Beta Thalassemia

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Outline

- Hemoglobin
- Anatomy of a gene
- Hemoglobinopathies
  - Alpha and Beta Thalassemias
- Beta Thalassemia in North America
- Beta globin gene mutations
- Concluding remarks
α-like and β-like Globin Genes

α-like genes

β-like genes

Chromosome 16

Chromosome 11

0 20 40 60 kb


Nusbaum et al: Thomson and Thompson's Genetics in Medicine 7e

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The Human α-Globin and β-Globin Gene Families

HBB at 11p15.5

LS: 1 2 3 4

LCR

HBA at 16pter

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Red Blood Cells

Red blood cells contain several hundred hemoglobin molecules which transport oxygen. Oxygen binds to heme on the hemoglobin molecule.

Hemoglobin

Beta chains

Heme units with iron atom

Alpha chains
Donor and Acceptor Sites

Splice donor site | Branch site | Splice acceptor site
--- | --- | ---
GU | CUPuA | Py

20 - 50 bases

Splicing Consensus Sequences

5' Exon | Intron | 3' Exon
--- | --- | ---
AG | GUAAUGU | CAG

Anatomy of an Intron

Standard gene

5' UTR | Exon 1 | Exon 2 | 3' UTR
--- | --- | --- | ---
ATG | GT | AG | STOP

Intron

5' splice site | Branch site | 3' splice site
--- | --- | ---
GU | A | U

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3.5
The conserved nucleotides in the transcript are recognized by small nuclear ribonucleoprotein particles (snRNPs), which are complexes of protein and small nuclear RNA. A functional splicing unit is composed of a team of snRNPs called a spliceosome.

U1 snRNP

U1 is a specialized, relatively short RNA (less than 200 nucleotides long) known as an snRNA (small nuclear RNA). It is complexed with proteins to form the U1 snRNP (small nuclear ribonucleoprotein). snRNPs form the core of the spliceosome. U1 snRNP base pairs with the 5’ splice junction.
Beta globin gene

Uppercase characters:
- mature mRNA
- introns
- flanking sequences

Lowercase characters:
- Red
  - cat box
  - tata box
  - polyadenylation sequence

General outline of mechanism by which disease-causing mutations produce disease

HPFH: hereditary persistence of fetal hemoglobin

Expression of gene: at wrong time in wrong place
Hemoglobinopathies

- Hemoglobinopathies are the most common inherited disorders in humans, resulting from mutations in the α globin and β globin gene clusters.
- Molecular defects in either regulatory or coding regions of the human α globin, or β globin genes can minimally or drastically reduce their expression, leading to α thalassemia or β thalassemia, respectively.

Where is Thalassemia Endemic?
**α or β Hemoglobin Chain Pairing**

- **Thalassemia** is caused by impaired production of either the α or β hemoglobin chain.
- Normally, beta chains pair only with alpha chains.
- α thalassemia occurs when one or more of the 4 alpha chain genes fails to function.

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**Alpha: Loss of One α Gene**

- The loss of one gene diminishes the production of the alpha protein only slightly.
  - Person is called a silent carrier
  - Condition close to normal
  - Can be detected only by specialized laboratory techniques.
**Two-Gene Deletion α Thalassemia**

- The loss of 2 α genes produces a condition with small RBCs, and at most mild anemia.
  - People with this condition look and feel normal.
  - Condition can be detected by routine blood testing.

**Three-Gene Deletion α Thalassemia**

- The loss of 3 α genes produces a serious hematological problem.
  - Patients have severe anemia, and often require blood transfusions to survive.
  - Severe imbalance between the alpha chain production (now powered by one gene, instead of 4) and beta chain production (which is normal) causes an accumulation of beta chains inside the RBCs.
Four-Gene Deletion α Thalassemia

- The loss of all 4 alpha genes during fetal life causes death in utero or shortly after birth.
- Rarely, 4 gene deletion alpha thalassemia has been detected in utero, usually in a family where the disorder occurred in an earlier child.
- Repeated transfusions can keep victims alive.

β Thalassemias

- Unlike α thalassemia, β thalassemia rarely arises from the complete loss of a beta globin gene that is present, but produces little beta globin protein.
- To date at least 200 molecular defects have been defined in β thalassemias.
- The types of genes can be analyzed in each case.
β Thalassemia from Parents (I)

Both Parents are Carriers

Patient

- 25% with thalassaemia
- 50% beta thal trait
- 25% Unaffected

β - Thalassaemia

Unaffected

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β Thalassemia from Parents (II)

Partner who carries beta thalassaemia

Partner who carries beta thalassaemia

Eggs:
- 2 types:
  - Half haemoglobin A
  - Half beta thalassaemia

Sperms:
- 2 types:
  - Half haemoglobin A
  - Half beta thalassaemia

Changes in each pregnancy

Child with beta thalassaemia major

Carrier of beta thalassaemia

Carrier of beta thalassaemia

Not a carrier

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### Major Intermedia and Minor

- Absence of beta chain causes **beta-zero-thalassemia**.
- Reduced amounts of detectable beta globin causes **beta-plus-thalassemia**.
- For clinical purposes, **beta-thalassemia** is divided into:
  - **thalassemia major** (transfusion dependent)
    - 8 or more transfusions per 12 months [TCRN]
  - **thalassemia intermedia** (of intermediate severity)
    - Less than 8 transfusions per 12 months [TCRN]
  - **thalassemia minor** (asymptomatic).

### Point Mutations Causing β-Thalassemia

The mutations are distributed throughout the β globin gene. The mutations affect virtually every process required for the production of normal β globin.
Molecular Defects in β Thalassemia

- **Large Deletions and Insertions**
  - Deletions
    - 14 deletions from 290 to over 60,000 bp
    - Most common: 619 bp deletion at 3’ of gene (Sind and Punjabi pop)
  - Insertion
    - Retrotransposon of the L1 family at 3’ end of intron 2

- **Non-deletional forms of β thalassemia**
  - Amounts for the vast majority of β Thalassemia alleles
  - They result from:
    - single base substitutions
    - small insertions within β globin gene or immediate flanking sequences
    - small deletions within β globin gene or immediate flanking sequences

β-globin Mutations Affecting Transcription

- **Promoter Mutations**
  - Single base substitutions in the conserved DNA sequences that form the β-globin promoter:
    - TATA-Box, CAT-Box, CACCC-TFBS
  - Example: -29 A→G (Africa and China)

- **Mutations of the 5’ UTR**
  - 5’ UTR is 50 bp long
  - Single base substitutions and minor deletions
  - Examples:
    - +1 A→C
    - +33 C→G leads to 33% of normal β-globin mRNA.
**β-globin Mutations Affecting mRNA Processing (1)**

- Exons and introns contain ‘cryptic’ splice sites: sequences which mimic the consensus sequence for a splice site but which are not normally used.
- Mutations can occur in these sites, creating a sequence that resembles more closely the normal splice site.
- During RNA processing, the newly created site is used preferentially, leading to mis-spliced mRNA.

**β-Globin Mutations Affecting mRNA Processing (2)**

- **Junctional mutations**
  - Mutations at the invariant dinucleotides in the splice junction: GT – AG, completely abolish normal splicing and produce phenotype of beta-zero thalassemia.
  - They transcribe normally, but the mis-spliced mRNA does not allow the translation of functional β-globin.
  - **Examples:**
    - IVS1-1 G→C (Mediterranean)
    - IVS2-1 G→C (Iran)
β-Globin Mutations Affecting mRNA Processing (3)

• **Consensus-sequence mutations**
  - Mutations within the consensus sequences at the splice junctions reduce the efficiency of normal splicing to varying degrees and produce a β-thalassemia phenotype that ranges from mild to severe.
  - single amino acid mutation leading to very unstable beta globin.
  - **Examples:**
    • IVS1-5 G→C (Asian Indian, south-east Asian, Melanesian)
    • IVS2-6 T→C (a.k.a Portuguese β Thalassemia, Mediterranean)

β-Globin Mutations Affecting mRNA Processing (4)

• **Cryptic splice-site mutations in introns**
  - Several splice mutations involve base substitutions within the introns rather than consensus splice sites.
  - **Examples:**
    • IVS1-110 G→A (Mediterranean)
**β-Globin Mutations Affecting mRNA Processing (5)**

- **Cryptic splice-site mutations in exons**
  - **Example:**
    - cd2 GGT → GGA (US Afro-American, Japanese)

- **3’UTR and Polyadenelation site mutations**
  - A few nucleotide substitutions and 2 minor deletions affecting the conserved AATAAA
  - **Examples:**
    - AATAAA → AATAAG (Kurd)
    - AATAAA → AACAAAA (African American)

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**β-Globin Mutations Affecting mRNA Translation (1)**

- **Start and Stop codon mutations:**
  - These are mutations that affect either the start codon or stop codons of the mRNA.
  - Several mutations of AUG have been found all of which produce beta-zero thalassemia.
  - **Example:**
    - AUG → AAG (North European).
• Missense (frameshift) and nonsense mutations
  – Around half of the β thalassemia alleles are characterized by premature β-chain termination (mainly in exons 1 and 2), produced by frameshift or nonsense mutations.
  – Examples:
    • Nonsense mutation:
      – cd17 AAG → TAG (Chinese, Japanese).
      – cd39 CAG → TAG (Mediterranean).
    • Frameshift mutation:
      – cd17 AAG → TAG (Chinese, Japanese).
Goals of the TCRN

- The goal of the Thalassemia Clinical Research Network (TCRN) of the National Heart, Lung, and Blood Institute is to provide information on the changing face of this disease and the implications for diagnosis, counseling, and treatment.
- TCRN examined the demography and natural history of 728 patients with thalassemia who are registered in the 5 largest treatment centers in North America.

TABLE 1. $\beta$ and $\alpha$ Mutations Associated With Each Thalassemia Phenotype

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Elliott P. Vichinsky et al., 2005
Epidemiology of Thalassemia in North America

- The epidemiology of thalassemia in North America reflects a heterogeneous group of diseases with new ethnicities, genotypes, and phenotypes.
- In these communities, physicians will need to provide education, prenatal diagnosis, counseling, and management of this newly diverse group of patients.

The Future

- Thalassemia, often considered a pediatric disease, has become a chronic adult illness with a median life span approaching 40 years in North America.
- Fertility and other complex medical problems associated with older patients need to be addressed.
- Linguistic isolation and socioeconomic barriers, often associated with immigrant populations, impair the ability to implement comprehensive care and necessitate trained counselors and translators.
- A multidisciplinary approach that addresses the changing treatment and epidemiology of thalassemia will ensure improved quality of life and survival.
Reference

• Changes in the Epidemiology of Thalassemia in North America: A New Minority Disease
  – Elliott P. Vichinsky, Eric A. MacKlin, John S. Waye, Fred Lorey and Nancy F. Olivieri
  – Pediatrics published online Nov 15, 2005
  – http://www.pediatrics.org/cgi/content/full/peds.2005-0843v1

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Elliott P. Vichinsky et al., 2005
cd 39 (C>T)

ATG GTG CAT CTG ACT CCT GAG GAG AAG TCT GCC GTT
ACT GCC CTG TGG GGC AAG GTG AAC GTG GAT GAA GTT
GGT GGT GAG GCC CTG GCC AG GTTGGTATCAAGGTATTACAA
GACAGGGTTAAGGAGACCAATAGAAACTGGGCATGGAGACAGAGA
AGACTGTGGGGTTCTGATAGGCCACTGACTCTCTCTGCTATTTGTC
ATTATTCCCACCCCTTAG G CTG CTG GTG GTC TAC CCT TGG
ACC CAG AGG TTC TTT GAG TCC TTT GGG GAT CTG TCC
ACT CCT GAT GCT GTT ATG GCC AAC CCT AAG GTG AAG
GCT CAT GCC AAG AAA GTG CTC GGT GCC TTT AGT GAT
GGC CTG GCT CAC CTG GAC AAC CTC AAG GCC ACC TTT
GCC ACA CTG AGT GAG CTG CAC TGT GAC AAG CTG CAC
GTG GAT CCT GAG AAC TTC AGG GTGAGTCTATGGGACGCTT
GATGTTTTCTTTTCCCCCTCTCTCTATGTGTTAAGGTCATGCTATAGG
AAGGGGATAAGTACAGGGTACAGTTTAGAATGGGAAACAGACGAAT

Stop Codon

cd 39 (C>T)

ATG GTG CAT CTG ACT CCT GAG GAG AAG TCT GCC GTT
ACT GCC CTG TGG GGC AAG GTG AAC GTG GAT GAA GTT
GGT GGT GAG GCC CTG GCC AG GTTGGTATCAAGGTATTACAA
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GCT CAT GCC AAG AAA GTG CTC GGT GCC TTT AGT GAT
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GATGTTTTCTTTTCCCCCTCTCTCTATGTGTTAAGGTCATGCTATAGG
AAGGGGATAAGTACAGGGTACAGTTTAGAATGGGAAACAGACGAAT
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Elliott P. Vichinsky et al., 2005

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**Consensus Sequence at Splice Sites**

![Consensus Sequence Diagram]

- GU
- A
- (Y/A)G

- 5’ splice site
- Branch site
- 3’ splice site

3 bases | 6 bases | 10 bases | 1 base

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**IVS-1-110 (G>A)**

**Consensus:** YYYYYYNYAGG

**Mutation:** TGCCCTATTAG | T

**Wild Type:** TGCCCTATTGG | T
YYYYYYNAG|G

**Consensus:** YYYYYYNAG|G

**Mutation:** TGCCTATTAG|T

**Wild Type:** TGCCTATTGG|T
cd 8/9 (+G)

ATG GTG CAT CTG ACT CCT GAG GAG AAG TCT GCC GTT
ACT GCC CTG TGG GGC AAG GTG AAC GTG GAT GAA GTT
GGT GGT GAG GCC CTC GGC AG GTGGTATCAAGGTTACAA
GACAGTGTAAAGGAGACCAATAGAAGACTGGGCATGTGGAGACAGAGA
AGACTCTTGGTTTCTGATAGGCACTGACTCTCTGCTGCTATTTGTC
TATTTTCCCACCCTTAG G CTG CTG GTG GTC TAC CCT TGG
ACC CAG AGG TTC TTT GAG TCC TTT GGG GAT CTG TCC
ACT CCT GAT GCT GTC GTT ATG GCC AAC CCT AAG GTG AAG
GCT CAT GGC AAG AAA GTG TCC GGT GCC TTT AGT GAT
GTC CTG GCT CAC CTG GAC AAC CTC AAG GCC ACC TTT
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### Cryptic Splice Sites

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<th>Mutations</th>
<th>Type</th>
<th>Wild-type</th>
<th>Mutant</th>
<th>Cryptic Splice Site</th>
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<td>JM</td>
<td>CAG</td>
<td>GTTGGT</td>
<td>1) Exon 1, 105: AAG</td>
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<td>2) Exon 1, 127: GTG</td>
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<td>3) Intron 1, 13: AAG</td>
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<td>2) IVS I-5 (G → C)</td>
<td>CSM</td>
<td>CAG</td>
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Some of the mutations of the beta globin gene that yield to Beta Thalassemia.
IVS: intervening sequence or intron; CSM: consensus-sequence mutation; JM: junctional mutation

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**TABLE 1. β and α Mutations Associated With Each Thalassemia Phenotype**

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<tr>
<td>Codon 39 (C → T) β0/codon 39 (C → T) β0</td>
<td>28</td>
</tr>
<tr>
<td>IVS I-5 (G → C) β+ /IVS-I-5 (G → C) β+</td>
<td>16</td>
</tr>
<tr>
<td>Codon 39 (C → T) β0/IVS-I-6 (T → C) β+</td>
<td>14</td>
</tr>
<tr>
<td>IVS I-6 (T → C) β+ /IVS-I-110 (G → A) β+</td>
<td>12</td>
</tr>
<tr>
<td>−28 (A → G) β+ /codon 41/42 (−TCTT) β0</td>
<td>11</td>
</tr>
<tr>
<td>Other genotypes</td>
<td>10</td>
</tr>
<tr>
<td>Thalassemia intermedia</td>
<td>154</td>
</tr>
<tr>
<td>IVS I-6 (T → C) β+ /IVS-I-6 (T → C) β+</td>
<td>5</td>
</tr>
<tr>
<td>Hb A/codon 39 (C → T) β0</td>
<td>4</td>
</tr>
<tr>
<td>Codon 41/42 (−TCTT) β0/codon 41/42 (−TCTT) β0</td>
<td>4</td>
</tr>
<tr>
<td>−28 (A → G) β+ /−28 (A → G) β+</td>
<td>3</td>
</tr>
<tr>
<td>Codon 39 (C → T) β0/IVS-I-6 (T → C) β+</td>
<td>3</td>
</tr>
<tr>
<td>IVS I-5 (G → C) β+ /IVS-I-5 (G → C) β+</td>
<td>3</td>
</tr>
<tr>
<td>Codon 8/9 (+G) β0/IVS II-1 (G → A) β0</td>
<td>2</td>
</tr>
<tr>
<td>Other genotypes</td>
<td>54</td>
</tr>
</tbody>
</table>

Elliott P. Vichinsky et al., 2005
IVS-II-1 (G→A)

- It has been experimentally demonstrated by Treisman et al. that the mutation results in 2 abnormally spliced mRNA transcripts.
  - The more abundant transcript utilizes a cryptic 5' splice site that is downstream of the original 5' splice site in IVS II (ATG|GTAAAG).
  - The other abnormal mRNA transcript is found at low levels and is obtained by completely skipping Exon 2 and splicing together Exon 1 and Exon 3.
Skipping Exon Two

- Recall that the other abnormal mRNA transcript is obtained by skipping exon 2 and splicing together exons 1 and 3. This also results in a frameshift in the CDS. As no stop codon is encountered in exons 1 and 3 in the new frame, if translation was carried out to produce a protein, translation would have to proceed into the 3’UTR. The resulting protein would be an 83 amino acid abnormal protein.
### TABLE 1. \( \beta \) and \( \alpha \) Mutations Associated With Each Thalassemia Phenotype

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thalassemia major</td>
<td>275</td>
</tr>
<tr>
<td>Codon 39 (C(\rightarrow)T) ( \beta^0/)IVS-1-110 (G(\rightarrow)A) ( \beta^+ )</td>
<td>30</td>
</tr>
<tr>
<td>IVS-1-110 (G(\rightarrow)A) ( \beta^+ /)IVS-1-110 (G(\rightarrow)A) ( \beta^+ )</td>
<td>28</td>
</tr>
<tr>
<td>Codon 39 (C(\rightarrow)T) ( \beta^0 /)codon 39 (C(\rightarrow)T) ( \beta^0 )</td>
<td>16</td>
</tr>
<tr>
<td><strong>IVS I-5 (G(\rightarrow)C) ( \beta^+ /)IVS I-5 (G(\rightarrow)C) ( \beta^+ )</strong></td>
<td>14</td>
</tr>
<tr>
<td>Codon 39 (C(\rightarrow)T) ( \beta^0 /)IVS-1-6 (T(\rightarrow)C) ( \beta^+ )</td>
<td>12</td>
</tr>
<tr>
<td>IVS-1-6 (T(\rightarrow)C) ( \beta^+ /)IVS-1-100 (G(\rightarrow)A) ( \beta^+ )</td>
<td>11</td>
</tr>
<tr>
<td>(-28 (A(\rightarrow)G) ( \beta^+ /)codon 41/42 (-TCTT) ( \beta^0 )</td>
<td>10</td>
</tr>
<tr>
<td>Other genotypes</td>
<td>154</td>
</tr>
<tr>
<td>Thalassemia intermedia</td>
<td>78</td>
</tr>
<tr>
<td>IVS-1-6 (T(\rightarrow)C) ( \beta^+ /)IVS-1-6 (T(\rightarrow)C) ( \beta^+ )</td>
<td>5</td>
</tr>
<tr>
<td>Hb A/codon 39 (C(\rightarrow)T) ( \beta^0 )</td>
<td>4</td>
</tr>
<tr>
<td>Codon 41/42 (-TCTT) ( \beta^0 /)codon 41/42 (-TCTT) ( \beta^0 )</td>
<td>4</td>
</tr>
<tr>
<td>(-28 (A(\rightarrow)G) ( \beta^+ /)28 (A(\rightarrow)G) ( \beta^+ )</td>
<td>3</td>
</tr>
<tr>
<td>Codon 39 (C(\rightarrow)T) ( \beta^0 /)IVS-1-6 (T(\rightarrow)C) ( \beta^+ )</td>
<td>3</td>
</tr>
<tr>
<td>IVS-1-5 (G(\rightarrow)C) ( \beta^+ /)IVS-1-5 (G(\rightarrow)C) ( \beta^+ )</td>
<td>3</td>
</tr>
<tr>
<td>Codon 8/9 (+G) ( \beta^0 /)IVS II-1 (G(\rightarrow)A) ( \beta^0 )</td>
<td>2</td>
</tr>
<tr>
<td>Other genotypes</td>
<td>54</td>
</tr>
</tbody>
</table>

Elliott P. Vichinsky et al., 2005

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**Exon 1-51**

- U1 snRNA 3’-CAUCA-5’
- ATG GTG CAT CTG ACT CCT GCT GAG GAG AAG TCT GCC GTC
- ACT GCC CTG TGG GGC AAG GTG AAC GTG GAT GAA
- GGT GGT GAG GCC CTG GGC AG GGGTGTATCAAGGTTTACA
- GACAGGTCTAAGGAGACCAATAGAAACTGGGCATGTGAGACAGAGA
- AGACTCTTGTTCTGATAGGCACCTGACTCTCTCTGCTATTG
- TATTTCCCACCCCTTAG G CTG CTG GTC TAC CCT TGG
- ACC CAG AGG TTC TTT GAG TCC TTT GGG GAT CTG TCC
- ACT CCT GAT GCT GAT ATG GGC AAC CCT AAG GAT AAG
- GCT CAT GCC AAG AAA GTC CTC GGT GCC TTT AGT GAT
- GGC CTG GCT CAC CTG GAC AAC CTC AAG GCC ACC TTT

**IVS-1-5 (G > C)**

3 criptic sites in red Exon-I-105, Exon-I-127 & IVS-I-13

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