Highlights on globin gene switch: new target for therapeutics

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ORGANIZATION AND DEVELOPMENTAL EXPRESSION OF GLOBIN GENES

CHROMOSOME 11

CHROMOSOME 16

LCR

ε

γ

Gγ

Aγ

δ

β

ζ

α

α

HS 40

4 weeks

birth

1 year

Mai P.
Type And Distribution Of β-Thalassemia Mutations

Promoter
5' & 3' UTR
RNA Processing
ATG
Nonsense codons
Frameshifts

Globin chain synthesis with β-thalassemia mutations

- 4 weeks
- Birth
- 1 year

α
γ
β
HbA
HbF

Moi P.
Pathophysiology of β-thalassemia

HbA  alpha 2/beta 2

Normal production alpha chains

Absent or decreased beta chains

Excess unstable alpha tetramers

Absent or decreased haemoglobin A

Ineffective erythropoiesis

ANEMIA

Hemolysis
Mechanisms Of $\beta$-Thalassemia Intermedia

Severe Phenotype

$\alpha$ thalassemia

ild/silent $\beta$ alleles

creased $\gamma$

Increased $\gamma$ globin

$\beta + \gamma$ globin
HOMOZYGOUS SARDINIAN $\delta^{\beta^0}$-THALASSEMIA

DNA analysis:
- $\beta^{39}$ C→T homozygous
- $\alpha^\gamma$ -196 C→T homozygous

Hb g/dl: 11.9
MCV pg: 63.4
MCH pg: 21.6
F %: 99.7
$A_2$ %: 0.3
$A_\gamma$ %: 72.0

MCV fl: 72.7
MCH pg: 24.2
F: 18.8
$A_2$ %: 2.8

Age: 3.5 y

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

- 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


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


F cells measure the presence of fetal hemoglobin, a haematopoietic 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 novel 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.
GWAS results for HbF

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, i, *
Hb F variation associated SNPs

Chr 2
- 2p16: BCL11A
  - rs766432
  - rs1427407
  - rs11886868
  - rs4671393

p = 9.14e-08

Chr 6
- 6q23: MYB
  - rs9399137
  - rs4895441

p = 0.018

Chr 11
- 11p15: HBB
  - rs4910742
  - rs7482144

p = 0.446
Bcl11A and HbF

- genetic association detected by G-WAS

- SNPs 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

KLF1

- Direct binding to β-promoter
  - Induces β-globin gene expression

- Direct binding to BCL11A promoter
  - Inhibition of γ globin gene expression
Delayed fetal hemoglobin switching in subjects with KLF1 gene mutation

Stefania Satta a, Lucia Perseu b, Liliana Maccioni a, Nicola Giagu c, Renzo Galanello a,*
γ-globin gene expression in β-talassemia and in HPFH

Diagram showing the expression of different globin genes over time:
- α-globin (red line)
- γ-globin (green line)
- β-globin (blue line)
- δ-globin (orange line)

Key points:
- 4 weeks
- Birth
- 1 year
- α-globin expression peaks and then decreases
- γ-globin expression peaks and then decreases
- β-globin remains constant
- δ-globin increases with age
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

<table>
<thead>
<tr>
<th>Locus</th>
<th>p</th>
<th>Hazards Ratio</th>
<th>Harrell’s C-index</th>
<th>Predictor for later transfusion start</th>
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<tbody>
<tr>
<td>HBG2:g.-58C&gt;T</td>
<td>&lt;0.001</td>
<td>0.081</td>
<td>0.54</td>
<td>+/-</td>
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<tr>
<td>α gene defects</td>
<td>&lt;0.001</td>
<td>0.514</td>
<td>0.61</td>
<td>class 2</td>
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<tr>
<td>BCL11A</td>
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<tr>
<td>rs1427407</td>
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<td>G allele</td>
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<td>HBS1L/MYB</td>
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<tr>
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<tr>
<td>rs6904897</td>
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<td>0.697</td>
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<td>TT genotype</td>
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<tr>
<td>Gender</td>
<td>0.016</td>
<td>0.738</td>
<td>0.52</td>
<td>Male</td>
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</table>
Clinically relevant fetal hemoglobin modifiers

• Genetic:
  - *in cis* variants:
    - $\gamma$ globin genes
    - $\beta$ globin cluster
    - LCR
  - *in trans* variants:
    - BCL11A
    - HBS1L-MYB
    - KLF1 mutations
    - GATA1

• Epigenetic:
  - trisomy 13
  - DNA methylation
  - histone modification (*acetylation, phosphorylation, methylation*)
  - erythropoiesis expansion (*perturbation of erythroid kinetics*)
Current hemoglobin switching model
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 with blood safety and availability in several countries

HbA = adult haemoglobin; HbF = fetal haemoglobin.
## HbF inducers

<table>
<thead>
<tr>
<th>Class</th>
<th>Compound</th>
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<tbody>
<tr>
<td>Hypomethylating/cytotoxic agents</td>
<td>5-azacytidine, decitabine</td>
</tr>
<tr>
<td>Cytotoxic agents</td>
<td>hydroxyurea</td>
</tr>
<tr>
<td>Histone Deacetylase Inhibitors</td>
<td>butyrate analogs, short chain fatty acids, adipicin, scriptaid, trichostatin A, valproic acid,</td>
</tr>
<tr>
<td>Antioxidants</td>
<td>resveratrol, angelicin, curcumin....</td>
</tr>
<tr>
<td>Others</td>
<td>Erythropoietin, thalidomide</td>
</tr>
</tbody>
</table>
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 (?)
Summary of clinical trials with HU in \( \beta \)-thalassemia syndromes

- 18 trials
- 7 thalassemia major, 8 intermedia, 3 HbE/\( \beta \)-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 **Xmn1** and **BCL11A** Single Nucleotide Polymorphisms May Help Predict Hydroxyurea Response in Iranian β-Thalassemia Patients


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 Najmabadi¹,⁵
Thalidomide therapy in patients with thalassemia major.

A genotype –28A→C/cd39

100 mg/kg/d
HbF=−100%

B genotype IVS1-6/cd44C

75 mg/kg/d
HbF=50 → 73%


Abbreviations: CTX, cyclophosphamide; PDN, prednisone; RBC, red blood cell transfusions
Pharmacologic induction of HbF: concerns and limitations

- potential toxicity → safety and long term
- unpredictable and variable response
- effectiveness and long term \((\text{loss of efficacy overtime})\)
- 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

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