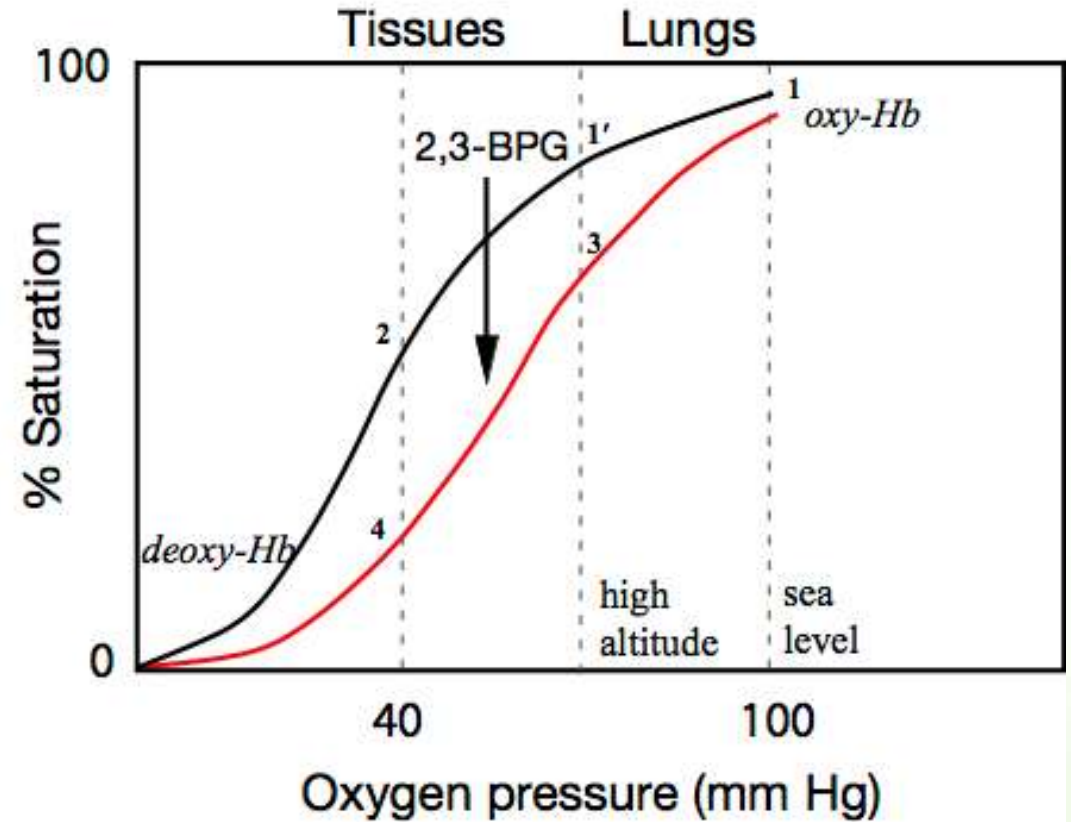


Better explanation of the role of 2,3-BPG

- 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.





Hemoglobinopathies

Prof. Mamoun Ahram

Edited by Dr. Motamed Qadan

Hematopoietic-lymphatic system

Resources



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

What we are covering in this lecture

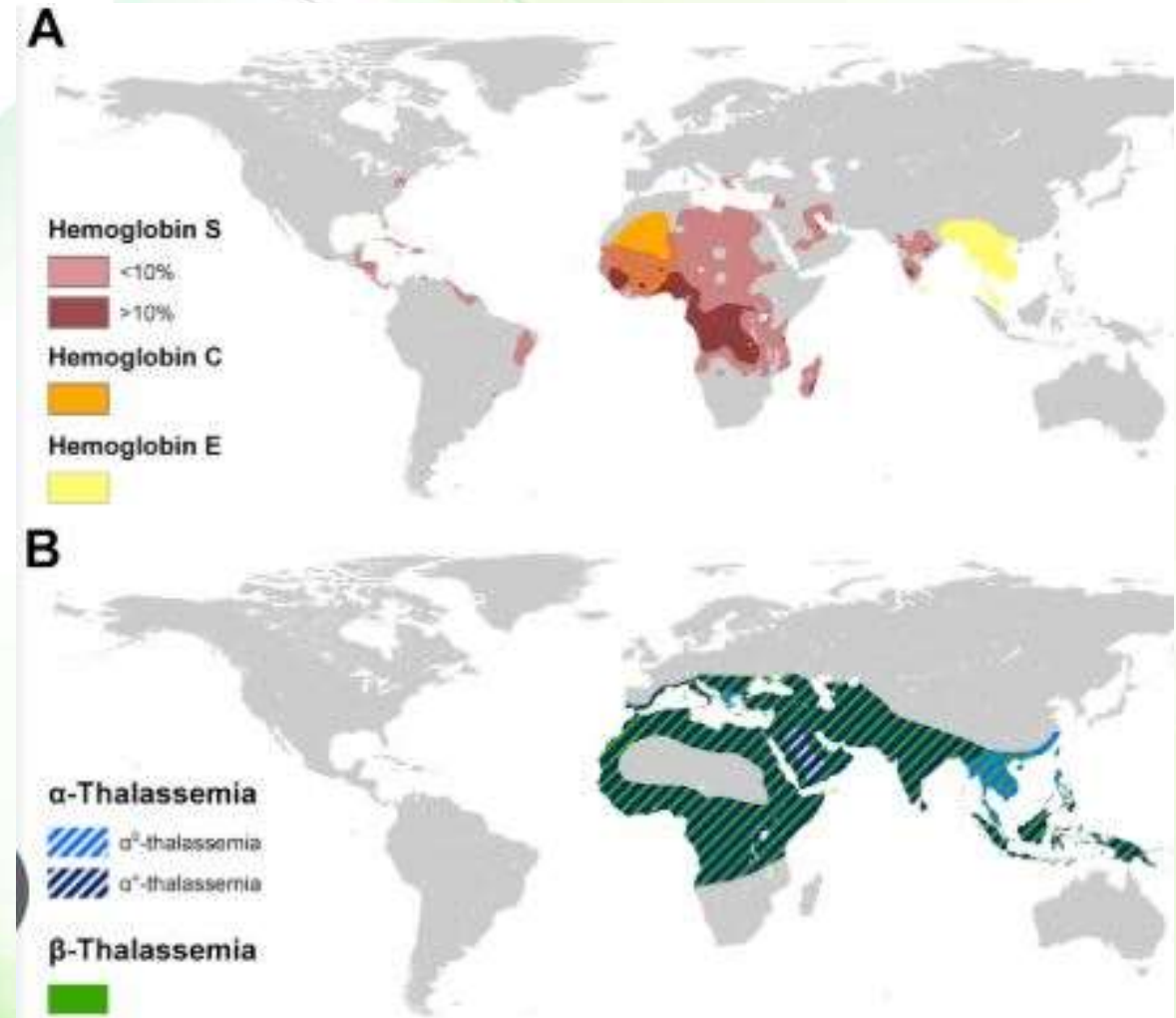


- 1- Hereditary persistence of fetal hemoglobin HPFH
- 2- Quantitative Hemoglobinopathies:
 - 1- Alpha Thalassemia
 - 2- Beta Thalassemia
 - 2- HbE and Constant Spring
- 3- Qualitative Hemoglobinopathies:
 - 1- Sickle cell HbS, HbC, HbSC
 - 2- Already covered (Yakima, Cowtown, Kansas, Hemmersmith,

What are hemoglobinopathies?



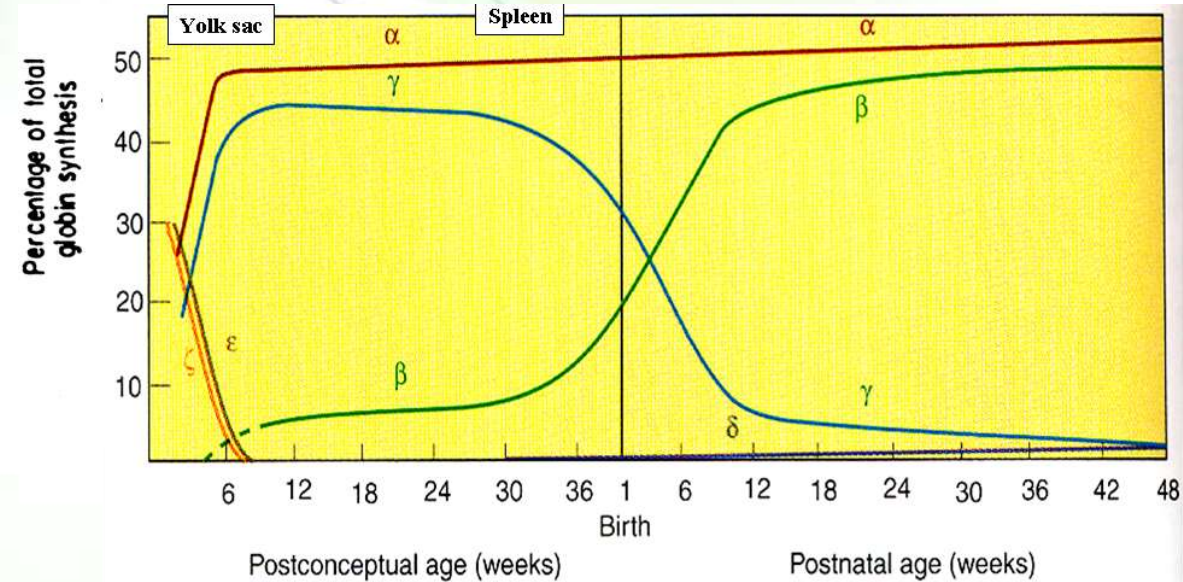
- Hemoglobinopathies: Disorders of human hemoglobin.
- The most common genetic group of genetic diseases in the world (5% of people have mutations whether carriers or have the disease)
- Hemoglobin disorders account for 3.4% of deaths in children < 5 years.



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.

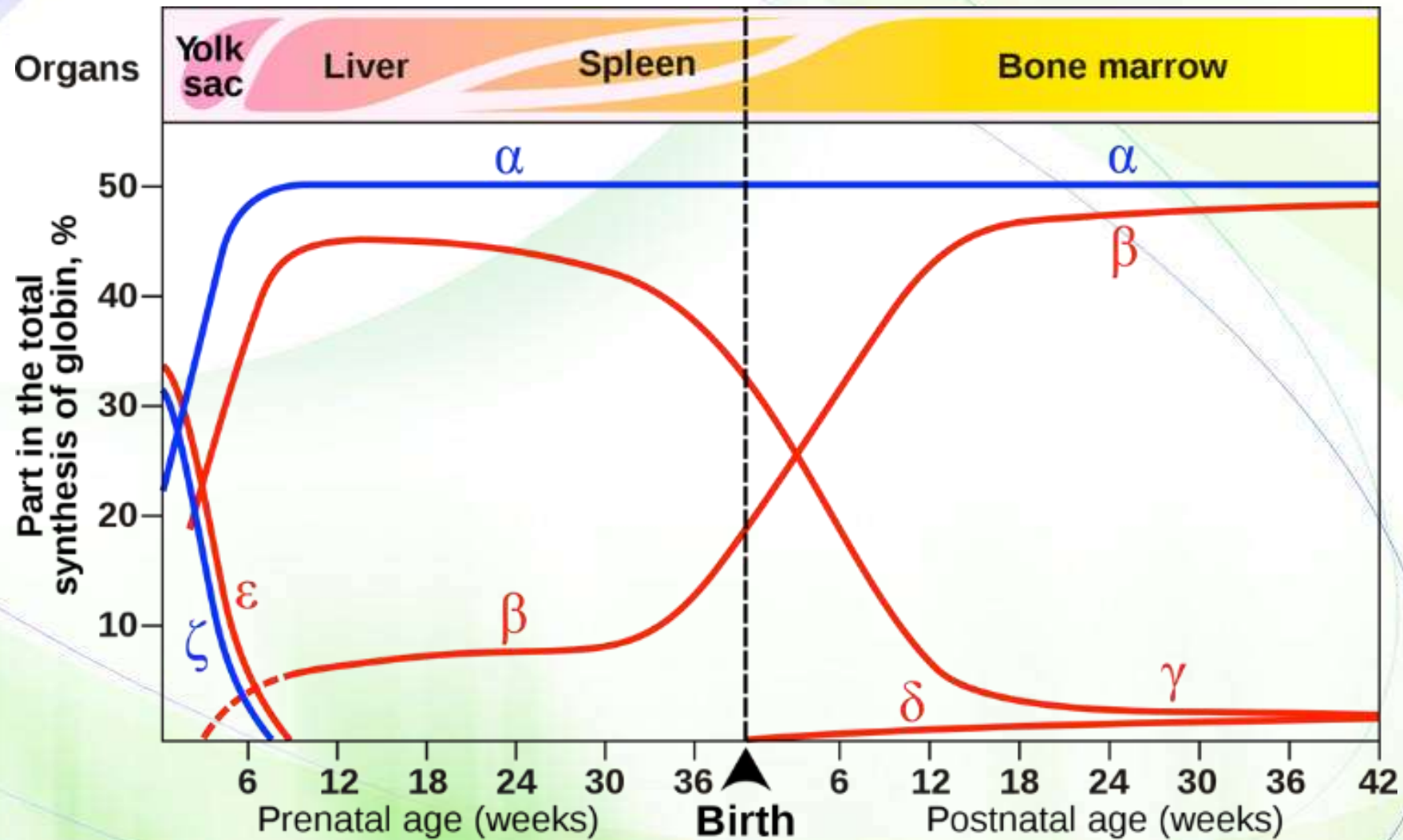


What we are covering in this lecture



- 1- Hereditary persistence of fetal hemoglobin HPFH

The embryonic stage

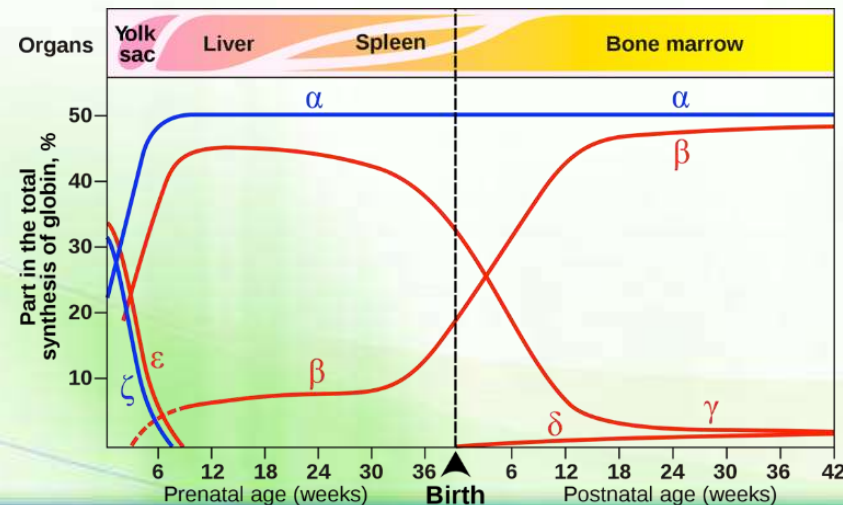


— α -similar globin chains
— β -similar globin chains

The embryonic stage

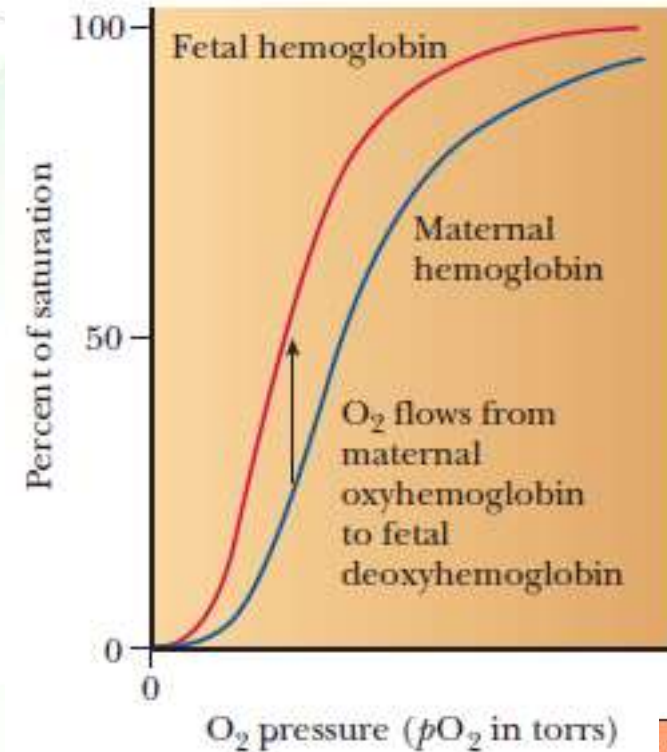
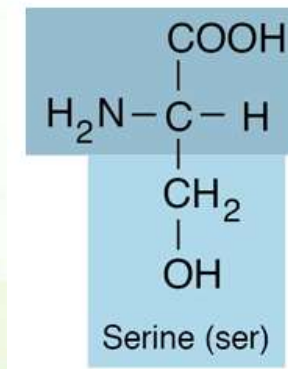
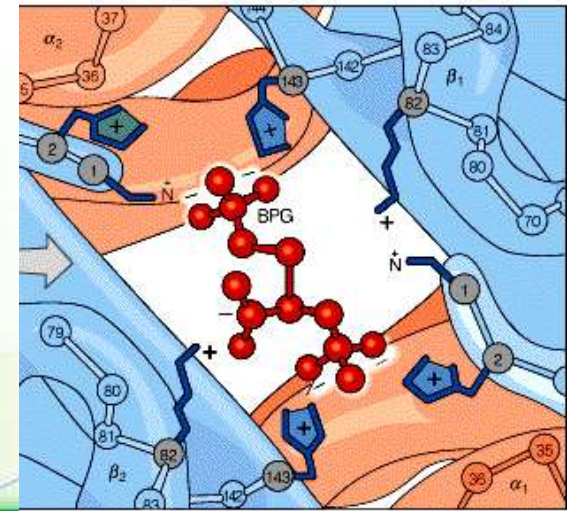


- Hemoglobin synthesis begins in the first few weeks of embryonic development within the yolk sac.
- The major hemoglobin (HbE Gower 1, week 3 – week 9 or 10) embryonic stage is a tetramer composed of 2 zeta (ζ) chains and 2 epsilon (ϵ) chains.
- Fetal hemoglobin (HbF) $\alpha_2\gamma_2$, week 10 to 40.
- Other embryonic hemoglobin forms exist (that show later in the embryonic stage as other globins are expressed): HbE Gower 2 ($\alpha_2\epsilon_2$) because alpha starts developing, HbE Portland 1 because gamma starts developing ($\zeta_2\gamma_2$), HbE Portland 2 because beta starts developing ($\zeta_2\beta_2$).



Fetal hemoglobin

- Fetal Hb (HbF) has higher affinity towards oxygen than adult hemoglobin (HbA).
 - $\text{HbA} = \alpha_2\beta_2$
 - $\text{HbF} = \alpha_2\gamma_2$
- 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.



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



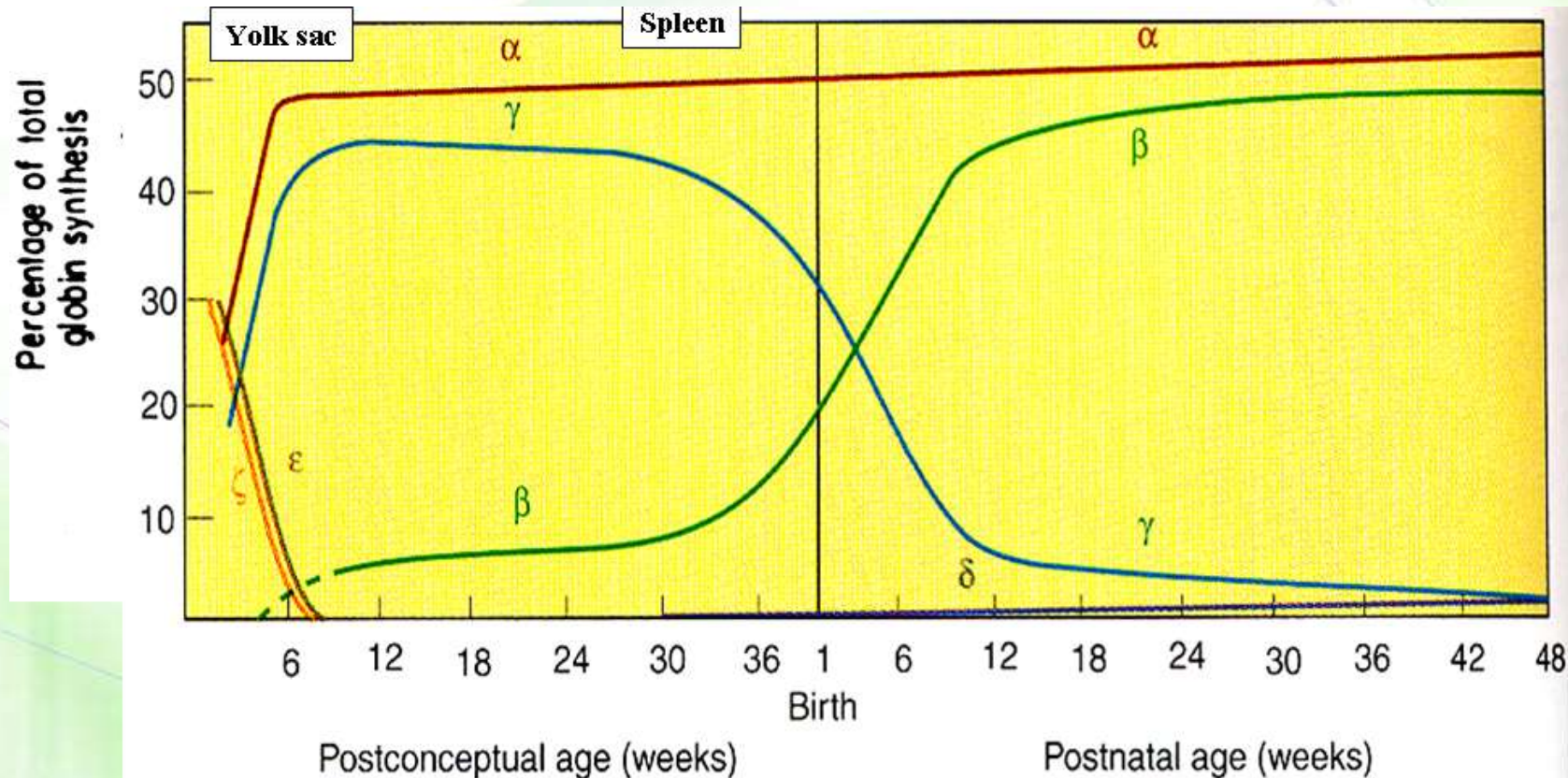
- Thalassemias: the most common human single-gene disorder.
- They are caused by a reduced amount of either the α or β protein, which alters the ratio of the $\alpha:\beta$ ratio.



The Alpha-Thalassemias



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



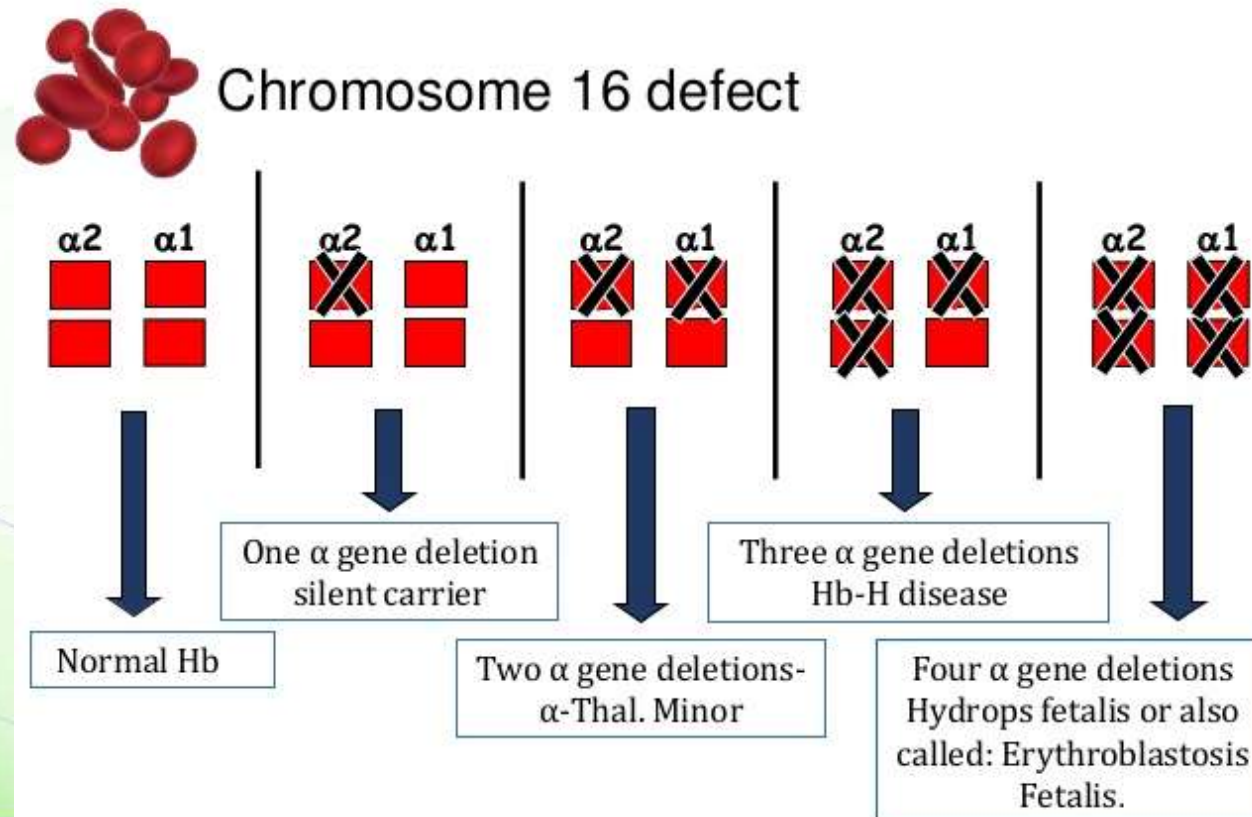


Alpha Thalassemia subtypes from the most severe to the least

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.

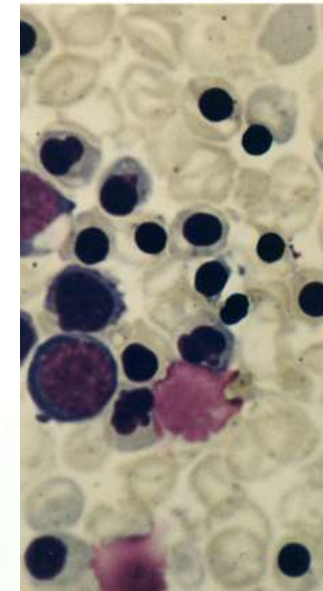


α -thalassemia major

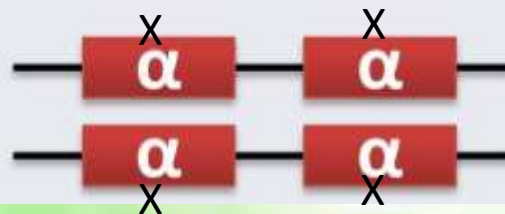


α -thalassemia major, Hydrops fetalis or Hb Bart (most severe)

- 4 of 4 genes are deleted.
- The predominant fetal hemoglobin is a tetramer of γ -chains.
- γ_4 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.



Incompatible with Life
Hydrops Fetalis



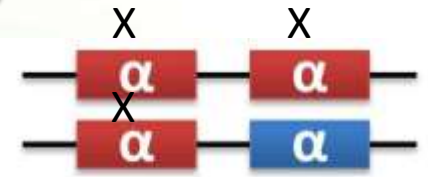
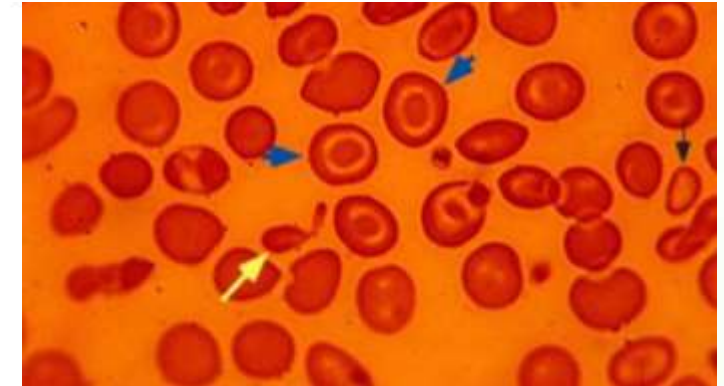
α -thalassemia intermedia



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 (β_4 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.

Hb H Disease: Symptomatic
Hemolytic and Microcytic anemia
Splenomegaly

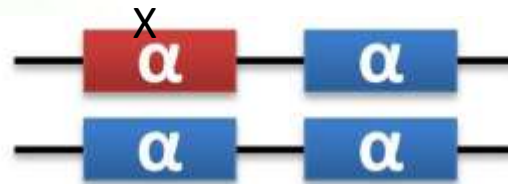


α -thalassemia minor and silent carrier

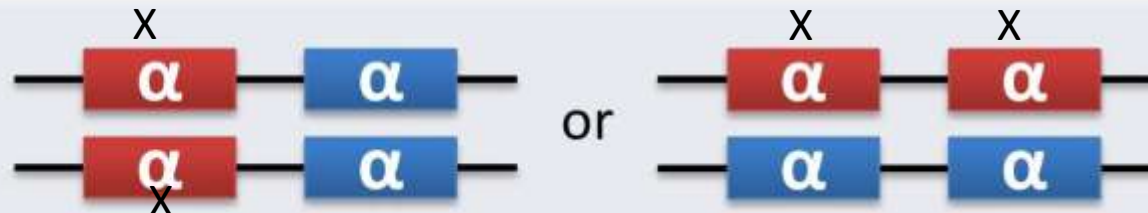


- α -Thalassemia trait: If 2 of the 4 genes are inactivated.
 - The individuals have mild microcytic anemia.
- Silent carrier: 1 of 4 genes deleted.
 - Individuals are completely asymptomatic.

Carrier: Asymptomatic
No abnormalities



α -thal minor: Asymptomatic
Mild microcytic anemia



Summary of α -thalassemias



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

^aNumber 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



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

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 β molecule that causes lower production of β chain.

Note:

a- that in the 2nd row the thalassemia 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 row there is some partial functional β thalassemia produced β^+ , however β^E/β^0 is more on the severe end.

c- homozygous β^E/β^E leads to mild microcytic anemia as it causes the production of β chain to be reduced.

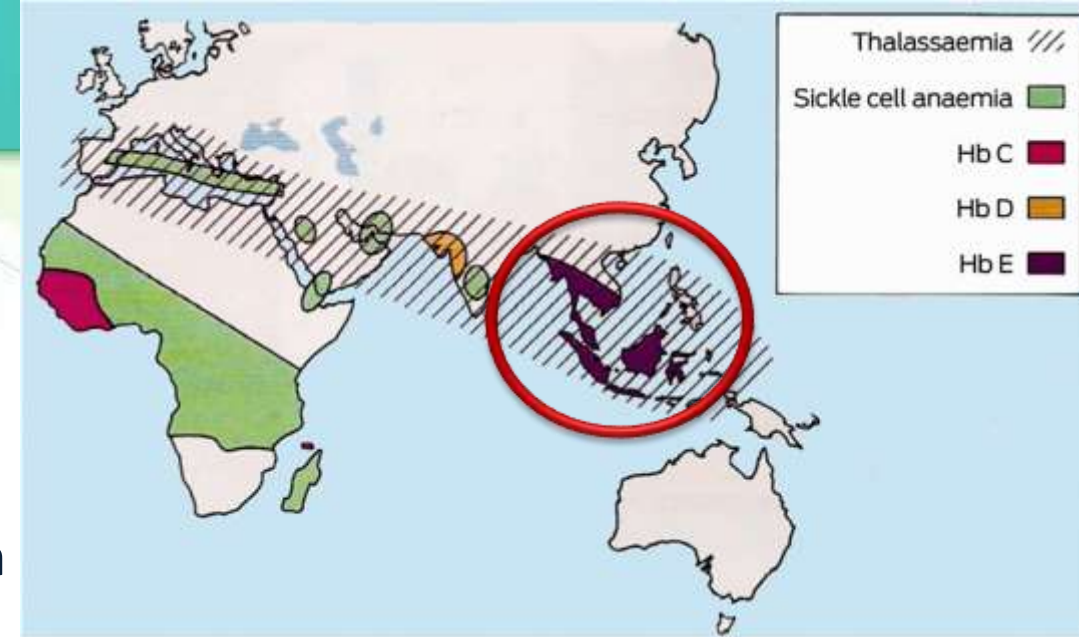
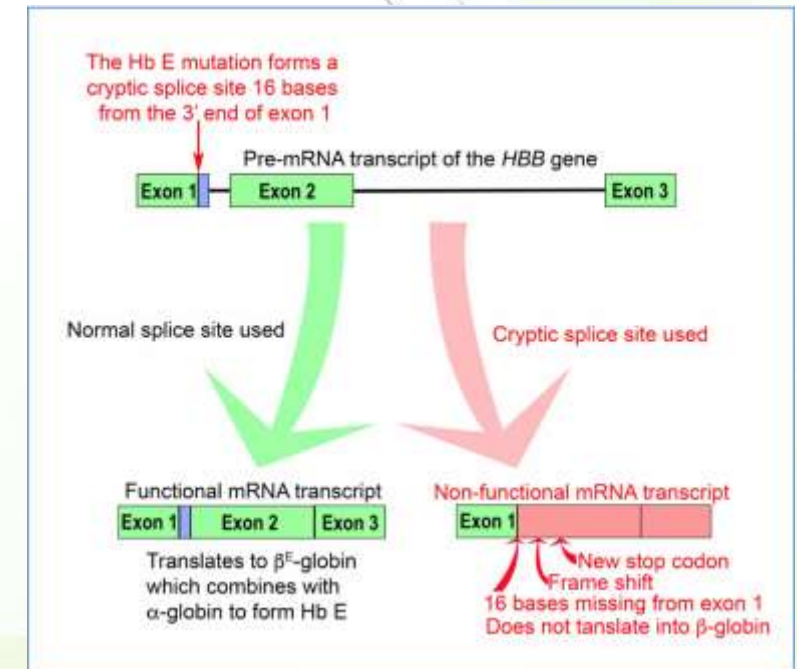
Classification and types of β -thalassemia



Beta thalassemia			Alpha thalassemia		
Common genotypes	Name	Phenotype	Genotype (alpha-globin gene number)	Name	Phenotype
β/β	Normal	None	4 or 3 ($\alpha\alpha / \alpha\alpha$ or $\alpha\alpha / \alpha-$)	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 ($-- / \alpha\alpha$ or $\alpha- / \alpha-$)	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 ($-- / \alpha-$)	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

Hemoglobin E

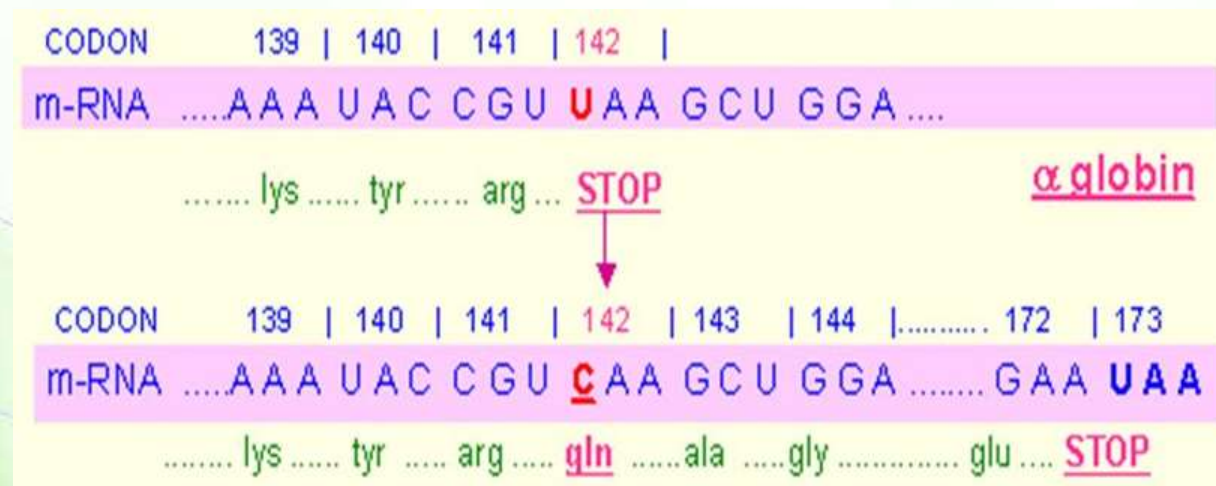
- It is common in Southeast Asia
- It has both quantitative and qualitative characteristics.
- The same mutation can have either one of the following consequences:
 - 1- It can cause alternative RNA splice site and an earlier stop codon which causes lower production of hemoglobin so it is similar somehow that mild β 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.



Hb Constant Spring (Hb CS)



- Hemoglobin Constant Spring (Hb CS) is an abnormal Hb caused by a mutation at the termination codon of the $\alpha 2$ -globin gene leading to the production of longer than normal unstable mRNA and protein products.
 - The anemia is usually moderate.
- Heterozygotes have the genotype ($\alpha\alpha/\alpha\alpha^{CS}$) and have α^0 -thalassemia trait phenotype.
- It is commonly found among Southeast Asian and Chinese people.
- If co-inherited with α -thalassemia, it leads to an α^0 -thalassemia intermedia syndrome.



Classification of molecular mutations

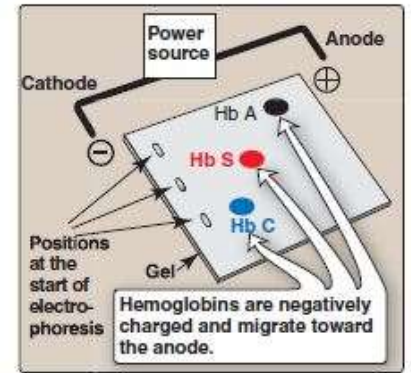
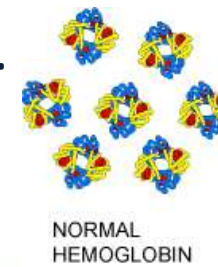
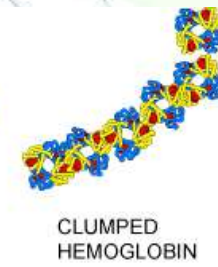
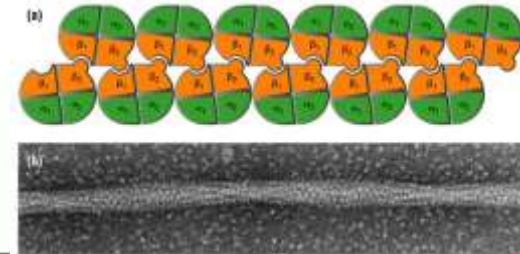
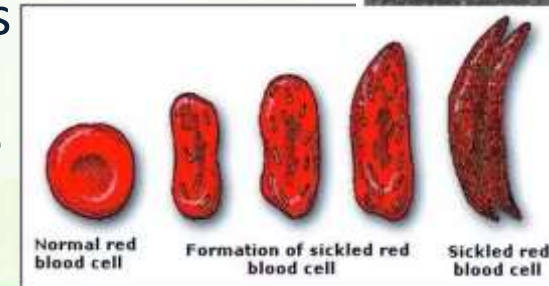


- HbS, HbC, HbSC and

Sickle cell hemoglobin (HbS)



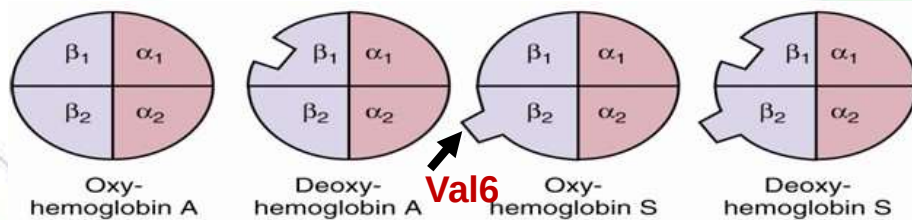
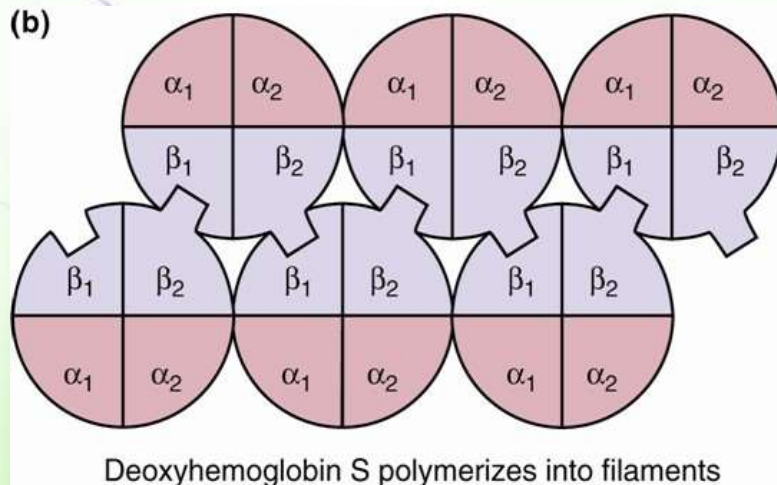
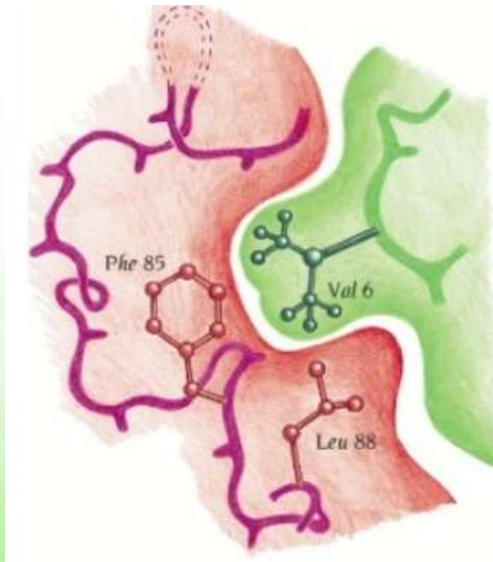
- It is caused by a change of amino acids in the 6th position of β globin (Glu negatively charged to Val hydrophobic). Therefore, during electrophoresis at alkaline pH, Hb S migrates more slowly toward the anode (positive electrode) than does Hb A. The hemoglobin is designated $\alpha_2\beta_s_2$ or HbS.
- The hemoglobin tetramers aggregate into arrays upon deoxygenation in the tissues, and increase their sickle trait upon conditions that increase the deoxygenation 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 changes due to marrow hyperplasia to replace hemolytic anemia, but the anemia supersedes. Bone pain also occurs due to occlusions to the blood vessels there.



How does the fiber form?



- Fiber formation only occurs in the deoxy- or T-state.
- The mutated valine of the β_2 chain is protruded and inserted into a hydrophobic pocket on the surface of β_1 chain.



Variables that increase sickling

- Decreased oxygen pressure (high altitudes)
- Increased $p\text{CO}_2$
- Decreased pH
- Increased 2,3-BPG
- Dehydration (why?)
- Because dehydration increases the concentration of the hemoglobin

Sickle cell hemoglobin (HbS) control

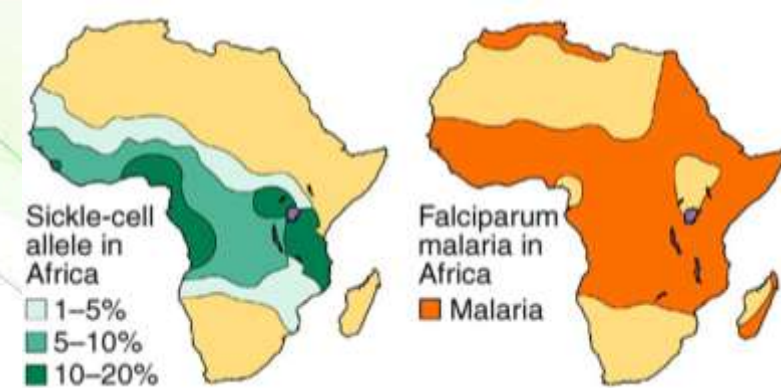


- Therapy involves adequate hydration, analgesics, aggressive antibiotic therapy if infection is present. Intermittent transfusions with packed red cells reduce the risk of stroke, but the benefits must be weighed against the complications of transfusion, which include iron overload (hemosiderosis), bloodborne infections, and immunologic complications.
- Hydroxyurea, an antitumor drug, is therapeutically useful because it increases circulating levels of Hb F, which decreases RBC sickling

Sickle cell trait



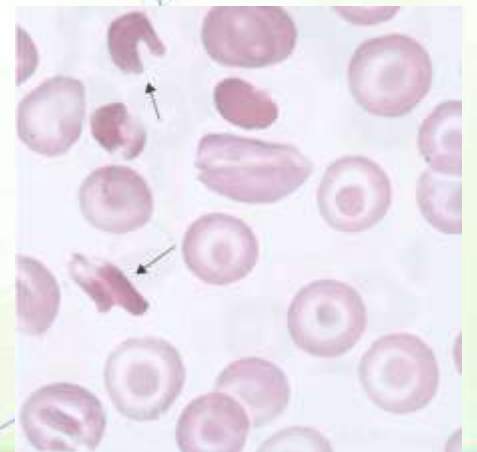
- It occurs in heterozygotes (individuals with both HbA and HbS), who are clinically normal, but they have few cells that sickle when subjected to low oxygen. They have lower potential to sickle than the homozygote trait.
- HBSA has a selective advantage over HBSS as HBSS has already caused some splenic 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.



Hemoglobin C (HbC)



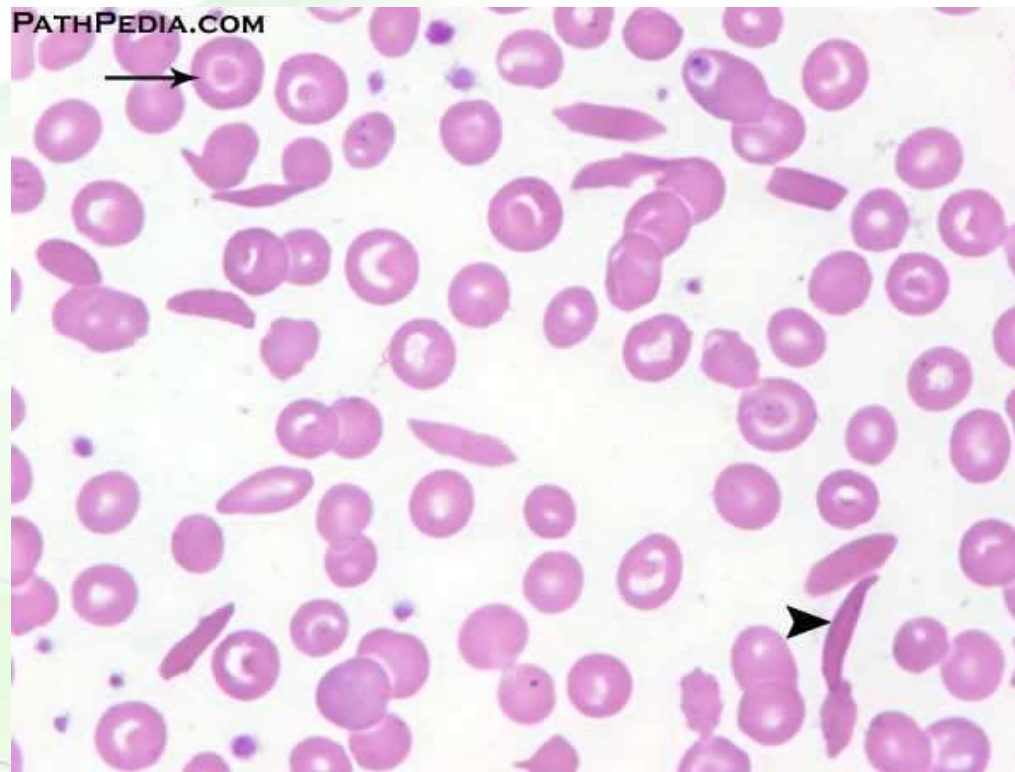
- (HbC) is also due to a change at the 6th position of β globin replacing the glutamate with lysine (designated as β^c).
- This hemoglobin is less soluble than HbA so it crystallizes in RBCs reducing their deformability in capillaries.
- HbC also leads to water loss from cells leading to higher hemoglobin 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.



HbSC disease



- Individuals with both β^c and β^s 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)

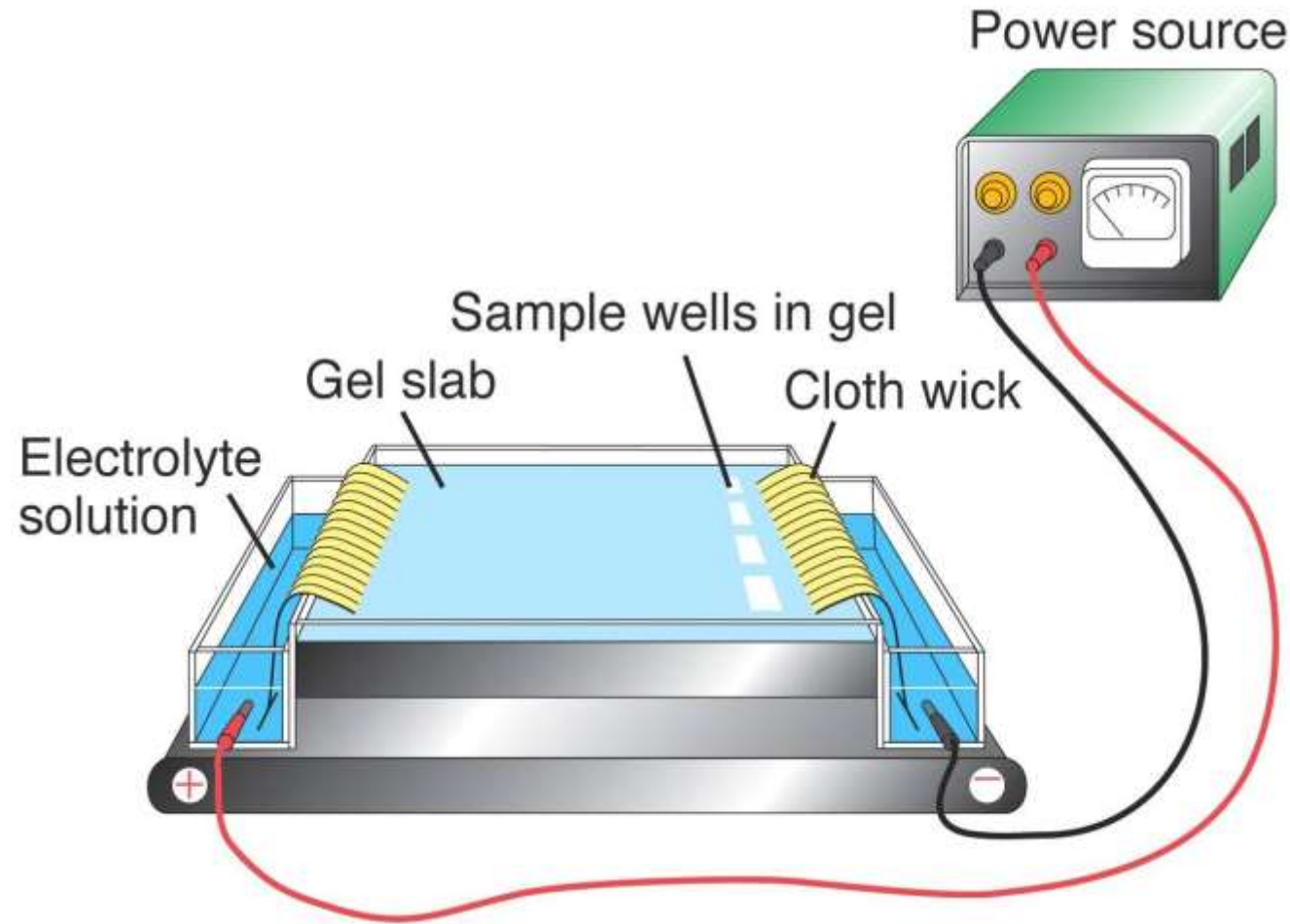


Mutation and migration



- 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



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

