بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

HLS BioChemistry | MID 3

Hemoglobinopathies



Written by : Sara Qatatshah
Omaima hjjaj

Reviewed by : Jamila Bouka

بسم الله الرحمن الرحيم،

نرجو منكم الدعاء لزميلنا في الدفعة
عون العدوان، الذي وافته المنية.

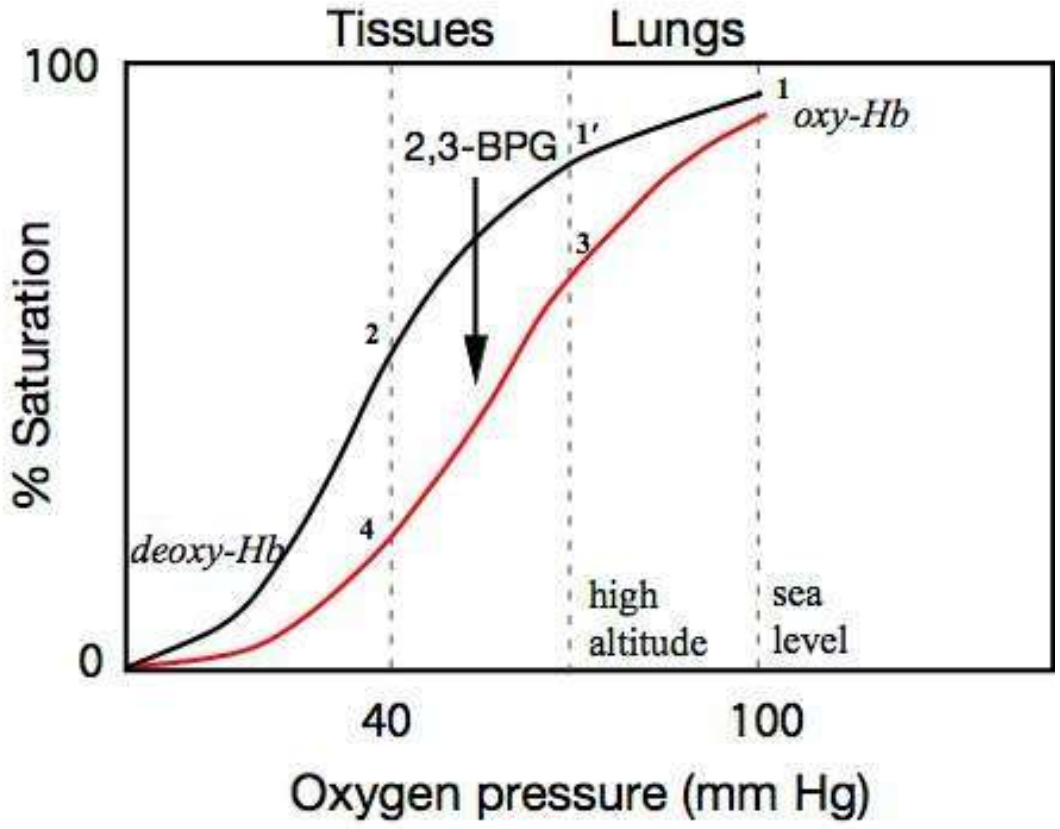
اللهم ارحمه واغفر له، وأسكنه فسيح جناتك، وأنر قبره وآنس وحشته، واجعل قبره روضةً من رياض الجنة.

اللهم اجبر قلوب أهله وذويه، وارزقهم الصبر والسلوان، وألهمهم جميل الصبر وحسن العزاء.

رحمه الله رحمةً واسعة وأسكنه الفردوس الأعلى من الجنة...

Better explanation of the role of 2,3-BPG

- At sea level the lungs pick up oxygen with 100% saturation of Hb (1) and when the oxygen pressure drops to 40 mm Hg in the tissues (2) the Hb will be 55% saturated.
  - They have released 45% of bound oxygen.
- At high altitudes (in case of no adaptation), Hb is only 80% saturated (1'). Thus at 40 mm Hg in the tissues (2) when Hb is only 55% saturated, it will only have released 25% of its oxygen.
- At high altitude (with increased 2,3-BPG production- in red), At the lungs (3) the Hb will be less bound with oxygen — only 70% saturation — but at 40mm Hg in the tissues (4) it will be much less saturated than on the black curve — 30%. Thus, it will have made available 40% of its oxygen.
- This is not a perfect solution, but over time there is increased production of red blood cells to provide more hemoglobin to compensate for the smaller amount of oxygen it can bind.



❑ Comparison of Two Hemoglobin Oxygen Saturation Curves:

➢ **Without 2,3-BPG (2,3-Bisphosphoglycerate):**

Shows a higher oxygen affinity / Oxygen saturation level at the lungs reaches around **80% - 90%**.

➢ **With 2,3-BPG:**

Binds to hemoglobin and stabilizes the **T-state (Tense state)**, lowering oxygen affinity / Oxygen saturation level at the lungs drops to around **70% - 75%**.

❑ Impact of High Altitude Environment:

At high altitudes, ambient oxygen availability is low. Due to lower oxygen levels, normal hemoglobin saturation decreases from ~95–98% at sea level down to 85%–88%.

❑ Oxygen Release at the Tissues (Delivery Mechanism)



➢ **Without 2,3-BPG:**

Hemoglobin releases less oxygen to the tissues (saturation drops only down to ~38%).

➢ **With 2,3-BPG:**

Oxygen saturation at tissues drops significantly further down to **50%–60%**.

This demonstrates that 2,3-BPG enhances oxygen delivery/release to peripheral tissues more readily, compensating for lower atmospheric oxygen.

❑ Clinical & Physiological Conclusion:

Mutations or physiological increases in **2,3-BPG** that stabilize the **T-state** are **less severe** than those stabilizing the R-state (Relaxed state).

Reason: Although stabilization of the T-state slightly reduces initial oxygen loading in the lungs, it significantly improves oxygen unloading at the tissue level, providing a overall net functional benefit.

Hemoglobinopathies

Prof. Mamoun Ahram
Edited by Dr. Motamed Qadan
Hematopoietic-lymphatic system

Resources

Resources

- This lecture
- Mark’s Basic Medical Biochemistry, Ch. 44

Before we start:



❑Hemoglobinopathies and Thalassemia Mutations

Definition of Thalassemia: Thalassemia is considered a quantitative mutation because it decreases the production rate of globin chains that make up hemoglobin.

Main Types of Thalassemia: The lecture focuses primarily on the deficiency of alpha-globin chains or beta-globin chains.

Associated Mutations: There are approximately 8-7 mutations responsible for globin deficiencies leading to thalassemia, alongside other mutations that reduce globin levels but are not directly linked to thalassemia unless they occur concomitantly, bringing the main disease-causing mutations to about 10.

Amino Acid Mutations (Sickle Cell Anemia): There are 3 remaining mutations occurring in the same amino acid (Glutamic Acid)—specifically seen in sickle cell anemia—where the amino acid changes from negatively charged to neutral, or negative to positive, or two mutations occur simultaneously within the same amino acid.

What we are covering in this lecture

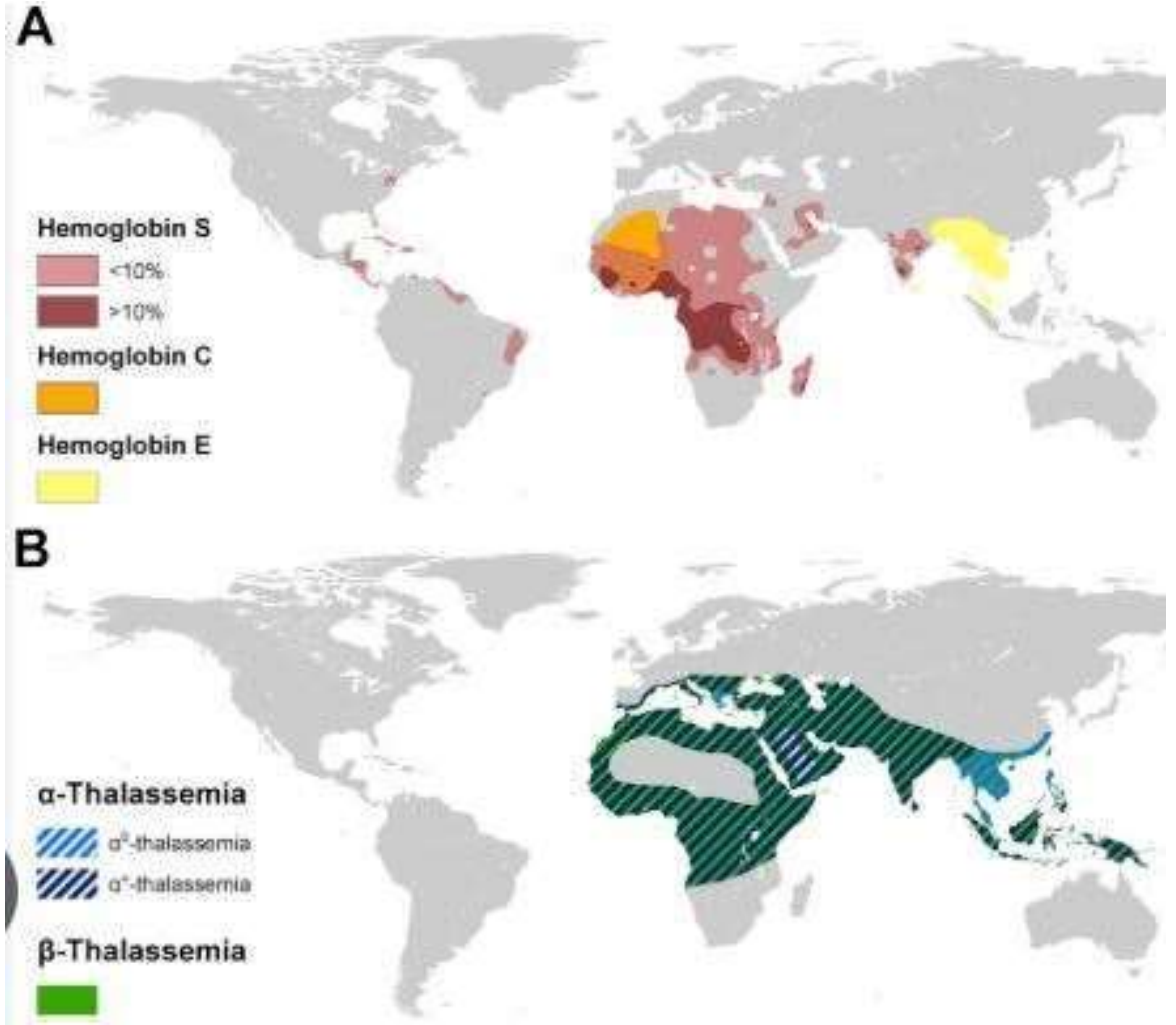
What we are covering in this lecture

- 1 Hereditary persistence of fetal hemoglobin HPFH
- 2 Quantitative Hemoglobinopathies:
  - 1 Alpha Thalassemia
  - 2 Beta Thalassemia
  - 2- HbE and Constant Spring
- 3- Qualitative Hemoglobinopathies:
  - 1 Sickle cell HbS, HbC, HbSC
  - 2 Already covered (Yakima, Cowtown, Kansas, Hemmersmith,

What are hemoglobinopathies?

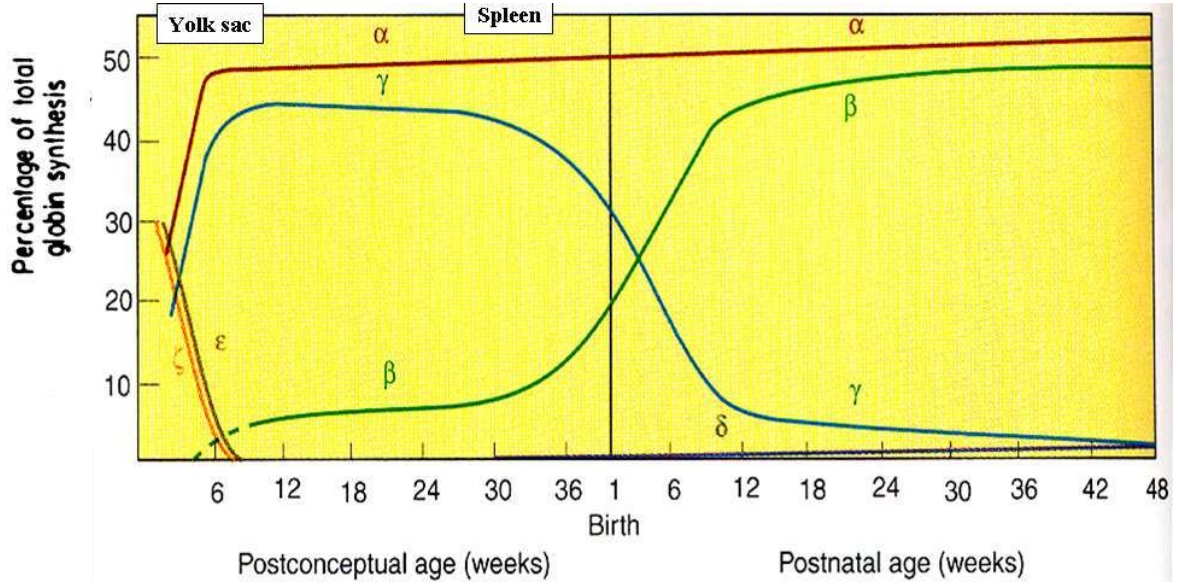
What are hemoglobinopathies?

- Hemoglobinopathies: Disorders of human hemoglobin.
- The most common genetic group of genetic diseases in the world (5% of people have mutations whether carriers or have the disease)
- Hemoglobin disorders account for 3.4% of deaths in children < 5 years.



Hereditary hemoglobins disorders

- Hemoglobin disorders are:
- 1 Hereditary persistence of fetal hemoglobin (HPFH): impairment of the perinatal switch from γ to β globin.
- 2 Quantitative abnormalities are abnormalities in the relative amounts of α and β subunits (thalassemias).
- 3 Qualitative abnormalities: mutations resulting in structural variants.
  - Over 800 variants have been identified.



What we are covering in this lecture

- 1- Hereditary persistence of fetal hemoglobin HPFH

The embryonic stage

- **The human body goes through three main stages for globin production**:

Embryo Stage: Zeta and epsilon globins appear initially and are considered primary types.

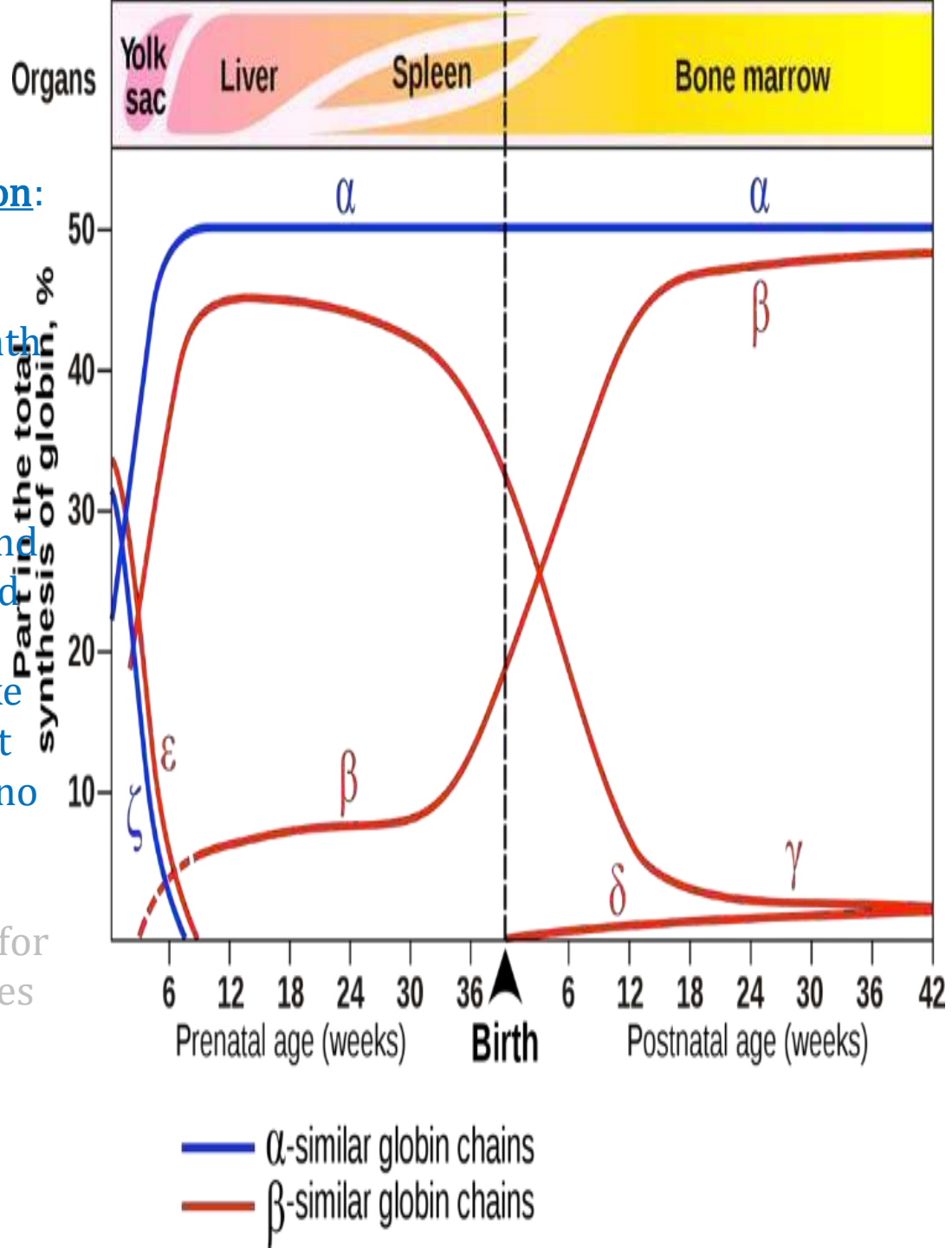
Fetus Stage: This spans roughly from the third month to the ninth month in utero, where alpha and gamma chains are predominant.

Adult Stage: The predominant chains are alpha and beta.

Dynamics and Interactions of Globin Types:

- **Interchangeability (Blue and Red Types):** Blue types (such as zeta and beta at certain stages) and red types (such as gamma, delta,beta, and epsilon) substitute for one another during development.
- **Compensation:** If alpha chains are missing, zeta can temporarily take their place during early stages (the first 6 weeks to 3 months), but it disappears quickly; however, if alpha is completely absent, there is no long-term substitute, whereas some red chains (like gamma, delta, beta, or epsilon) can compensate to varying degrees.

The main focus should be on Alpha & Beta for adults, Alpha & Gamma for fetuses, and Zeta & Epsilon for early embryonic stages, while other types are produced in very small, mixed quantities.



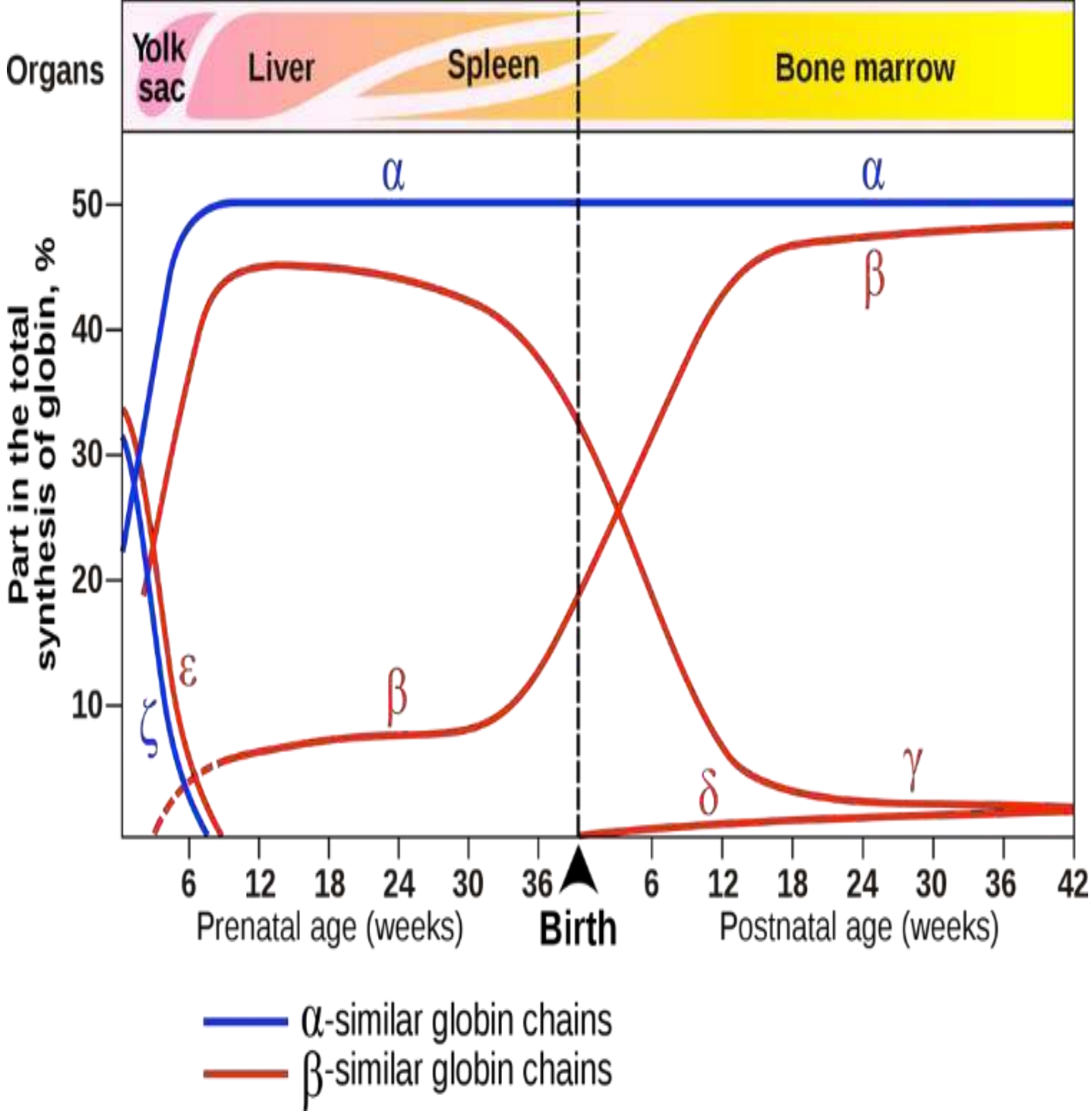
The embryonic stage

- **Switching and Control System:** Beta chain production begins in small amounts and then increases, whereas gamma production starts high and then decreases.
- **Silencing Region:** A silencing region exists within the beta gene cluster that suppresses gamma production while activating and increasing beta production, which explains the continuous developmental switch from gamma to beta.
- **Persistence of Gamma:** In certain pathological conditions, a deletion occurs within the beta gene including its silencing region. Because of this loss of the silencing mechanism, gamma production remains high and persistent, which prevents the worsening of beta deficiency by substituting it with continued gamma production.
- **Gamma's Oxygen Demand:** The "Gamma" type of globin requires a greater amount of oxygen compared to others. Physiological Reason: This is because a fetus in utero depends on oxygen derived from the mother's blood, thus requiring a higher oxygen affinity to meet the fetus's physiological needs.
- **Release Rate:** The issue with certain types is that their oxygen release rate to tissues is lower, though not to a fatal or critical degree; rather, falls within an acceptable, normal range to handle various body functions.



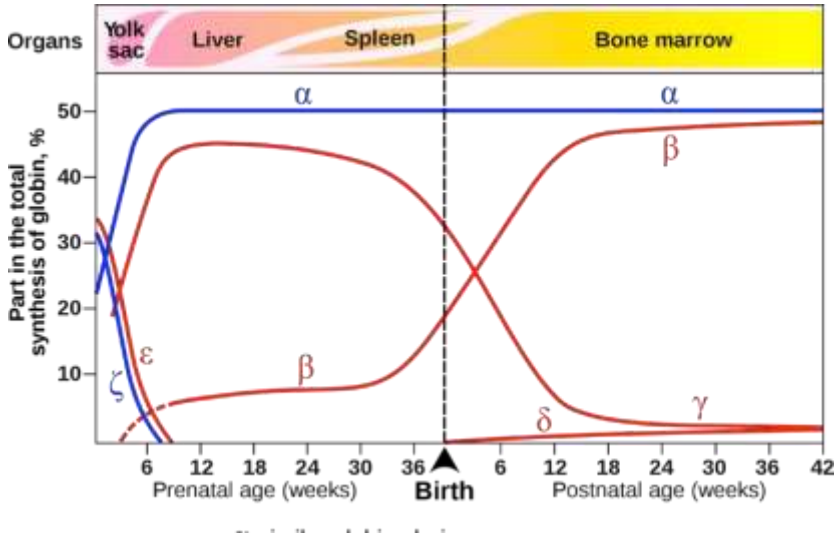
The embryonic stage

- **Beta Mutation Alone:** If a mutation occurs only in the beta gene without any production of beta chains, it leads to thalassemia.
- **Thalassemia Types & Deletions:** This results in a complete deletion of the beta genes, and if the repression area of the beta gene (the repression area of gamma) is also affected, the gamma chains will continue to remain present.
- **Differentiation based on Mutation:** Therefore, you can differentiate whether the mutation occurred in the beta gene alone or in both the beta gene and the repression area.
- **Gamma and Oxygen:** Gamma prefers oxygen more.



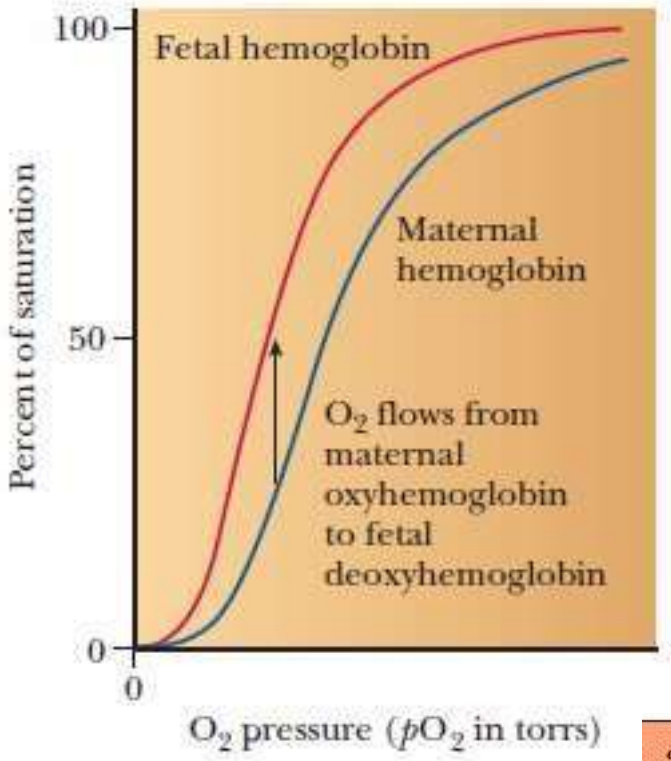
The embryonic stage

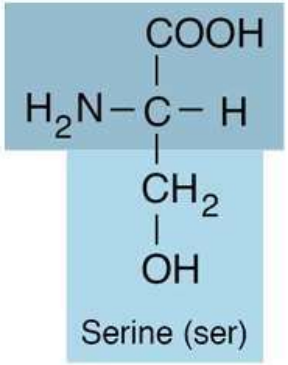
- Hemoglobin synthesis begins in the first few weeks of embryonic development within the yolk sac.
- The major hemoglobin (HbE Gower 1, week 3 – week 9 or 10) embryonic stage is a tetramer composed of 2 zeta (ξ) chains and 2 epsilon (ε) chains.
- Fetal hemoglobin (HbF) alpha 2 gamma 2 α2γ2, week 10 to 40.
- Other embryonic hemoglobin forms exist (that show later in the embryonic stage as other globins are expressed): HbE Gower 2 (α2ε2) because alpha starts developing, HbE Portland 1 because gamma starts developing (ζ2γ2), HbE Portland 2 because beta starts developing(ζ2β2).

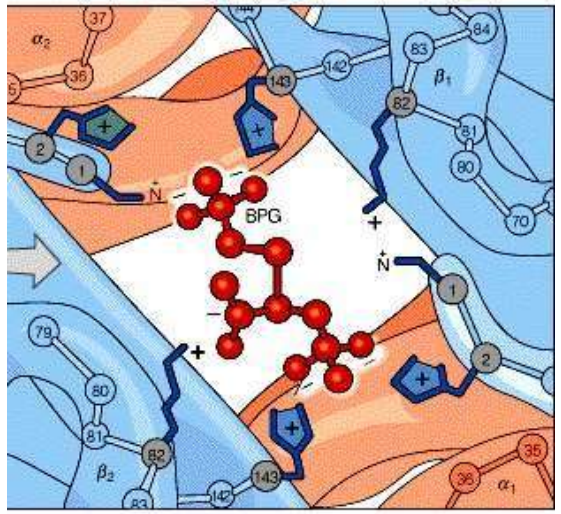


Fetal hemoglobin

- Fetal Hb (HbF) has higher affinity towards oxygen than adult hemoglobin (HBA).
  - HbA = $\alpha 2\beta 2$
  - HbF = $\alpha 2\gamma 2$
- His143 residue in the β subunit is replaced by a serine residue in the γ subunit of HbF.
  - Since serine cannot form a salt bridge with 2,3-BPG, it binds weaker to HbF than to HbA.







Fetal hemoglobin

❑ Gamma-Globin and Oxygen Affinity:

Gamma-globin has a higher affinity for oxygen because it does not bind well to 2,3-Bisphosphoglycerate (2,3-BPG).

Normally, 2,3-BPG facilitates the transition from the R state to the T state. Since 2,3-BPG binds to positive charges—which are absent in gamma-globin—it fails to bind to gamma, keeping it in the high-oxygen-affinity state.

❑ Reactivation Strategy for Gamma-Globin:

A potential therapeutic strategy for beta-globin mutations is to reactivate gamma-globin expression to compensate for defective beta-globin.

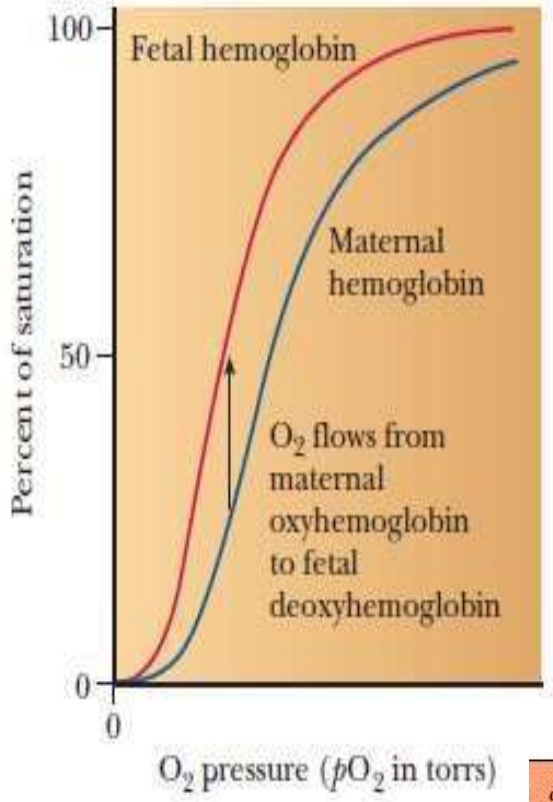
To achieve this, the primary challenge is to overcome the silencer region that represses the gamma-globin gene.

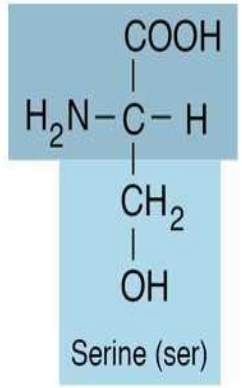
Once this repression is removed or bypassed, gamma-globin expression can be successfully reactivated. This approach is particularly useful because many mutations occur in the beta-globin gene.

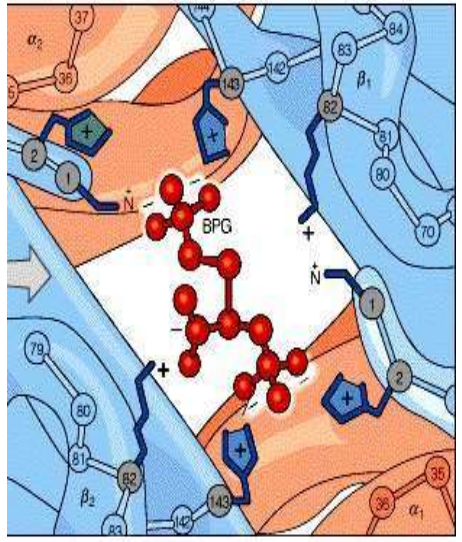
Beta vs. Gamma Activation Dynamics:

While gamma is naturally active, when beta is activated, it typically suppresses gamma.

Researchers have noted that bypassing or removing the repression area of the gamma gene is the main hurdle, whereas silencing the beta gene or dealing with promoters is less problematic.







Hereditary persistence of fetal hemoglobin (HPFH)



- Persons with HPFH continue to make HbF as adults.
- Because the syndrome is benign most individuals do not even know they carry a hemoglobin abnormality (HbF has higher affinity but it still binds to 2,3 bfg, it may cause a little production of extra rbcs, it is not as strong as Yakima for example).
- Many HPFH individuals harbor large deletions of the δ- and β-coding region of the cluster (Usually the B gene has a silencer region for the Hbf and it remains active in thalassemia, so no Hbf is produced, however, in HPFH the silencer is deleted, thus the fetal hemoglobin can transcribe)
- Think: treatment for β-thalassemia!!!!

GENE REGULATION

Switching from fetal to adult hemoglobin

Xunde Wang & Swee Lay Thein

Nature Genetics **50**, 478–480(2018) | Cite this article

1102 Accesses | **5** Citations | **9** Altmetric | Metrics

The switch from fetal to adult hemoglobin relies on repression or silencing of the upstream γ-globin gene, but identification of the transcriptional repressors that bind to the sites at which a cluster of naturally occurring variants associated with HPFH (hereditary persistence of fetal hemoglobin) are found has been elusive. A new study provides mechanistic evidence for the direct binding of BCL11A and ZBTB7A, two previously identified γ-globin gene repressors.

What we are covering in this lecture

- 1 Hereditary persistence of fetal hemoglobin HPFH
- 2 Quantitative Hemoglobinopathies:
  - 1- Alpha Thalassemia



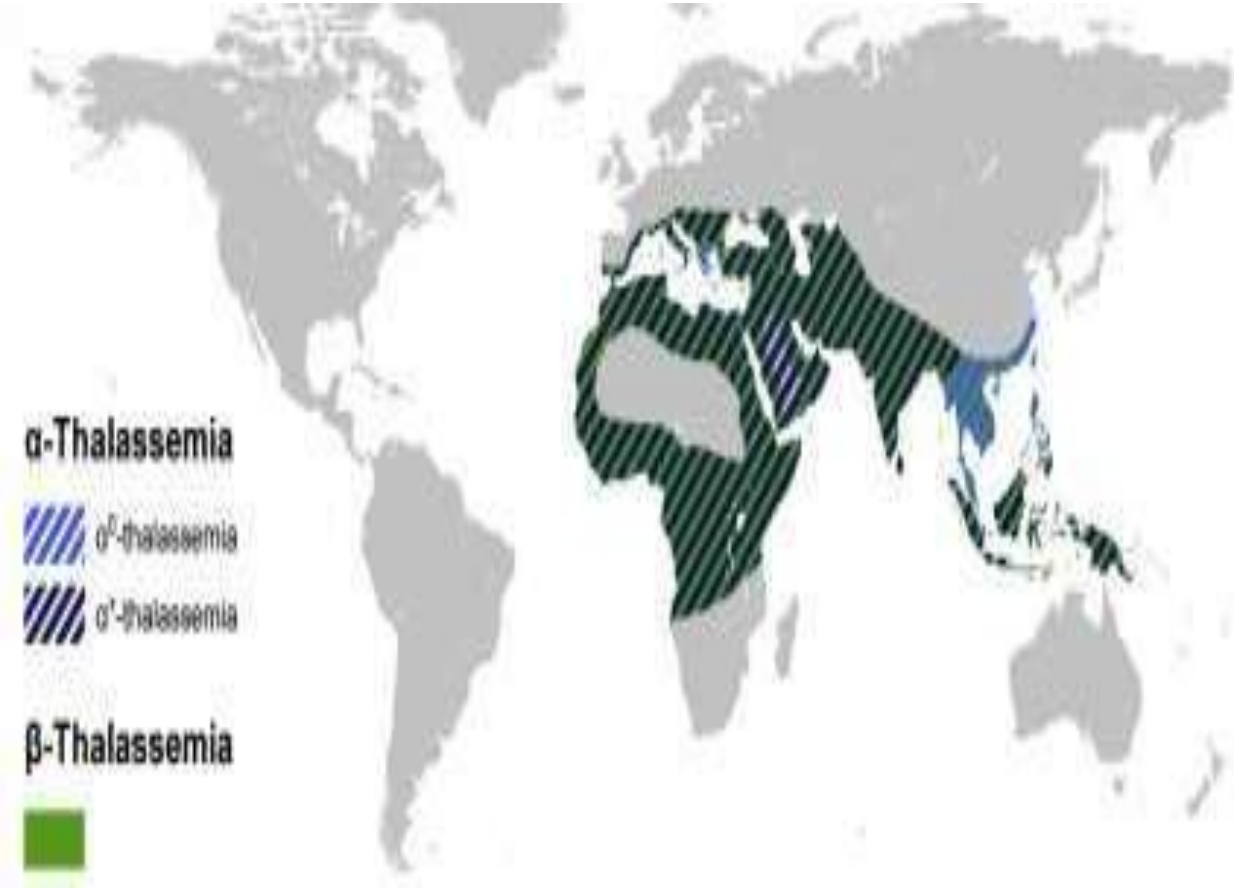
Thalassemias

- Thalassemias: the most common human single-gene disorder.
- They are caused by a reduced amount of either the α or β protein, which alters the ratio of the α:β ratio.

The discussion then shifted to Thalassemia disorders. The first condition mentioned is not strictly a disease state in terms of simple regulation, where gamma remains active.

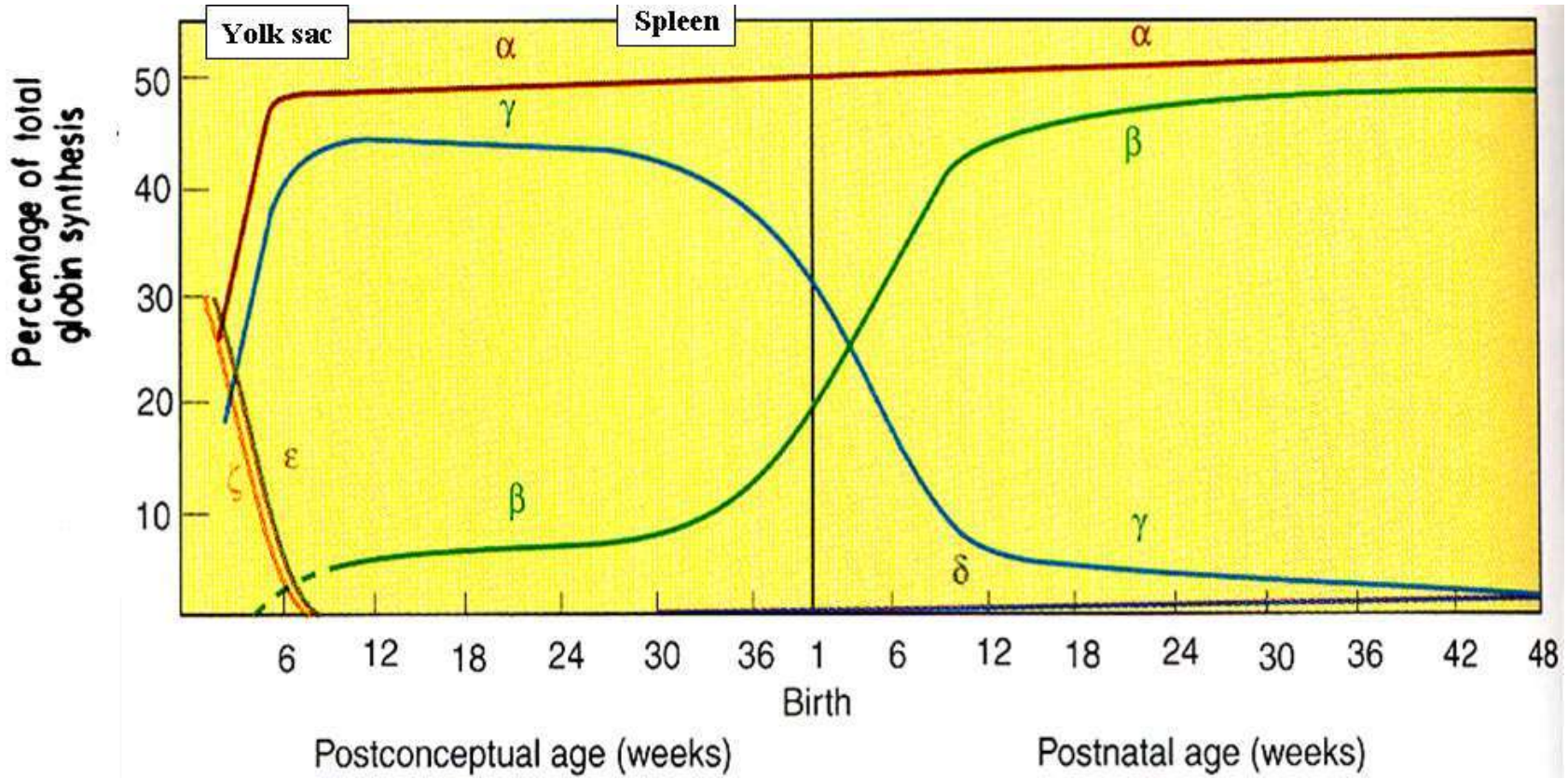
Alpha-thalassemia involves a deficiency or lack of alpha-globin chains.

This condition is notably prevalent in specific regions, including Africa and South Asia (such as India).



The Alpha-Thalassemias

- Alpha-thalassemia: underproduction of the α-globin chains.
- Mainly a deletion mutation that causes the gene to be non-functional.



Alpha Thalassemia subtypes from the most severe to the least



Variable severity

- With α-thalassemia, the level of α-globin production can range from none to very nearly normal levels.
- This is due to the fact that each individual has 4 genes, 2 on each chromosome.

❑ Fundamental Rules & Genetic Basis:

➢ Alpha Genes:

There are **4 copies** of the alpha-globin gene. Mutation can affect 1, 2, 3, or all 4 copies.

During embryonic/fetal development alpha-chains are substituted/replaced by **Zeta chains**.

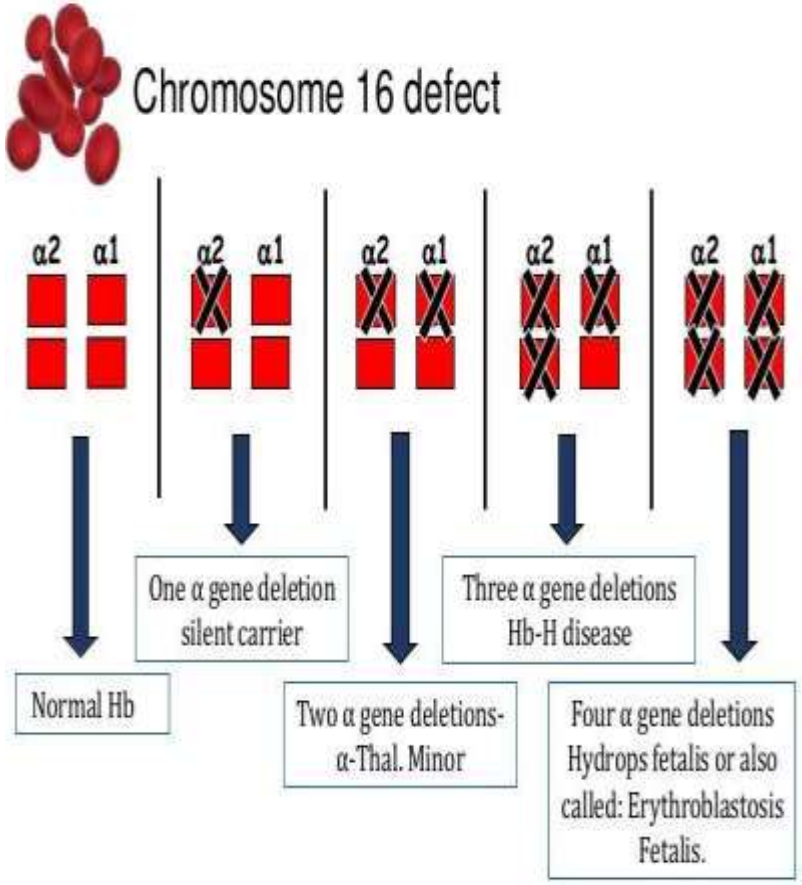
➢ Beta Genes:

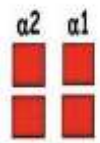
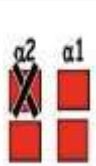
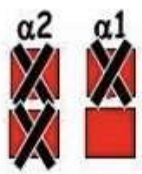
There are **2 copies** of the beta-globin gene. Thus, the range/margin for gene deletion or mutation is smaller (either 1 or 2 copies mutated).

✓ General Rule of Severity:

As long as **half (50%) of the genes** are intact and functional, the condition remains relatively mild (minor category).

- alpha-**Thalassemia:** Having 2 intact alpha-genes out of 4 keeps the condition in the minor category.
- beta-**Thalassemia:** Having 1 intact beta-gene out of 2 keeps the condition in the minor category.

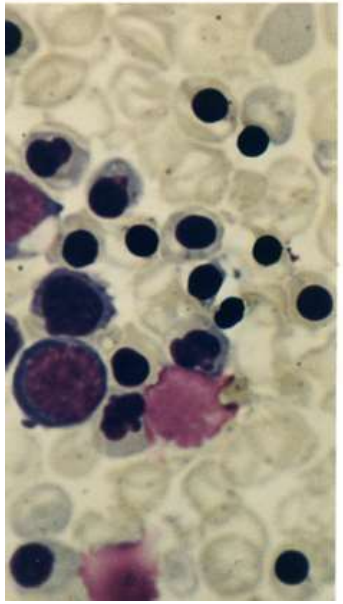


FUNCTIONAL GENES	CLINICAL STATUS / NAME	PATHOPHYSIOLOGY & CLINICAL PICTURE
4 Functional (0 mutated) 	Normal	Healthy individual with normal hematologic profile and hemoglobin synthesis. no mutations.
3 Functional (1 mutated) 	Silent Carrier	Asymptomatic carrier; normal hematologic parameters with no clinical complications.
2 Functional (2 mutated) 	α-Thalassemia Minor	Microcytic hypochromic anemia; mild clinical manifestation; excellent survival without regular transfusions.
1 Functional (3 mutated) 	α-Thalassemia Intermedia (**HbH Disease**)	Moderate-to-severe hemolytic and microcytic anemia. Forms **Beta tetramers (β_4)** forming **Heinz bodies**. Requires occasional blood transfusions.
0 Functional (4 mutated) 	α-Thalassemia Major (**Hydrops Fetalis / Hb Bart's**)	Fatal *in utero* or shortly after birth. Complete absence of α/ζ chains causes excess γ chains to form **Gamma tetramers (γ_4)**.

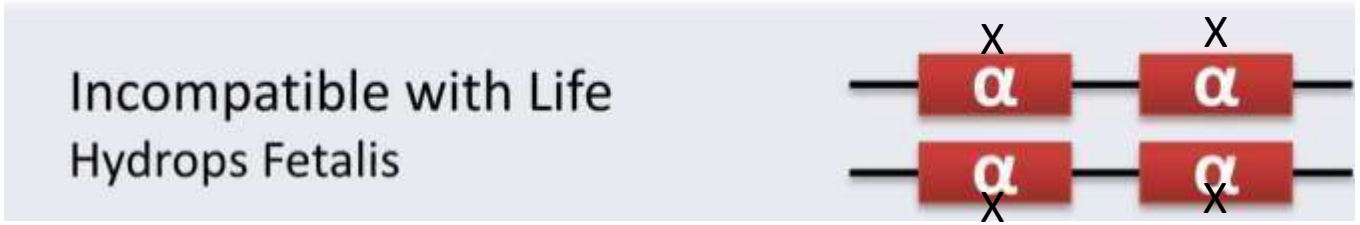
α-thalassemia major

α-thalassemia major, Hydrops fetalis or Hb Bart (most severe)

- 4 of 4 genes are deleted.
- The predominant fetal hemoglobin is a tetramer of γ-chains.
- γ4 or Hb Bart: a homotetramer of γ.
- Hb Bart has a high affinity towards oxygen.
- This condition is called hydrops fetalis.
- Stillbirth (death in the womb) or death shortly after birth occurs.







α-thalassemia major

α-thalassemia major, Hydrops fetalis or Hb Bart (most severe)

- **4 Mutated alpha-Genes (0 Functional) — *Hydrops Fetalis / Hb Bart's /* alpha-*Thalassemia Major:***



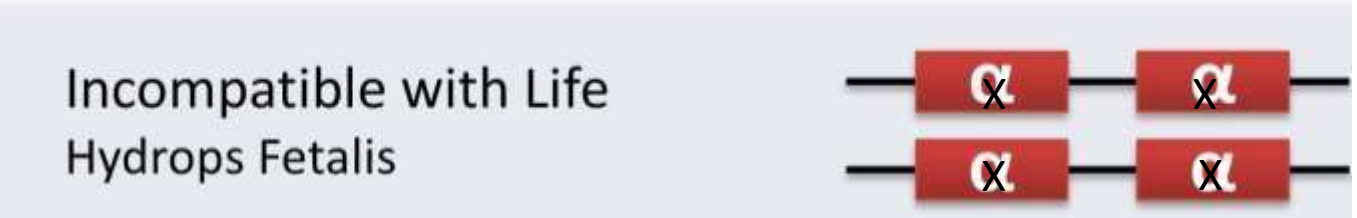


Genetics: Complete loss of all 4 alpha-globin genes.
Pathophysiology: Neither alpha-chains nor substituting zeta-chains are available.

As a result, excess gamma-chains pair together to form **Gamma tetramers** (gamma4), known as **Hemoglobin Bart's (Hb Bart's)**.
Oxygen Binding: gamma4 tetramers have an extremely high affinity for oxygen and **do not release oxygen** to fetal tissues efficiently.

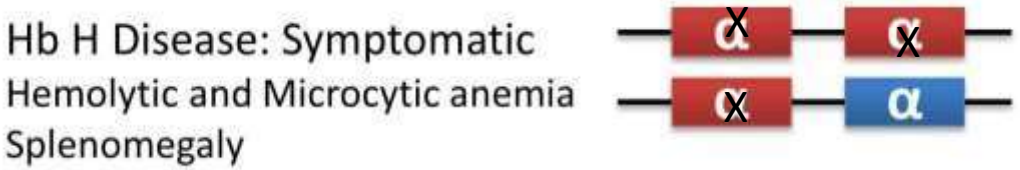
- **Clinical Outcome:**

Causes severe fluid accumulation/swelling throughout the body (**Hydrops Fetalis**).
It is **fatal to the fetus** (*in utero* or at birth).
Even if a fetus theoretically survives, it cannot properly deliver oxygen while growing inside the mother's womb



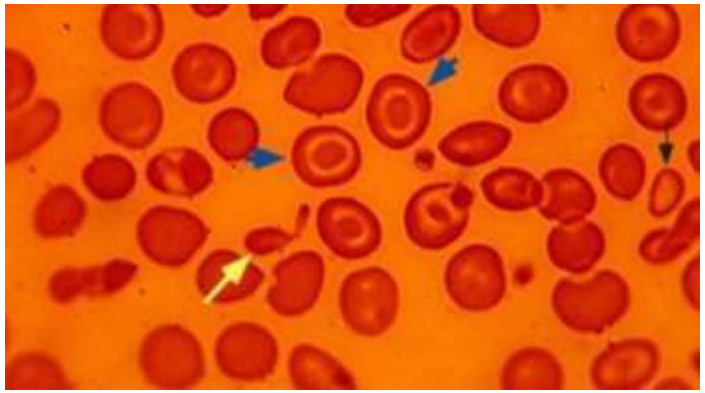
α-thalassemia intermedia

α-thalassemia intermedia or Hemoglobin H (HbH) disease



3 of 4 genes deleted.

- Clinically, it is known as hemoglobin H disease.
- With the reduction of α chain production, and β-chain production is established, homotetramers of β (β4 or HbH) can form.



- The HbH tetramers have a high affinity towards oxygen and are highly unstable (meaning that they denature, aggregate and precipitate resulting in the formation of Heinz bodies. It is less stable than hb bart).
- Mild to moderate microcytic and hemolytic anemia in adults, may need blood transfusions.
- The disease is not fatal.
- The main type of mutation is deletion.

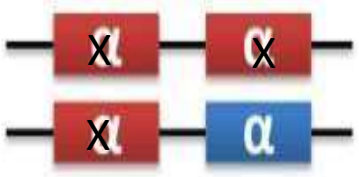
α-thalassemia intermedia

α-thalassemia intermedia or Hemoglobin H (HbH) disease

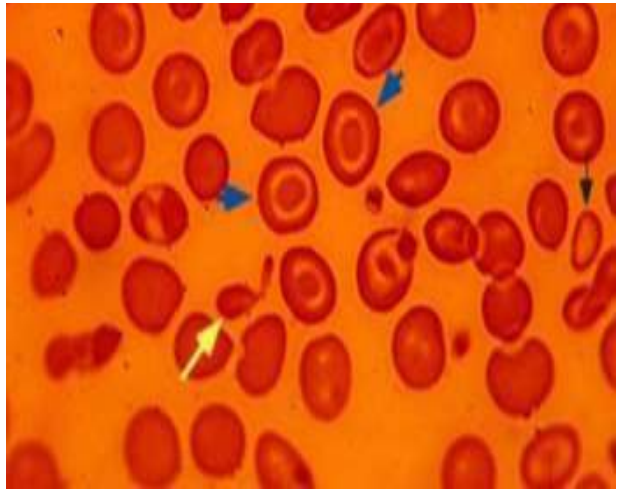
- **3 Mutated alpha-Genes (1 Functional) — *HbH Disease / alpha-Thalassemia Intermedia:***

Genetics: Loss of 3 alpha-globin genes; only 1 functional gene remains.
Pathophysiology: Some beta-chains are produced as the patient grows, but beta-chains far exceed the available alpha-chains.

Hb H Disease: Symptomatic
Hemolytic and Microcytic anemia
Splenomegaly



The excess beta-chains aggregate to form **Beta tetramers** (beta4), known as **Hemoglobin H (HbH)**.
HbH is unstable, precipitates inside red blood cells, and forms **Heinz bodies**.
These tetramers bind oxygen tightly and do not release it efficiently to tissues.



- **Clinical Outcome:**

Patients suffer from **hemolytic anemia** and **microcytic anemia**.
Patients can survive, but they require **blood transfusions**.

α-thalassemia minor and silent carrier

- α-Thalassemia trait: If 2 of the 4 genes are inactivated.

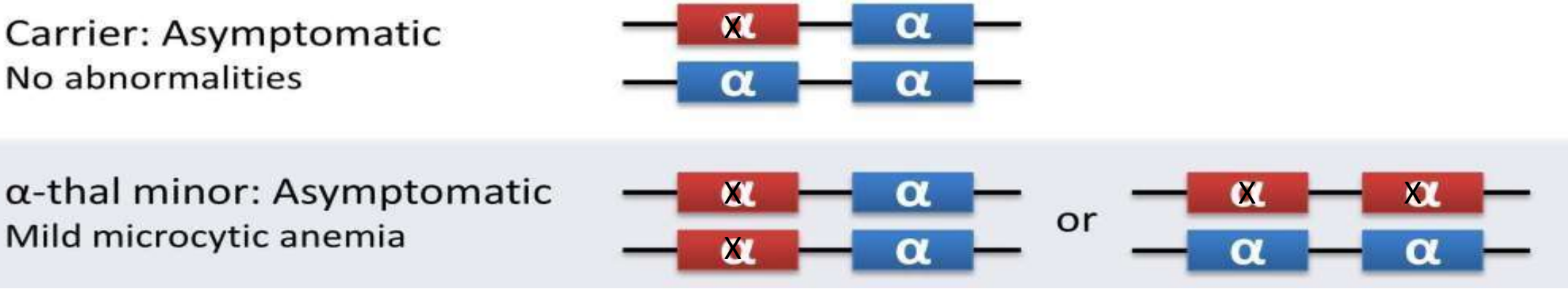
  The individuals have mild microcytic anemia.

Pathophysiology & Clinical Outcome:

Sufficient alpha-chains are produced to cover basic needs.
Presents with **microcytic anemia** and mild clinical signs.
Patients survive well and require minimal to no blood transfusions.

- Silent carrier: 1 of 4 genes deleted.

  Individuals are completely asymptomatic.



Summary of α-thalassemias

Genotype	α-globin gene number[a]	Name	Phenotype
αα / αα	4	Normal state	None
αα / α–	3	Silent carrier	None (values for Hb and MCV may be near the lower limits of normal)
– – / αα or α– / α–	2	Thalassemia trait	Thalassemia minor: asymptomatic, mild microcytic anemia
– – / α–	1	Hb H disease	Thalassemia intermedia: mild to moderate microcytic anemia
– – / – –	0	Alpha thalassemia major	Thalassemia major: hydrops fetalis

[a]Number of normal alpha globin genes

Note microcytic anemia has to do with the shape of the RBC while hemolytic has to do with lysis of the RBCs.

What we are covering in this lecture

What we are covering in this lecture

1. Hereditary persistence of fetal hemoglobin HPFH
2. Quantitative Hemoglobinopathies:
    1. Alpha Thalassemia
    2. Beta Thalassemia

It is caused by decreased β-globin chain synthesis,and since we have only two copies of this gene, the ability to compensate for defective synthesis is limited.

- Most mutations occur in the β-globin gene

The beta-thalassemias

The beta-thalassemias

- β-globins are deficient and the α-globins are in excess and will form α-globin homotetramers.
- The main types of mutation are point mutations that lead to non-functional protein.
  - The mutations occur within the promoter or LCR (enhancer), the exon, translation initiation codon, splicing positions, or poly-adenylation termination signal.
- The α-globin homotetramers are extremely insoluble, which leads to premature red cell destruction in the bone marrow and spleen.
- Delta and gamma are common globin become apparent.

β-thalassemia major and minor

β-thalassemia major (Cooley's anemia)

- A complete lack of HbA is denoted as β^0-thalassemia or β-thalassemia major.
- Affected individuals suffer from severe anemia beginning in the first year of life and need blood transfusions.
  - Long-term transfusions lead to the accumulation of iron in the organs, particularly the heart, liver and pancreas and, finally, death in the teens to early twenties. Can be treated with iron chelators which improve the prognosis.

β-thalassemia minor

- Individuals heterozygous for β-thalassemia with one normal β-globin gene and a mutated gene are termed β-thalassemia minor.
- Individuals with beta-thalassemia minor are generally asymptomatic with mild anemia.

Classification and types of β-thalassemia

Common genotypes	Name	Phenotype
β/β	Normal	None
β/β^0 β/β^+	Beta thalassemia trait	Thalassemia minor: asymptomatic, mild microcytic hypochromic anemia
β^+/β^+ β^+/β^0 β^E/β^+ β^E/β^0	Beta thalassemia intermedia	Variable severity Mild to moderate anemia Possible extramedullary hematopoiesis Iron overload
β^0/β^0	Beta thalassemia major (Cooley's Anemia)	Severe anemia Transfusion dependence Extramedullary hematopoiesis Iron overload

β^0: complete lack of β chain
β^+: some expression of β chain
β: normal expression of β chain
β^E: point mutation that is characterized by substitution of a.a. 26 from Glu to Lys on the surface of the b molecule that causes lower production of B chain.

Note:

a. that in the 2nd raw the thalesemia is minor when half the beta is functional. The same as in the alpha thalassemia table when half of the alpha were functional the symptoms were mild.

b. In the third raw there is some partial functional b thalesemia produced B+, however $\beta^E \beta^0$ is more on the severe end.

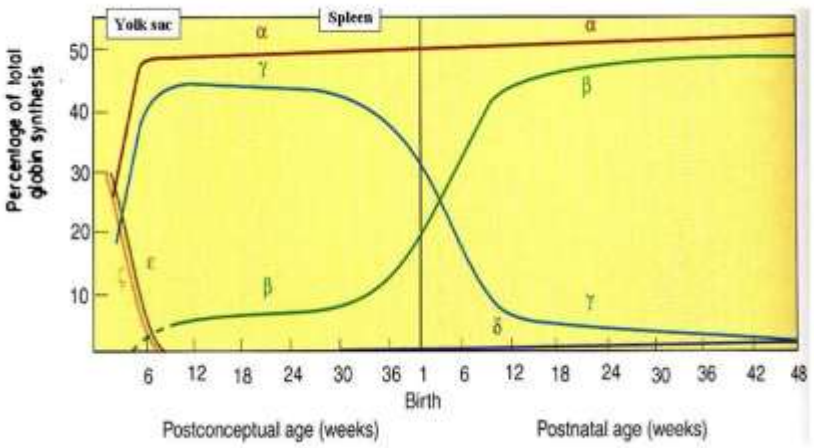
C- homozygous $\beta^E \beta^E$ leads to mild microcytic anemia as it causes the production of B chain to be reduced.

1. **Beta-thalassemia major** is characterized by the complete absence of β-globin synthesis (β^0/β^0). The mutations are point mutations that result in the absence of functional β-globin chains, rather than actual gene deletions.

β^0/β^0	Beta thalassemia major (Cooley's Anemia)	Severe anemia Transfusion dependence Extramedullary hematopoiesis Iron overload

due to repeated blood transfusions

- it is a severe, potentially life-threatening condition that requires frequent blood transfusions.



Unlike α-thalassemia, in which the deletion of all four α-globin genes is usually fatal, individuals with beta-thalassemia major can survive because γ-globin and δ-globin chains can still be produced. Therefore, the condition is severe, but the absence of β-globin chains is not as severe as the complete absence of α-globin chains.
Although γ-globin production is normally repressed after birth, some γ-globin synthesis persists because the regulatory region responsible for its repression remains intact.

β/β^0 β/β^+	Beta thalassemia trait	Thalassemia minor: asymptomatic, mild microcytic hypochromic anemia

β^+: Reduced β-globin synthesis.
β^0: Complete absence of β-globin synthesis from the affected gene.

2. **beta-thalassemia minor**, one β-globin gene is functional while the other is affected, resulting in reduced β-globin production

β^+/β^+	Beta thalassemia intermedia	Variable severity
β^+/β^0		Mild to moderate anemia
β^E/β^+		Possible extramedullary hematopoiesis
β^E/β^0		Iron overload

3. **Beta-Thalassemia Intermedia:** Both βglobin genes are affected by mutations, but some β-globin production persists; however, it is insufficient. For example, β^E/β^+ can result in some βglobin production, but not enough to meet the body's needs. β^0/β^E and β^+/β^0 are associated with more severe disease because one allele produces no β-globin, while the other produces either an abnormal variant or a reduced amount.

Classification and types of β-thalassemia

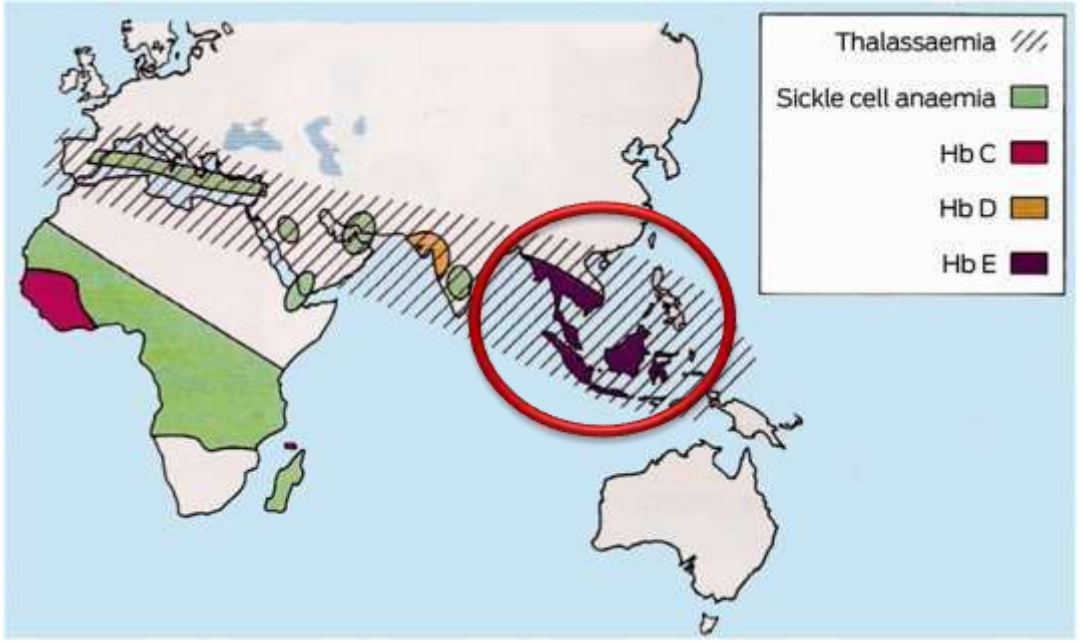
Beta thalassemia			Alpha thalassemia		
Common genotypes	**Name**	**Phenotype**	**Genotype** (alpha-globin gene number)	**Name**	**Phenotype**
β/β	Normal	None	4 or 3 (αα / αα or αα / α–)	Normal state / Silent carrier	None (values for Hb and MCV may be near the lower limits of normal)
* β/$β^0$ or β/$β^+$	Beta thalassemia trait	Thalassemia minor: asymptomatic, mild microcytic hypochromic anemia	2 (– – / αα or α– / α–)	Thalassemia trait	Thalassemia minor: asymptomatic, mild microcytic anemia
$β^+/β^+$ $β^+/β^0$ $β^E/β^+$ $β^E/β^0$	Beta thalassemia intermedia	Variable severity Mild to moderate anemia	1 (– – / α–)	Hb H disease	Thalassemia intermedia: mild to moderate microcytic anemia
$β^0/β^0$	Beta thalassemia major (Cooley's anemia)	Severe anemia, transfusion dependent	0 (– – / – –)	Alpha thalassemia major	Thalassemia major: hydrops fetalis

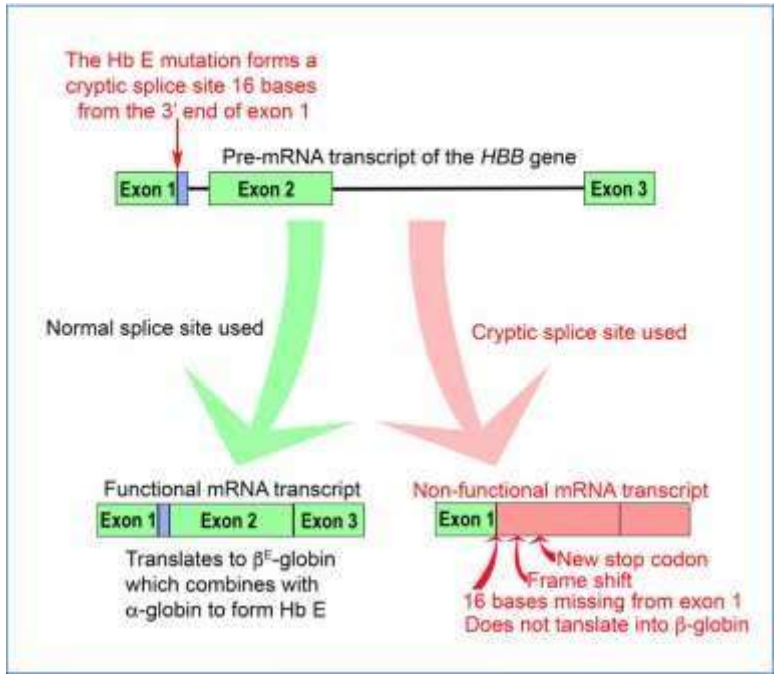
*The common feature of both **α-thalassemia minor and β-thalassemia minor** is that approximately half of the globin gene production capacity is affected. Both conditions are typically associated with **mild microcytic anemia**

Hemoglobin E

- It is common in Southeast Asia and china
- It has both quantitative and qualitative characteristics.
- The same mutation can have either one of the following consequences:
- 1 It can cause alternative RNA splice site and an earlier stop codon which causes lower production of hemoglobin so it is similar somehow that mild b thalassemia.
- 2 It can cause a point mutation in codon 26 that changes glutamic acid (GAG) to lysine (AAG) creating a slightly different hemoglobin that binds oxygen more (R state).

- individuals with HbEE generally have mild microcytosis and sometimes mild anemia, whereas HbE combined with a severe β-thalassemia allele can produce significant disease.





Doctor's explanation of the previous slide:

❑ Hemoglobin E (HbE)

A point mutation in the DNA is transcribed into mRNA, and the body may respond in two ways:

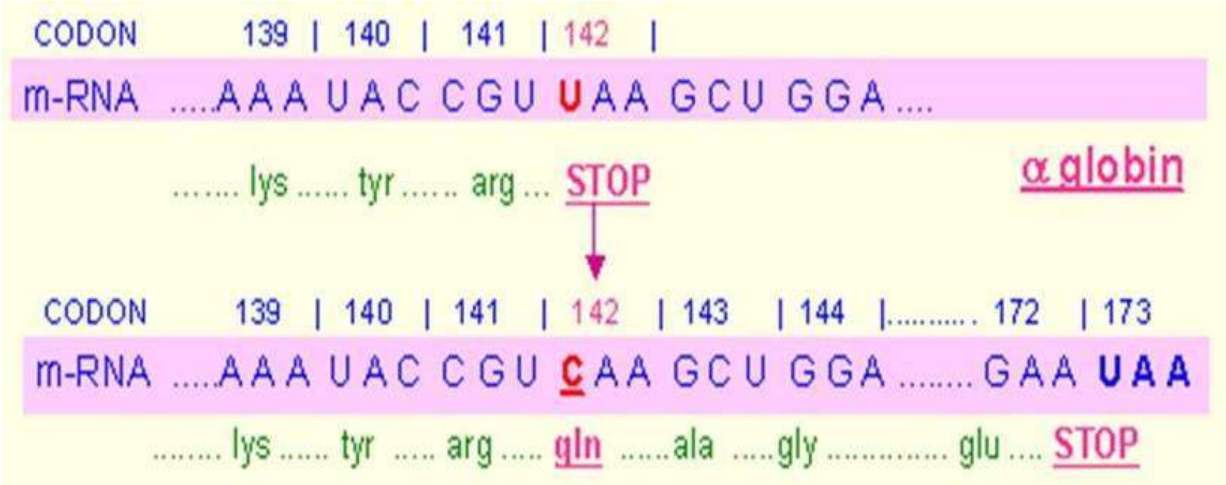
1. Abnormal mRNA splicing: The mRNA undergoes abnormal splicing, resulting in some nonfunctional mRNA and reduced β-globin production. However, the condition is usually mild when HbE is present alone.

2. Production of an abnormal protein: The mRNA is translated, producing an abnormal β-globin variant (βE), which forms Hemoglobin E (HbE). This condition is also usually mild when HbE is present alone.

Clinical significance: HbE is generally associated with a mild condition when present alone. However, when inherited together with a β^0-thalassemia mutation, it can result in **beta-thalassemia major**.

Hb Constant Spring (Hb CS)

- Hemoglobin Constant Spring (Hb CS) is an abnormal Hb caused by a mutation at the termination codon of the α2-globin gene leading to the production of longer than normal unstable mRNA and protein products.
  - The anemia is usually moderate.
- Heterozygotes have the genotype (αα/αα^{CS}) and have α°-thalassemia trait phenotype.
- It is commonly found among Southeast Asian and Chinese people.
- If co-inherited with α-thalassemia, it leads to an α°-thalassemia intermedia syndrome.



Doctor's explanation of the previous slide:

❑ Hemoglobin Constant Spring (Hb Constant Spring)

Hemoglobin Constant Spring is an α-globin variant caused by a mutation in the stop codon. The stop codon is not recognized normally, so translation continues, producing an abnormally elongated α-globin chain.

When Hb Constant Spring is present alone, even in the homozygous state, it usually causes a mild condition

However, when two Constant Spring mutations are inherited together with two α-thalassemia gene deletions, the condition can become more severe, resulting in HbH–Constant Spring disease.

- Hb Constant Spring can cause mild hemolytic anemia

Classification of molecular mutations

Among the three conditions, **HbC is the least severe, while HbS is the most severe**. However, individuals with all three conditions can survive.

- HbS, HbC,HbSC and

❑ **Point Mutations in the β-Globin Gene**

All three point mutations occur at the same amino acid position, the 6th amino acid of the β-globin chain, where glutamic acid, a negatively charged amino acid, is replaced by another amino acid.

- In sickle cell anemia **(HbS),** glutamic acid is replaced by a hydrophobic amino acid, valine.

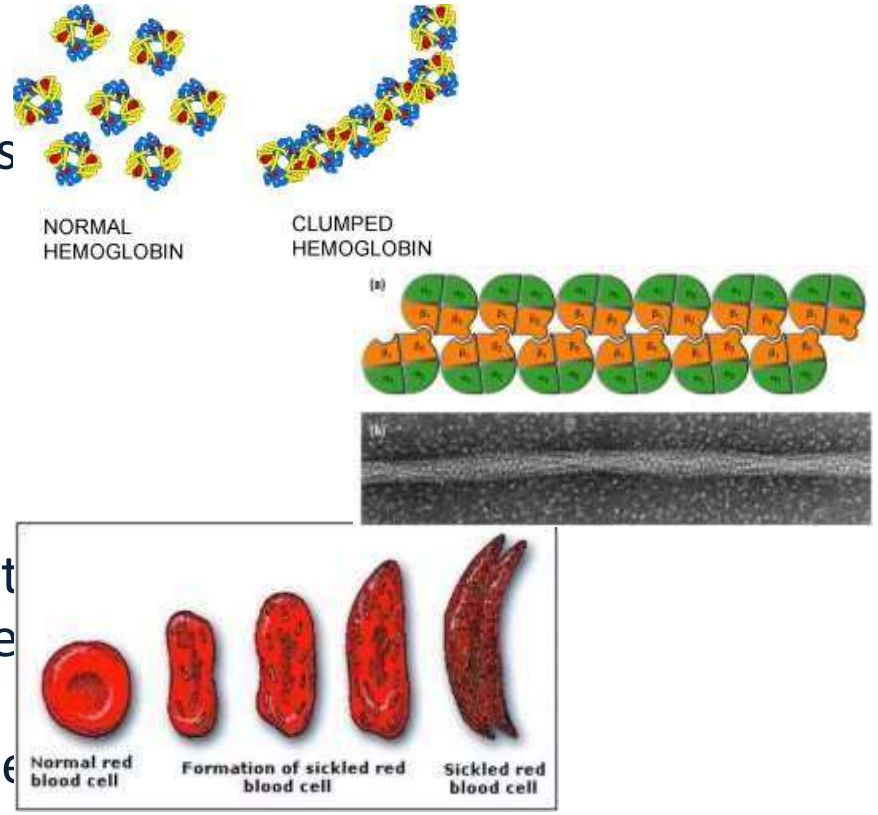
When only one β-globin gene is affected, the condition is usually mild, and carrying the HbS mutation can provide protection against severe malaria, which explains its prevalence in parts of Africa.

However, when both β-globin genes carry the mutation, the condition becomes clinically significant and can cause sickle cell disease.

Sickle cell hemoglobin (HbS)

- It is caused by a change of amino acids in the 6th position of β globin (Glu negatively charged to Val hydrophobic). Therefore, during electrophoresis at alkaline pH, Hb S migrates more slowly toward the anode (positive electrode) than does Hb A. The hemoglobin is designated α2βs2 or HbS.
- The hemoglobin tetramers aggregate into arrays upon deoxygenation in the tissues, and increase their sickle trait upon conditions that increase the dexoygentation such as high altitudes
- This aggregation leads to deformation of the red blood cell.
- It can also cause hemolytic anemia (life span of RBCs is reduced from 120 days to <20 days).
- Sickle cell anemia is characterized by lifelong episodes of pain ("crises"), and increased susceptibility to infections, usually beginning in early childhood. Other symptoms include acute chest syndrome, stroke, splenic and renal dysfunction, and bone change due to marrow hyperplasia to replace hemolytic anemia, but the anemia supersedes. Bone pain also occurs due to occlusions to the blood vessels there.

- In sickle cell disease, sickled RBCs can obstruct small blood vessels, causing vaso occlusion. This leads to painful vaso occlusive crises, particularly in the bones.



❑ Gel Electrophoresis of Hemoglobin

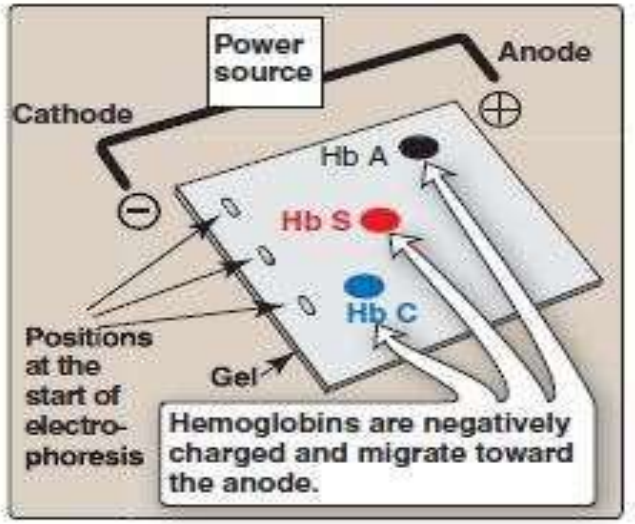
In native gel electrophoresis, proteins migrate according to their charge and other properties, without the addition of SDS.

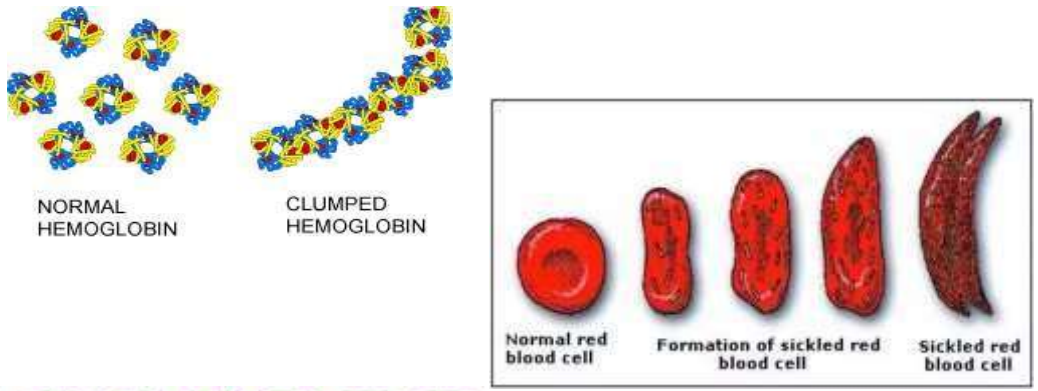
HbA: Glutamic acid at position 6 carries a negative charge, so HbA migrates fastest toward the positive electrode.

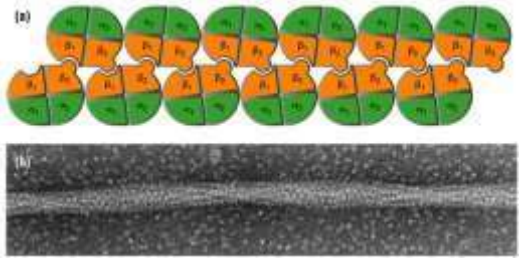
HbS: Glutamic acid is replaced by valine, a hydrophobic amino acid, reducing the negative charge and resulting in slower migration through the gel compared with HbA.

HbC: Glutamic acid is replaced by lysine, a positively charged amino acid, resulting in even slower migration toward the positive electrode.

Thus, the migration order is HbA → HbS → HbC, from fastest to slowest.



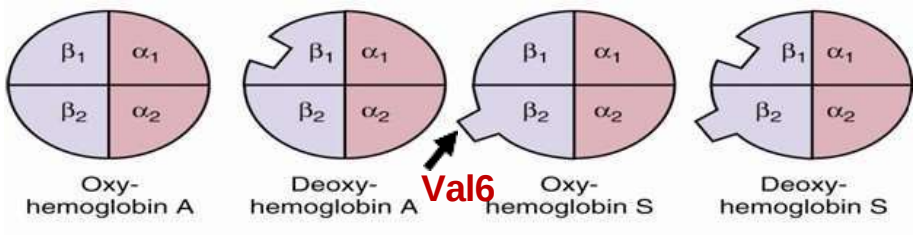


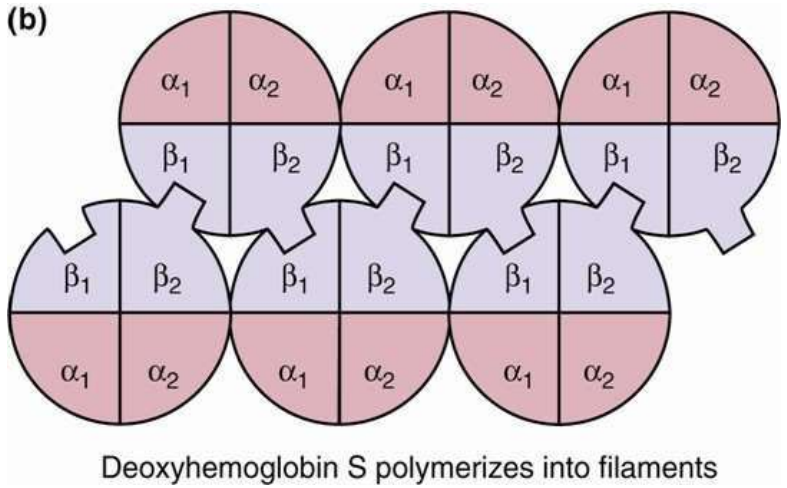


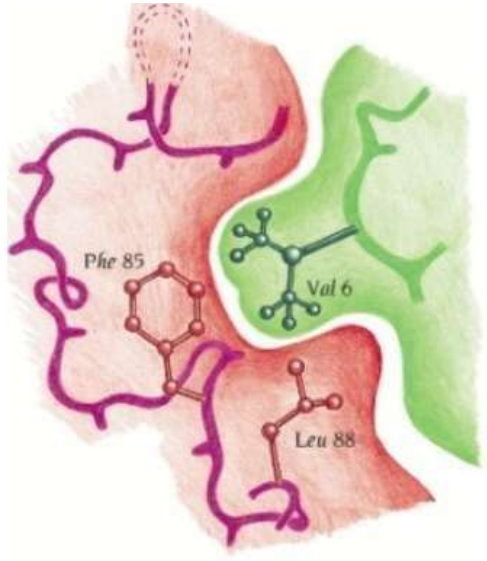
In HbS, low oxygen levels and RBC dehydration promote the polymerization of hemoglobin molecules, causing them to form long, rigid fibers. This changes the shape of RBCs into the characteristic sickle shape.

How does the fiber form?

- Fiber formation only occurs in the deoxy- or T-state.
- The mutated valine of the β2 chain is protruded and inserted into a hydrophobic pocket on the surface of β1 chain.







Variables that increase sickling

- Decreased oxygen pressure (high altitudes)
- Increased pCO_2
- Decreased pH
- Increased 2,3-BPG
- Dehydration (why?)
- Because dehydration increases the concentration of the hemoglobin

Sickle cell hemoglobin (HbS) control

- Therapy involves adequate hydration, analgesics, aggressive antibiotic therapy if infection is present. Intermittent transfusions with packed red cells reduce the risk of stroke, but the benefits must be weighed against the complications of transfusion, which include iron overload (hemosiderosis), bloodborne infections, and immunologic complications.

- Hydroxyurea, an antitumor drug, is therapeutically useful because it increases circulating levels of Hb F, which decreases RBC sickling

  It can remove the repression of γ-globin production,Therefore, even in the presence of a β-globin gene mutation, increased γ-globin production can compensate for the reduced β-globin production. This is one of the possible treatment approaches.

Sickle cell trait

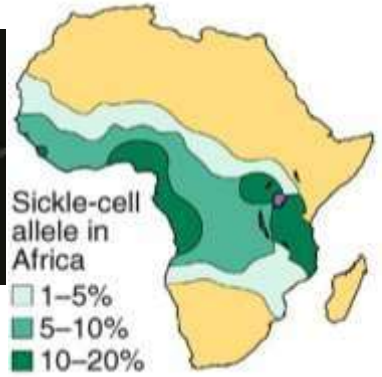
It occurs in heterozygotes (individuals with both HbA and HbS), who are clinically normal, but they have few cells that sickle when subjected to low oxygen. They have lower potential to sickle than the homozygote trait.

HBSA has a selective advantage over HBSS as HBSS has
already caused some splenic dysfunction.

In HBSA the parasite consumes oxygen and alters the RBC's internal environment. These changes promote localized HbS polymerization and oxidative stress, making infected RBCs more susceptible to damage.

The spleen preferentially removes some of these infected RBCs.



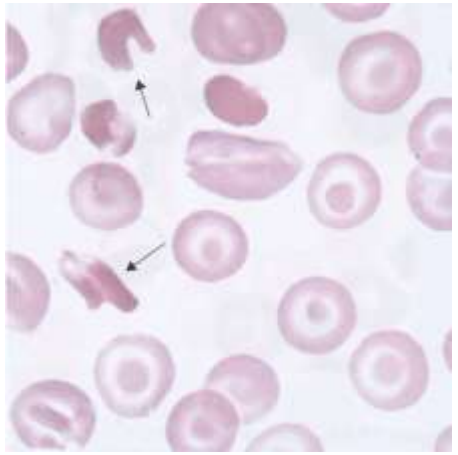




- When the HbS mutation is present in only one β-globin gene (sickle cell trait), it provides partial protection against severe malaria.
- Malaria is caused by Plasmodium, a unicellular parasite transmitted by mosquitoes. Part of its life cycle occurs inside RBCs, where it feeds on hemoglobin and uses its components for growth and development.
- In individuals with sickle cell trait, when the parasite infects RBCs, changes in oxygen availability can promote sickling of the affected cells. These RBCs are then more likely to be destroyed or removed from circulation, interfering with the parasite's life cycle and providing a survival advantage in malaria endemic regions, particularly in Africa.

Hemoglobin C (HbC)

- (HbC) is also due to a change at the 6th position of β globin replacing the glutamate with lysine (designated as βc).
- This hemoglobin is less soluble than HbA so it crystallizes in RBCs reducing their deformability in capillaries.
- HbC also leads to water loss from cells leading to higher hemoglobin concentration. This dehydration occurs because the HbC mutation leads to a positive charge that leads to an interaction between the HbC and the membrane proteins which eventually activate a potassium-chloride cotransporter (KCC) leading to increased cellular loss of potassium and chloride. Water follows to maintain to the osmotic gradients.
- This problem causes only a minor hemolytic disorder.

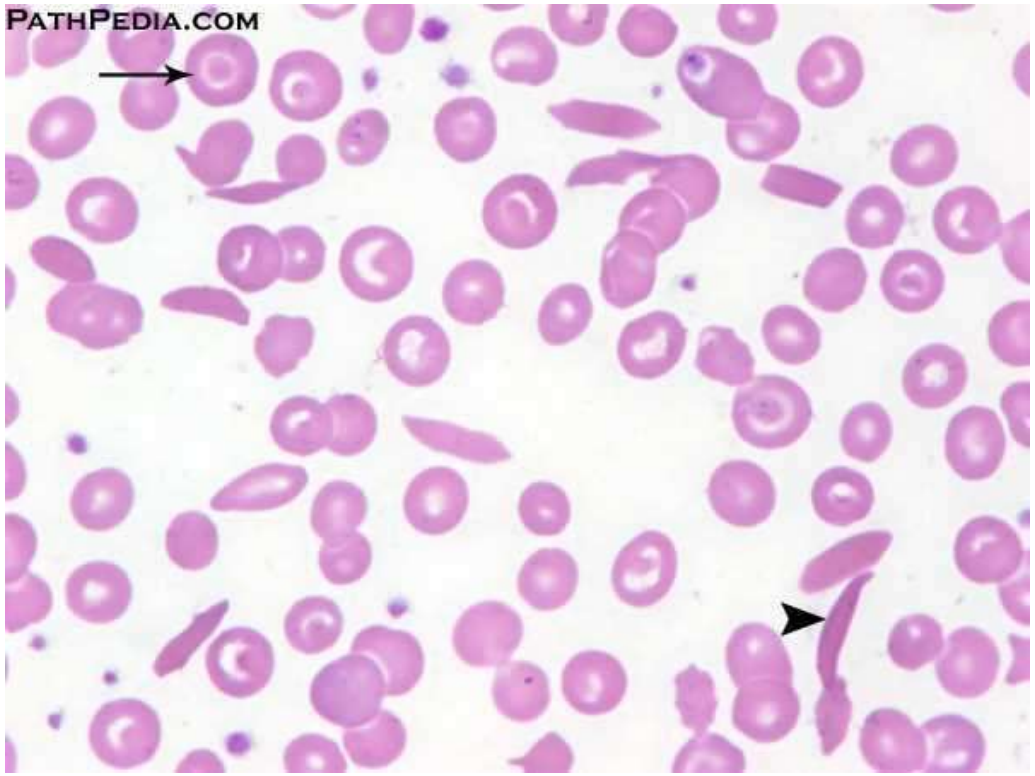


- HbC results from a point mutation in the β-globin gene at the same amino acid position as HbS, where **glutamic acid (negatively charged) is replaced by lysine (positively charged).**
- HbC is generally less severe than HbS. It activates an ion transport mechanism that causes sodium and chloride to move out of the RBCs, followed by water loss. This leads to RBC dehydration and hemoglobin crystallization, making the hemoglobin less functional.

HbSC disease

- Individuals with both βc and βs mutations have HbSC disease, a mild to severe hemolytic disorder that may especially manifest when there is dehydration leading to sickling (which is initiated by HBC after activating the pump)

Hemoglobin SC (HbSC) results from the combination of HbS and HbC and is considered an intermediate condition in terms of severity.

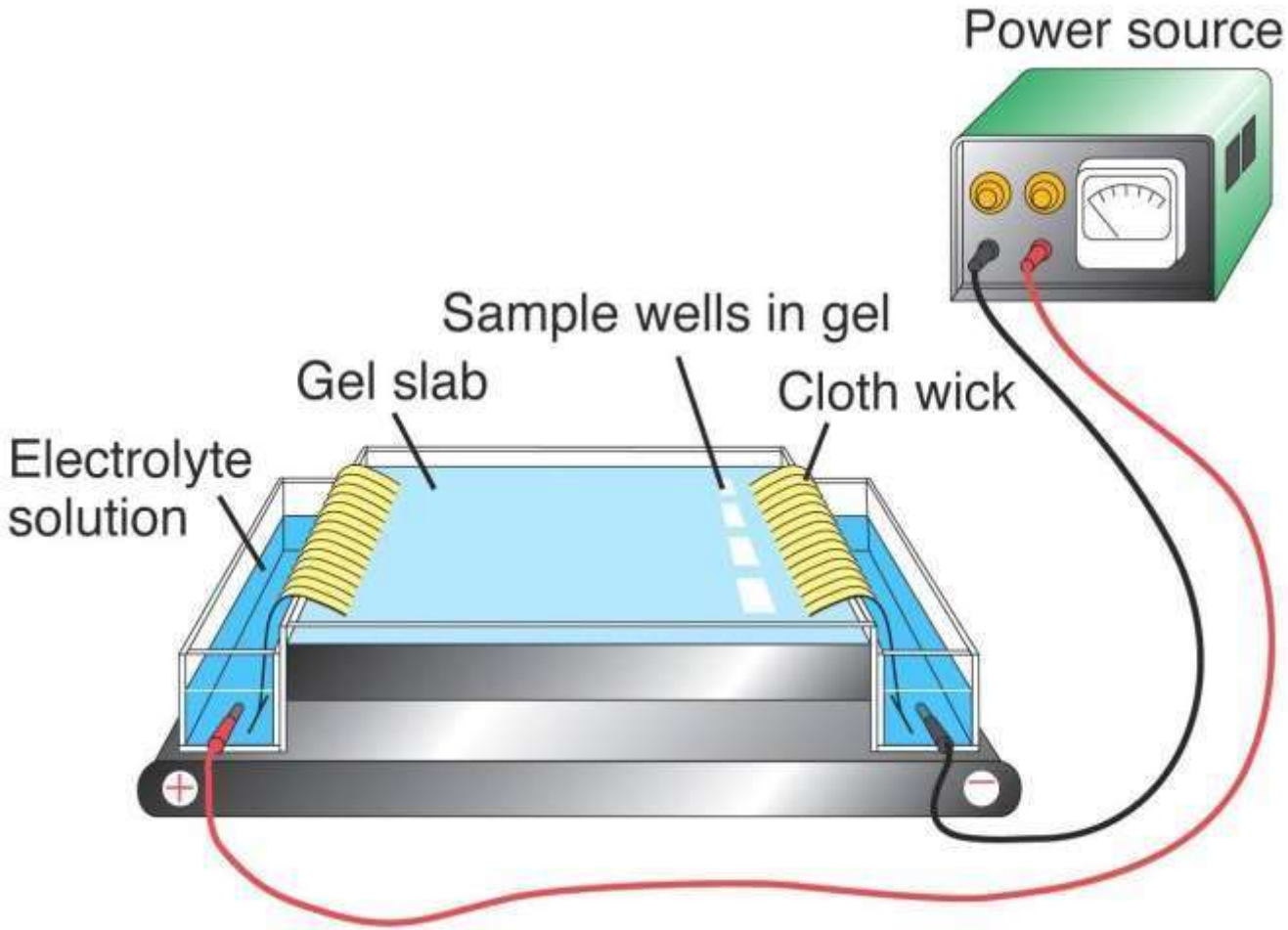


Mutation and migration

- • Amino acid substitution in abnormal Hbs results in an overall change in the charge of the molecule.
- • Therefore, Hb migration in a voltage gradient is altered.
- • Electrophoresis of hemoglobin proteins from individuals is an effective diagnostic tool in determining if an individual has a defective hemoglobin and the relative ratios of the patient's hemoglobin pattern.

Hemoglobin Electrophoresis

Hemoglobin Electrophoresis



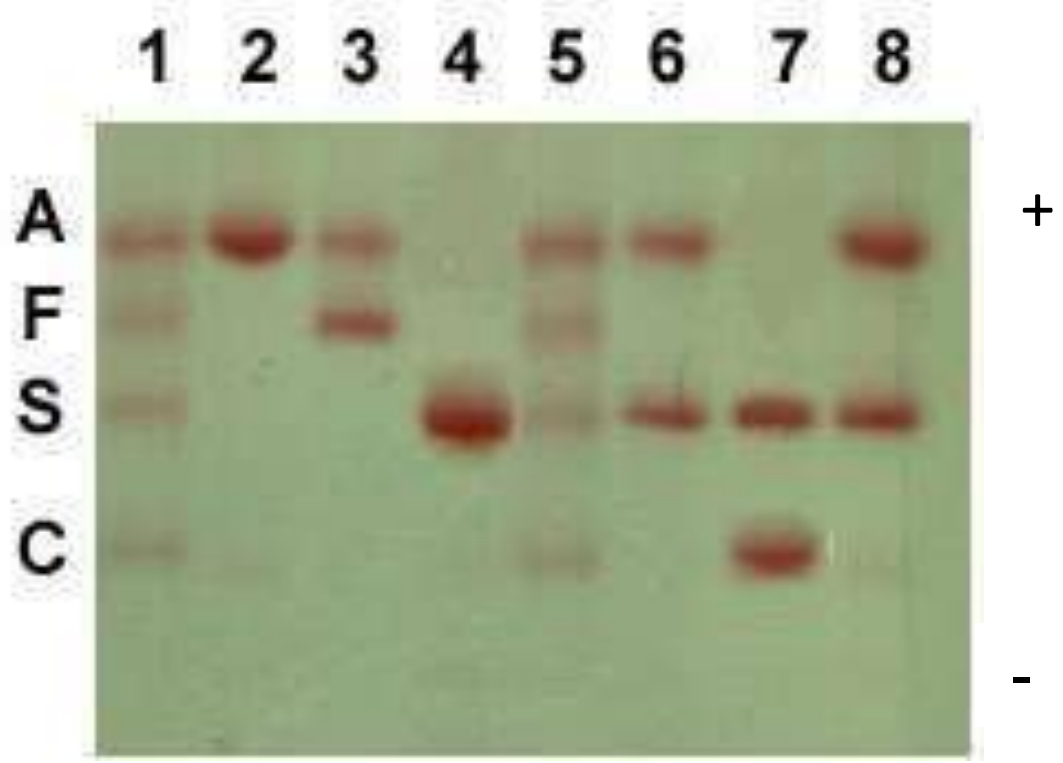
Examples

Examples

- In Sickle Cell hemoglobin, replacement of a negatively-charged glu in the standard HbA by a neutral val in HbS results in a protein with a slightly reduced negative charge.
- In homozygous individuals, the HbA tetramer electrophoreses as a single band, and the HbS tetramer as another single band.
- Hemoglobin from a heterozygous individual (with both alleles) appears as two bands.
- Since HbC contains a lysine instead of the normal glutamate, HbC will travel even faster to the cathode.

Results

- Lanes 1 and 5: Hb standards
- Lane 2: normal adult
- Lane 3: normal neonate
- Lane 4: homozygous HbS
- Lanes 6 and 8: Sickle cell trait
- Lane 7: HbSC disease



رسالة من الفريق العلمي:

"اللهم بارك لنا في علمنا، وانفعنا بما علّمتنا، وعلّمنا ما ينفعنا، وزدنا علمًا وفهمًا وتوفيقًا .اللهم يسّر لنا دروبنا، ووفّقنا في دراستنا، واجعل لنا من كل تعب أجرًا، ومن كل جهد ثمرة، وارزقنا النجاح الذي يرضيك عنّا .اللهم اجعل علمنا نافعًا، وعملنا صالحًا، وأثرنا طيبًا، واكتب لنا أجر كل من انتفع بما تعلّمناه .آمين يا رب العالمين."

For any feedback, scan the code or click on it

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			