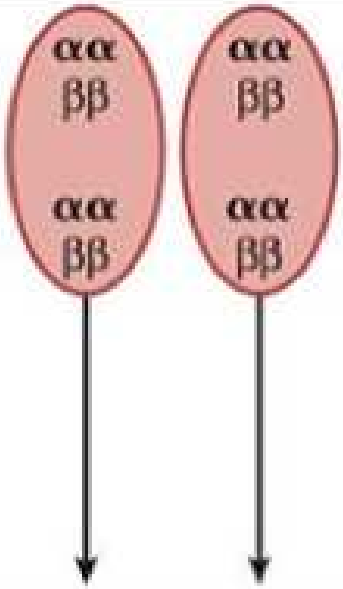
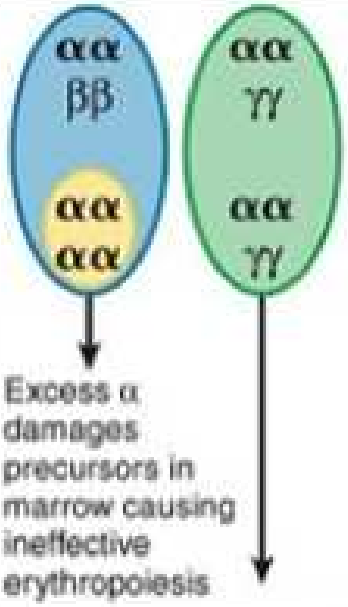
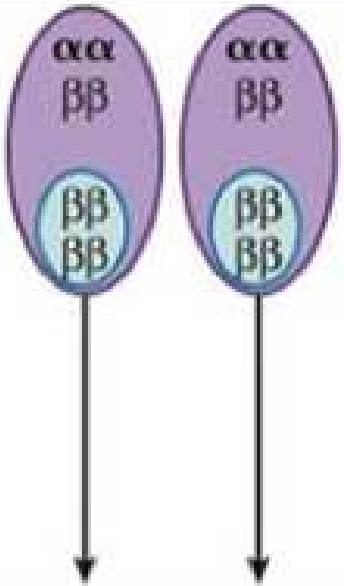
Mutazioni nei geni globinici → fenotipo



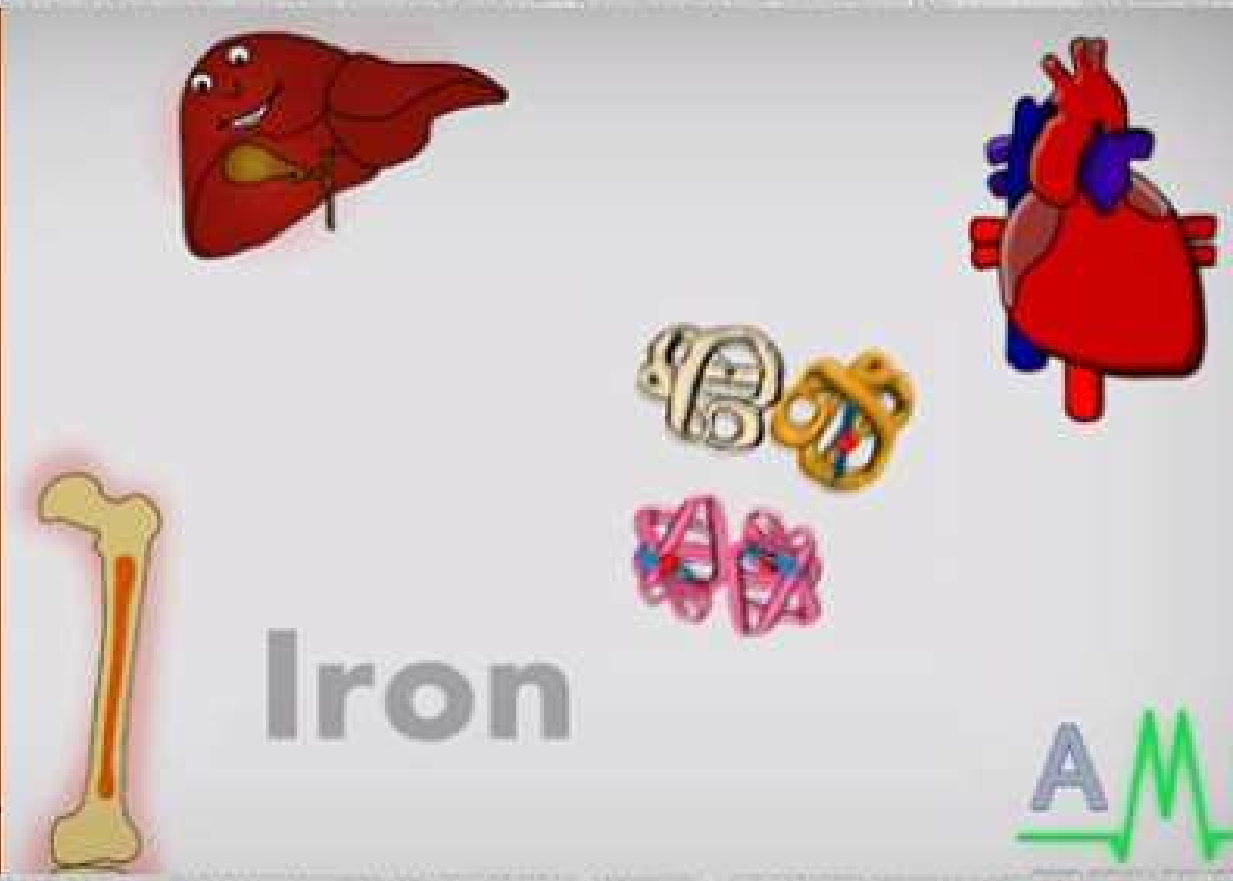
Diverse mutazioni → Diverse globine

- **Hemoglobin S** ($\alpha_2\beta^S_2$, severe). This the predominant hemoglobin in people with sickle cell disease.
- **Hemoglobin Constant Spring** named after isolation in a Chinese family from the Constant Spring district of Jamaica (severe). In this variant, a mutation in the alpha globin gene produces an alpha globin chain that is abnormally long. Both the mRNA and the alpha chain protein are unstable.
- **Hemoglobin H** (β_4, mild). This is a tetramer composed of four beta globin chains: it occurs only with extreme limitation of alpha chain availability. Hemoglobin H forms in people with three-gene alpha thalassemia as well as in people with the combination of two-gene deletion alpha thalassemia and hemoglobin Constant Spring.
- **Hemoglobin Barts** (γ_4, lethal). With four-gene deletion alpha thalassemia no alpha chain is produced. The gamma chains produced during fetal development combine to form gamma chain tetramers. Individuals with four-gene deletion thalassemia and consequent hemoglobin Barts die in utero (hydrops fetalis).

Alterata sintesi delle globine - patogenesi

	Normal	Severe β thalassemia	Severe α thalassemia (hemoglobin H disease)
Globin production	Balanced α and β production	Decreased β production	Decreased α production
Bone marrow			
Peripheral blood	Normal hemoglobin composition (97% hemoglobin A)	Selective survival of cells producing hemoglobin F	Hemoglobin H detectable in blood. Hemoglobin H precipitates and damages RBCs, causing hemolysis

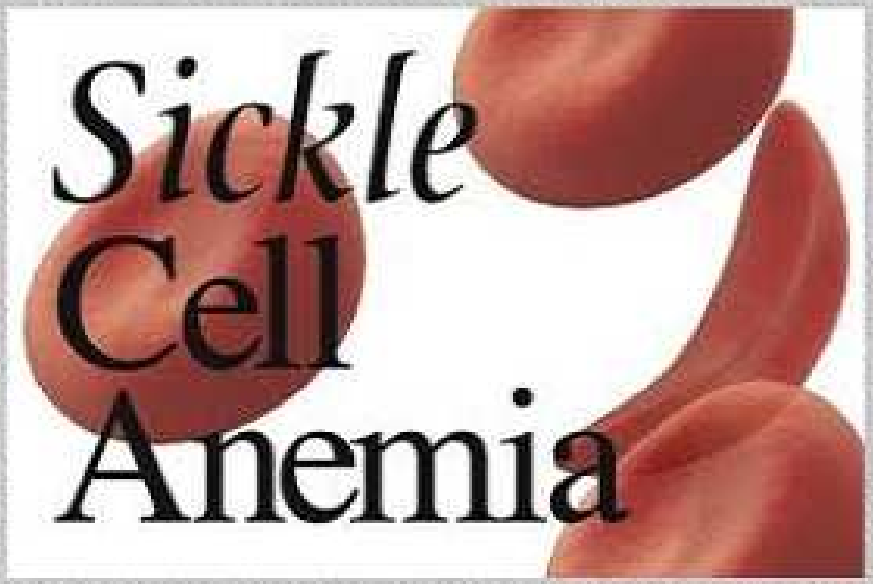
Alterata sintesi delle globine - patogenesi

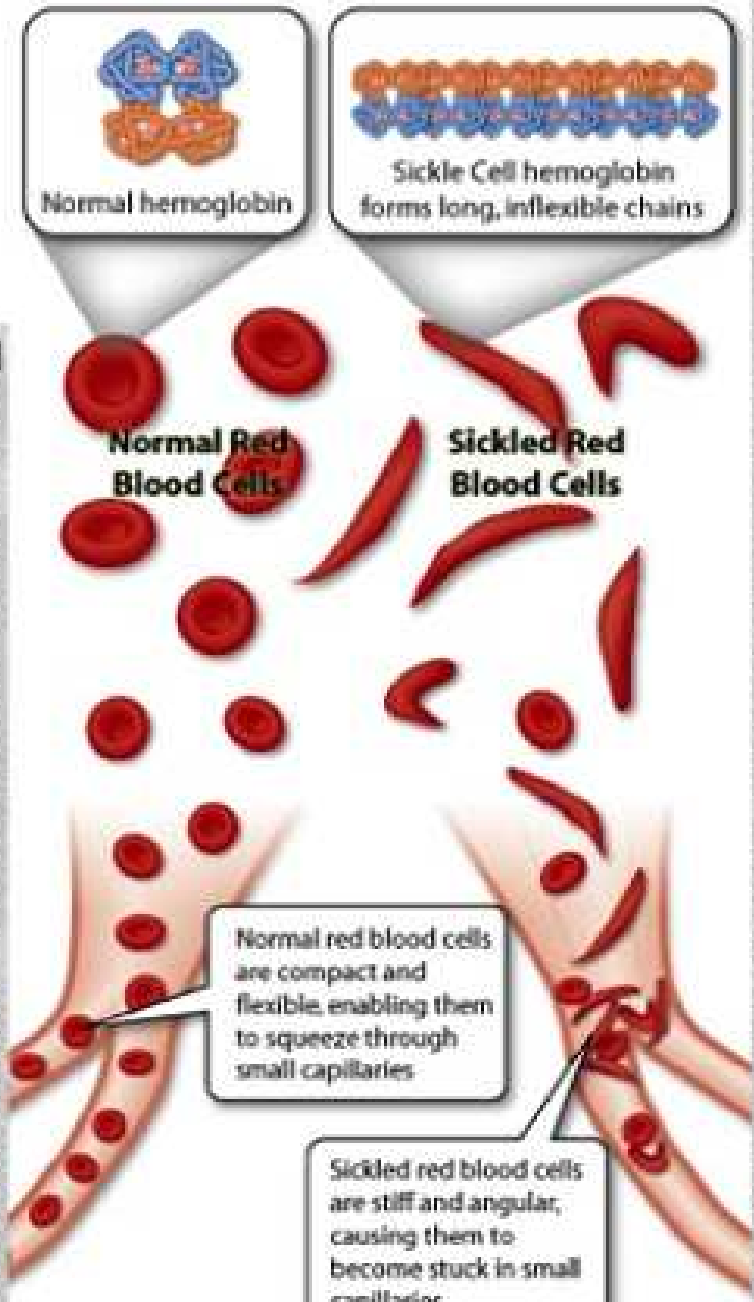


Anemia falciforme - **HbS**

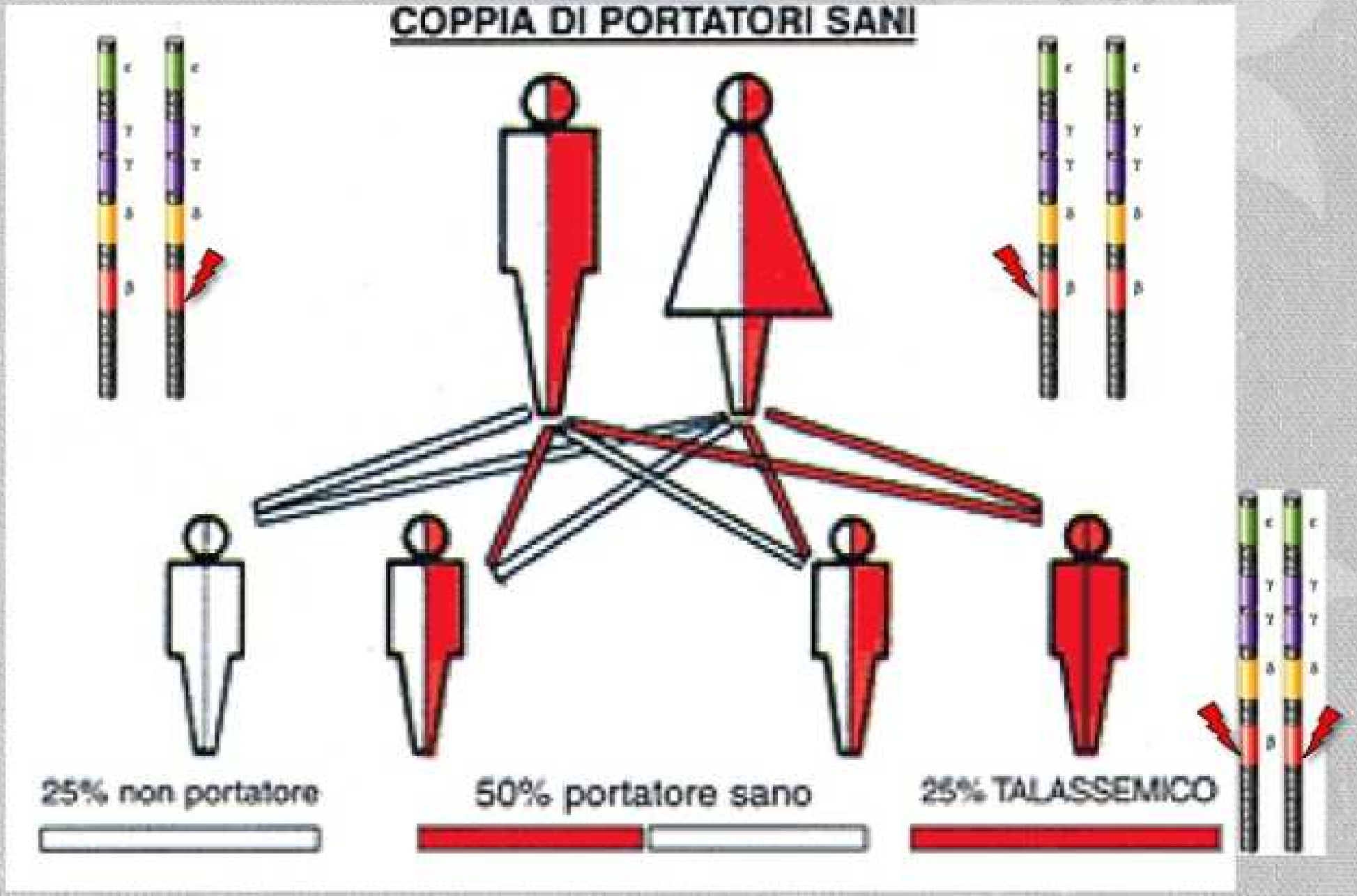
	Val	His	Leu	Thr	Pro
NORMAL	GTG	CAT	CTG	ACT	CCT
SICKLE	GTG	CAT	CTG	ACT	CCT
	Val	His	Leu	Thr	Pro

In sickle cell disease an A>T nucleotide substitution causes glutamic acid the β-globin protein.

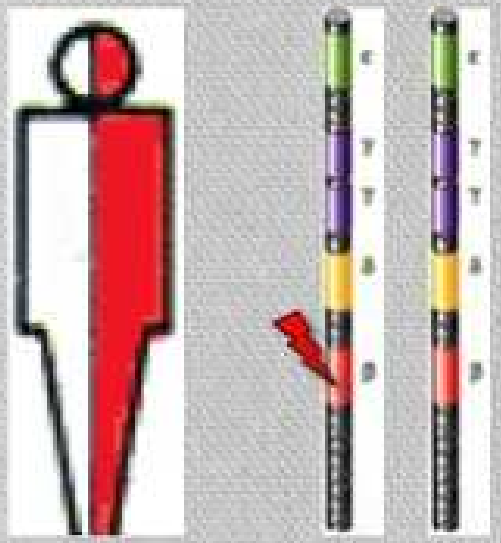




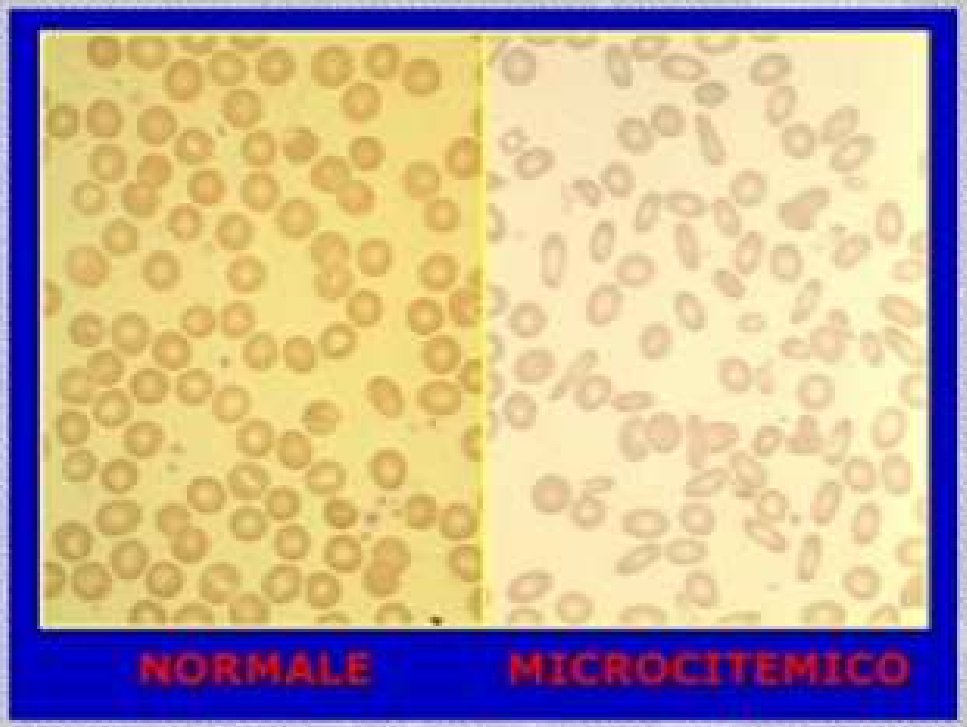
Modello di ereditarietà della talassemia



Endofenotipo talassemia: **microcitemia**

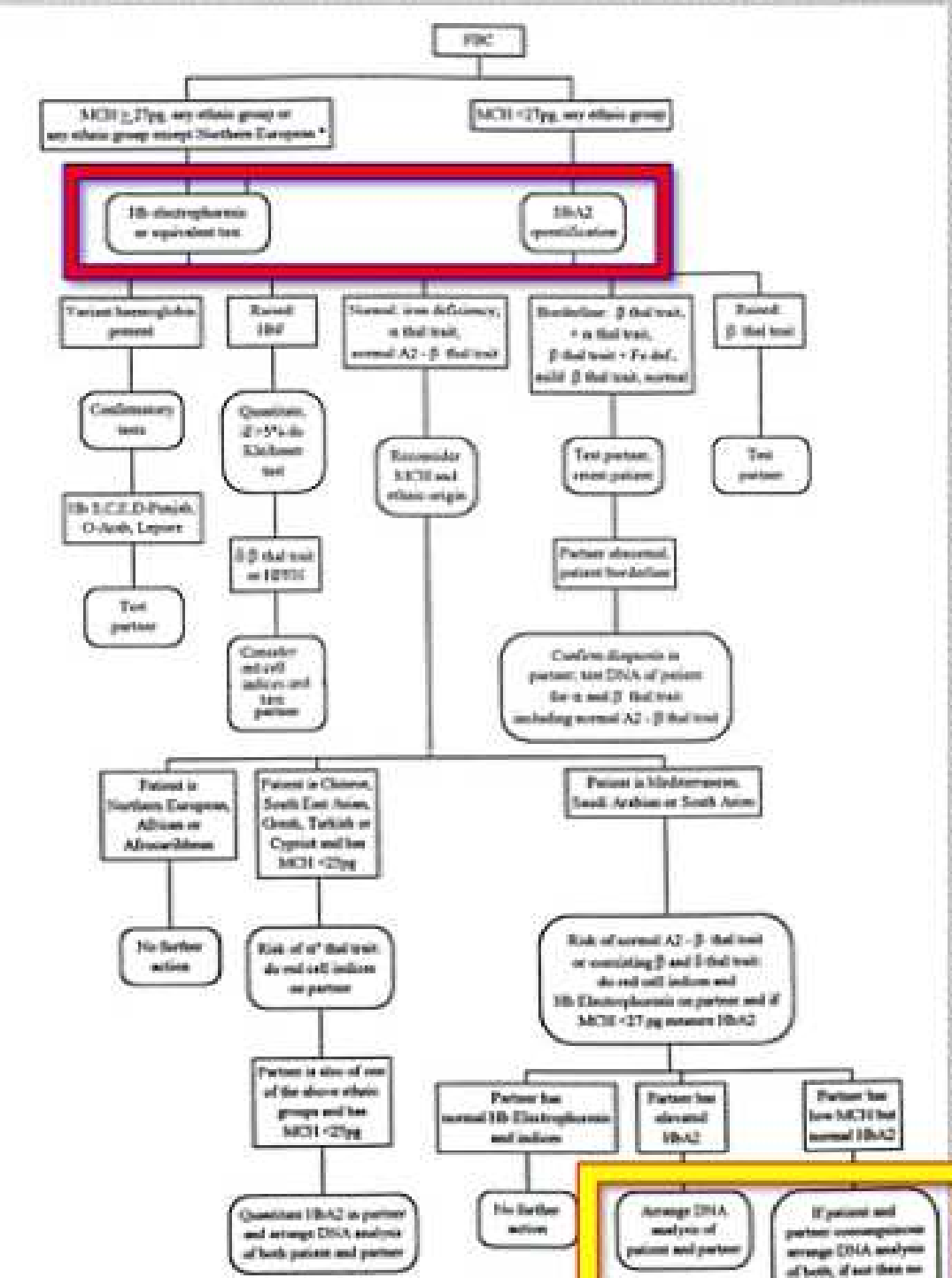


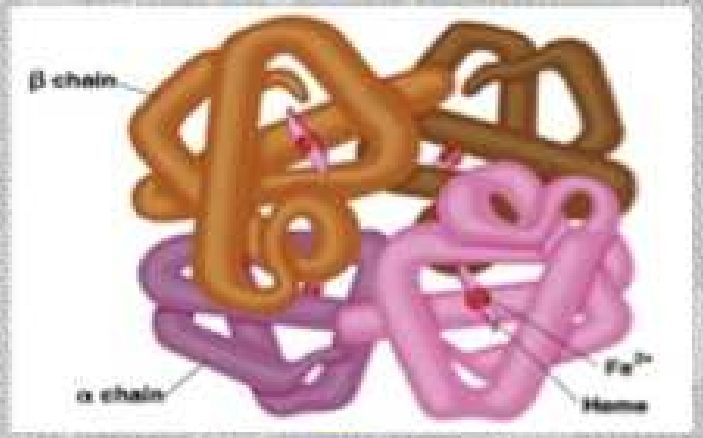
Fenotipo cellulare



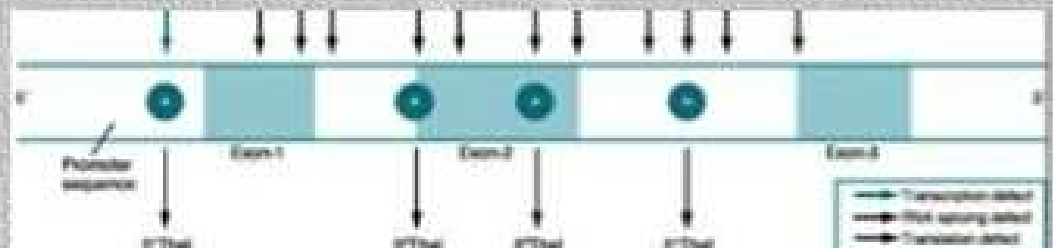
Possibile identificare i portatori sani mediante test NON GENETICI !

Flow-chart diagnostica no genetics without chemistry!





elettroforesi



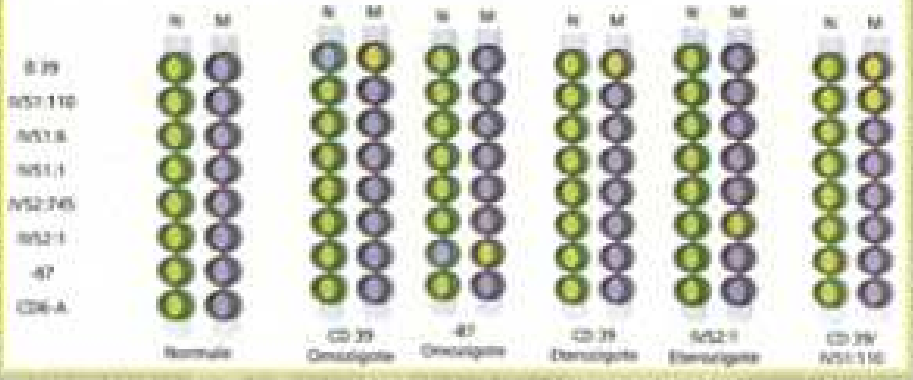
Analisi genetica: **non una** ma **diversi tipi**

Table 5.1 The principal methods of DNA diagnosis of the haemoglobinopathies.

DISORDER AND MUTATION TYPE	DIAGNOSTIC METHOD
α^0-thalassaemia	Gap-PCR, MLPA
α^+-thalassaemia: deletion non deletion	Gap-PCR, MLPA ASO, RE, DGGE, Sanger sequencing
β-thalassaemia: deletion non deletion	Gap-PCR, MLPA ASO, RDB, ARMS, RE-PCR, Sanger sequencing
δβ-thalassaemia	Gap-PCR, MLPA
HPFH deletion non deletion	Gap-PCR, MLPA ASO, ARMS, RE-PCR, Sanger sequencing
Hb Lepore	Gap-PCR, MLPA
HbS	ASO, RDB, ARMS, RE-PCR, pyrosequencing
HbC	ASO, RDB, ARMS, pyrosequencing
HbE	ASO, RDB, ARMS, RE-PCR, pyrosequencing
Hb D-Punjab	ASO, RDB, ARMS, RE-PCR, Sanger sequencing
Hb O-Arab	ASO, ARMS, RE-PCR, Sanger sequencing
Hb variants	Sanger sequencing

Reverse dot-blot

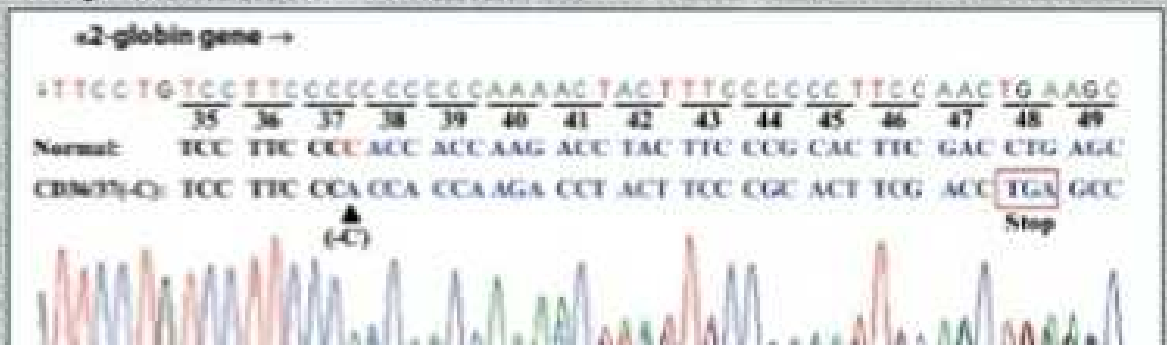
La rivelazione dell'avvenuta ibridazione attraverso una reazione enzimatico-colorimetrica (colore giallo), indica quali sequenze (normali o mutate) sono presenti nel campione.

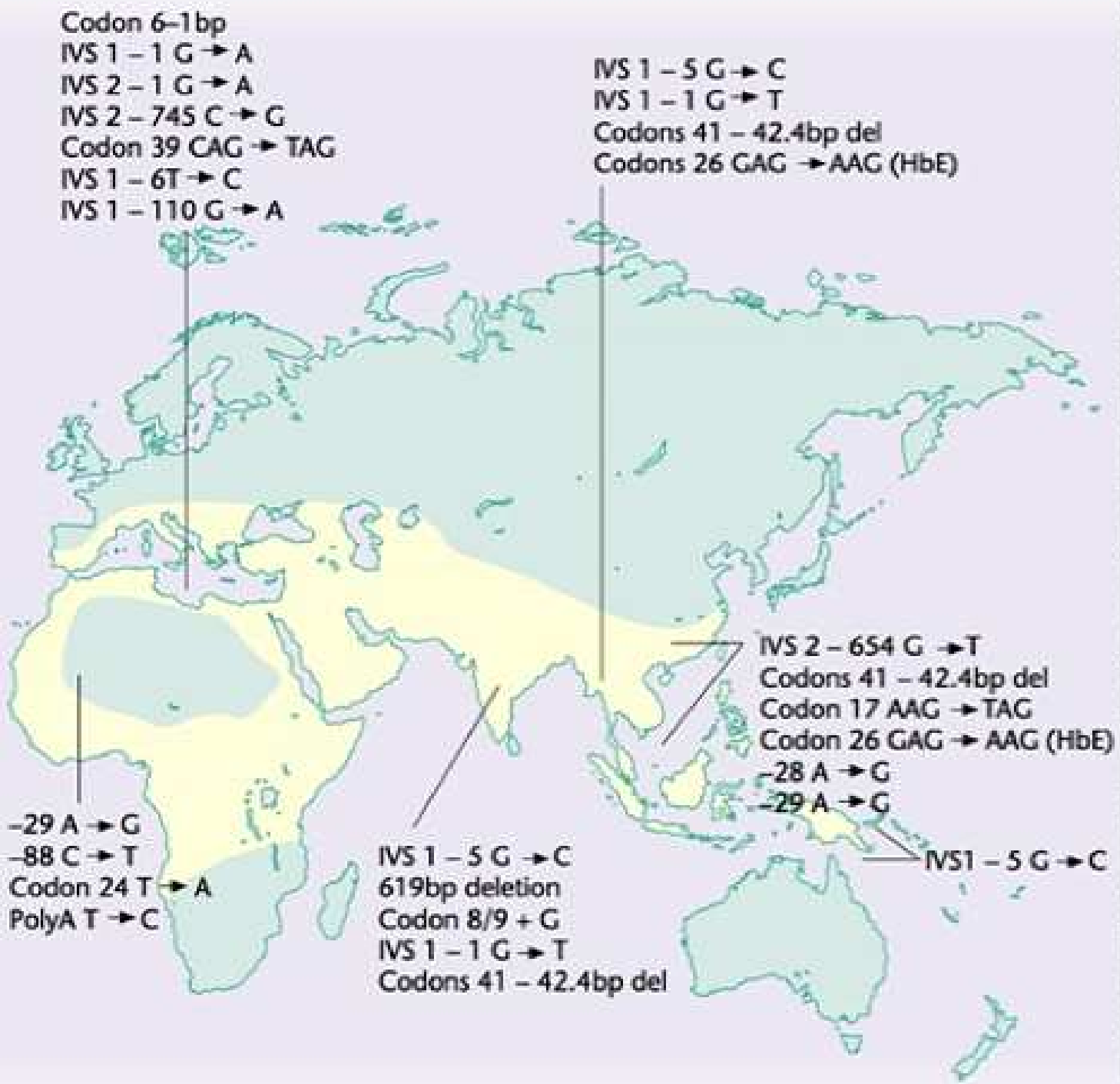


MLPA

(...)

Sequenziamento diretto





Distribuzione nel mondo delle mutazioni della talassemia

Letteratura Talassemia – Falcemia (Albania)

Boletini E, *Svobodova M, Divoky V, Curuk M, Dimovski A, Liang R, Adekile AD, Huisman THJ.*
Sickle cell anemia, sickle cell β-thalassemia, and thalassemia major in Albania: characterization of mutations. Hum Genet. 1994; 93: 182-187.

Angioletti M, *Lacerra G, Boletini E, Di Noce F, Musollino G, Carestia C.*
b- and α-globin genotypes in Albanian patients affected by b-globin disorders. Haematologica 2002; 87(9): 1002-1003.

Mokini V, *Duka D, Rosatelli C, Tuveri T, Demurtas M, Babameto-Laku A, Cao A.*
Molecular characterization of b-thalassemia mutations in Albania. The UNEPSA and European Congress of Paediatrics, 2000. Abstract Book, Haematology and Oncology. 2000; HO-265: 143.

Babameto-Laku A, *Mitre A, Berisha S, Mokini V, Roko D*
Molecular genetic characterization of b-thalassemia and sickle cell syndrome in the Albanian population. Balkan J Med Genet 2011; 14: 45–50.

Letteratura Talassemia – Falcemia (Albania)

	β and α globin genotypes β	α	Patients no. and age (yrs)	Transfusions
A	β-IVS-I-110 homozygosis	αα/αα	5 (18m, 3)	regular
			3 (14, 3)	sporadic
	β cod 39/β-IVS-II-1	αα/αα	1 (17)	sporadic
			1	regular
	β⁰ homozygosis	αα/αα	2 (14, 6)	sporadic
			1	regular
	β cod 39/β⁰	αα/αα	3 (18, 15, 7)	sporadic
			1 (10)	regular
	β-IVS-I-110/β⁰	αα/αα	7 (24, 27, 10, 8)	sporadic
			1 (8)	regular
B	β-IVS-I-110/β cod 39	αα/αα	4	regular
	β-IVS-I-110/β cod 44	αα/αα	2	regular
	β-IVS-I-110 homozygosis	ααα$^{anti3.7}$/αα	1	regular
	β-cod 39 homozygosis	αα/αα	1	regular
	β-IVS-I-110/β cod 82-83	αα/αα	1	regular
	β cod 39/β cod 5	αα/αα	1	regular
	β cod 39/β-IVS-I-1	αα/αα	1	regular
	β-IVS-II-745/β-IVS-I-1	αα/αα	1	regular
	β-IVS-I-6/β cod 37	αα/αα	1	regular
C	β-IVS-I-6/β IVS-I-110	αα/αα	5 (6, 13, 15, 3)	sporadic
	β-IVS-I-6 homozygosis	αα/αα	2 (7, 43)	sporadic
	β poly A/ β IVS-I-110	αα/αα	2	sporadic
	β-IVS-I-6/β cod 39	αα/αα	1 (15)	sporadic
	β-IVS-I-6/β cod 44	αα/αα	1 (4)	sporadic
	β-cod 39 homozygosis	α$^{-(\alpha)3.4}$/αα	1 (10)	sporadic
	β⁰ homozygosis	-α$^{3.7}$/αα	2 (23, 24)	sporadic
	β IVS-I-1/β⁰	αα/αα	1	sporadic
D	β cod 39/β⁰	ααα$^{anti3.7}$/αα	1 (9)	none
	β cod 44/β⁰	ααα$^{anti3.7}$/αα	1 (45)	none
	β⁰/β⁰	αα/αα	1 (2)	none
	β IVS-I-110/β⁰	αα/αα	1	none
	β IVS-II-1/β⁰	αα/αα	1	none

… alta eterogeneità allelica nei pazienti Albanesi con emoglobinopatie…

Angioletti M b- and α-globin genotypes in Albanian patients affected by b-globin disorders.

Studi pilota in Albania - **Lushnjë**

Acta Haematologica

A Pilot Beta-Thalassaemia Screening Program in the Albanian Population for a Health Planning Program

Leila Baghermajad-Salehi[a] Maria Rosaria D'Apice[a] Anila Babameto-Laku[b]
Michela Biancolella[a, c] Anila Mitre[b] Silvia Russo[c] Nicola Di Daniele[d]
Federica Sangiuolo[a, c] Vahe Mokini[b] Giuseppe Novelli[a, c, e]

217 students

Saliva DNA extraction (DNA only)

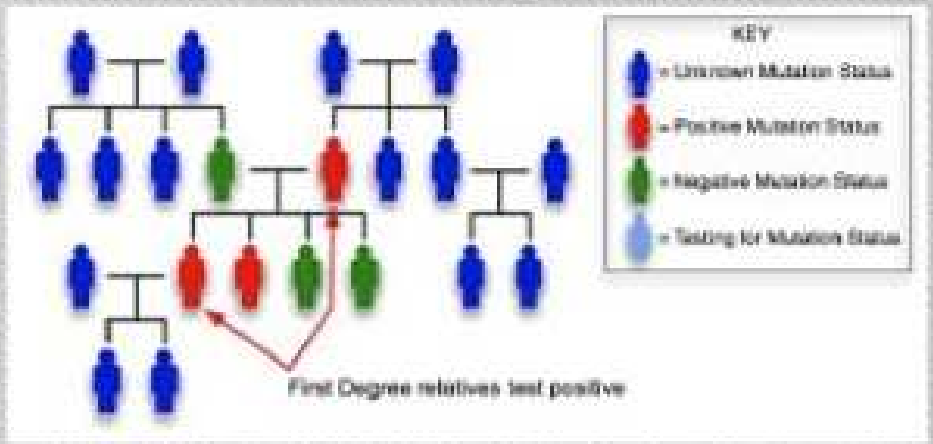
Results:

- HbS c.20A→T 3.2%
- IVS-I-110 (G→A) 1%

DNA only (selected mutations)

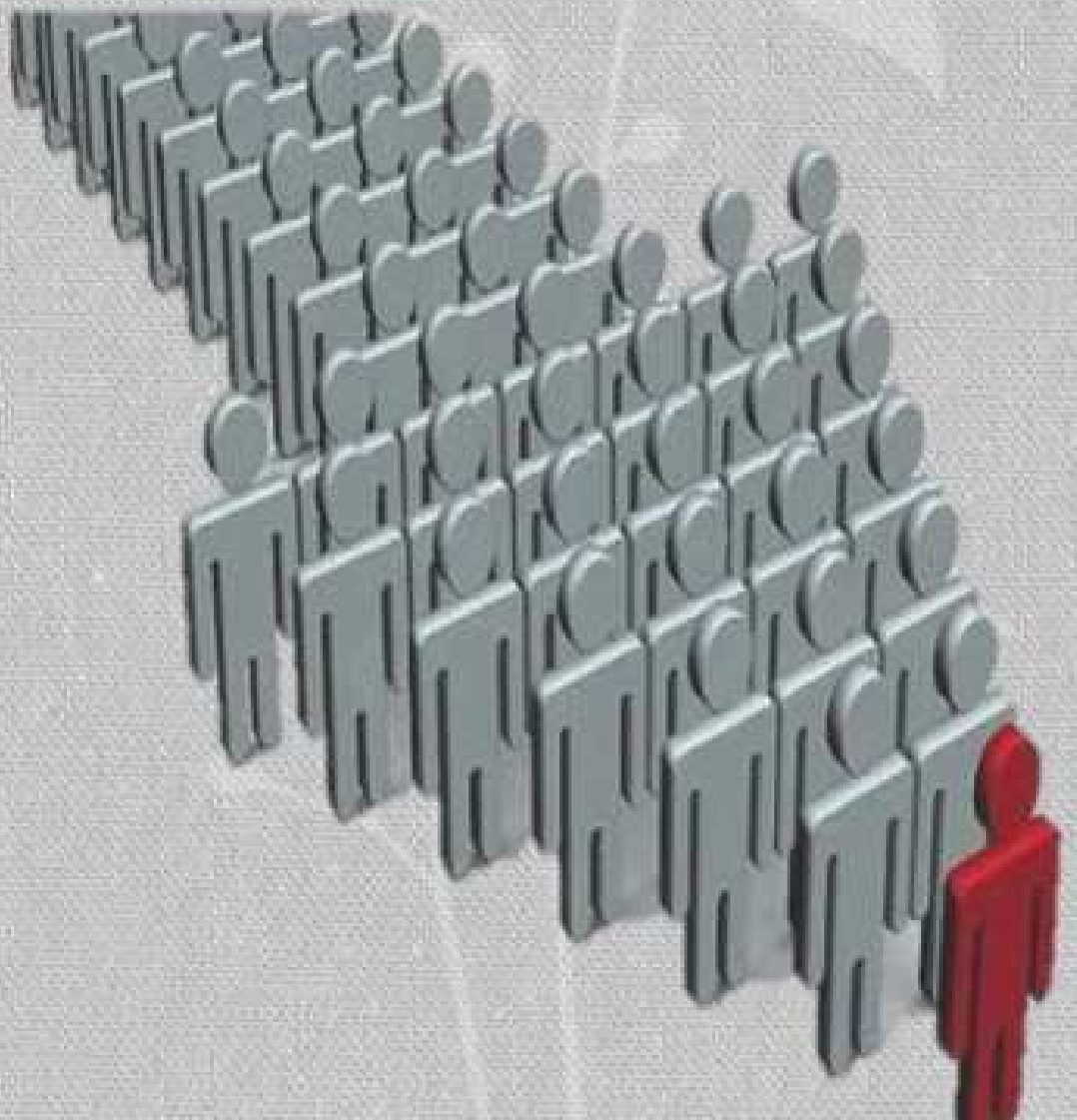
Strategie di SCREENING genetico

a cascata



CONSULENZA GENETICA

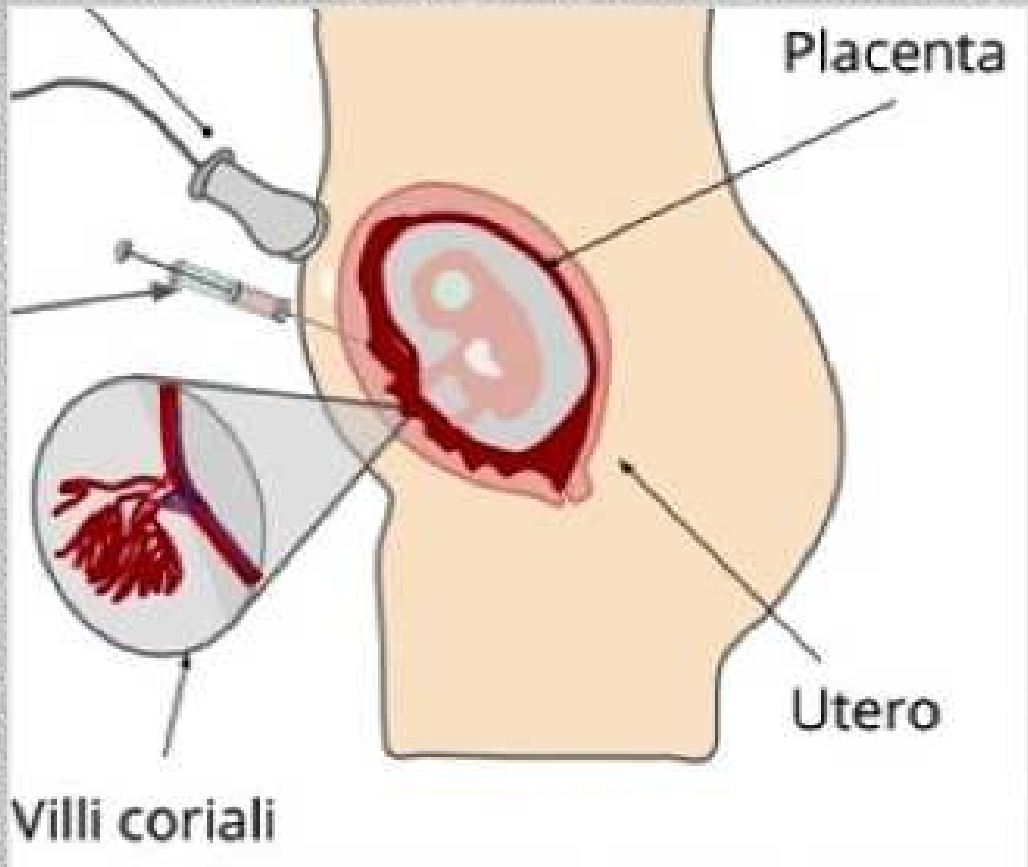
di popolazione



Diagnosi prenatale

... ricerca delle specifiche mutazioni CONFERMATE NEI GENITORI su prelievo di villi coriali a 11 settimane di gestazione...

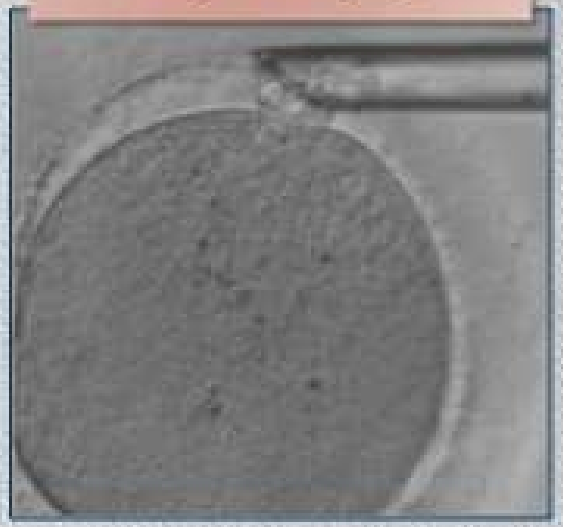
LABORATORIO ALTA SPECIALIZZAZIONE



Diagnosi Genetica Preimpianto

progressi, ricerca e tecnologia

Day 0 biopsy



- Paternal and post-zygotic errors not detected
- Need of 2°PB biopsy
- High false positive diagnostic rate
- Impact on embryo development
- Most expensive and time-consuming approach

Day 3 biopsy

- High worldwide experience
- Small reduction in embryo viability
- High impact of mosaicism
- Single cell analysis

Day 5 biopsy

- More robust genetic analysis
- No impact of biopsy
- Low impact of mosaicism
- Reduced number of embryos/cycles
- Less expensive

Courtesy: Dr. Laura Rienzi

PGD: take home message

Non *laboratori* ma Servizi di Medicina della Riproduzione

Effetti dello SCREENING genetico

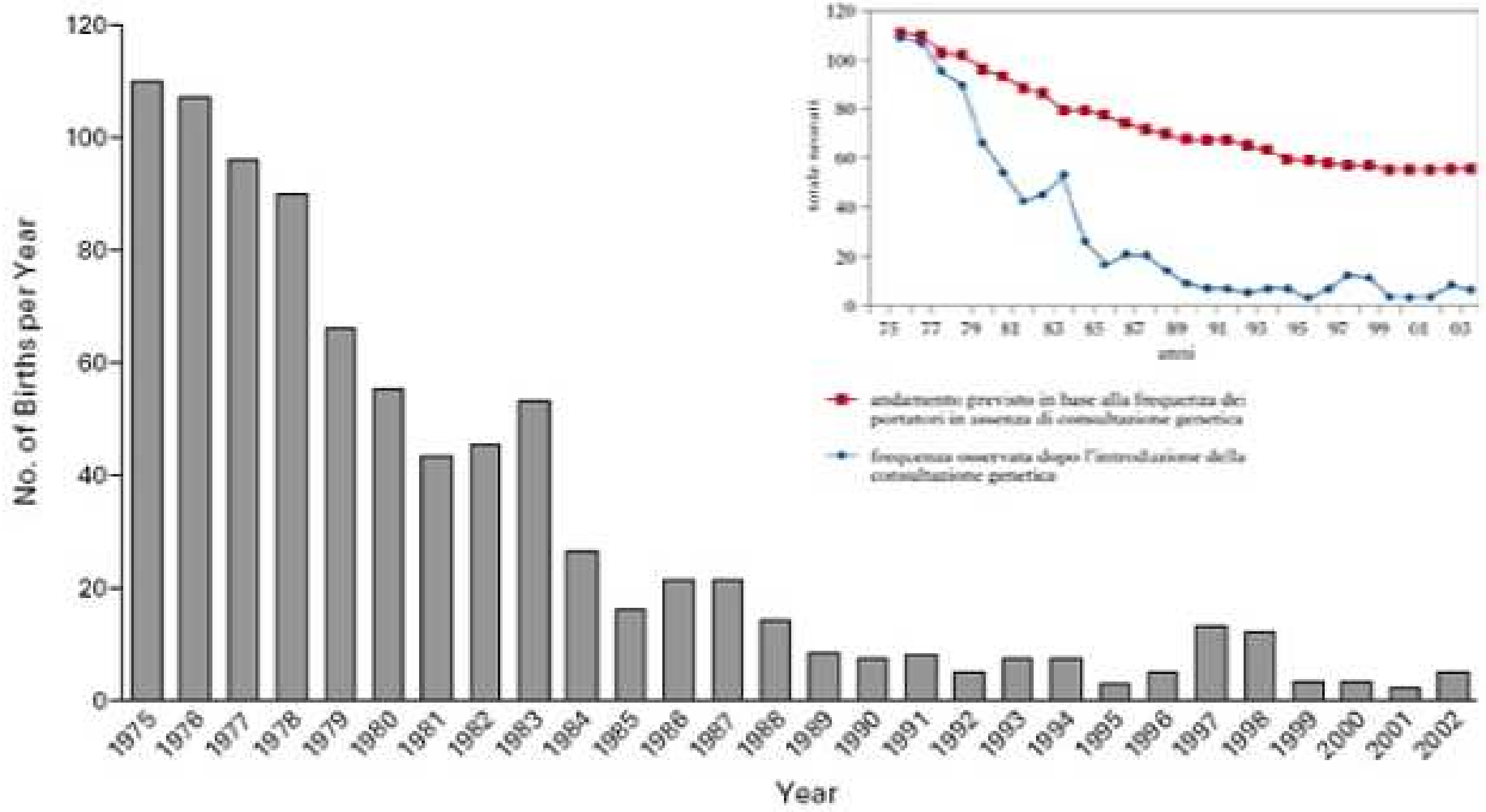


Figure 1. Declining Rate of Birth of Infants Homozygous for β-Thalassemia in Sardinia since 1975, When the Screening Program Began.

Cao & Galanello, NEJM 347: 1202, 2002

Grazie !

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