بسم الله الرحمن الرحيم

Hemoglobinopathies

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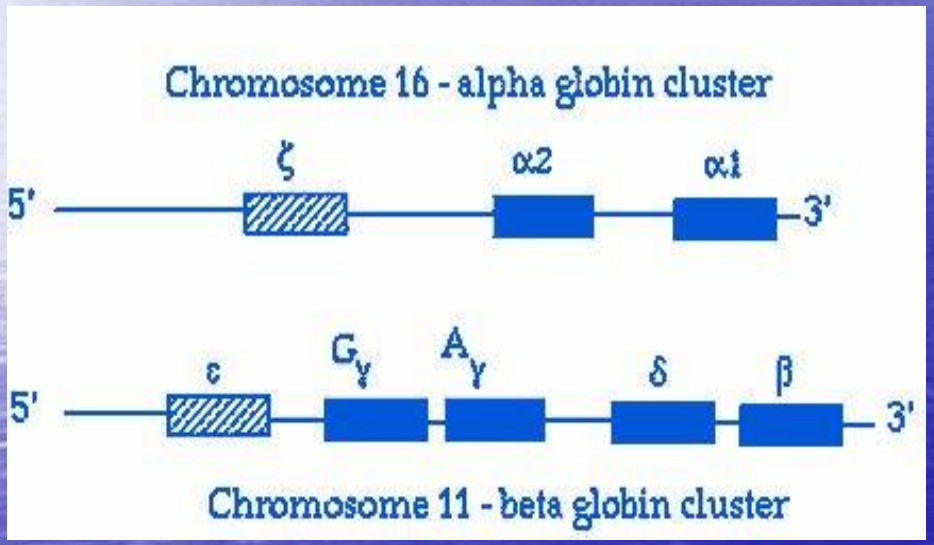
Introduction.

- Haemoglobinopathies are 'inherited abnormalities of globin chain synthesis'
- clinical spectrum from asymptomatic findings on blood film to death in utero.

Hemoglobin Structure.

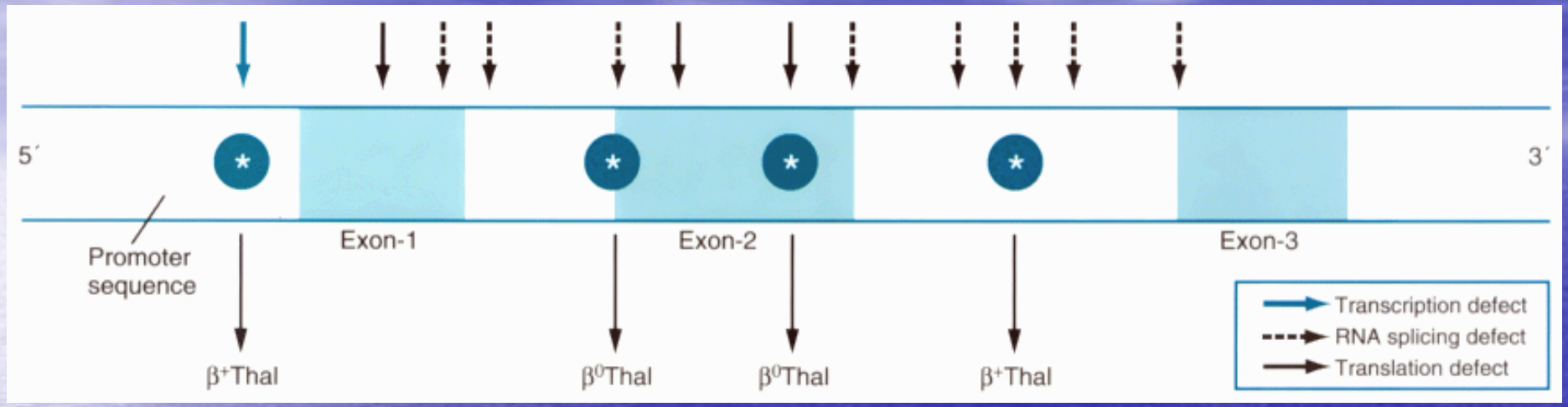
- Each RBC contains hemoglobin.
- A normal hemoglobin molecule consists of:
  - Four globin chains (2 alpha, 2 beta).
  - Each globin chain has an iron containing heme molecule.

Globin Genetics



- The genes directing the synthesis of the α globin chains are found on Chromosome 16; there are two genes on each chromosome
- The genes directing the non-α globins are found on Chromosome 11; there is one pair of genes for each non-α chain

the β-globin Gene



Promoter region mutations

produce β^+ thal by reducing transcription

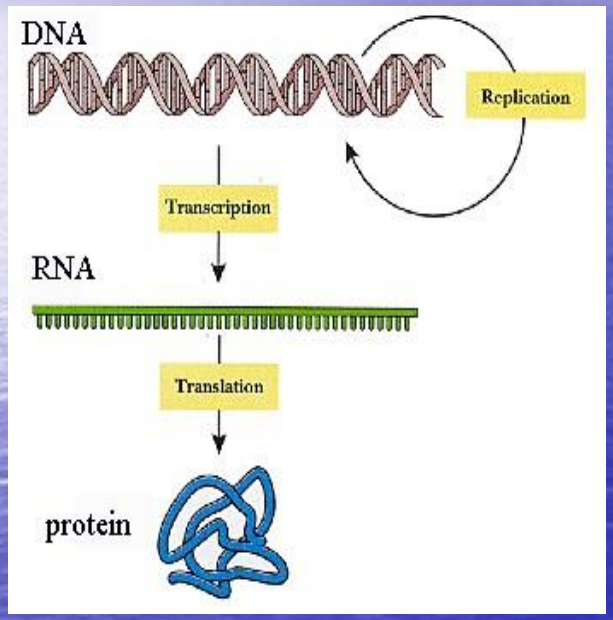
Chain terminator mutations

stop codon or frameshift leading to stop codon

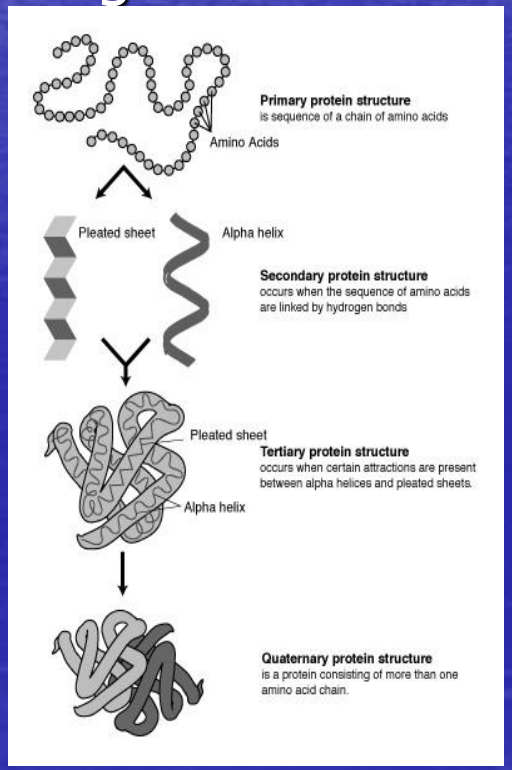
Splicing mutations

most common defect, usually in introns

Hemoglobin Synthesis/Assembly

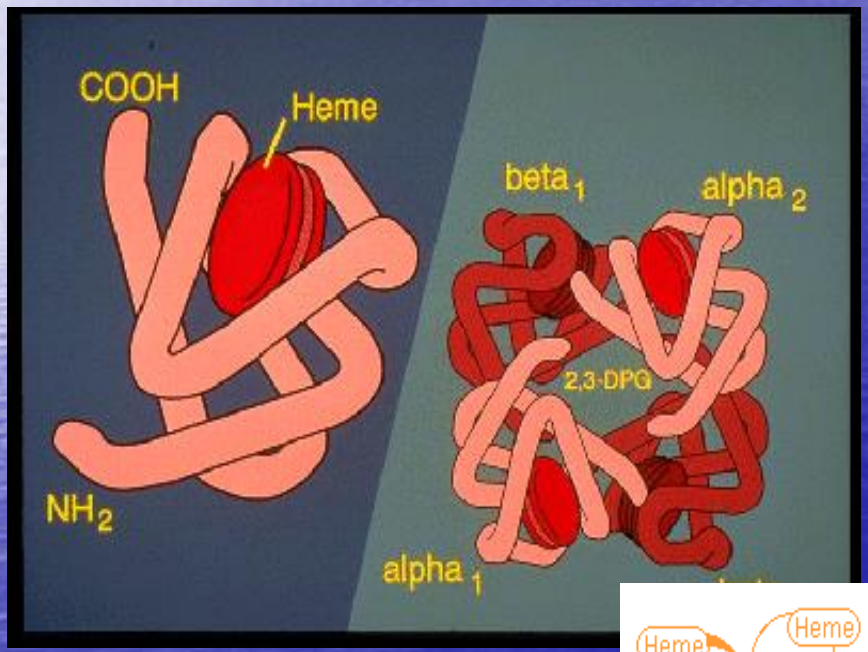


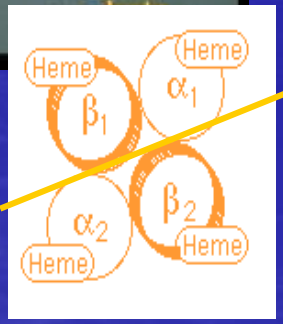
- Like all proteins, the "blueprint" for hemoglobin exists in DNA



- The protein chain assumes its conformation
- The individual globin chains then aggregate in precise molecular orientation

Hemoglobin Composition





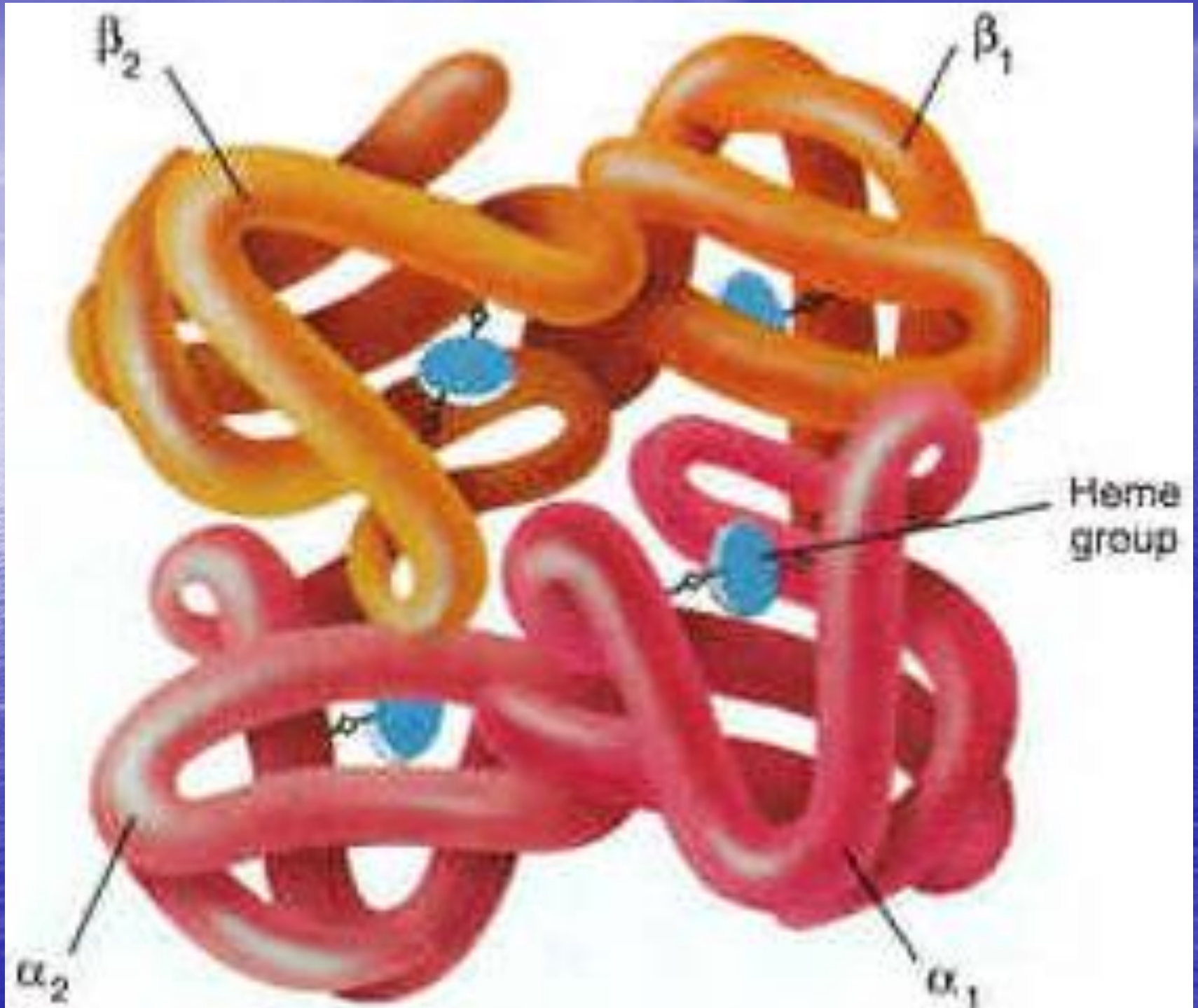
Hemoglobin A

- Both proteins must be present for the hemoglobin to pick up and release oxygen normally
- One of the component proteins is called
  - "alpha"
- The other
  - "non-alpha"

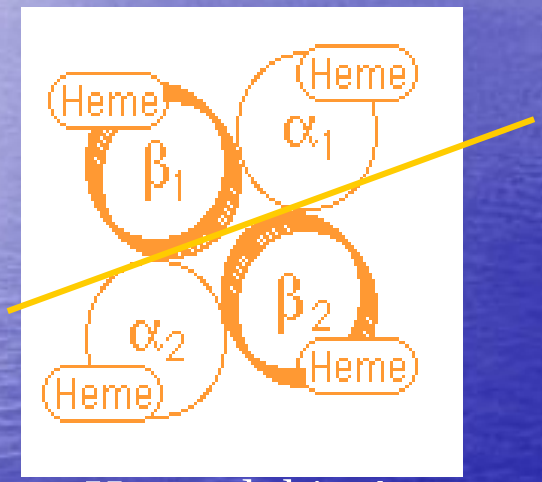
  Commonly
  - "gamma"
  - "beta" (β)
  - "delta"

Hemoglobin Synthesis

- Depends on:
  - Adequate iron supply
  - Adequate synthesis of protoporphyrin (synthesized in the mitochondrion)
  - Adequate globin chain synthesis


β2
β1
Heme group
α2
α1

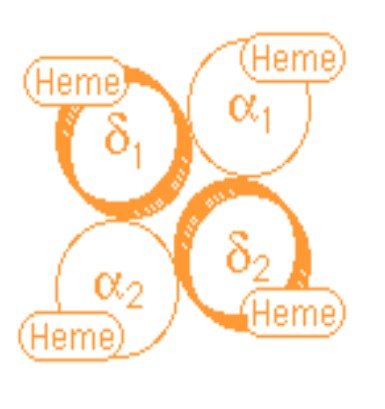
Normal Hemoglobin



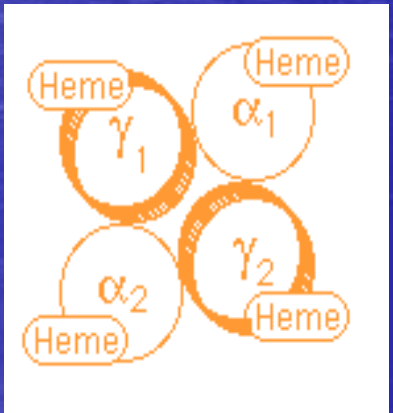
Hemoglobin A

- In the normal adult, Hemoglobin A ($\alpha_2\beta_2$) is the most prevalent, comprising about 95% of all hemoglobin

Normal Hemoglobin



Hemoglobin A2



Hemoglobin F

- Two minor hemoglobins also occur:
  - Hemoglobin A2, composed of two alpha and two delta globins ($\alpha_2\delta_2$) comprises 2-3.5% of hemoglobin
  - Hemoglobin F, two alpha and two gamma globins ($\alpha_2\gamma_2$), makes up less than 2% of hemoglobin

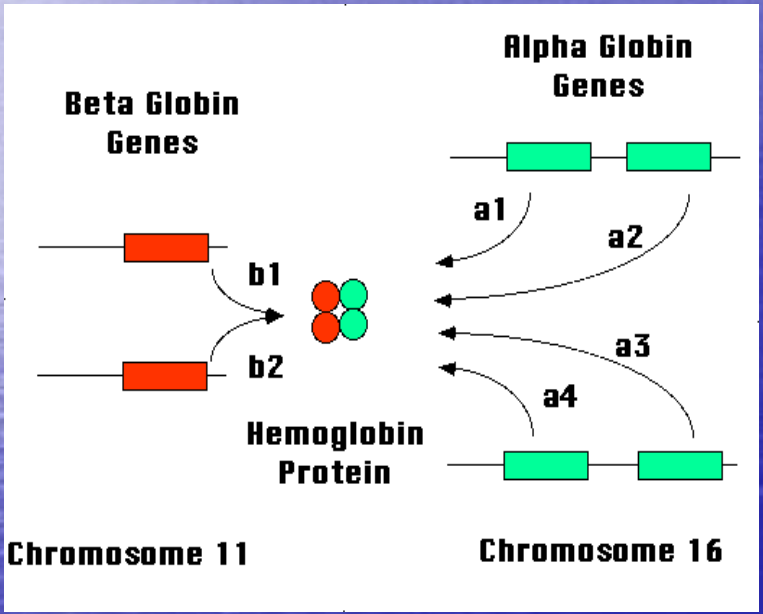
Review of Hemoglobin Structure

- A globular protein with a mass of 64.4 kd constituting 95% of RBCs dry weight

In the the adult there are three hemoglobin types normally present:

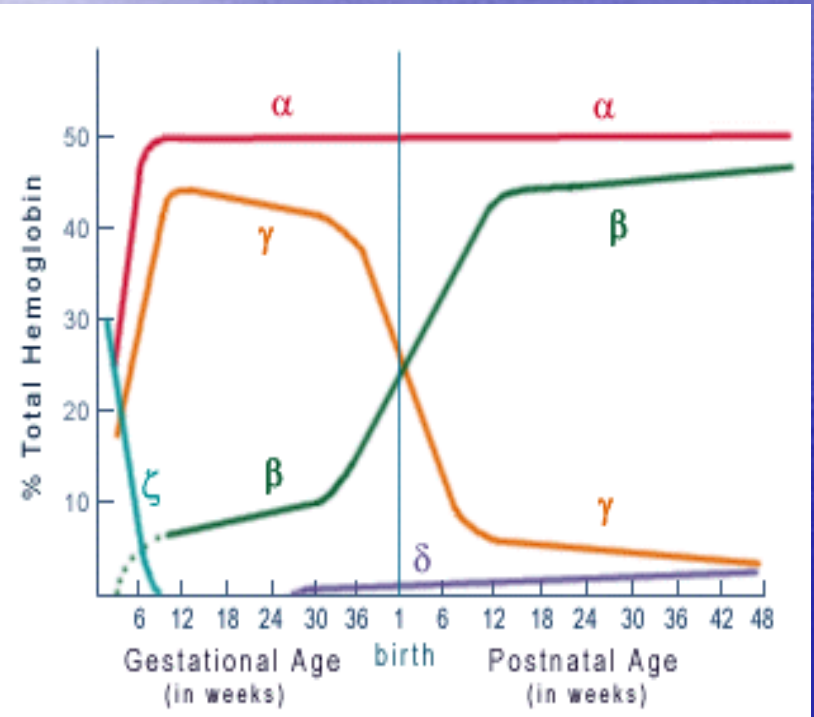
- Hgb A: 2 alpha & 2 beta chains ($\alpha_2\beta_2$) 95%
- Hgb A2: 2 alpha & 2 delta chains ($\alpha_2\delta_2$) 3%
- Hgb F: 2 alpha & 2 gamma chains ($\alpha_2\gamma_2$) 2%

Assembly of Globin Molecules



- The alpha chains and the non-alpha chains are made in precisely equal amounts, despite the differing number of genes

Globin Synthesis
Gestational Factors



- Alpha (α) and gamma (γ) globin are generated during fetal development
- The beta (β) protein is not expressed in large quantities before birth
- After birth the γ-globin diminishes, replaced by a rising β-globin.
- Delta (δ) globin is an adult globin seen in the older infant

Abnormalities of Hemoglobin

- Disorders of abnormal hemoglobin synthesis are called *hemoglobinopathies*
- Hemoglobinopathies may result in hemolysis because of changes in hemoglobin *solubility* or because of *instabilities* in the hemoglobin molecule
- Such changes are caused by either qualitative or quantitative defects

Abnormalities of Hemoglobin (II)

- Structural abnormalities are due to alterations in the polypeptide sequence changing the molecular structure and, often, the function of the globin chains
  - Examples
    - Hgb SS (Sickle cell disease)
    - Hgb AS (Sickle cell trait)
    - Hgb C disease
    - Hgb SC disease

Abnormalities of Hemoglobin (III)

- Quantitative abnormalities are secondary to decreased rates of globin chain synthesis
  - Examples
    - β-thalassemia
    - α-thalassemia
    - Hgb E

Thalassemias
Historical Perspective

- 1925 Thalassemia was first described by Cooley and Lee
- Most of Mediterranean ancestry
- Hence this group of anemias are also known as:
  - Cooley's Anemia
  - Mediterranean Anemia
- Thalassemia is a genetically determined defect in hemoglobin synthesis
- There is an inability to manufacture sufficient quantities of globin chains

Thalassemia Syndromes (I)

- Some mutations may result in inability to produce normal amounts of hemoglobin
- Usually, only one of the sets of hemoglobin genes is affected
  - For example, one of the two beta globin genes may fail to produce a normal quantity of beta chain protein
  - The alpha globin gene set will continue to produce a normal quantity of alpha chain protein
  - An imbalance develops in the amount of alpha chain and beta chain protein in the cell
  - There is too much alpha chain for the amount of beta chain that is present
  - This imbalance is called "thalassemia"
    - In this example, it would be beta thalassemia, because it is the beta chain gene that has failed

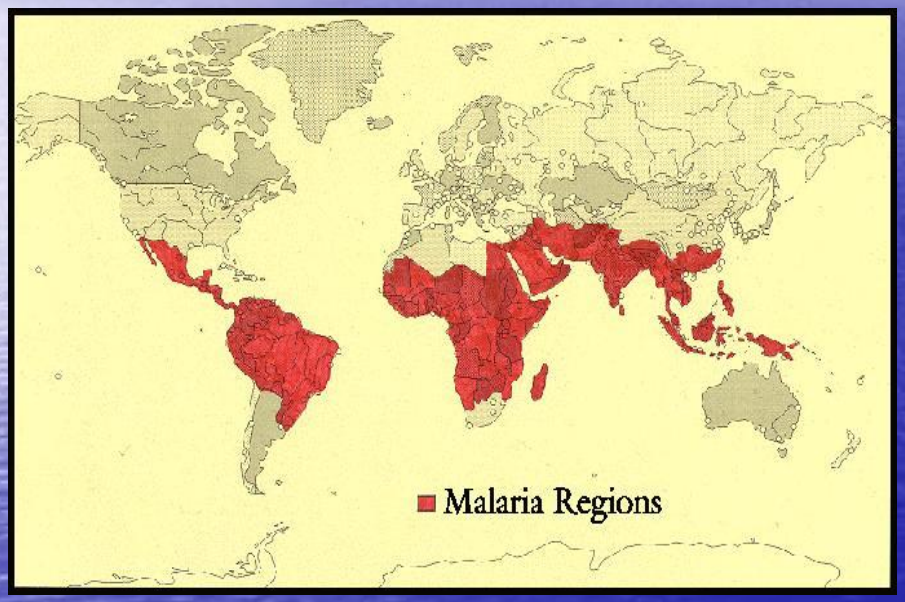
Imbalance

In Globin Biosynthesis

β Thalassemia

- β^0 Thalassemia
  - defective allele produces no beta chains
- β^+ Thalassemia
  - defective allele produces some beta chains
- Permutations
  - β^0 β, β^+ β – thalassemia minor or trait
  - β^0 β^+ , β^+ β^+ , β^0 β^0 – thalassemia major

Thalassemia Syndromes



- The thalassemias are a diverse group of genetic blood diseases by absent or decreased production of normal hemoglobin, resulting in a microcytic anemia of varying degree
- The thalassemias have a distribution concomitant with areas where *P. falciparum* malaria is common

Beta Thalassemia Minor (I) (Heterozygous) (β^+)

- This is the most common of the thalassemias
- Beta chain production is less than normal due to the failure of one of the genes coding for beta chains
- Alpha chain production continues at a near normal rate
- The alpha chains combine with the available beta chains resulting in decreased levels of hemoglobin A

Beta Thalassemia minor (II) (Heterozygous) (β^+)

- Alpha and delta chains combine form increased Hgb A2
- If there is an excess of alpha chains normal gamma chain production does not function correctly; the rate of gamma chain production is greater than normal, resulting in increased amounts of Hgb F
- The importance of identifying heterozygous beta thalassemia is to prevent costly investigations and appropriate genetic counseling

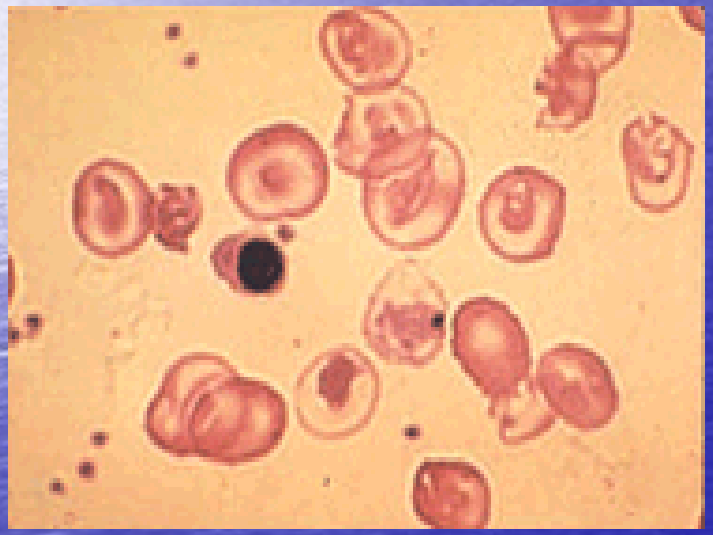
Laboratory Diagnosis
β Thalassemia minor (I)

- Hemoglobin, Hematocrit are decreased but the RBC count is usually normal or increased
- Hemoglobin seldom below 9.5 g/dL
  - If Hgb is <9.3 g/dL, unlikely β thalassemia minor
- Morphology: microcytic, hypochromic
- Anisocytosis slight; reflected in near normal RDW
- Slight poikilocytosis with an occasional target cell
- Moderate basophilic stippling
- Indexes characteristically discordant
  - MCV slightly decreased
  - MCH decreased
  - MCHC normal
  - RBC number usually normal or increased

Beta Thalassemia major (I) (Homozygous) (β^0)

- Well at birth
- Develop life threatening anemia by one or two months of age
- Supported with blood transfusions; result in iron overload
- In beta thalassemia major there is a complete failure of beta chain production; no Hgb A present
- Delta and gamma chain production is increased
- As a result there is raised levels of Hgb A2 and Hgb F
- Excess erythropoietin stimulates marrow
  - Extramedullary hematopoiesis
  - Splenomegaly

Laboratory Diagnosis
β Thalassemia major (I)

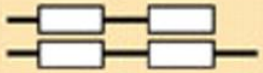
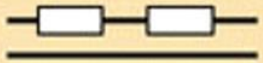
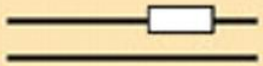


- Anemia is severe - Hgb 2.0 to 3.0 g/dL
- Hematocrit and RBC count decreased; indexes uniformly depressed
  - The MCV, MCH and MCHC are all decreased
  - ***The RDW is increased***
- Morphology is severe hypochromia, microcytosis, marked anisocytosis and poikilocytosis
  - Many target cells, schistocytes, basophilic stippling; many NRBCs
- Retic count - relative increase 5 to 10%

Alpha Thalassemia

- There are four genes coding for alpha chain production
- These genes are located on chromosome 16
- As a result there are at least five forms of alpha thalassemia depending on the number and location of the abnormal genes

α-Thalassemia Syndromes

α Gene Map	α Genotype	α Clinical Syndrome
	Normal	Normal
	Heterozygous α - Thal - 2 (also called α^+)	Silent Carrier of α Thalassemia
	Heterozygous α - Thal - 1 (also called α^o)	α - Thalassemia Trait
	Homozygous α - Thal - 2 (also called homozygous α^+)	α - Thalassemia Trait
	Compound Heterozygous α - Thal - 1 & 2 (also called α^+/α^o)	Hb - H Disease
	Homozygous α - Thal - 1	Hydrops Fetalis

Schrier, S. ASH Image Bank 2002;2002:100327