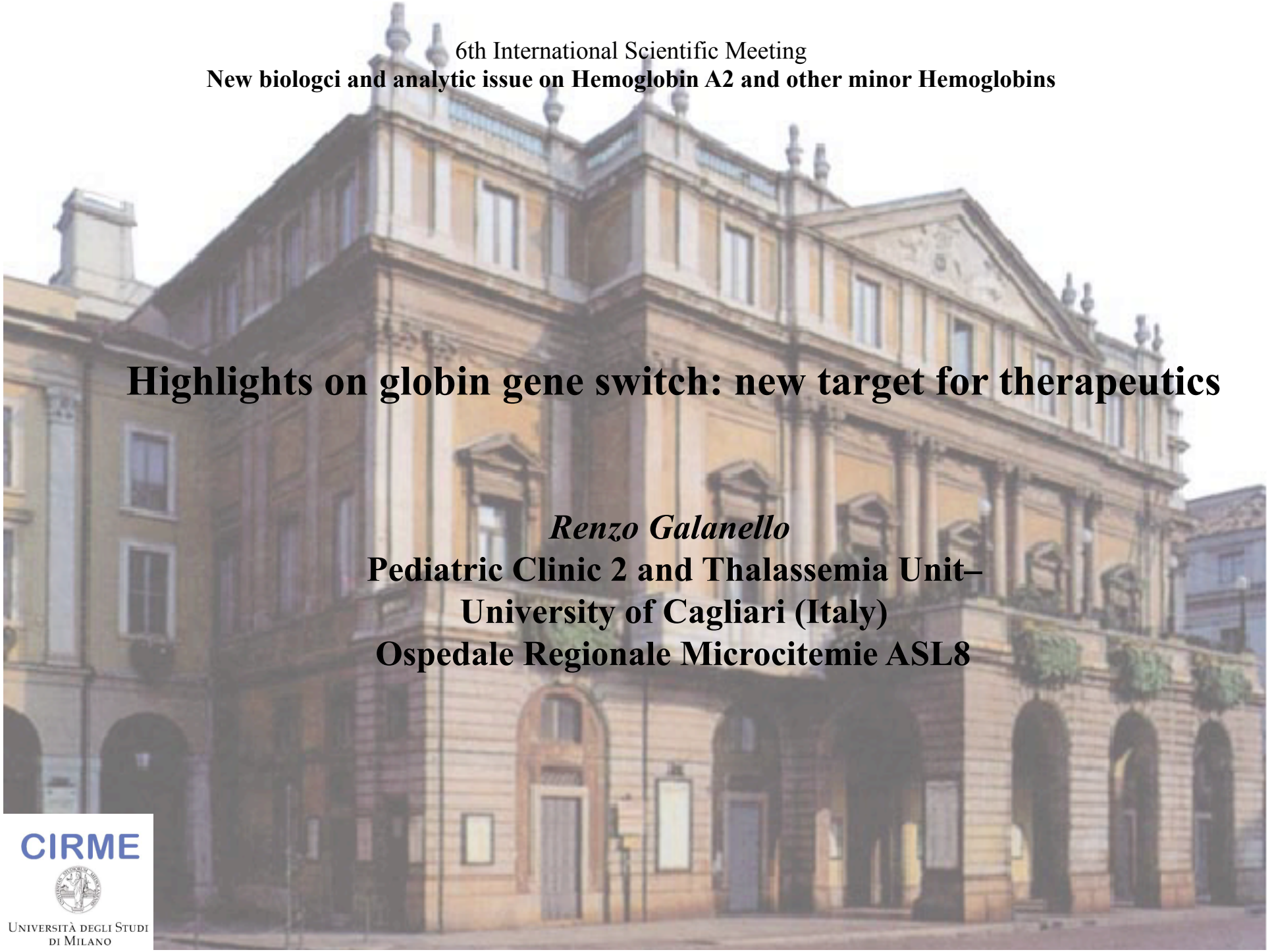
6th International Scientific Meeting

New biologci and analytic issue on Hemoglobin A2 and other minor Hemoglobins



Highlights on globin gene switch: new target for therapeutics

Renzo Galanello

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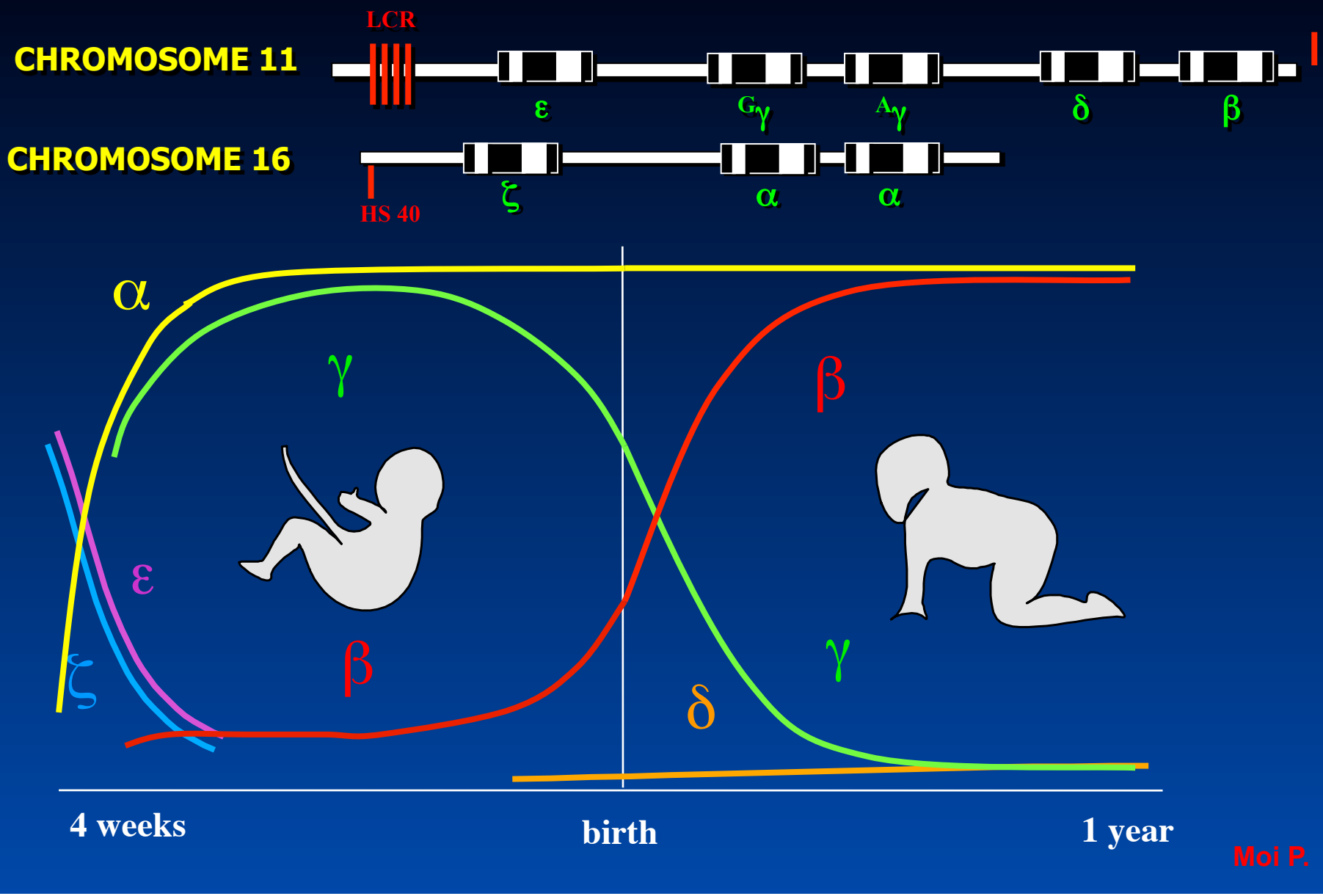
University of Cagliari (Italy)
Ospedale Regionale Microcitemie ASL8

CIRME

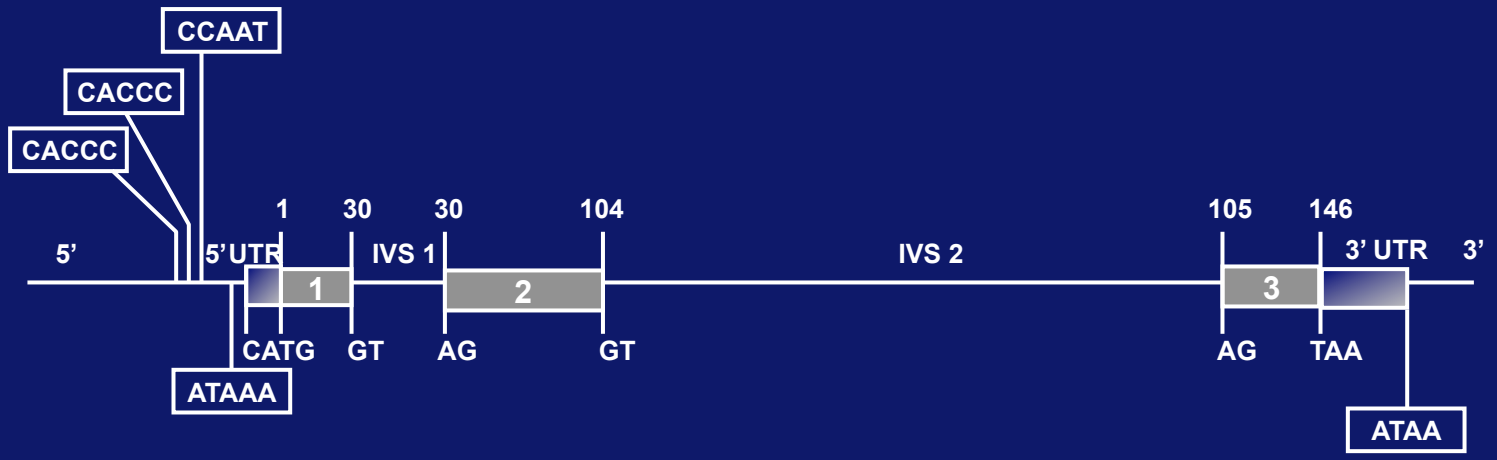
Università degli Studi di Milano

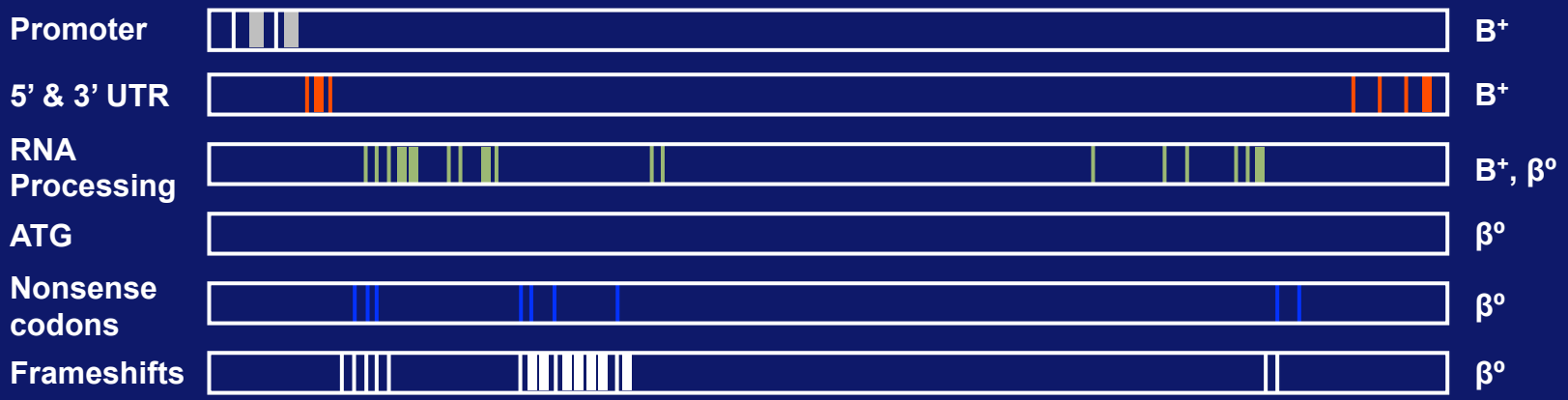
ORGANIZATION AND DEVELOPMENTAL EXPRESSION OF GLOBIN GENES



Moi P.

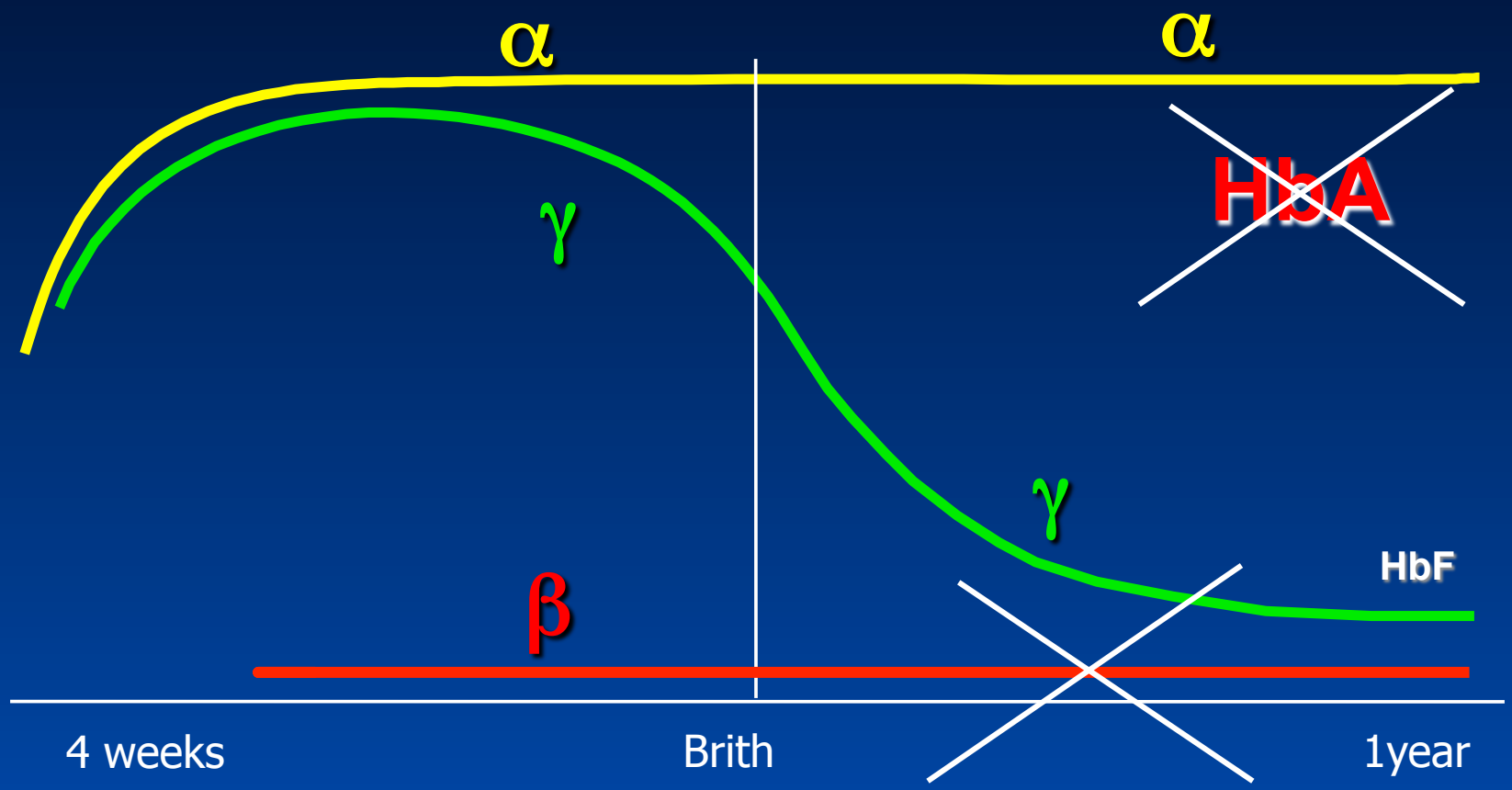
Type And Distribution Of β-Thalassemia Mutations





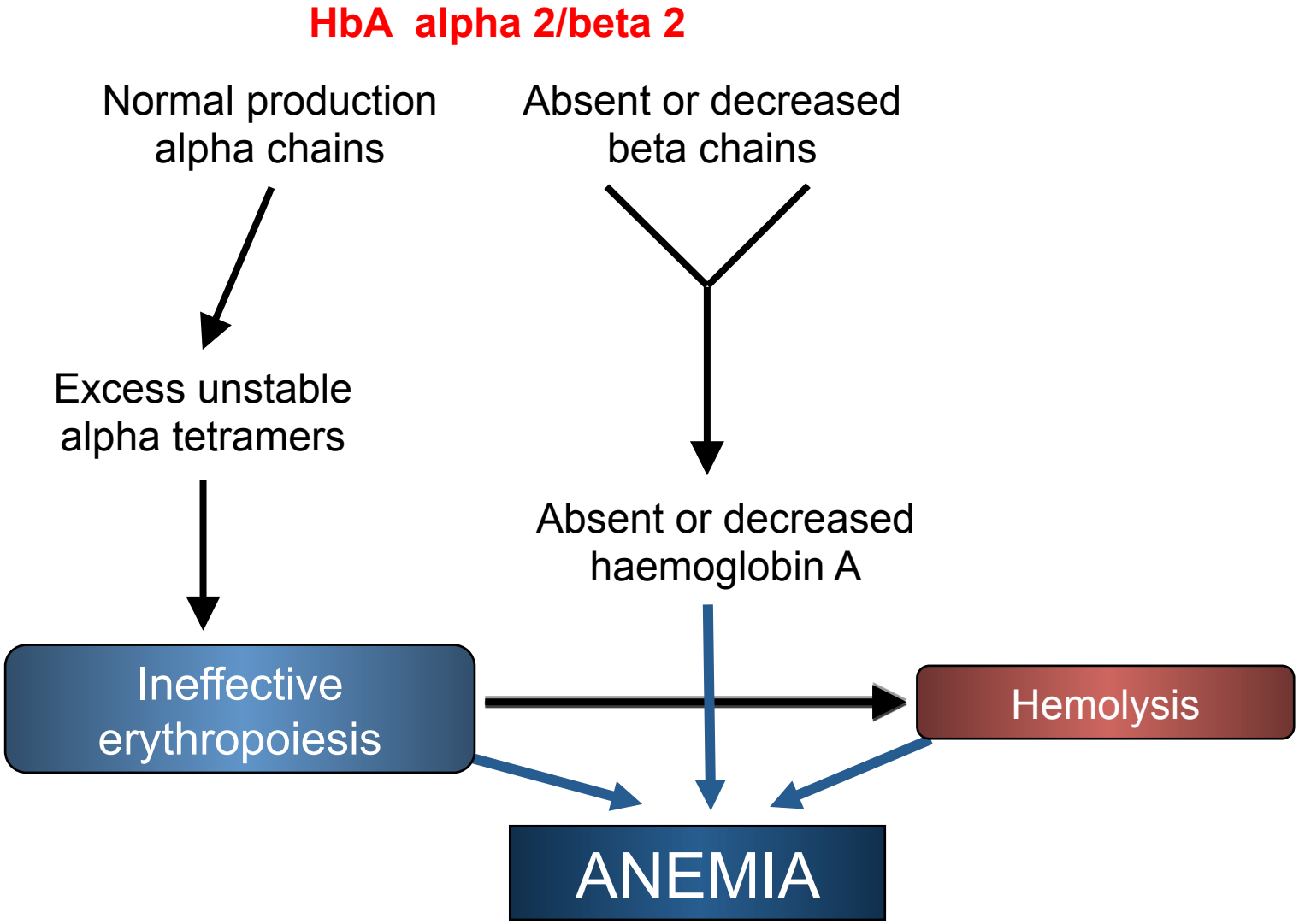
Thein et al. 2004.

Globin chain synthesis with β-thalassemia mutations



Moi P.

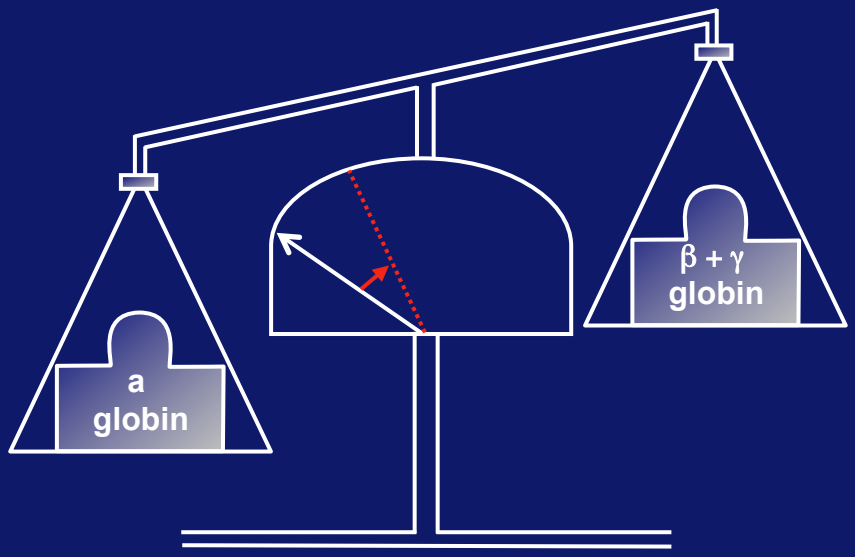
Pathophysiology of β-thalassemia



Mechanisms Of β-Thalassemia Intermedia

Severe Phenotype

α thalassemia



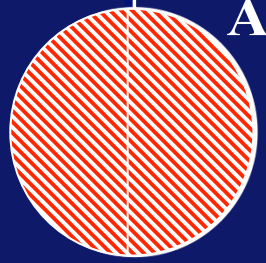
ild/silent β alleles

creased γ

HOMOZYGOUS SARDINIAN δβ°-THALASSEMIA

MCV fl 72.7
MCH pg 24.2
F 18.8
A_2 % 2.8

Age 3.5 y



Hb g/dl 11.9
MCV pg 63.4
MCH pg 21.6
F % 99.7
A_2 % 0.3
Aγ % 72.0

DNA analysis:
β39 C→T homozygous
Aγ -196 C→T homozygous

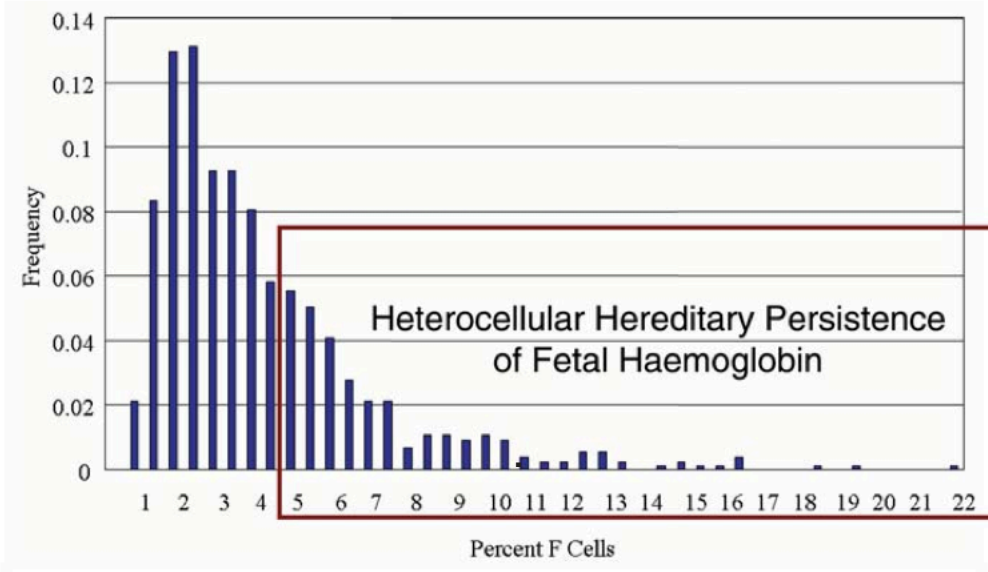
δβ°-thalassemia

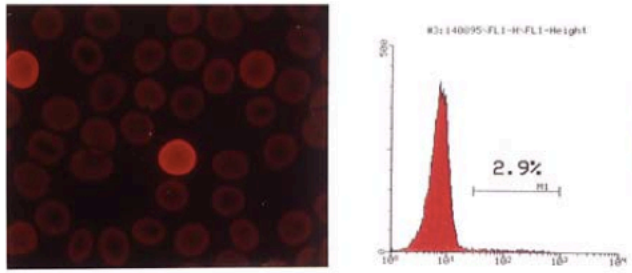
Galanello et al,Blood 2002

Why interest in fetal hemoglobin and hemoglobin switching?

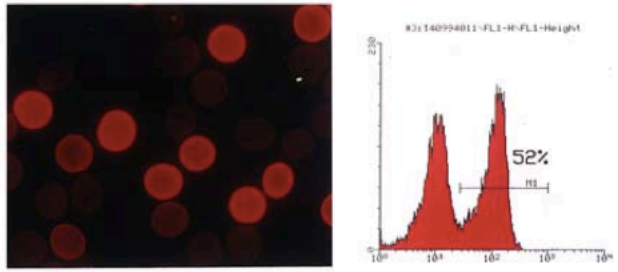
- ✓ Hb F is a strong modifier of hemoglobinopathies' severity
- ✓ level of HbF is a variable and inducible quantitative trait in humans
- ✓ to understand the mechanisms of thalassemia intermedia
- ✓ to understand the general mechanisms of gene expression and of developmental gene regulation
- ✓ to develop targeted therapeutic approaches for ameliorating the severity of the beta-hemoglobinopathies

Population distribution of fetal hemoglobin





Hb F 0.4%, F cells 2.9%



Hb F 4.8%, F cells 52%

- interindividual HbF variation is highly heritable
- genetic investigation is expected to identify genetic factors that controls HbF production

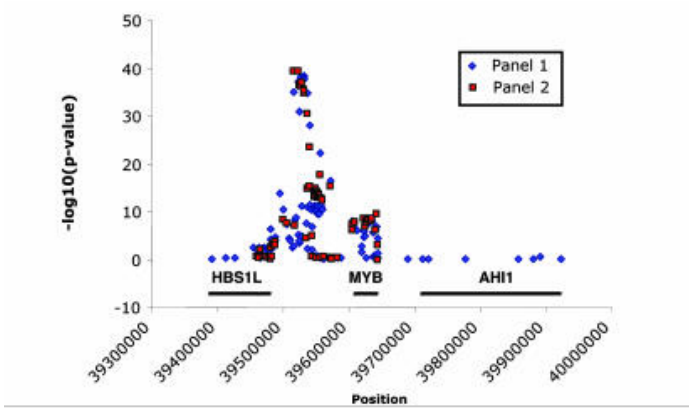
Thein SL,2009

QTLs map to HBS1L-MYB and BCL11A

Intergenic variants of *HBS1L-MYB* are responsible for a major quantitative trait locus on chromosome 6q23 influencing fetal hemoglobin levels in adults

Swee Lay Thein*†‡, Stephan Menzel*, Xu Peng§, Steve Best*, Jie Jiang*, James Close*¶, Nicholas Silver*, Ageliki Gerovassili*, Chen Ping§, Masao Yamaguchi§, Karin Wahlberg*, Pinar Ulug*, Tim D. Spector||, Chad Garner**, Fumihiko Matsuda§, Martin Farrall††, and Mark Lathrop§

11346–11351 PNAS July 3, 2007 vol. 104 no. 27



A QTL influencing F cell production maps to a gene encoding a zinc-finger protein on chromosome 2p15

Stephan Menzel[1], Chad Garner[2], Ivo Gut[3], Fumihiko Matsuda[3], Masao Yamaguchi[3], Simon Heath[3], Mario Foglio[3], Diana Zelenika[3], Anne Boland[3], Helen Rooks[1], Steve Best[1], Tim D Spector[4], Martin Farrall[5], Mark Lathrop[3] & Swee Lay Thein[1,6]

F cells measure the presence of fetal hemoglobin, a heritable quantitative trait in adults that accounts for substantial phenotypic diversity of sickle cell disease and β thalassemia. We applied a genome-wide association mapping strategy to individuals with contrasting extreme trait values and mapped a new F cell quantitative trait locus to *BCL11A*, which encodes a zinc-finger protein, on chromosome 2p15. The 2p15 *BCL11A* quantitative trait locus accounts for 15.1% of the trait variance.

NATURE GENETICS VOLUME 39 | NUMBER 10 | OCTOBER 200

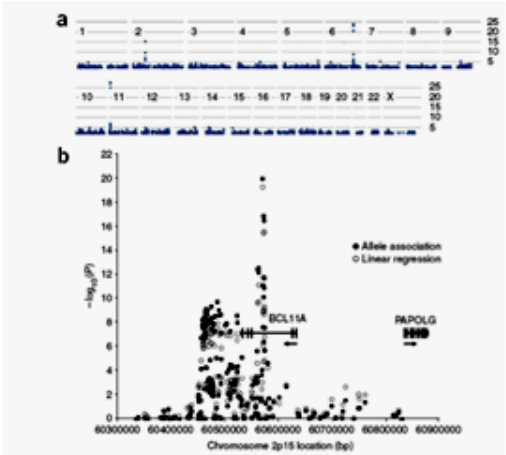
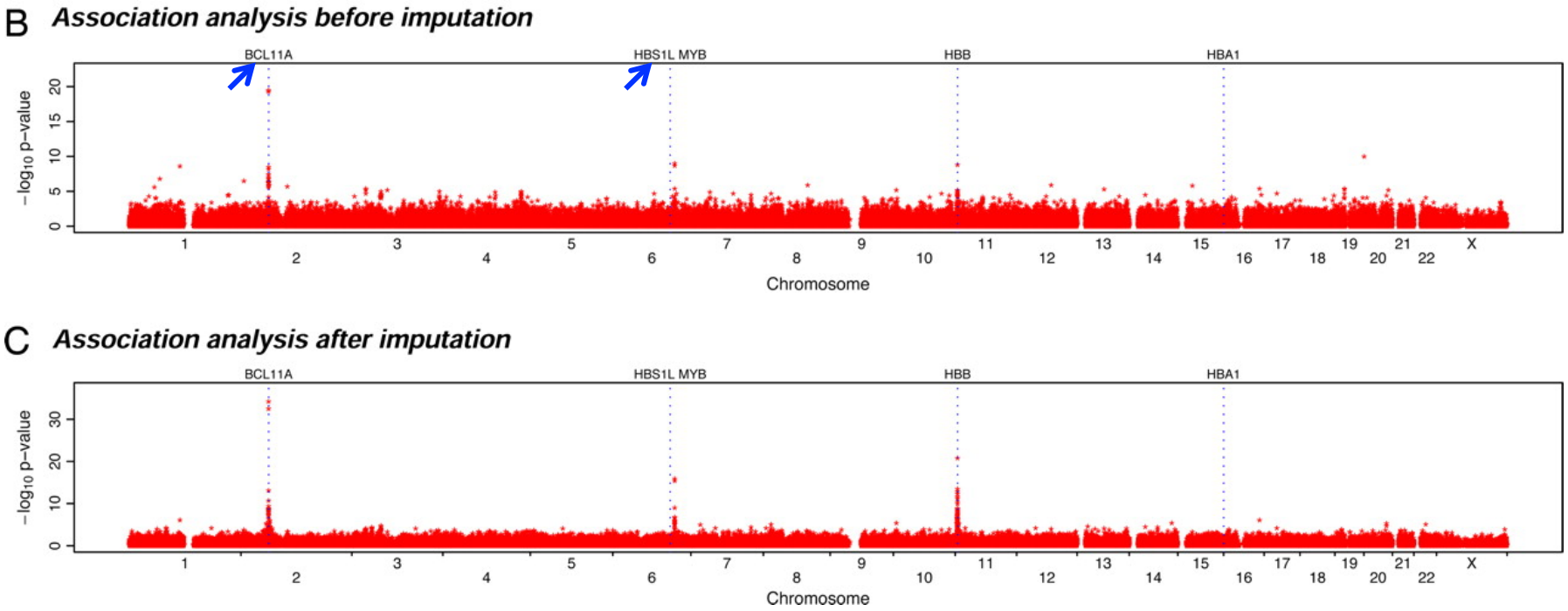


Figure 1 Association statistics ($-\log_{10}(P)$) for individuals included in the genome-wide screening panel. (a) Association statistics for 3,225 markers genome-wide with $P < 10^{-2}$. (b) Association statistics for 211 markers across the 2p15 region of association.

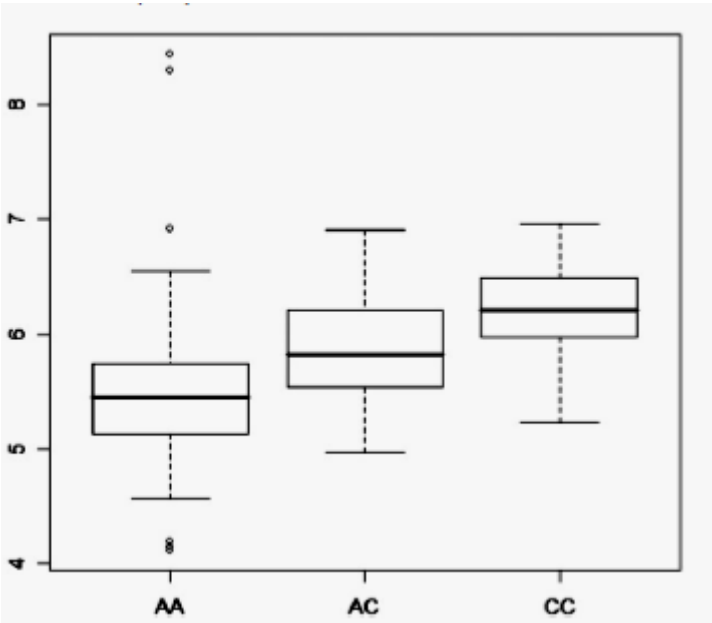
GWAS results for HbF



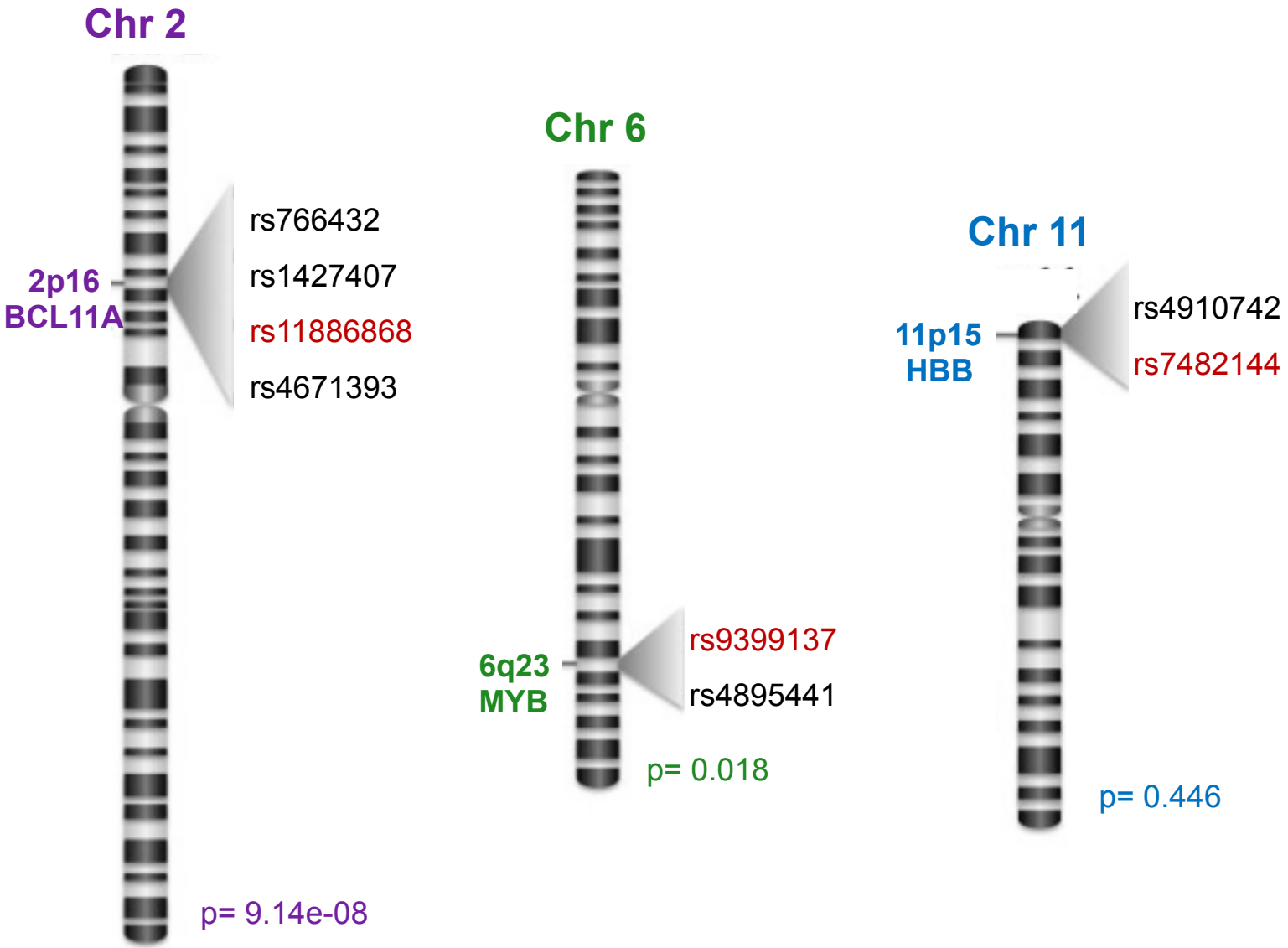
Uda M.,Galanello R et al, Proc. Natl. Acad. Sci. USA 105, 1620-1625 (2008)

BCL11A is a major HbF quantitative trait locus in three different populations with β-hemoglobinopathies ☆

Amanda E. Sedgewick [a,1], Nadia Timofeev [a,1], Paola Sebastiani [a], Jason C.C. So [b], Edmond S.K. Ma [b], Li Chong Chan [b], Goonnapa Fucharoen [c], Supan Fucharoen [c], Cynara G. Barbosa [d], Badri N. Vardarajan [e], Lindsay A. Farrer [a,e,f,g,h], Clinton T. Baldwin [e,i], Martin H. Steinberg [d], David H.K. Chui [d,*]



Hb F variation associated SNPs

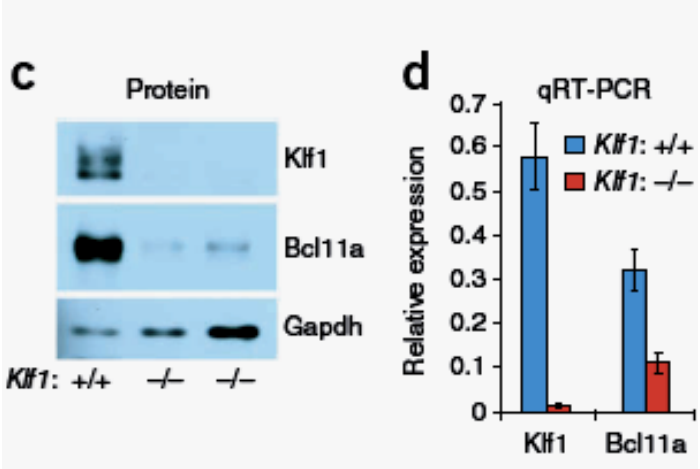


Bcl11A and HbF

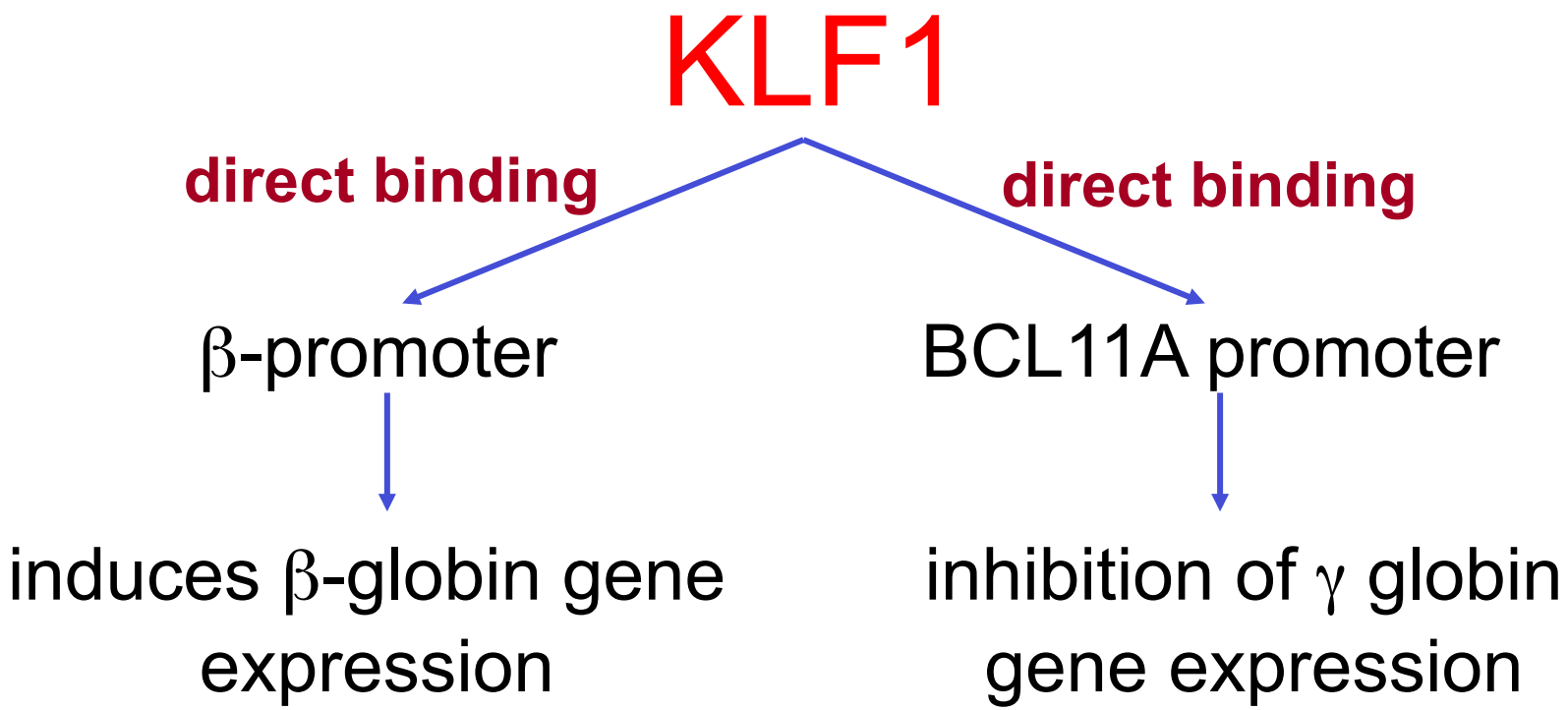
- genetic association detected by G-WAS
- SNP_s in IVS2 described in different populations, in HPFH, beta thalassemia, HbE, SCD
- High HbF is associated with low Bcl11A expression
- Bcl11A expression varies at different developmental stages
- Bcl11A is down-regulated by KLF1 gene

KLF1 regulates BCL11A expression and γ- to β-globin gene switching

Dewang Zhou, Kaimao Liu, Chiao-Wang Sun, Kevin M Pawlik & Tim M Townes



Schematic mechanism of KLF1 action



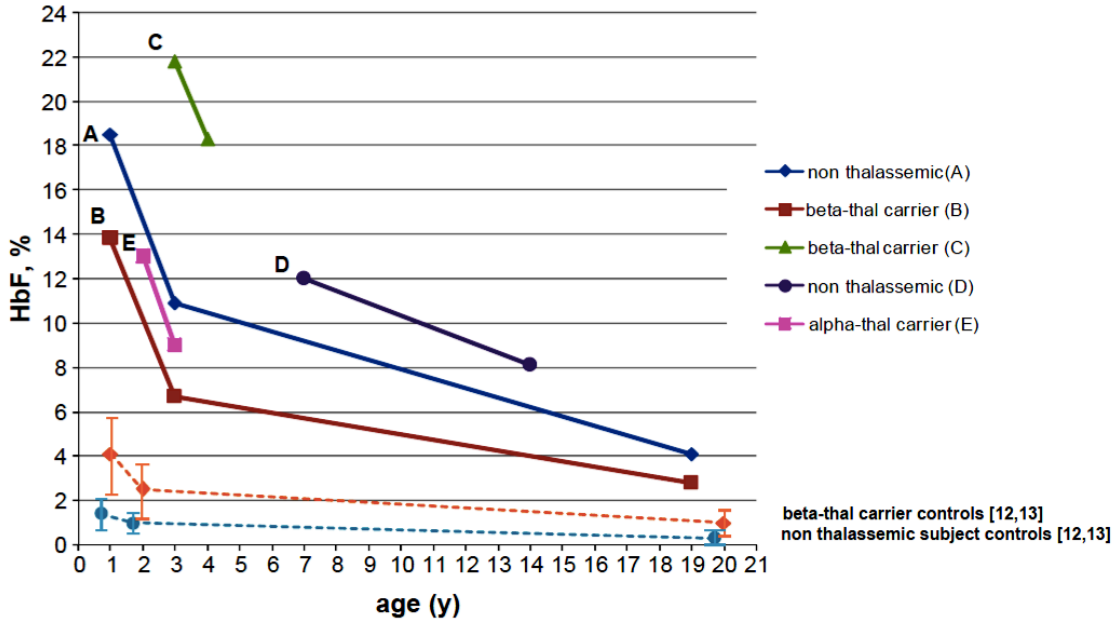
Contents lists available at SciVerse ScienceDirect

Blood Cells, Molecules, and Diseases

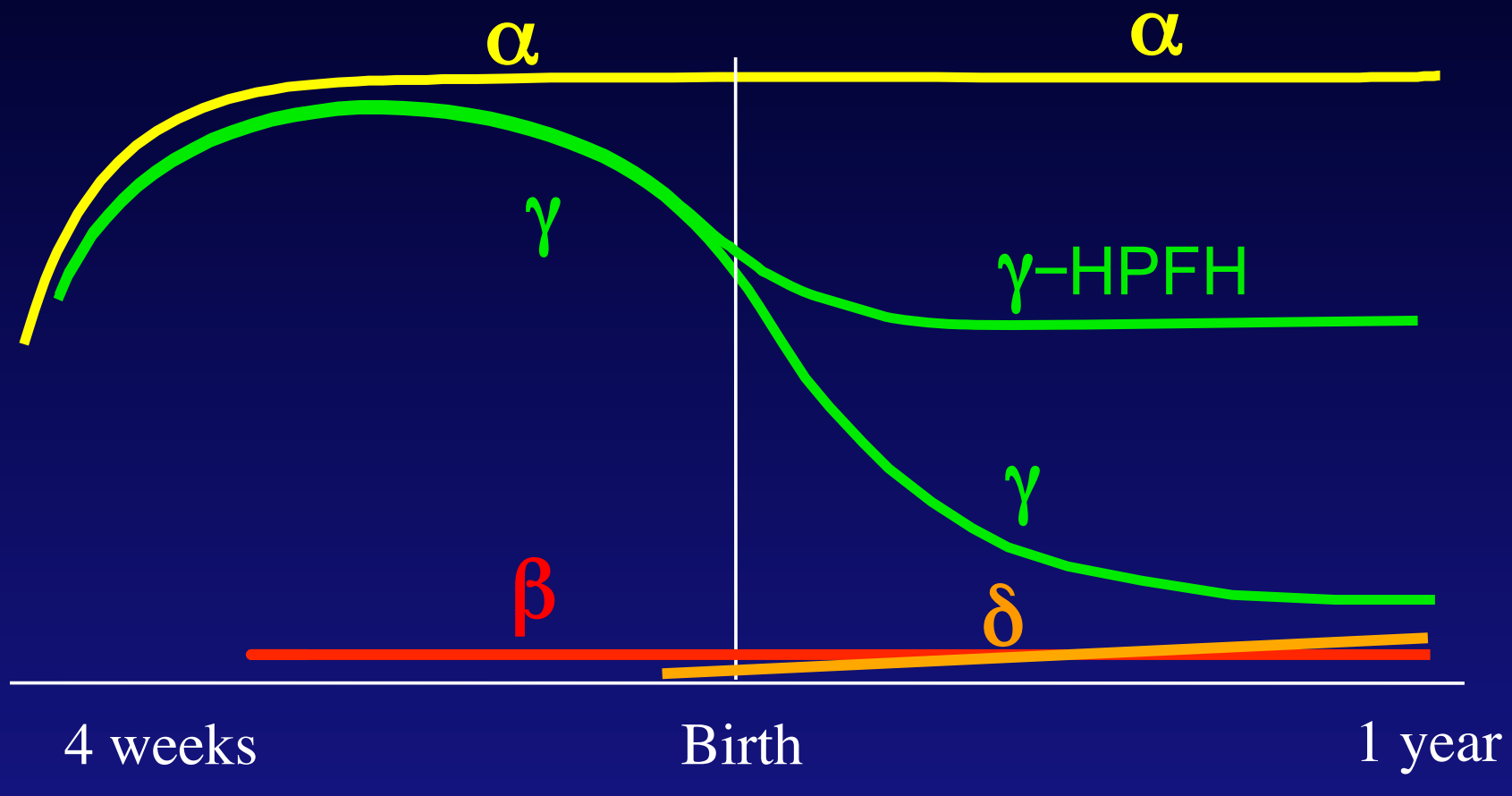
journal homepage: www.elsevier.com/locate/ybcmd

Delayed fetal hemoglobin switching in subjects with KLF1 gene mutation

Stefania Satta [a], Lucia Perseu [b], Liliana Maccioni [a], Nicolina Giagu [c], Renzo Galanello [a,*]



γ-globin gene expression in β-talassemia and in HPFH

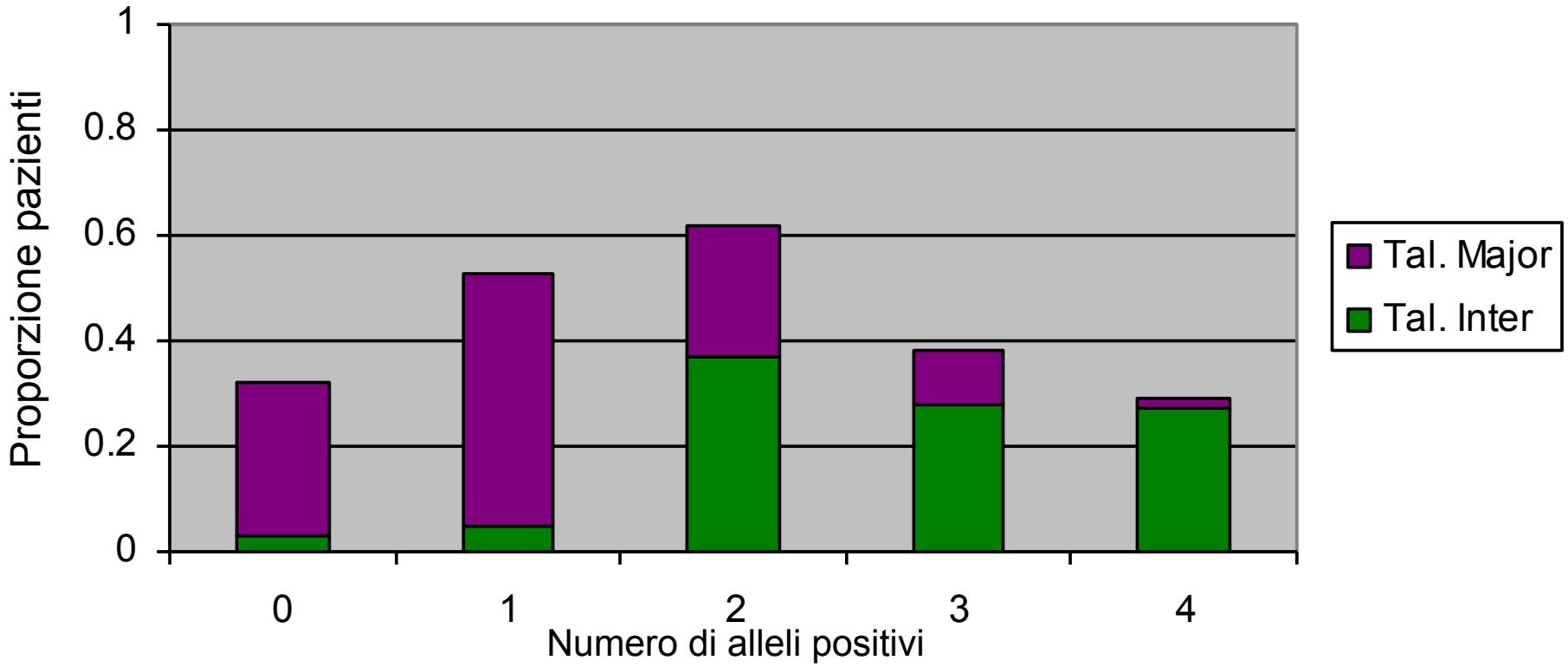


Moi P.

Brief report

Amelioration of Sardinian β^0 thalassemia by genetic modifiers

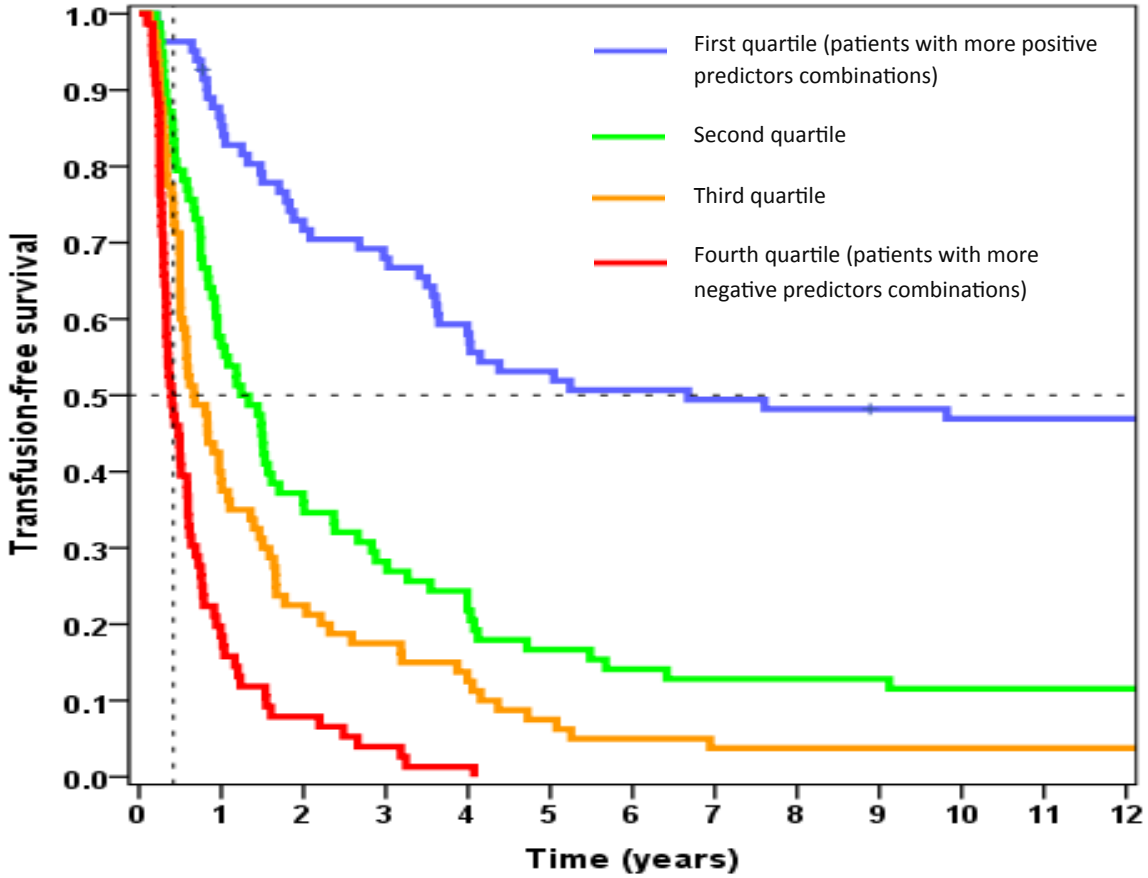
Renzo Galanello,[1] Serena Sanna,[2] Lucia Perseu,[2] Maria Carla Sollaino,[1] Stefania Satta,[1] Maria Eliana Lai,[3] Susanna Barella,[1] Manuela Uda,[2] Gianluca Usala,[2] Goncalo R. Abecasis,[4] and Antonio Cao[2]



CONTRIBUTO DI ALCUNI ALLELI NEL DETERMINARE IL FENOTIPO DI TALASSEMIA MAJOR E INTERMEDIA

Blood,2009

Survival curves for 316 patients with different combinations of predictors for later and earlier time to transfusion



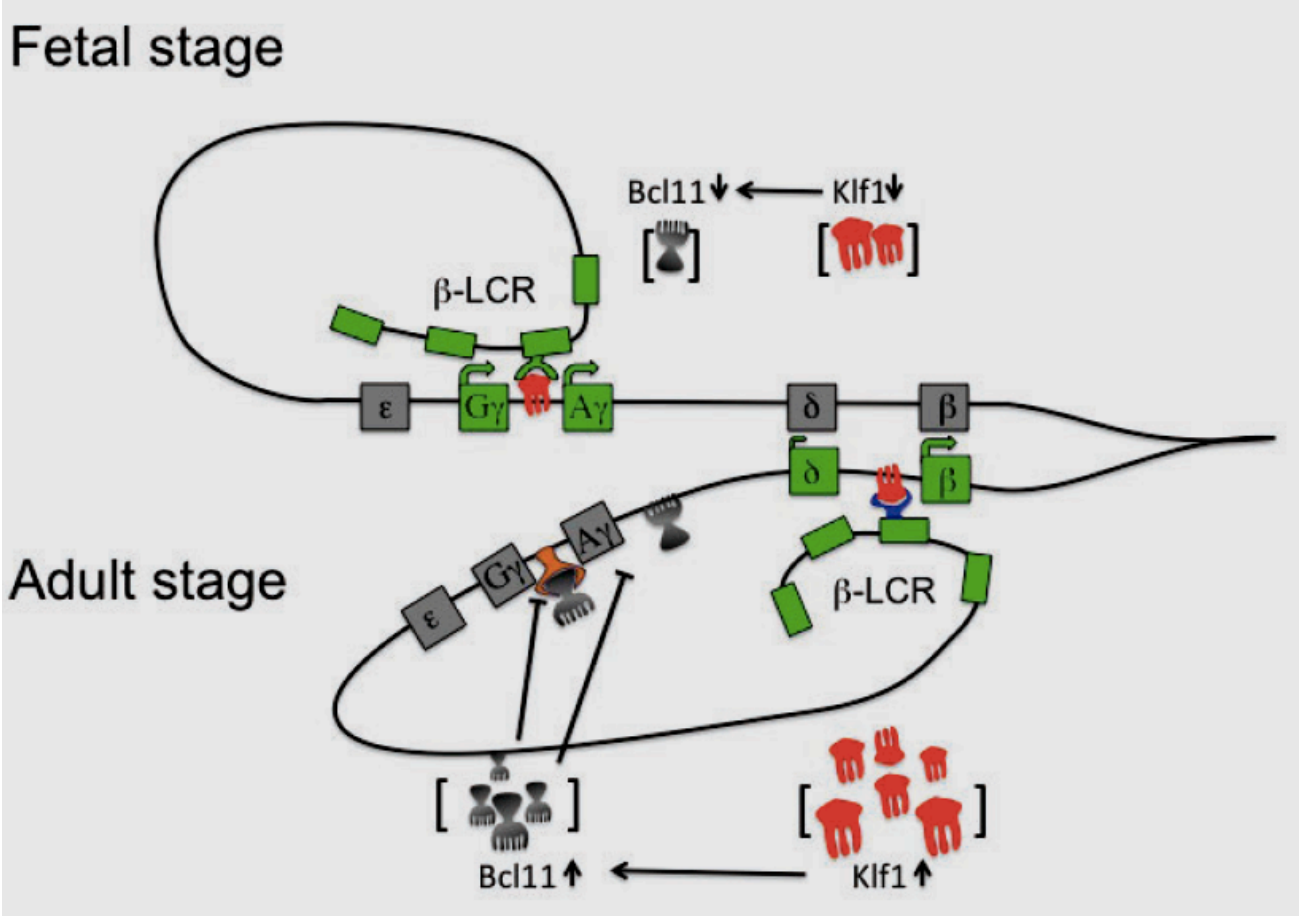
Danjou F et al.,2012

Locus		p	Hazards Ratio	Harrell's C-index	Predictor for later transfusion start
HBG2:g.-58C>T		<0.001	0.081	0.54	+/-
α gene defects		<0.001	0.514	0.61	class 2
BCL11A	rs1427407	<0.001	2.391	0.63	T allele
	rs10189857	0.005	1.312		G allele
HBS1L/MYB	rs4895441	<0.001	1.979	0.57	G allele
	rs6904897	0.020	0.697		TT genotype
Gender		0.016	0.738	0.52	Male

Clinically relevant fetal hemoglobin modifiers

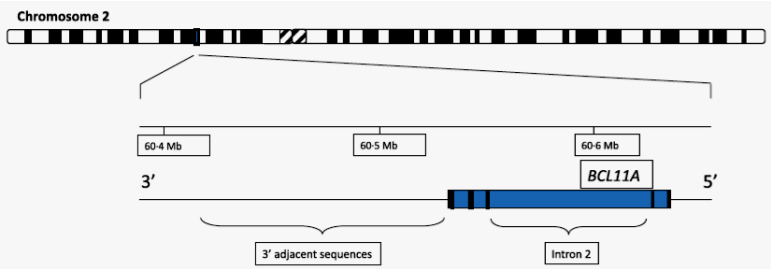
- Genetic:
  - *in cis* variants:
    - γ globin genes
    - β globin cluster
    - LCR
  - *in trans* variants:
    - BCL11A
    - HBS1L-MYB
    - KLF1 mutations
    - GATA1
    - trisomy 13
- Epigenetic:
  - DNA methylation
  - histone modification *(acetylation, phosphorylation, methylation)*
  - erythropoiesis expansion *(perturbation of erythroid kinetics)*

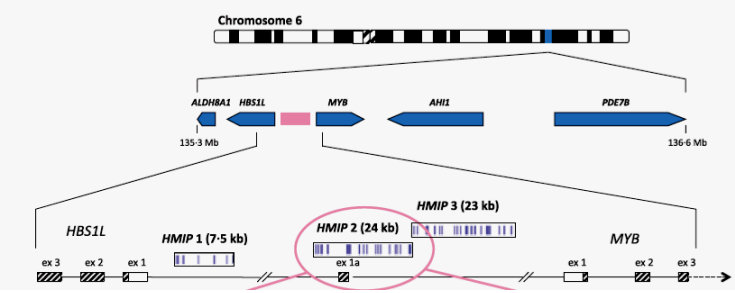
Current hemoglobin switching model

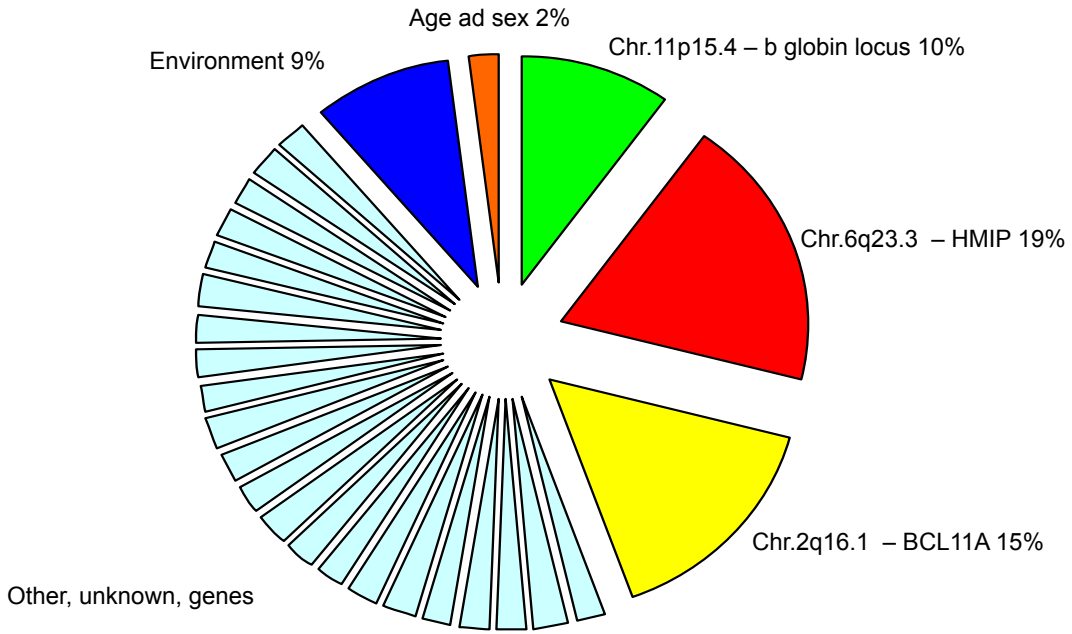


Cao A Moi P. Galanello R.: Pediatric Reports 2011

Variance components of the F-cell trait







(MenzelS, 2009)

Rationale for HbF induction

- ✓ Gamma globin genes are intact
- ✓ HbF will functionally compensate for the absence of HbA (HPFH homozygotes, genetic compounds (β-thal/HPFH)
- ✓ HbF in post-natal life is influenced by physiological and genetic factors (pregnancy, acute erythroid expansion, haematological diseases, haemoglobinopathies)
- ✓ High cost of conventional treatment
- ✓ Problems wit blood safety and availability in several countries

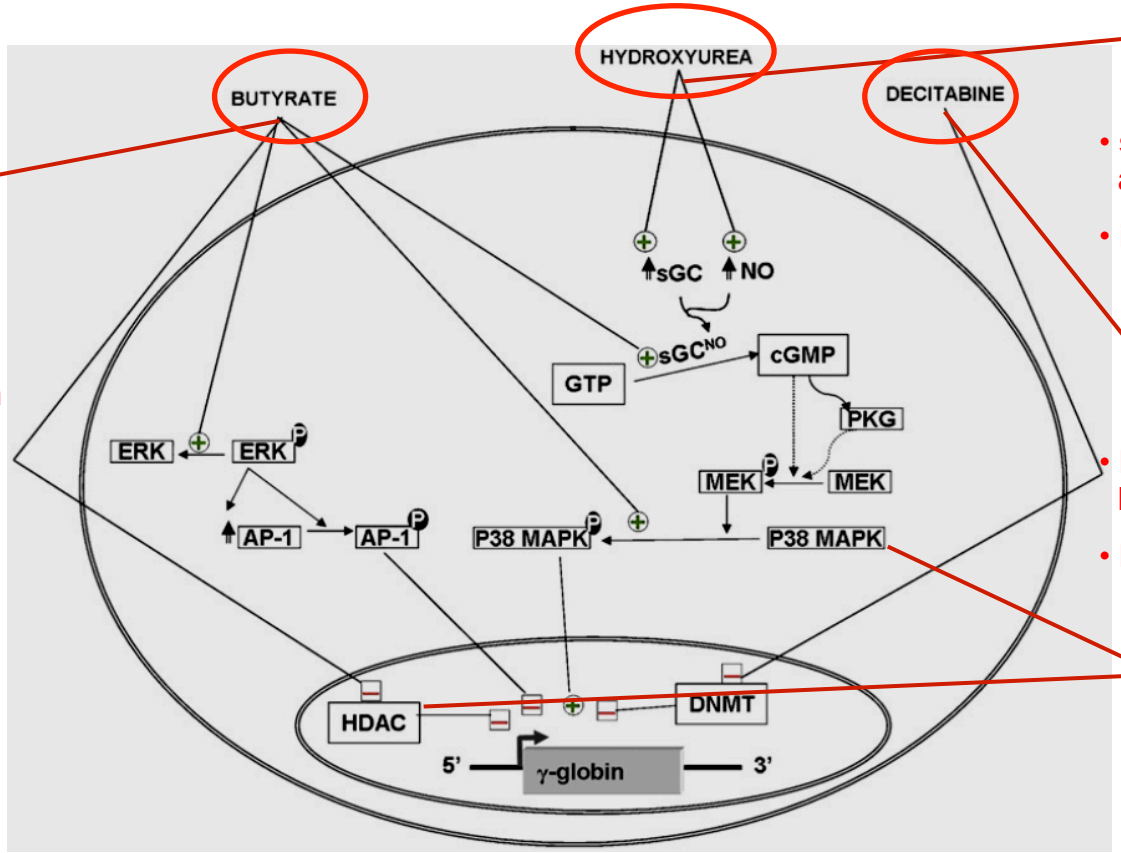
HbA = adult haemoglobin; HbF = fetal haemoglobin.

HbF inducers

Class	Compound
Hypomethylating/cytotoxic agents	5-azacytidine, decitabine
Cytotoxic agents	hydroxyurea
Histone Deacetylase Inhibitors	butyrate analogs, short chain fatty acids, adipicin, scriptaid, trichostatin A, valproic acid,
Antioxidants	resveratrol, angelicin, curcumin……
Others	Erythropoietin, thalidomide

Mechanisms for γ-globin induction by pharmacologic agents

- histone hyperacetylation
- p38-MAPK phosphorylation
- ERK MAPK dephosphorylation
- sGC/cGMP pathway activation
- increased γmRNA translation



- sGC/cGMP pathway activation
- increase of CAMP
- hypomethylation by inhibition of DNA
- hyperacetylation (?)

thalidomide

Atweh g eta l Hematol Incol Clin N Am 2010

Summary of clinical trials with HU in β-thalassemia syndromes

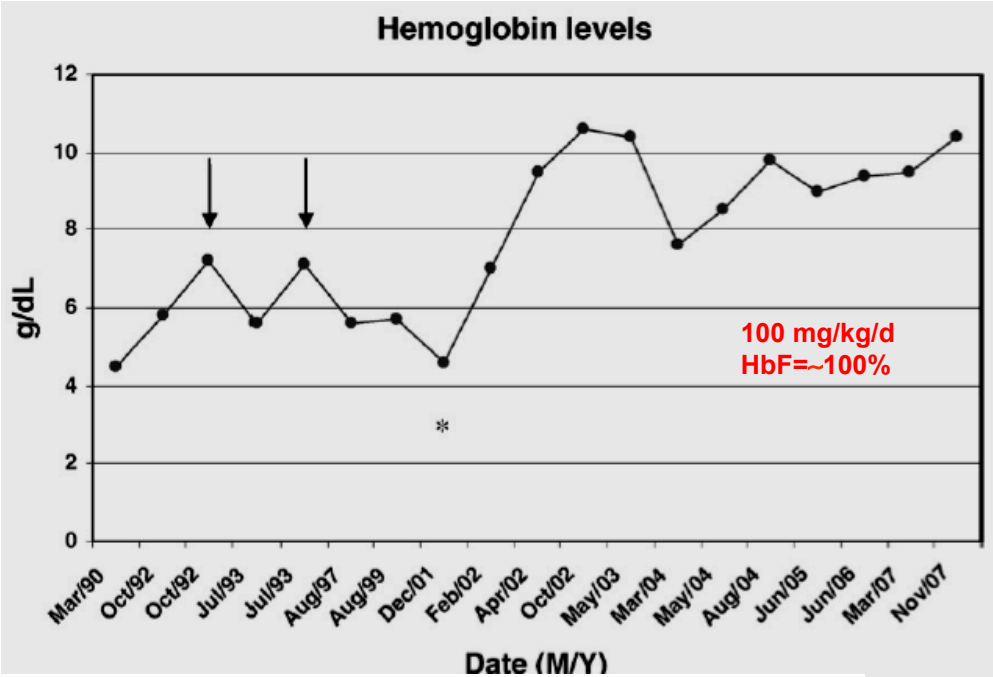
- ✓ 18 trials
- ✓ 7 thalassemia major, 8 intermedia, 3 HbE/β-thal
- ✓ treated patients 573
- ✓ number of patients/trial: 2 to 163
- ✓ variable doses 3 to 30 mg/kg/day
- ✓ responding patients: 25 to 100%
- ✓ total hemoglobin increase: <1 g up to 4g/dl
- ✓ best response in thalassemia intermedia
- ✓ response correlates with XmnI polymorphism

The *Xmn*I and *BCL11A* Single Nucleotide Polymorphisms May Help Predict Hydroxyurea Response in Iranian β-Thalassemia Patients

2012, Vol. 36, No. 4 , Pages 371-380 (doi:10.3109/03630269.2012.691147)

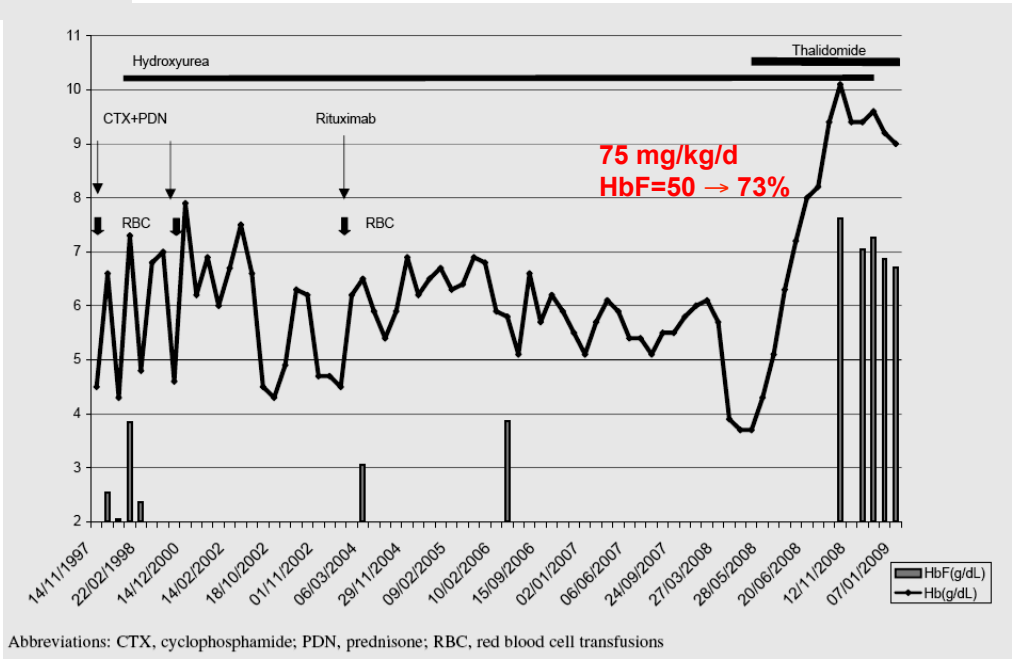
Mehdi Banan,[1] Hadi Bayat,[1] Azita Azarkeivan,[2,3] Saeid Mohammadparast,[1] Koorosh Kamali,[4] Samaneh Farashi,[1] Nooshin Bayat,[5] Masumeh Hadavand Khani,[3] Maryam Neishabury,[1] and Hossein Najmabadi[1,5]

Thalidomide therapy in patients with thalassemia major.



A *genotype –28A→C/cd39*

B *genotype IVS1-6/cd44C*



Aguilar-Lopez et al Blood Cell Mol Dis 2008

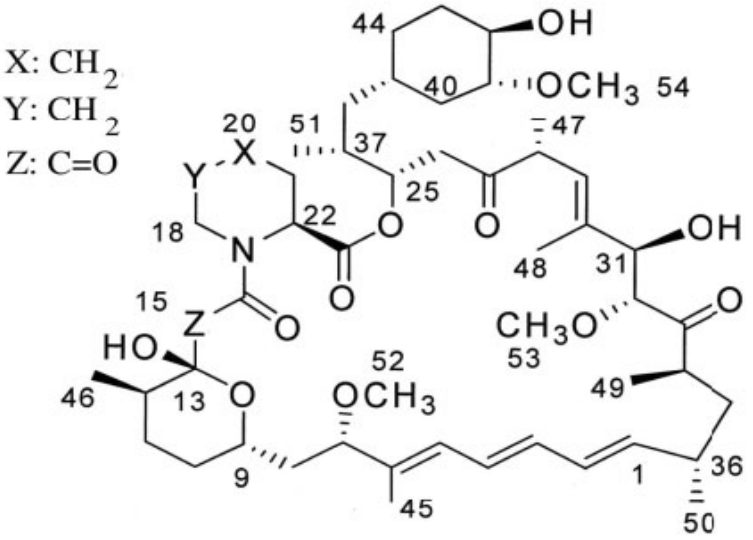
Pharmacologic induction of HbF:*concerns and limitations*

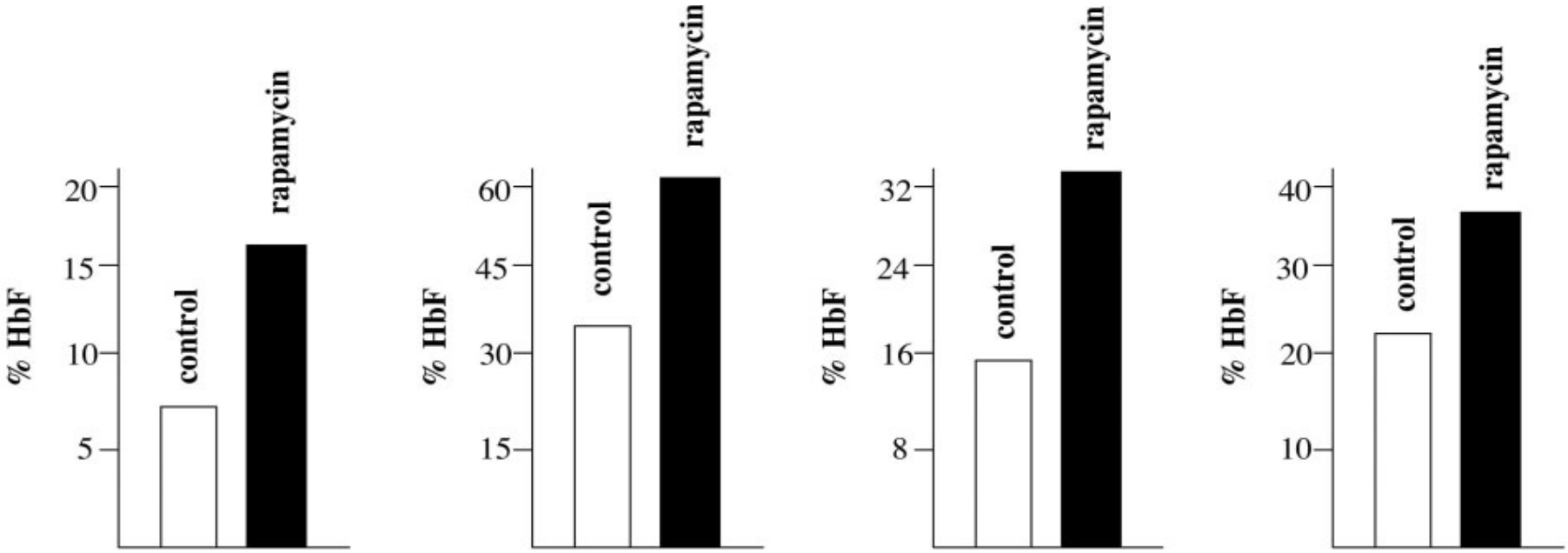
- potential toxicity→ safety an long term
- unpredictable and variable response
- effectiveness and long term *(loss of efficacy overtime)*
- compliance

Future directions

- other safer and more effective compounds
- better understanding of molecular mechanisms
- combination therapies
- correlation with individual genetic determinants
- personalized therapies
- impact on natural history

Induction of HbF by rapamycin





Bianchi N et al

Conclusions

- second generation DNA technologies have improved the knowledge of globin gene modifiers
- well phenotyped cohorts of patients are essential for genetic research:

  your genetics is only as good as your phenotype

- gene modifiers are useful in the follow-up of disease -related complications and in drug selection and dosing
- genetic studies of disease modifiers can identify new potential targets for therapy
- an integrated approach by clinicians, geneticists, clinical researchers and basic scientists is needed to further improve the knowledge of variability and the treatment of patients with hemoglobinopathies