Thalassemias

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Today's Discussion

- Background
- Categories of Thalassemia
- α-thalassemia
- β- thalassemia
- Thalassemia associated with structural hemoglobin variants

Definitions and History

- Thalassemia- group of inherited disorders caused by genetic mutations affecting the globin chain component of hemoglobin

- High incidence of patients of Mediterranean descent with this disorder
  - Disease was called *Thalassic* (Greek for “great sea”) anemia

- Results from a reduced or absent synthesis of one or more of the globin chains of hemoglobin
  - Diminished hemoglobin synthesis and production of microcytic hypochromic RBCs

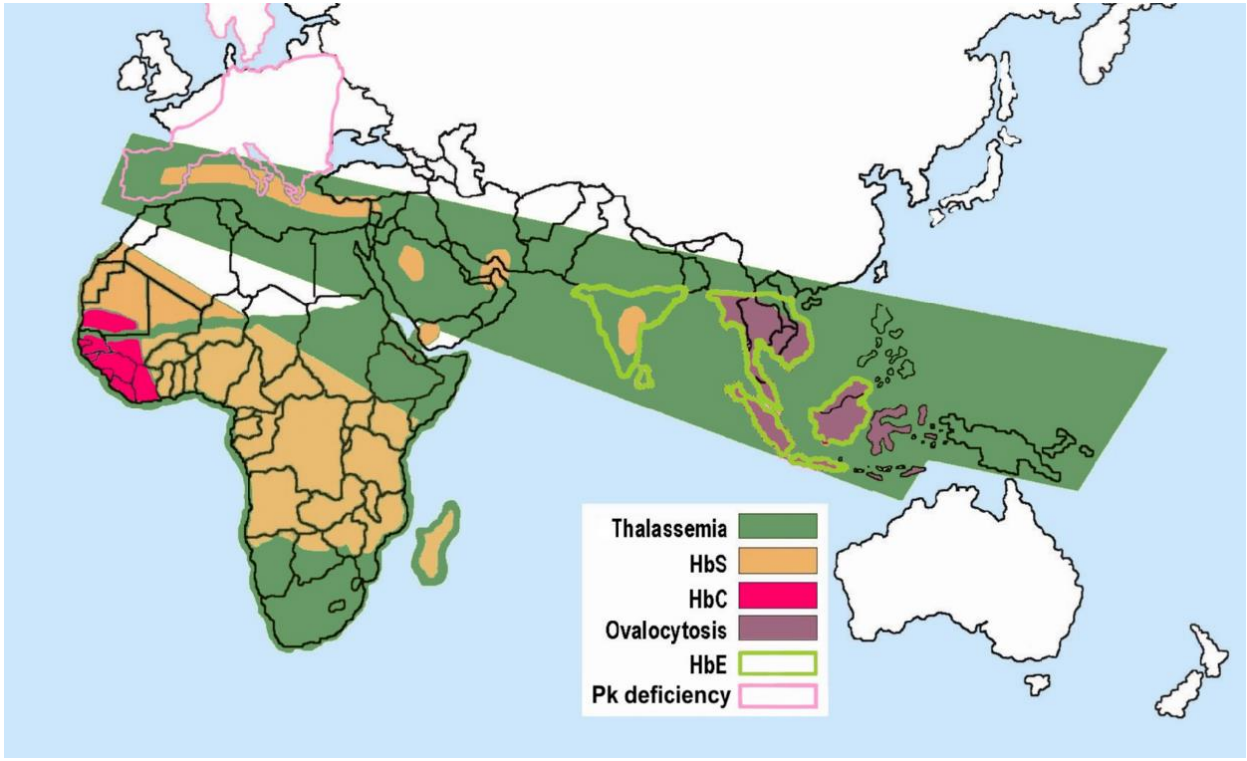
- Mutations affecting the alpha and beta chains are the most significant
  - Decrease or absence of one of these chains leads to decrease production of Hb and imbalance of α/β chain ratio

Diagnosis of Thalassemia

- History and physical examination
  - Ethnic background should be evaluated
    - Increase prevalence of specific gene mutations in certain populations
  - Clinical examination
    - Pallor (due to anemia)
    - Jaundice (due to hemolysis)
    - Splenomegaly
      - Caused by sequestration of abnormal RBCs, excessive extravascular hemolysis, some extramedullary erythropoiesis
    - Skeletal deformities
      - Massive expansion of BM cavities

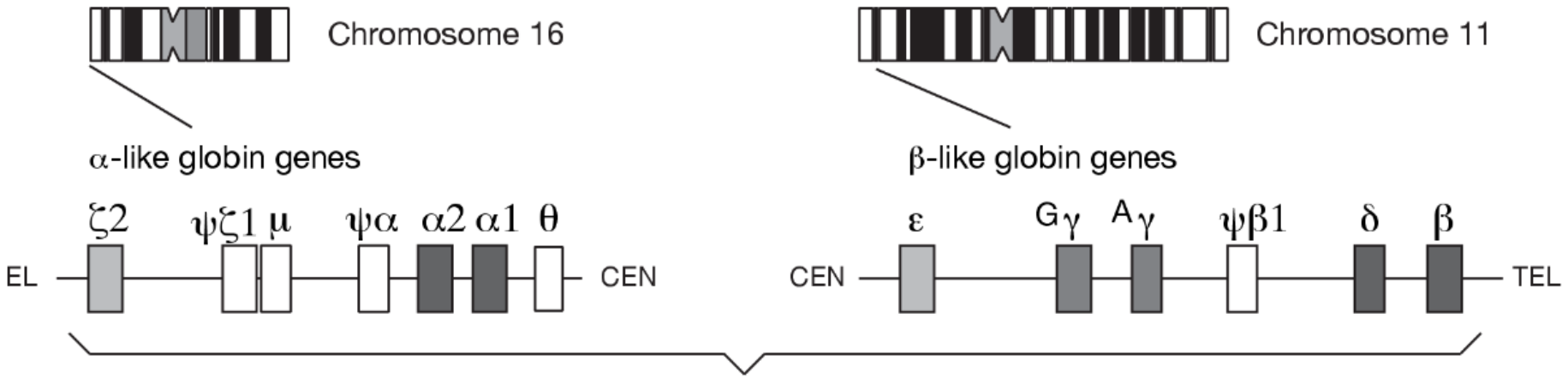
Epidemiology

- 56,000 infants conceived or born with significant thalassemia each year
  - More than ½ require transfusion
- Distribution concentrated in the "thalassemia belt"
  - Mediterranean east through the Middle East and India to Southeast Asia and south to Northern Africa
  - Coincides with areas in which malaria is prevalent
    - The resistance and protective effect of thalassemia against malaria are not clear



Compendium of Hemoglobinopathies, SERBIA Education Library

Genetics Review



- Normal hemoglobin tetrameter is two α-like chains (α or ζ) and two β-like chains (β,γ,δ, and ε)
- An individual will inherit one cluster of the functional genes on chromosome 11 and 16 from each parent

Genotype for normal β chain synthesis is designated β/ β

Genotype for normal α chain synthesis is designated αα/αα

https://www.semanticscholar.org/paper/Disorders-of-Hemoglobin%3A-The-Normal-Structure-and-Forget-Hardison/289d153350857db9c43b552cd34430a3f1c20fed/figure/0

Genetic Defects Causing Thalassemia

- Types of genetic defects that can cause a reduced or absent production of the particular globin chain
  - Single nucleotide (or point) mutation
  - small insertions or deletion
  - Large deletions
- Mechanisms by which mutation interferes with chain production
  - Reduced or absent transcription of mRNA
  - mRNA processing errors
  - Translation errors
  - Deletion of one or more globin genes

Symptomatic Thalassemia

Divided into 2 broad groups based on transfusion requirements

- Transfusion-dependent thalassemia (TDT)
  - β thalassemia major
  - Severe E-β thalassemia
  - α thalassemia major (Hb Barts hydrops fetalis)
- Non-transfusion- dependent thalassemia
  - β thalassemia intermedia
  - Mild- moderate Hb E- Thalassemia
  - α thalassemia intermedia (Hb H disease)

Categories of Thalassemia

- α-thalassemia
- β-thalassemia

Categories of Thalassemia

α-thalassemia

α-thalassemia

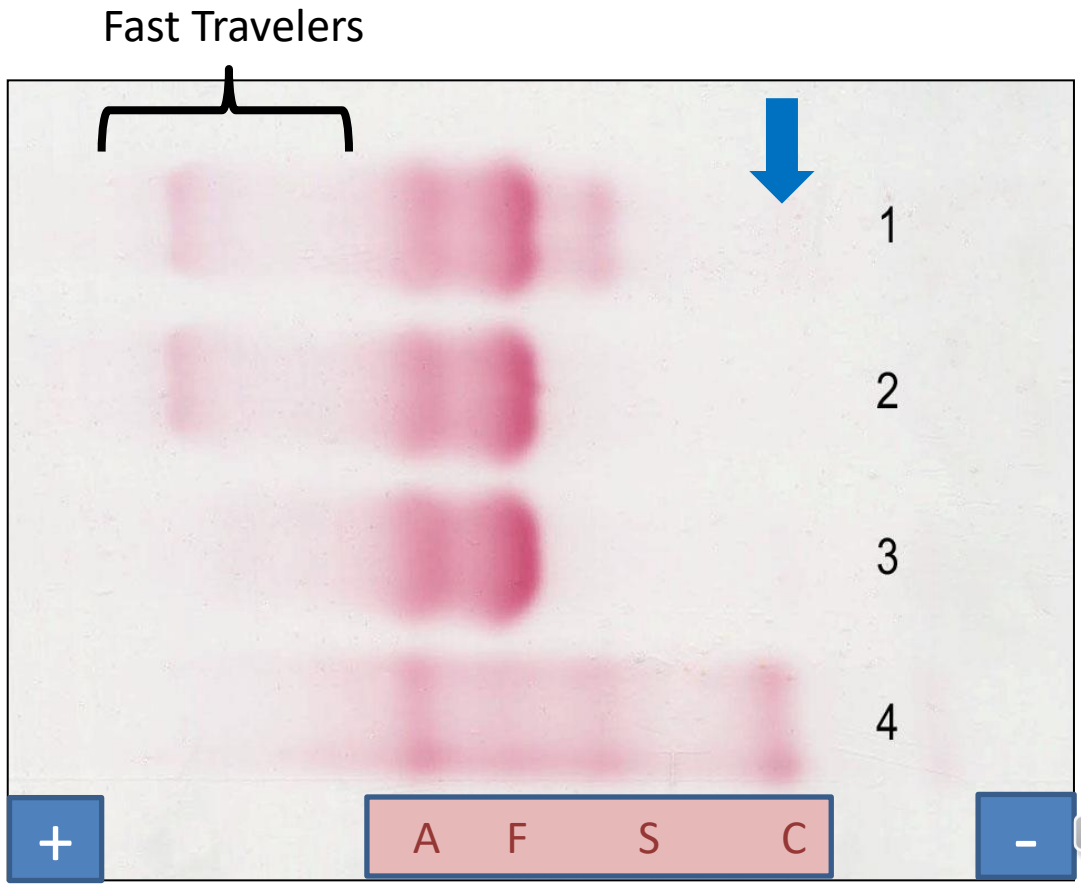
- Disorders or reduced globin chain production arise from α_1 and α_2 chains on chromosome 16
- Most common mutations are deletions involving the α_1 and/or α_2 globin genes
- Severity of symptoms depends on specific mutation, number of genes affected, and whether the affected gene is α_1 or α_2
  - α_2 gene produces 75% of the α chains in RBCs

Nomenclature

αα/αα	• Normal (no disorders or clinical effect)
-α/αα	• Heterozygous α-thal-2 (silent carrier/asymptomatic)
-α/-α	• Homozygous α-thal-2 (Thalassemia Minor/ microcytosis, mild anemia)
--/αα	• Heterozygous α-thal-1 (Thalassemia Minor/ microcytosis, mild anemia)
--/-α	• α-thal-1 / α-thal-2 (hemoglobin H disease/chronic hemolytic anemia)
--/--	• Homozygous α-thal-1 (Bart's hydrops fetalis/lethal)

α-Thalassemia Mechanism

- α- chain apart of fetus, newborn, and adult hemoglobin
- ↓ in α chain in fetus and newborn
  - Results in γ chains excess
  - γ chains stable, form a tetramer (γ_4)
    - Hb Bart
- ↓ in α chain in adults
  - β chain excess
  - β chain stable and form tetramer (β_4)
    - Hb H
- Hb chain "fill in" are fast travelers on electrophoresis



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α- Thalassemia

2 haplotypes:

- **α ^{0}**:
  - deletion of both α_1 and α_2 no production of α chains from that chromosome

- **α $^{+}$** (2 types):
  - deletion of either α_1 and α_2 on chromosome 16
    - Most common
  - non-deletional mutation in α globin gene. Less common
    - **Constant Spring (α^{CS} chain)**
      - $\alpha_2^{142Stop->Gln}$
        - Additional bases are added to the end of the mRNA during transcription until a stop codon is reached
      - Enlongated mRNA very unstable and process small amount of α^{CS} chain
        - Longer α chain makes tetramer unstable
      - Instability of both mRNA and tetramer causes the circulating Hb Constant Spring to be very low (<1%)

Four Clinical Syndromes of α - Thalassemia

- Silent carrier state
- α- thalassemia minor
- Hb H disease
- Hb Bart hydrops fetalis syndrome

Silent Carrier State

- Deletion of one α-globin gene (- αα/αα)
  - α/β chain ratio nearly normal
  - No hematologic abnormalities
  - Slight excess of γ at birth that form tetramers
    - Hb Barts (γ_4) 1-2%
- Non-deletional α^+ mutation in one α globin gene ($\alpha^T\alpha/\alpha\alpha$)
  - If heterozygous mutation ($\alpha^{CS}\alpha/\alpha\alpha$), Hb Constant Spring is less than 1% of total hemoglobin

α - Thalassemia Minor

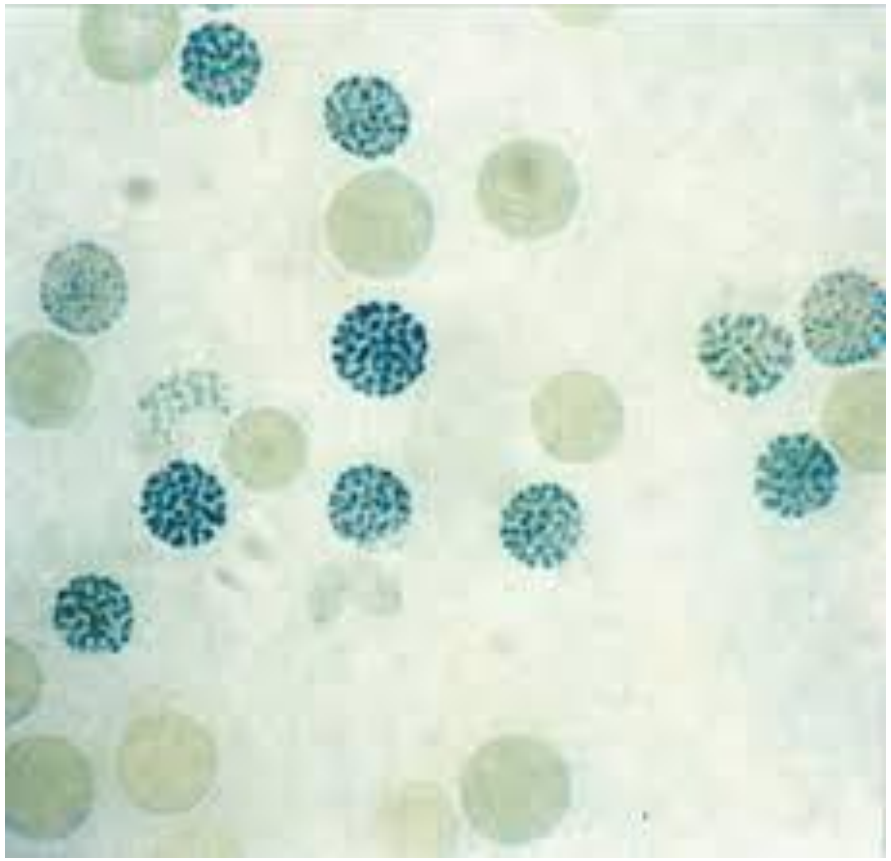
- AKA α- Thalassemia Trait
- Major cause is the deletion of two α- globin genes
  - Homozygous (- α/-α) and heterozygous (--/αα)
  - Asymptomatic with mild microcytic anemia, MCV less than 80 fL, and a MCH less than 27 pg
  - At birth → Hb Bart (5%-15%)
  - Adults → α and β usually balanced. Hb H is not present
- Homozygous non-deletional mutation in both α_2 globin ($\alpha^T\alpha/\alpha^T\alpha$)
  - Mild to moderate hemolytic anemia
  - Jaundice and hepatosplenomegaly
  - If homozygous mutation ($\alpha^{CS}\alpha/\alpha^{CS}\alpha$), Hb Constant Spring is 5%-6% of total hemoglobin

Hemoglobin H Disease

- α Thalassemia Intermedia
- Deletion of 3 α globin genes (--/- α)
  - Only one α-globin gene to produce all the α chains
  - Common in Asian population
  - Excess unpaired β chains form tetramers
    - Newborns: Hb Bart's (10-40%)
    - Adults: Hb H
      - After γ to β switch, Hb H replaces most of the Hb Barts
      - Hb H comprises 1-40% with decreased Hb A and Hb A_2

Hemoglobin H Disease

- Symptoms
  - Mild to moderate, chronic hemolytic anemia with reticulate count 3-10%
  - Usually non-transfusion dependent
  - Enlarged spleen
  - Peripheral Blood Smear
    - Hb H inclusions inside the RBC (vulnerable to oxidation)
    - Microcytic and hypochromic RBCs
    - Poikilocytosis



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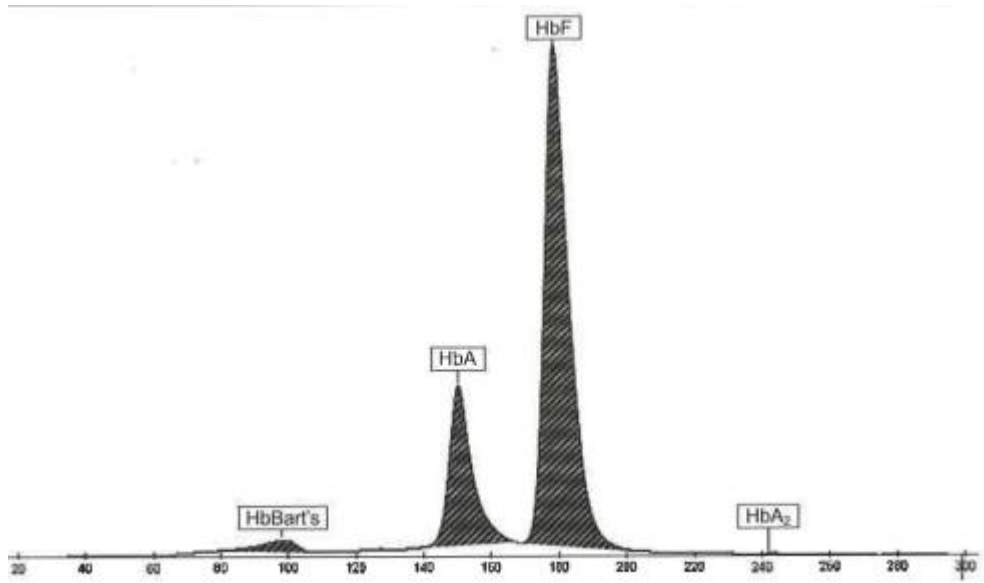
Hb Bart Hydrops Fetalis Syndrome

- α Thalassemia Major
- Homozygous α^0- thalassemia (--/--) results in absence of all α chain production
- Usually results in death in utero or shortly after birth
- Transfusion dependent thalassemia
- Severe anemia in the fetus
  - Cardiac failure and edema in subcutaneous tissues (hydrops fetalis)
- Hb Bart (γ_4) predominant Hb
  - Small amounts of Portland ($\zeta_2\gamma_2$) and traces of Hb H
  - Has a high oxygen affinity and does not deliver oxygen to the tissues



HbBart's = 2,3 %; HbA = 22,0 %; HbF = 75,6 %; HbA_2 = 0,1 %

Compendium of Hemoglobinopathies, SERBIA Education Library

Table 14: Diagnosis, genotypes, hematological data and cardinal symptoms of α-thalassemias

Genetic status/diagnosis	Structure of the α-globin genes	Red blood cell count	Qualitative hemoglobin pattern	Cardinal symptoms
Normal finding	■■ / ■■ αα / αα	Hb normal MCH normal	normal	no symptoms
Heterozygous α^+-thalassemia = α-thalassemia minima	■■ / ■■ –α / αα	Hb normal MCH < 27 pg	normal	no symptoms, minor changes in the blood count
Homozygous α^+-thalassemia = α-thalassemia minor	■■ / ■■ –α / –α	Hb low MCH < 26 pg	normal	mild anemia, noticeable changes in the blood count
Heterozygous α^0-thalassemia = α-thalassemia minor	■■ / ■■ – – / α α	Hb low MCH < 24 pg	normal	mild anemia, noticeable changes in the blood count
Compound heterozygosity α^+/α^0-thalassemia = HbH disease	■■ / ■■ – – / –α	Hb 8-10 g/dl MCH < 22 pg	HbH ≈ 10 - 20 %	variable, chronic hemolytic anemia
Homozygous α^0-thalassemia = HbBart's Hydrops fetalis syndrome	■■ / ■■ – – / – –	Hb < 6 g/dl MCH < 20 pg	HbBart's 80 - 90 % HbPortland ≈ 10 - 20 % HbH < 1 %	life-threatening fetal anemia, generalized hydrops

■ active α-genes ■ deleted α-genes

Categories of Thalassemia

β-thalassemia

β Thalassemia

- Disorders of reduced globin chain production arise from the β globin gene cluster on chromosome 11
- Mainly affect β chain production but can also involve γ,δ, and ε chains
  - More than 300 mutations known (more than 280 mutations in β-globin gene alone)
- Types:
  - **β^0**: No β chain is produced (commonly found in Mediterranean areas)
  - **β^+**: partial deficiency of β chains
  - **β^{silent}**: silent carrier
  - **$\delta\beta^0$**: mutations in δ or β genes in which no δ or β chains are produced
  - **$\delta\beta^{Lepore}$**: fusion of the δ and β globin genes that produce Hb Lepore

Mechanism of β Thalassemia

- Unpaired, excess α chains precipitate in developing erythroid precursors
  - Form inclusion bodies → cause oxidative stress and damage to cellular membrane
    - Apoptosis is triggered
- Ineffective erythropoiesis
  - Premature death of erythroid precursors in BM
  - BM attempt to produce RBCs but not able to release viable cells into circulation
    - If RBCs released, they contain inclusions and are destroyed in the spleen (extravascular hemolysis)
- Symptoms
  - Fetal life asymptomatic until 6 month
  - Will begin 6-34 months
    - Completion of the γ-β switch

Nomenclature

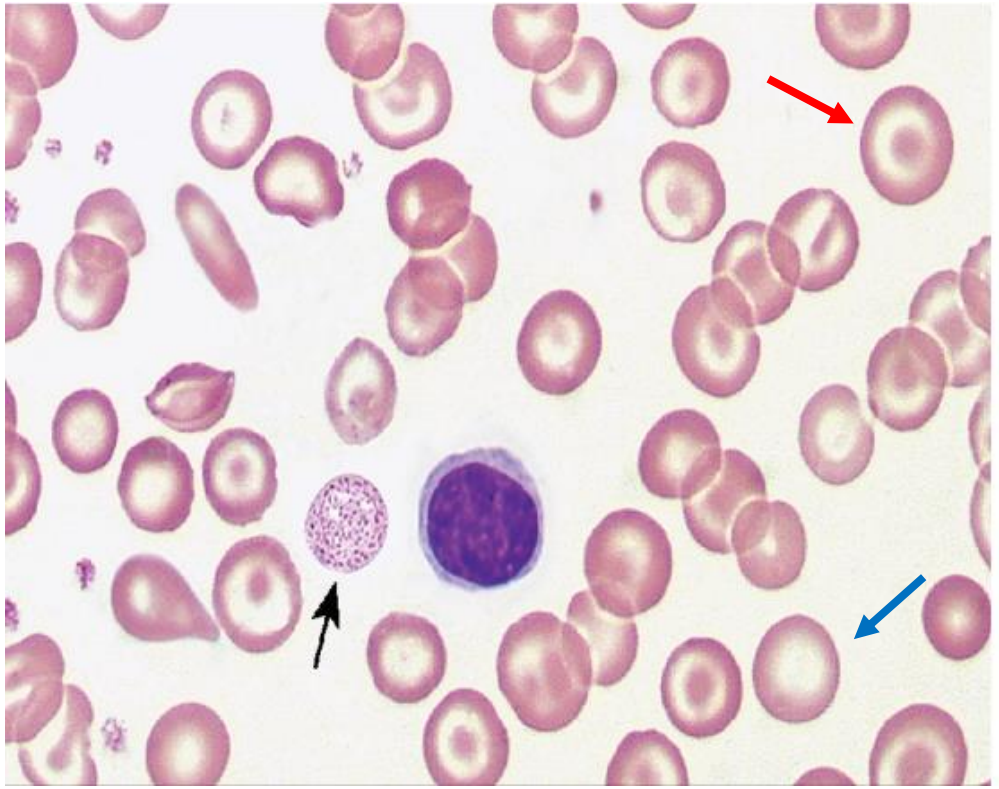
β /β	• Normal (Hb normal)
β_{silent} /β	• Silent Carrier (Hb normal)
$\beta^{+}\beta$ or β^{0}/β	• Minor (heterozygous) • Hb 10-13 g/dL
β^{+}/β^{+} or β^{+}/β^{0} or β^{0}/β^{0}	• Major (homozygous) • Hb 2-3 g/dL
β thalassemia intermedia	• Intermediate severity (Hb 7 g/dL) • Example: Parent with silent carrier state (β^{silent}/β) and parent with β-thalassemia trait (β^{+}/β or β^{0}/β) →Compound heterozygosity (β^{0}/β^{silent} or β^{+}/β^{silent})

β - Thalassemia Silent Carrier

- Various heterogeneous β globin gene mutations that produce small decrease in production of β chains
- Silent state (β^{silent}/ β)
  - Nearly normal α/β ratios
  - No hematologic abnormalities
  - Recognized when unknown silent carrier and β thal trait have a child with symptoms of β thal intermedia (compound heterozygosity)
- Some individuals who are Homozygous (β^{silent} /β^{silent}) have been described
  - Mild β-thalassemia intermedia phenotype with ↑ Hb F and ↑ Hb A_2

β - Thalassemia Minor

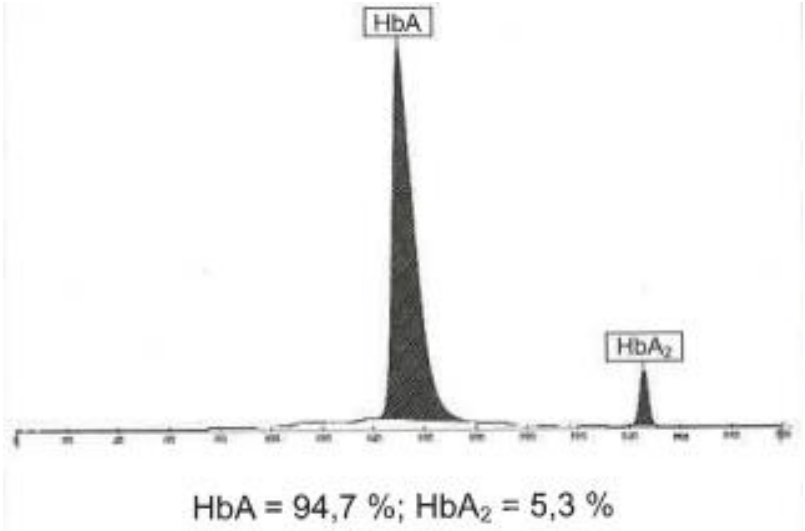
- β thalassemia trait (heterozygous state)
- 1 mutated β gene (↓ or abolished expression) and 1 normal gene
- Mild, asymptomatic anemia
- Hb levels: 11-15 g/dL in Men and 10-13 g/dL in Woman
- Peripheral smear:
  - Microcytic, hypochromic RBCs
  - Target cells
  - Elliptocytes
  - Basophilic stippling



https://doctorlib.info/hematology/rodak-hematology-clinical-principles-applications/29.html

β - Thalassemia Minor

- Extra α chains combine with δ to form more Hb A2 and with γ to form more Hb F
  - β^{+}/β or β^{0}/β
    - Hb A level of 92-95%
    - Hb A2 elevated to 3.5-7.0%
    - Hb F ranges from 1-5%
- Less common types: $\delta\beta^{Lepore}/\beta$ or $\delta\beta^{0}/\beta$
- Screening for β-Thalassemia minor
  - Very important
    - High carrier frequency
  - Mass screening in Italy and Greece



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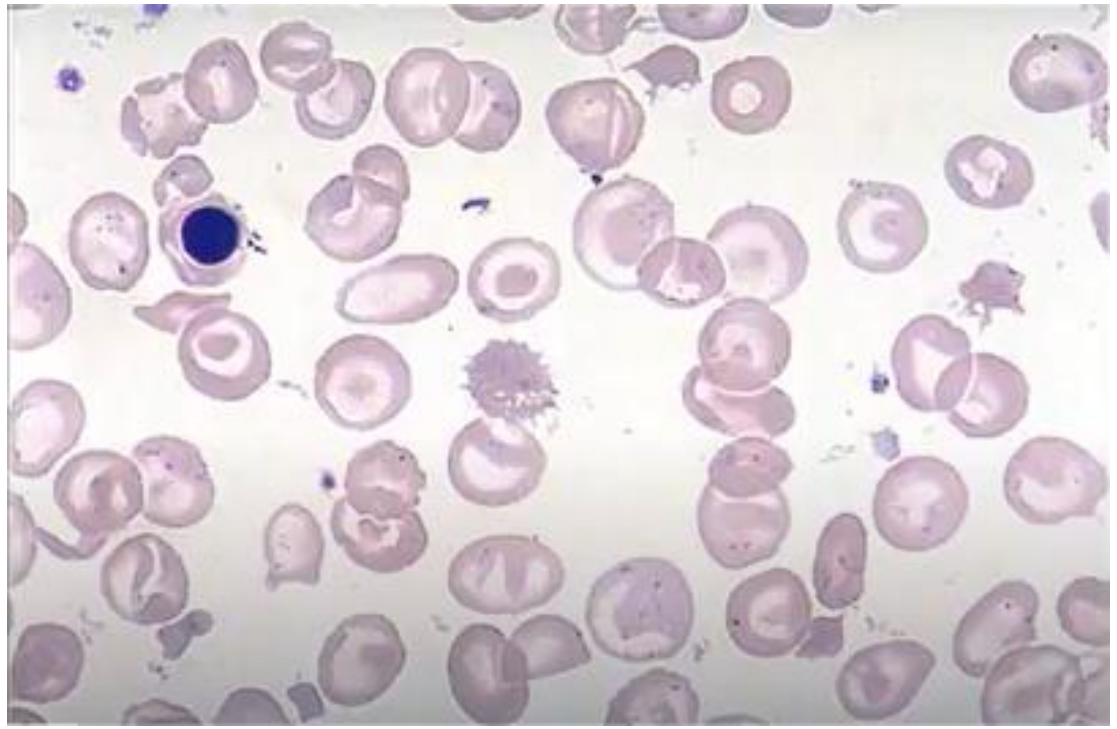
β - Thalassemia Major

- β^+/β^+ or β^+/β^0 or β^0/β^0
- Homozygous or compound state
- Characterized by severe anemia, microcytic and hypochromic RBCs, severe clinical symptoms and transfusion dependence
- Diagnosed between 6 month-2 years of age
- Requires regular transfusion therapy
  - Hemoglobin can go from 7 g/dL (treated) to as low as 2-4 g/dL (untreated)
- Increased Hb F and Hb A_2 with little to no Hb A

β - Thalassemia Major

- Decreased MCV and reticulocyte is 2-8%
- Bone Marrow:
  - Increased RBC so M:E ratio is 1:20
- Peripheral Blood:
  - Marked microcytosis, hypochromia, anisocytosis, and poikilocytosis
  - Target cells, teardrop cells, and elliptocytes
  - Basophilic stippling, Howell-Jolly and Pappenheimer bodies
  - nRBCs may be present
- Profound anemia stimulates an increase in EPO and results in a massive (but ineffective) erythroid hyperplasia



β - Thalassemia Major Without Treatment

- Enlarged liver and spleen
- Massive bone marrow expansion gives prominence of forehead, cheek bones, and upper jaw
- Extramedullary erythropoiesis causes hepatosplenomegaly
  - Enlarged spleen can cause worsening anemia with neutropenia and thrombocytopenia
- Increased RBC destruction leads to excess hemoglobin and increased indirect bilirubin
  - Can lead to jaundice

http://www.thistle.co.za/pdf_files/education/differential_slides/differential_slide_legends/cycle-48/Cycle%2048%20Slide%205%20-%20Thalassemia%20Major.pdf

β - Thalassemia Major: With Treatment

- Requires transfusion regimen
  - Hypertransfusion- correct anemia, suppress marked erythropoiesis
- Must watch for iron overload
  - Iron chelation therapy used to prevent growth restriction in children and cardiomyopathy or cirrhosis of liver in adults
  - Iron excreted in urine and stool
- Hematopoietic stem cell transplantation (HSCT)
  - Only curable therapy for thalassemia major

https://depositphotos.com/stock-photos/blood-transfusion.html

β - Thalassemia Intermedia

- Syndrome in which the α/β chain imbalance and symptoms fall between β Thalassemia minor and β Thalassemia major but without a need for regular transfusion therapy to maintain Hb level*
- Non transfusion dependent thalassemia with Hb between 7-10 g/dL
- Genotypes show great heterogeneity: Many possible mutations
- Patients experience Iron overload even though they do not receive regular transfusions
  - Marked accelerated ineffective erythropoiesis suppresses hepcidin production by the liver
    - Results in more iron absorption by intestinal enterocytes
  - Regular monitoring for iron overload recommended: regular chelation therapy
  - Can result in an increased risk of thrombosis

Thalassemias caused by defects in β -Globin Gene Clusters

- Can be caused by deletion, inactivation, or fusion of a combination of the β-globin gene cluster

- Includes
  - Hereditary Persistence Fetal Hemoglobin (HPFH)
  - $\delta\beta^0$-Thalassemia
  - Hb Lepore Thalassemia

HPFH and $\delta\beta^0$-Thalassemia

- Heterogeneous conditions in which Hb F is expressed at increased levels beyond infancy into adulthood
- Normal MCV
- Anemia is usually not present
- **Hereditary persistence fetal hemoglobin (HPFH)**
  - β-globin gene cluster
  - Deletion in δβ region or non-deletional mutations in γ-chain promoter region*
  - Trait: Hb F = 15-30% (heterozygous)
  - Disease: Hb F = 100% (homozygous-no switch to adult Hb)
  - When looking at Hb distribution of Hb F can be pancellular (deletion type) or heterocellular (non-deletional type)
- **$\delta\beta^0$-Thalassemia**
  - Deletions of δ and β globin genes and increase in Hb F
  - 10-20% of thalassemia minors
  - Hb A_2 normal and increased Hb F (5-15%)
  - If homozygous, no Hb A or Hb A_2

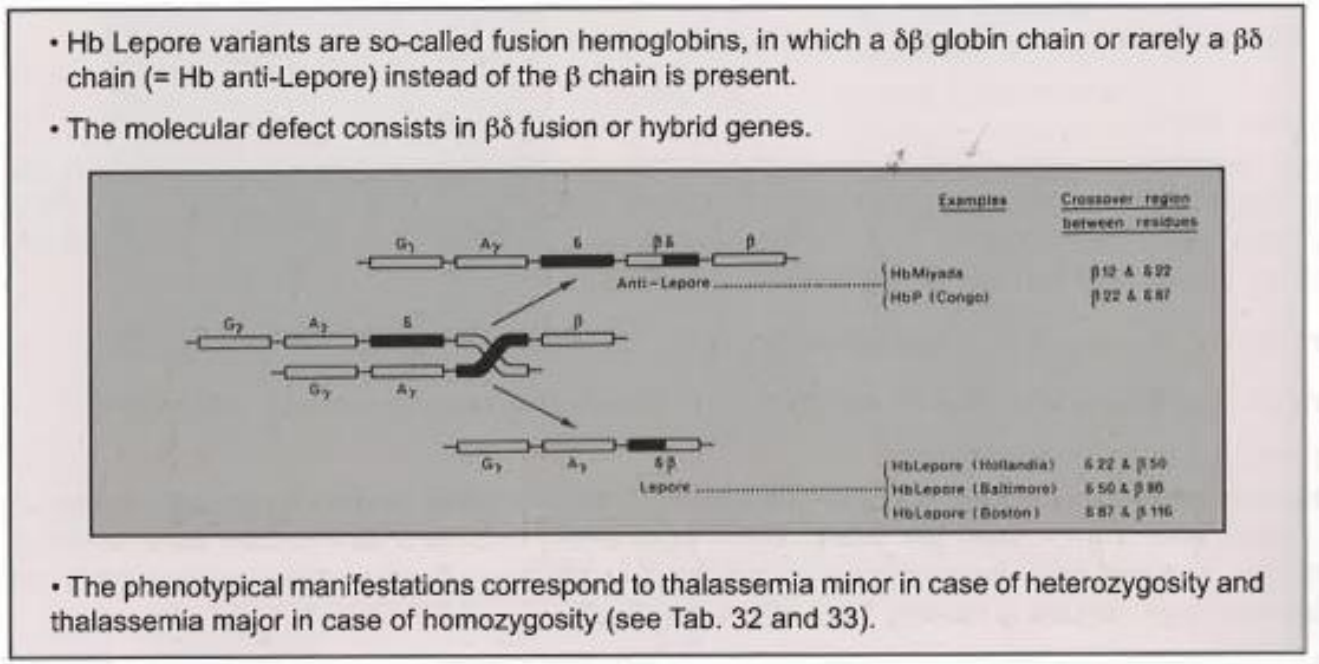
Hemoglobin Lepore Thalassemia

- $\delta\beta^{Lepore}$

- Rare structural variant in which there is a fusion of δβ-globin genes
  - During meiosis, from nonhomologous crossover on different chromosomes

- Heterozygous- Clinical manifestations are similar to β-thalassemia minor

- Homozygous- Clinical manifestations are similar to β-thalassemia major



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Thalassemia Associated with Structural Hemoglobin Variants

- Hemoglobin S- Thalassemia
  - Hb S-α- Thalassemia
    - Common in populations of African ancestory
    - Milder anemia with ↑ Hb levels and ↓ Retic count than those with sickle cell anemia alone
  - Hb S-β- Thalassemia
    - Seen in Africa, Mediterranean, Middle East, and India
    - Expression depends on type of β Thalassemia mutation inherited

Thalassemia Associated with Structural Hemoglobin Variants

- Hemoglobin C- β-Thalassemia
  - Produces moderately severe hemolysis, splenomegaly, hypochromia, microcytosis, and numerous target cells
  - Hb electrophoresis pattern varies- depends on type of β Thalassemia gene defect
- Hemoglobin E- β-Thalassemia
  - Significant concern in SE Asia and E India
  - Hb E is due to a point mutation
  - Homozygous E (EE)- clinical symptoms similar to mild β Thalassemia
  - Severe E-β Thalassemia- transfusion dependent
  - Mild-Moderate Hb E-β Thalassemia- non transfusion dependent

References

Rodak's Hematology, Clinical Principles and Applications 6th Edition

Additional material Courtesy of Barbara Martien, MLS