Hemoglobinopathies

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DISCLOSURES

Off-Label Usage
  • None

Interests
  • Incyte Corp.
  • Teva
3-D Ribbon Structure of Hemoglobin A
Stick Structure of Hemoglobin A

Close-up view of heme with Fe at center
Normal Hbs found in Adults

<table>
<thead>
<tr>
<th>Hb A: $\alpha_2\beta_2$</th>
<th>Hb A$_2$: $\alpha_2\delta_2$</th>
<th>Hb F: $\alpha_2\gamma_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>97%</td>
<td>3%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>$\alpha$-globin+heme</td>
<td>$\alpha$-globin+heme</td>
<td>$\alpha$-globin+heme</td>
</tr>
<tr>
<td>$\beta$-globin+heme</td>
<td>$\delta$-globin+heme</td>
<td>$\gamma$-globin+heme</td>
</tr>
<tr>
<td>$\alpha$-globin+heme</td>
<td></td>
<td>$\alpha$-globin+heme</td>
</tr>
<tr>
<td>$\beta$-globin+heme</td>
<td></td>
<td>$\gamma$-globin+heme</td>
</tr>
</tbody>
</table>
DNA hypersensitive sites:
- β-globin LCR [locus control region]
- α-globin HS-40 [hypersensitive site-40]

(Weatherall and Proven, Lancet 2000;355:1169-1175)
Geographical overlap in distributions of malaria & thalassemias/Hbopathies

- provides compelling evidence that these red cell disorders protect against malarial infection.
Hemoglobinopathies and Thalassemias

- Mankind's most common single gene, mendelian diseases
- Disorders of the synthesis or structure of Hb
- Almost 1500 described
Hemoglobinopathies and Thalassemias

- Thalassemias: reduced amounts or absence of structurally normal globin chain
  - α-thalassemia
  - β-thalassemia

- Hemoglobinopathies: amino acid substitutions; structurally abnormal globin
  - Hb S, Hb E, Hb C, Hb G-Philadelphia, Hb D, Hb O-Arabia
Hemoglobinopathies and Thalassemias

- Interactions among thalassemias and hemoglobinopathies are common
  - Hemoglobin S / beta thalassemia
  - Hb S and alpha thalassemia
  - Hemoglobin E / beta thalassemia
Hemoglobinopathies: Lab Dx

- Hb electrophoresis
  - cellulose acetate (alkaline)
  - Citrate agar (acidic)
- HPLC
- Molecular biology
  - PCR; gene sequencing
Thalassemia Mutations

α-Thalassemia
- clinically expressed in fetus and at birth
- mostly caused by gene deletion

β-Thalassemia
- expressed after several mos because of switching from γ- to β-globin
- mostly caused by point mutations
Thalassemia: Molecular Mechanisms

**Gene deletion**
- entire gene
- portions of a gene

**Transcriptional defects**
- promoter mutants
- Locus Control Region mutants
- trans-acting mutations

**Translational defects**
- splice junction mutants
- reading frameshifts
- new splice sites
- polyA site mutants
- nonsense mutations

**Hyper-unstable globins**
Beta Thalassemias

- ↓ synthesis of \( \beta \)-globin chains
- Excess of \( \alpha \)-globin chains
  - \( \alpha \)-globin aggregates to form insoluble inclusions in erythroid precursors
  - highly toxic
  - intramedullary death of erythroid precursors: ineffective erythropoiesis
Membrane Defects in β-Thalassemia

Excess cellular Fe and unstable unpaired α-globin chains cause:

- membrane lipid oxidation
- membrane protein damage
- decreased RBC deformability
- removal from the circulation

Membrane damage leads to PS exposure and hypercoagulability.
Ineffective Erythropoiesis

- High degree of erythropoietic activity
- Death of erythroid precursors in BM
- Blood tests look like hemolysis, but retics not ↑ for degree of anemia
  - ↑ or high nl LDH, indirect bilirubin
  - ↓ haptoglobin
- Thal major and intermedia
  - both ineff. erythropoiesis & hemolysis
**β-Thalassemia Mutations**

**β^0-thal mutations**

- totally abolish expression of affected gene by critical point mutation or deletion

**β^+-thal mutations**

- partially abolish gene expression
- mild, moderate, severe—depending on amount of Hb A produced
**Clinical Classification of β-Thalassemia**

**β-Thalassemia trait**
- uncomplicated heterozygous β-thal
- β-thalassemia minor

**β-Thalassemia intermedia**
- no firm definition; many different genotypes

**β-Thalassemia major**
- Cooley's anemia
- homozygous or compound heterozygous β-thal.

*genotype-phenotype correlations often difficult to make: 100s of mutations, frequent interactions, role of other modifying genes and environment.*
Clinical Diagnosis of β-Thalassemia

β-Thalassemia trait
- microcytosis, hypochromia, +/- mild anemia
- elevated level of HbA₂ (>3.5%)

β-Thalassemia intermedia
- microcytic anemia, +/- Tx requirement
- many different genotypes, high Hb F
- bone disease, iron loading, splenomegaly, pulmonary hypertension
Clinical Diagnosis of β-Thalassemia

β-Thalassemia major

• transfusion-dependent microcytic anemia
• very high Hb F (approaching 100%)
• bone disease, iron loading, splenomegaly, pulmonary hypertension
# Beta Thalassemias

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
<th>Hematologic Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterozygote $(\beta^+/\beta)$</td>
<td>Silent carrier/thal minima</td>
<td>Normal</td>
</tr>
<tr>
<td>Heterozygote $(\beta^0/\beta$ or $\beta^+/\beta)$</td>
<td>Thal minor</td>
<td>Mild hypochromic anemia</td>
</tr>
<tr>
<td>Homozygote or compd hetero. $(\beta^+/\beta^+)$</td>
<td>Thal intermedia</td>
<td>Moderate hemolysis &amp; ineffective erythropoiesis</td>
</tr>
<tr>
<td>Homozygote or compd hetero. $(\beta^0/\beta^0)$</td>
<td>Thal major</td>
<td>Severe hemolysis &amp; ineffective erythropoiesis</td>
</tr>
</tbody>
</table>
### Clinical Features of $\beta$-Thal Syndromes

<table>
<thead>
<tr>
<th></th>
<th>Major</th>
<th>Intermedia</th>
<th>Minor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severity</td>
<td>4+</td>
<td>2+</td>
<td>±</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>4+</td>
<td>2-3+</td>
<td>0</td>
</tr>
<tr>
<td>Skeletal changes</td>
<td>2-4+</td>
<td>0-1+</td>
<td>0</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>&lt;7 g/dL</td>
<td>7-10 g/dL</td>
<td>&gt;10 g/dL</td>
</tr>
<tr>
<td>Hypochromia</td>
<td>4+</td>
<td>3+</td>
<td>2+</td>
</tr>
<tr>
<td>Microcytosis</td>
<td>3+</td>
<td>2+</td>
<td>1+</td>
</tr>
<tr>
<td>Nucleated RBC’s</td>
<td>3+</td>
<td>0-1+</td>
<td>0</td>
</tr>
</tbody>
</table>
# Hb Fractions in β-Thal Syndromes

<table>
<thead>
<tr>
<th></th>
<th>NI</th>
<th>Minor</th>
<th>Intermedia</th>
<th>Major</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb A</td>
<td>97%</td>
<td>&gt;90%</td>
<td>15-65%</td>
<td>0%</td>
</tr>
<tr>
<td>Hb A2</td>
<td>2.2-3.5%</td>
<td>3.5-8%</td>
<td>5.4-10%</td>
<td>1-5.9%</td>
</tr>
<tr>
<td>Hb F</td>
<td>&lt;1%</td>
<td>1-2%</td>
<td>30-75%</td>
<td>&gt;94%</td>
</tr>
</tbody>
</table>
Pathophysiology of β-Thalassemia

**NORMAL**
- HbA ($\alpha_2\beta_2$)
- Normal erythroblast
- Normal red blood cells

**β-THALASSEMIA**
- Reduced β-globin synthesis, with relative excess of α-globin
- Insoluble α-globin aggregate
- Abnormal erythroblast
- Few abnormal red cells leave
- Hypochromic red cell
- Destruction of aggregate-containing red cells in spleen

**ANEMIA**
- Increased iron absorption
- Blood transfusions
- Tissue anoxia
- Erythropoietin increase
- Marrow expansion
- Skeletal deformities

Dietary iron

**Systemic iron overload** (secondary hemochromatosis)

Heart

Liver

Pappas, AA
β-Thalassemia Major: Clinical Features

**Hematologic**
- Severe microcytic anemia
- Splenomegaly, extramedullary hematopoiesis

**Skeletal changes**
- expanded marrow cavity; thalassemic facies
- osteopenia, thin cortex

**Growth retardation**

**Thromboembolism**
β-Thalassemia Major: Clinical Features

Cardiopulmonary

- Myocardial Fe overload with arrhythmia
- CHF
- Hemolytic pulmonary hypertension

Liver

- Hepatic iron-loading with fibrosis, cirrhosis
- Pigmented gall stones
β-Thalassemia Major: Clinical Features

Endocrinopathies
- diabetes mellitus
- hypoparathyroidism
- hypogonadism and delayed puberty

Transfusion related
- infection
- Alloimmunization
Peripheral Smear in β-Thalassemia

Thalassemia minor

Thalassemia major
Skull and Face in Poorly Treated β-Thal
β-Thalassemia Major: Prognosis

• No Rx:
  – death by age 5 from infections, cachexia

• Episodic blood Tx’s:
  – survival into 2nd decade

• Aggressive blood Tx’s:
  – death ~age 20 from iron overload (cardiac)

• Aggressive blood Tx’s plus iron chelation:
  – prolonged survival
**β-Thalassemia Major: Treatment**

- **Management in comprehensive center:**
  - endocrinology
  - cardiology
  - social services

- **Hypertransfusion beginning 2nd or 3rd year:**
  - maintain Hb 9-10.5 g/dL

- **Splenectomy for increasing Tx requirement**
β-Thalassemia Major: Treatment

- Fe chelation starting after age 3 years-
  - keep liver Fe <5 mg/g
- Also:
  - Consider stem cell transplantation
  - ? Increase synthesis of fetal Hb with hydroxyurea or other agents
  - Genetic counseling
  - Prenatal diagnosis
Iron Chelators

*Deferoxamine (Desferal)*
- Given by prolonged infusion

*Deferasirox (Exjade)*
- Once daily oral dosing (20-40 mg/kg)
- Can remove cardiac Fe

*Deferiprone (Ferriprox)*
- Orally active; limited approval in US
- Removes cardiac iron
Potential Toxicity of Iron Chelation

- Skin reactions
- Renal, hepatic, bone, BM, otologic, retinal damage
- Yersinia infection
- Growth delay, misc others
Hemoglobin E (β26 glu → lys)

- Thalassemic hemoglobinopathy (decreased β^E^-mRNA production)
- RBC cytoplasm: precipitated α-chains, increased oxidant stress
- Second most prevalent Hb variant: 30,000,000 worldwide; >80% in SE Asia
- Carriers clinically silent; low MCV
<table>
<thead>
<tr>
<th>Condition</th>
<th>Genotype</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbE Trait</td>
<td>A/E</td>
<td>30% Hb E ±</td>
</tr>
<tr>
<td>HbE Disease</td>
<td>E/E</td>
<td>90% Hb E,</td>
</tr>
<tr>
<td>HbE-β-thal</td>
<td>E/beta(^0,^+)</td>
<td>Hb E 40-85%, Hb F 10-60%, (\downarrow) MCV, Hb</td>
</tr>
<tr>
<td>Hb SE disease</td>
<td>S/E</td>
<td>resembles Hb S-β(^+)-thal</td>
</tr>
</tbody>
</table>
Hemoglobin E/β-Thalassemia

- SE Asia
- Hb E 60-85%, Hb F 15-40%
- Mild to moderate microcytic hemolytic anemia
- Ineffective erythropoiesis and iron-loading
Alpha Thalassemia

• Decreased synthesis of α-globin chains
• Excess of beta-like globin chains
• Potential formation of abnl Hbs:
  – Hemoglobin Barts: $\gamma_4$
  – Hemoglobin H: $\beta_4$
Gene Deletion α-Thalassemia*

α⁺-thalassemia

• deletion of a single gene on one chromosome 16 allele

α₀ thalassemia

• deletion of both genes on one chromosome 16 allele

*Point mutations less common cause of α-thalassemia; often associated with severe θ in α-globin synthesis
Pathophysiology of α-thalassaemia

Fetus

\[ \gamma_4 \] Hb Bart’s

\[ \alpha_2 \gamma_2 \] (excess)

\[ \alpha \]-globin synthesis

Adult

\[ \beta_4 \] Hb H

\[ \alpha_2 \beta_2 \] (excess)

- High O2 affinity- Hypoxia
- Instability of homotetramers
- Inclusion bodies. Membrane damage
- Shortened RBC survival- Hemolysis
- Splenomegarly- Hypersplenism.

(Weatherall and Proven, Lancet 2000;355:1169-1175)
Genetics of $\alpha$-Thalassemia: SE Asia
Genetics of $\alpha$-Thalassemia: Africa

Diagram showing the genetics of $\alpha$-Thalassemia with different genotypes:

- **Normal**
  - \(\alpha\alpha\alpha\alpha\)

- **\(\alpha^+\) thal silent carrier**
  - \(\alpha\alpha\alpha\)
  - \(\alpha\alpha\alpha\x\)

- **\(\alpha^+\) thal silent carrier**
  - \(\alpha\alpha\alpha\x\)

- **\(\alpha^+\) thal trait**
  - \(\alpha\alpha\x\x\)

*
# Alpha Thalassemias

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Pheno-type</th>
<th>Hb Barts (γ₄)</th>
<th>Hb H (β₄)</th>
<th>Heme Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>αα/αα</td>
<td>Normal</td>
<td>---</td>
<td>---</td>
<td>Normal</td>
</tr>
<tr>
<td>αα/α⁻</td>
<td>Silent carrier</td>
<td>---</td>
<td>---</td>
<td>Normal</td>
</tr>
<tr>
<td>αα/-- or α⁻/α⁻</td>
<td>α-thal trait</td>
<td>2-10% newborn</td>
<td>---</td>
<td>Mild anemia</td>
</tr>
<tr>
<td>α⁻/--</td>
<td>Hb H disease</td>
<td>20-40% newborn</td>
<td>5-40% adults</td>
<td>Hemolysis, ineff. erythro.</td>
</tr>
<tr>
<td>--/--</td>
<td>Hydrops fetalis</td>
<td>~100% cord blood</td>
<td>---</td>
<td>Anemic stillborn</td>
</tr>
</tbody>
</table>
α-Thalassemia ‘Silent Carrier’

- heterozygous $\alpha^+$ thalassemia
- 3 of 4 alpha genes present and functional
- +/- mild anemia
- ↓ MCV (age dependent)
Alpha Thalassemia Trait

2 of 4 alpha genes present and functional
- Homozygous $\alpha^+$ thal ($\alpha-/\alpha-$): ~7% of Africans
- Heterozyg. $\alpha^0$ thal ($\alpha\alpha/--$): common SE Asia

Clinical features:
- +/- mild anemia
- MCV <78 fL
- Hb Barts ($\gamma_4$) 2-10% in newborns
Alpha Thalassemia Trait

Usually Dx of exclusion
- Compatible ethnicity and clinical picture
- Exclude Fe def, β-thal, hereditary sideroblastic anemia

Do not confuse with Fe def or treat with iron
Hemoglobin H Disease

- Genotype $\alpha/-/-$ (SE Asia)
  - $\alpha^+$-thal one allele
  - $\alpha^0$-thal other allele
- 20-40% Hb Barts ($\gamma_4$) in newborn
- 5-40% Hb H ($\beta_4$) in adults
  - visualized by brilliant cresyl blue
  - Hb electrophoresis
  - HPLC
Dx of Hb H Disease

RBC inclusions generated by brilliant cresyl blue

Fast moving peak on HPLC
Hemoglobin H Disease

• Clinical features
  – hemolytic anemia of varying degrees
  – microcytosis
  – splenomegaly
  – ineffective erythropoiesis
  – Fe-loading
Hemoglobin Bart's Hydrops Fetalis

Homozygous $\alpha^0$ thalassemia (-/-/-/-)
No functional $\alpha$-globin genes: Hb Barts ($\gamma_4$)
Eclampsia in mother
Stillbirth
Erythroblastosis in infant
## RBC Indices in Alpha-Thalassemia

<table>
<thead>
<tr>
<th></th>
<th>NI</th>
<th>Silent Carrier</th>
<th>Trait</th>
<th>Hb H Disease</th>
<th>Hydrops Fetalis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hb (g/dL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F: 12-16</td>
<td>F: 10-14</td>
<td>F: 7-11</td>
<td></td>
<td>F: 3-8</td>
<td></td>
</tr>
<tr>
<td><strong>MCV (fL)</strong></td>
<td>79-99</td>
<td>67-95</td>
<td>64-79</td>
<td>53-69</td>
<td>126-146</td>
</tr>
<tr>
<td><strong>MCH (pg)</strong></td>
<td>27-35</td>
<td>22-30</td>
<td>21-25</td>
<td>16-20</td>
<td>22-42</td>
</tr>
</tbody>
</table>
Atypical α-Thalassemias

α-Thalassemia-mental retardation syndromes

• ATR-16 (alpha thal. retardation associated with Chr. 16): large deletions involving α-globin genes
• X-linked- mutations in ATRX on Chr. X, which encodes a chromatin-associated protein

α-thalassemia-MDS

• acquired α-thalassemia in myelodysplastic syndrome
Management of α-Thal Syndromes

Hb Bart’s
• Screening, genetic counseling in populations at high risk

Hb H disease
• Regular medical follow-up
• Blood Tx and Rx of Fe overload as needed

Mild α-thalassemias
• Dx important for genetic counseling and avoiding misguided Rx like Fe
Other Conditions

• **Hemoglobin Lepore**
  - Fusion of $\beta$ and $\delta$ globin genes
  - ↓ synthesis of $\beta$-like globins
  - Homozygote: $\beta$-thal major phenotype
    • 8-30% Hb Lepore
    • 70-92% Hb F
  - Heterozygote: $\beta$-thal minor phenotype
Other Conditions

- **Hb Constant Spring**
  - non-deletional form of α-thalassemia
  - mutation in stop codon of α2-globin
  - poor output (1% of nl) of α-globin with 31 additional amino acids
  - homozygosity leads to Hb H type clinical picture but nearly nl MCV
Other Conditions

• Hereditary persistence of fetal Hb
  – Up-regulation of $\gamma$ chain synthesis
  – $\sim$100% Hb F in homozygotes
  – Clinically silent
  – Causes:
    • deletions involving $\beta$ and $\delta$ genes
    • expression of $KLF1$, transcription factor
      that activates the Hb F suppressor, $BCL11A$
Unstable Hemoglobin Disease

- Congenital Heinz body anemia
- Rare, autosomal dominant mutations → defective binding of heme by globin
- About 200 ‘unstable’ variants: phenotype heterogeneous
- Heinz bodies, peroxidant membrane damage, hemolysis
Unstable Hemoglobins
Unstable Hemoglobins

Diagnosis

- Normocellular to microcytic anemia
- ± Distinct electrophoreoretic pattern
- Isopropopanol precipit. test: Heinz bodies
- mutation detection
- Hb Köln most common
  - anemia; retics 10-25%
  - splenomegaly
Unstable Hemoglobins

Treatment

• avoid oxidant drugs
• blood Tx’s
• splenectomy in severe cases
Hemoglobin M Disorders

Hereditary methemoglobinemia and cyanosis

Autosomal dominant

Amino acid substitution in heme pocket and allows Fe oxidation (ferrous heme $\rightarrow$ ferric heme)

Clinical: asymptomatic cyanosis, slate grey/brownish skin, no dyspnea, nl life expectancy
Hemoglobin M Disorders

Diagnosis
- abnormal pulse oximeter saturation
- distinguish from other methemoglobinemias
- Hb electrophoresis, Hb spectra
- Methemoglobin < 30%
- Cyanosis not reversible with Vit C, Meth Blue

Treatment: major hazard is misdiagnosis and untoward treatment
Other Forms of Methemoglobinemia

1. Congenital deficiency methemoglobin reductase (cytochrome b5 reductase)
   - Heterozygous or homozygous
   - Defective enzymatic reduction of Fe$^{+3}$ to Fe$^{+2}$
   - Sometimes neurologic abnormalities
   - Methemoglobin usually < 30%
   - No Rx usually required; cyanosis improves with methylene blue, ascorbic acid
Other Forms of Methemoglobinemia

2. Oxidation of Fe$^{+2}$ Hb to Fe$^{+3}$ Hb by drugs or chemicals
   - Nitrites, trinitrotoluene, sulfanilamide, PAS, dapsone, primaquine, chloroquine, lidocaine, naphthoquinone, resorcinol, phenylhydrazine
   - Methemoglobin $> 30\%$ symptoms; $> 50\%$ lethal
   - Emergency treatment: 1-2 mg/kg methylene blue as 1\% solution IV over 10-15 minutes
Hemoglobin O$_2$ Dissociation Curve

Arterial point:
- $pO_2$ 100
- $SaO_2$ 98%

Mixed venous:
- $pO_2$ 40
- $SaO_2$ 75%

$p50$:
- $pO_2$ 26
- $SaO_2$ 50%

The curve has a sigmoid shape because of positive cooperativity.

Standard Conditions:
- Temp = 37°C
- pH = 7.40
- BE = 0
Hbs with Altered O₂ Affinity

The diagram shows the oxygen saturation (%) versus oxygen pressure (mmHg) for different hemoglobins (Hbs).

- **High affinity Hb**: saturates at lower oxygen pressures.
- **Hb A**: intermediate affinity between High and Low.
- **Low affinity Hb**: saturates at higher oxygen pressures.

Key values:
- At 2.5 mmHg, High affinity Hb is at 5% saturation.
- At 5.3 mmHg, Hb A is at 50% saturation.
- At 9.5 mmHg, Low affinity Hb is at 100% saturation.
Hemoglobins with High $O_2$ Affinity

- Familial erythrocytosis; autosomal dom.
- $\alpha$ or $\beta$-chain can be affected; ± distinct electrophoretic pattern
- Left shift $O_2$ dissociation curve (low $P_{50}$)
- Normal 2,3-DPG levels
- Dx: $P_{50}$ and finding globin gene mutation
- Erythrocytosis usually mild; phlebotomy not necessary
Hemoglobins with Low $O_2$ Affinity

- Cyanosis
- Right shift $O_2$ dissociation curve (high P50)
- No Rx required