Talassemia intermedia: Fisiopatologia e diagnosi

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Piemonte e Valle d’Aosta
Thalassemia intermedia: definition

- Patients not requiring regular RBC transfusion for survival
- Patients requiring sporadic/not regular transfusions as
  - Disease severity progresses
  - Clinical complications manifest
Global distribution of β-thalassaemia intermedia

<table>
<thead>
<tr>
<th>Hb disorder</th>
<th>Annual births</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-TM</td>
<td>22,989</td>
</tr>
<tr>
<td>β-TI</td>
<td>Ill-defined</td>
</tr>
</tbody>
</table>

β-TI occurs at a low and varying frequency in every population with a high frequency of β-thalassaemia and is particularly common in parts of Africa where mild β-thalassaemia alleles predominate.

Imbalance Of Globin Chain Synthesis In Beta Thalassemia

Severity Of Clinical Phenotype
**NTDT: diagnosis**

- **Clinical phenotype:**
  Child older than two years of age with microcytic anemia, mild jaundice and hepatosplenomegaly.

- **Hematological phenotype:**
  Hb >7 < 10 g/dl, MCV > 50 < 80 fl and MCH > 16 < 24 pg.
  Morphologic changes [microcytosis, hypochromia, anisocytosis, poikilocytosis (spiculated tear-drop and elongated cells)], nucleated RBC.

- **Hemoglobin pattern:**

<table>
<thead>
<tr>
<th>Hemoglobin Type</th>
<th>Normal 1</th>
<th>Affected</th>
<th>Carrier</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>β°-Thal Homozygotes 2</td>
<td>β⁺-Thal Homozygotes or β⁺/β° Compound Heterozygotes 3</td>
</tr>
<tr>
<td>HbA</td>
<td>96%-98%</td>
<td>0</td>
<td>10%-30%</td>
</tr>
<tr>
<td>HbF</td>
<td>&lt;1%</td>
<td>95%-98%</td>
<td>70%-90%</td>
</tr>
<tr>
<td>HbA₂</td>
<td>2%-3%</td>
<td>2%-5%</td>
<td>2%-5%</td>
</tr>
</tbody>
</table>

  1. Data from Telen & Kaufman [1999]

- **Molecular analysis**

- **Globin chain synthesis analysis:** α/β+γ generally less imbalanced than in thalassemia major
Molecular basis of thalassemia intermedia

Homozygous or compound heterozygous state for $\beta$ thalassemia

- Inheritance of mild $\beta$ thalassemia alleles
- Co-inheritance of $\alpha$ thalassemia
- Increased Hb F response
  - Xmn1 $G_\gamma$ polymorphism
  - $\beta$ globin gene promoter mutations
  - Trans-acting HPFH genetic determinants

Heterozygous state for $\beta$ thalassemia

- Co-inheritance of excess $\alpha$ globin genes
  - ($\alpha\alpha\alpha/\alpha\alpha$, $\alpha\alpha\alpha/\alpha\alpha\alpha$, $\alpha\alpha\alpha\alpha/\alpha\alpha$)
- Dominantly inherited $\beta$ thalassemia
  - (Hyperunstable $\beta$ globin chain variants)

Compound heterozygotes for $\beta$ thalassemia and $\beta$ chain variants

  e.g. Hb E/ $\beta$ thalassemia

Compound heterozygotes for $\beta$ thalassemia and mendelian HPFH or $\delta\beta$ thalassemia
Genotypes of 122 Sardinian patients with thalassemia intermedia
Famiglia G.

Hb, g/dl 15.2
MCV, fl 85.5
HbA2, % -
HbF, % -
α/β 1.2

Hb, g/dl 8.2
MCV, fl 61.0
HbA2, % 2.8
HbF, % 27.0
α/(β+γ) 4.16

Tratto silente
Mild thal. Intermedia
Severe thal. Intermedia

XmnI -/- +/
Bcl11A rs1427407 GG GT
HBS1L-Myb TT TT

β° 39
Genotypes of 122 Sardinian patients with thalassemia intermedia

- $\beta^{039}/\beta^{039}$: 56% (68 pts)
- Other genotypes: 44% (54 pts)
G-WAS looks for differences in SNPs between genomes to detect variants more common in cases than in controls.
Hb F variation associated SNPs

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

Chr 6
- 6q23
- MYB
- rs9399137
- rs4895441

Chr 11
- 11p15
- HBB
- rs4910742
- rs7482144

\[ p = 9.14 \times 10^{-8} \]
\[ p = 0.018 \]
\[ p = 0.446 \]
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

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 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.
Genome-wide linkage and association scan 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,g,h, Clinton T. Baldwin e,i, Martin H. Steinberg d, David H.K. Chui d,*
Human Fetal Hemoglobin Expression Is Regulated by the Developmental Stage-Specific Repressor BCL11A

Vijay G. Sankaran,1,2 Tobias F. Menne,1 Jian Xu,1 Thomas E. Akie,1 Guillaume Lettre,3,4 Ben Van Handel,5 Hanna K. A. Mikkola,5 Joel N. Hirschhorn,3,4 Alan B. Cantor,1 Stuart H. Orkin1,2,6*

A

Human Proerythroblasts

Exon:

1 2 3 4 5

XL (835 aa)

L (773 aa)

B

Relative Expression (Normalized to β-Actin)

p = 0.013

p = 0.004

XL Isoform

p = 0.018

p = 0.009

L Isoform

0.004

0.008

0.012

0.016

GG

AG

AA

“Low” HbF

“High” HbF

Genotype at rs4671393

C

BM Erythroblasts

FL Erythroblasts

Primitive Erythroblasts

K562 Cells

xl/l

Isoforms

Shorter

Variants

GAPDH

Sankaran et al, 2008
Bcl11A and HbF

- Genetic association detected by G-WAS
- SNPs in IVS2 described in different populations, in HPFH, beta thalassemia, HbE, SCD
- Bcl11A is a repressor of fetal hemoglobin
- High HbF is associated with low Bcl11A expression
- Bcl11A expression varies at different developmental stages
- Bcl11A is an essential component of hemoglobin switching in human and mouse
Amelioration of Sardinian $\beta^0$ thalassemia by genetic modifiers

Ameliorating alleles:

- BCL11A rs 11886868
- HBS1L-Myb rs 9389268
- $-\alpha/\alpha\alpha\alpha$
- $-\alpha/\alpha\alpha$
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>
</tr>
</thead>
<tbody>
<tr>
<td>HBG2:g.-58C&gt;T</td>
<td>&lt;0.001</td>
<td>0.081</td>
<td>0.54</td>
<td>+/-</td>
</tr>
<tr>
<td>α gene defects</td>
<td>&lt;0.001</td>
<td>0.514</td>
<td>0.61</td>
<td>class 2</td>
</tr>
<tr>
<td>BCL11A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1427407</td>
<td>&lt;0.001</td>
<td>2.391</td>
<td>0.63</td>
<td>T allele</td>
</tr>
<tr>
<td>rs10189857</td>
<td>0.005</td>
<td>1.312</td>
<td></td>
<td>G allele</td>
</tr>
<tr>
<td>HBS1L/MYB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs4895441</td>
<td>&lt;0.001</td>
<td>1.979</td>
<td>0.57</td>
<td>G allele</td>
</tr>
<tr>
<td>rs6904897</td>
<td>0.020</td>
<td>0.697</td>
<td></td>
<td>TT genotype</td>
</tr>
<tr>
<td>Gender</td>
<td>0.016</td>
<td>0.738</td>
<td>0.52</td>
<td>Male</td>
</tr>
</tbody>
</table>
## Extended genotype and starting of transfusion therapy

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patient: A</th>
<th>Patient: B</th>
</tr>
</thead>
<tbody>
<tr>
<td>beta globin genotype</td>
<td>$\beta 39 / \beta 39$</td>
<td>$\beta 39 / \beta 39$</td>
</tr>
<tr>
<td>alpha globin genotype</td>
<td>$-\alpha / \alpha \alpha$</td>
<td>$-\alpha / \alpha \alpha$</td>
</tr>
<tr>
<td>Xmn1 G$\gamma$</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Bcl11A rs 11886868</td>
<td>T/C</td>
<td>T/T</td>
</tr>
<tr>
<td>HBS1L-MYB rs 9399137</td>
<td>T/T</td>
<td>T/T</td>
</tr>
<tr>
<td>12 months not transfused</td>
<td></td>
<td>5 months first transfusion</td>
</tr>
<tr>
<td>Hb 9.2 g/d</td>
<td></td>
<td>Pre tfx Hb=7.1 g/dl</td>
</tr>
</tbody>
</table>
THE XmnI AND BCL11A SINGLE NUCLEOTIDE POLYMORPHISMS MAY HELP PREDICT HYDROXYUREA RESPONSE IN IRANIAN β-THALASSEMSIA PATIENTS

Mehdi Banan, Hadi Bayat, Azita Azarkeivan, Saeid Mohammadparast, Koorosh Kamali, Samaneh Farashi, Nooshin Bayat, Masumeh Hadavand Khani, Maryam Neishabury, and Hossein Najmabadi

1 Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, 2 Blood Transfusion Organization, 3 The Adult β-Thalassemia Clinic, 4 Reproductive Biotechnology Research Center, Avicenna Research Institute, 5 Kariminejad-Najmabadi Pathology & Genetics Center, Tehran, Iran
Thalassemia intermedia: physiopathology

Normal erythropoiesis → Erythroid precursor → Erythrocyte → Anemia → Epo

β-Thalassemia → Ineffective erythropoiesis → Epo

Increased pool of erythroid progenitor cells & presence of abnormal erythrocytes

Bone abnormalities → EMH-splenomegaly → Thrombosis

Rivella S et al, Blood Reviews 2012;
Schematic representation of normal and ineffective erythropoiesis.
The Janus Kinase 2- STAT5 pathway

Patnaik et al, Leukemia 2009;
Thalassemia intermedia: physiopathology

Rivella S et al, Blood Reviews 2012;
JAK inhibitors: beyond spleen and symptoms?
Mechanism of iron overload in non-transfused patients

Ineffective erythropoiesis
Chronic anaemia
Hypoxia

↑ Erythropoietin
↑ Duodenal iron absorption
↑ Ferroportin

↑ GDF15

↑ Release of recycled iron from RES macrophages

↓ Hepcidin

↓ Hepcidin
↓ Serum ferritin

↑ LIC

↑ LIC

↑ Serum ferritin

GDF15 = growth differentiation factor 15; HIF = hypoxia-inducible transcription factor; LIC = liver iron concentration; RES = reticulo-endothelial system.

### Liver iron concentrations and urinary hepcidin in β-thalassemia

Raffaella Origa, Renzo Galanello, Tomas Ganz, Nicolina Giagu, Liliana Maccioni, Gavino Faa and Elizabeta Nemeth

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Hb (g/dL)</th>
<th>Serum ferritin (μg/L)</th>
<th>LIC (ng Fe/g dry wt)</th>
<th>Cardiac T2* (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>37.2 ± 10</td>
<td>8.8 ± 1.3</td>
<td>558 ± 697</td>
<td>5.6 ± 7.8</td>
</tr>
</tbody>
</table>

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No evidence of cardiac iron in 20 never- or minimally-transfused patients with thalassemia intermedia

Raffaella Origa, Susanna Barella, Giovanni Maria Argiolas, Patrizio Bina, Annalisa Agus and Renzo Galanello

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![Graph showing hepatic hepcidin levels](chart.png)
Ineffective erythropoiesis and hepcidin regulation in β-thalassemia

GdF15 in human blood

Twsg1 mRNA in murine thalassemia

(Chou St et al, 2007; Tanno T et al, 2009)
Jak2 is required for hepcidin-mediated Ferroportin internalization.
Stat5 regulates cellular iron uptake of erythroid cells via IRP-2 and Tfr-1

Pathophysiology of NTDT vs clinical sequelae

Disrupted α:β globin ratio

Ineffective erythropoiesis

Hemolysis

Gut iron absorption↑

Iron and free radicals↑

Anaemia

Tissue oxygenation↓

Erythroid marrow expansion

- "Flip-flop" phenomenon
- Depletion of proteins C and D
- Red cell degenerative products

Diabetes mellitus
Growth hormone deficiency
Hypothyroidism
Hypoparathyroidism
Hypogonadism
HCC
Renal dysfunction

1. Leg ulcers
2. Thrombotic events

1. Pulmonary hypertension
2. Congestive heart failure

VDR, OesR, COL1A1

1. Facial deformities
2. Osteopenia

1. Hepatosplenomegaly and jaundice

APOEε4

UGT1A1

HCC = hepatocellular carcinoma.

Platelets
• Increased platelet aggregation
• Increased expression of activation markers
• Presence of platelet morphologic abnormalities

Peripheral blood elements
• Expression of endothelial adhesion molecules and tissue factor on endothelial cells
• Formation of microparticles

Nitric oxide
• Hallmark of haemolysis
• ↓ Levels leading to vasoconstriction

RBCs
• Formation of reactive oxygen species
• Expression of negatively charged phospholipids
• Enhanced cohesiveness and aggregability

Hypercoagulability

Other factors
• Cardiac dysfunction
• Hepatic dysfunction
• Endocrine dysfunction

Thrombophilia
• No role for prothrombotic mutations
• Decreased levels of antithrombin III, protein C, and protein S
• Anti-phospholipid antibodies

Splenectomy
• High platelet counts and hyperactivity
• High levels of negatively charged RBCs

Grazie