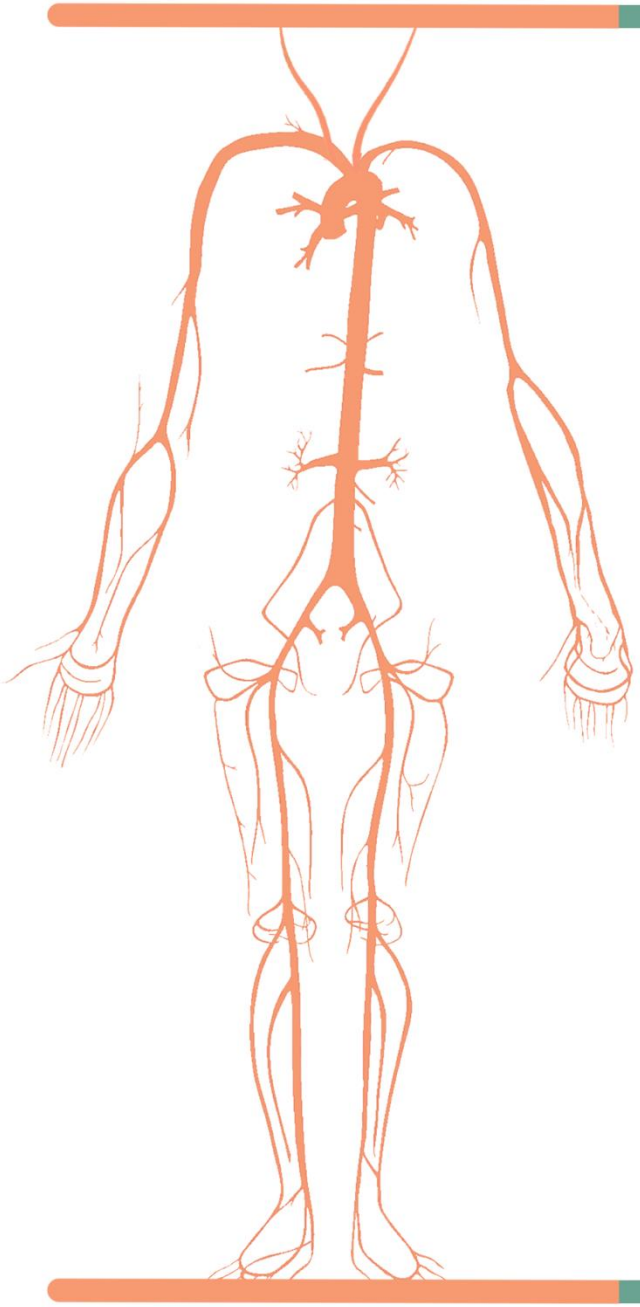
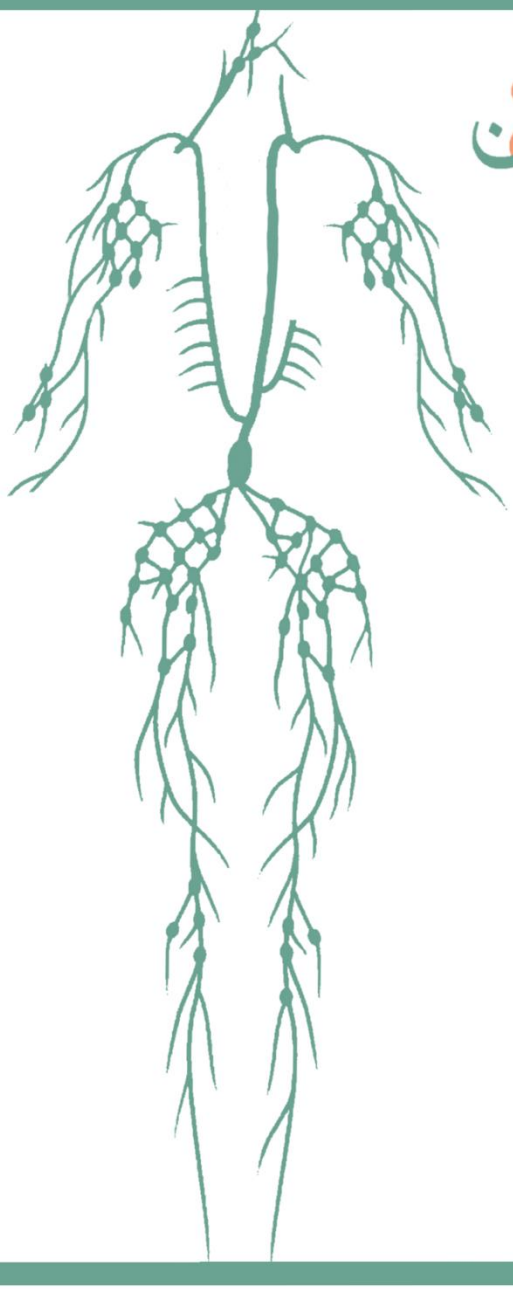
Biochemistry

HematoLymphatic





Title: Sheet 3 – Hemoglobinopathies

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Hemoglobinopathies are defined as disorders of human hemoglobin leading to a deficiency in the oxygen transport system.

- ✓ They're the most common genetic disease group in the world (5% of people are carriers) with substantial morbidity (about 300,000 born each year).
- ✓ They account for 3.4% of deaths in children < 5 years.
- ✓ The Middle East has a significant share of hemoglobinopathies.

Hemoglobinopathies are divided into:

1. Qualitative abnormalities: Genetic disorders caused by production of a structurally abnormal hemoglobin molecule; mutations result in **structural** variants (different Hb molecules), and over 700 variants have been identified.
2. Quantitative abnormalities: Abnormalities in the relative **amounts** of α and β subunits (aka. *thalassemias*).
   [Jordan has a high prevalence of thalassemia.]
3. Hereditary persistence of fetal hemoglobin (HPFH): Impairment of the perinatal switch from γ to β globin.

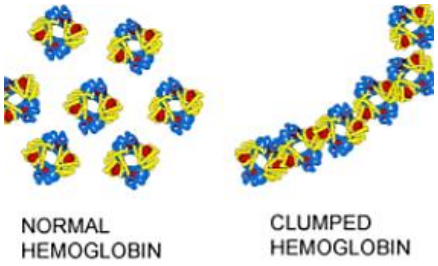
In **qualitative abnormalities,** we're basically talking about point mutations which can take place anywhere in the Hb molecule, and these abnormalities (mutations) are divided into:

1) Mutations in <u>surface</u> residues which are usually asymptomatic (e.g. HbE). An exception is HbS –which is produced in people with sickle cell disease-.
2) Mutations in <u>internal</u> residues in which unstable* hemoglobin is produced, producing *Heinz* bodies and causing hemolytic anemia (e.g. *Hb Hammersmith*).
   * remember that Hb is a globular protein with hydrophilic AA's outside & hydrophobic AA's inside. The Hb will be unstable if this environment is changed
3) Mutations stabilizing **<u>methemoglobin</u>** (in which heme is bound to ferric iron [heme-Fe^{+3}]) decreasing its capacity to bind to oxygen and resulting in cyanosis.
4) Mutations at **α1-β2 contacts** which alter the equilibrium of the T-state and R-state. This affects oxygen affinity (mainly becomes higher; a condition known as polycythemia).

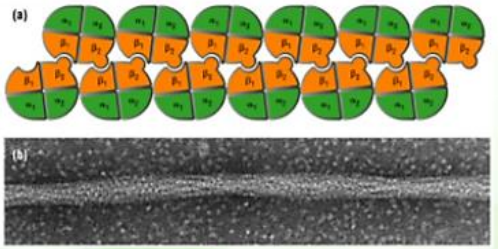
A. Sickle Cell Anemia (Hemoglobin S disease)

Sickle cell anemia, the most common of the RBC sickling diseases, is a genetic disorder of the blood caused by a single nucleotide substitution (a point mutation) in the gene for β-globin, resulting in a change of the amino acid in the 6th position of β globin (Glu "-" to Val "nonpolar"). The mutant β-globin chain is designated as βs, and the resulting hemoglobin, α2βs2, is referred to as HbS.

- At low oxygen tension, HbS tetramers **aggregate (clump/polymerize)** into arrays upon **deoxygenation** in tissues.
  This **fibrous aggregation (clumping)** leads to **deformation** of the red blood cell. It can also cause **hemolytic anemia** (destruction of RBCs) in which the half-life of RBCs is reduced from 120 days to <20 days.

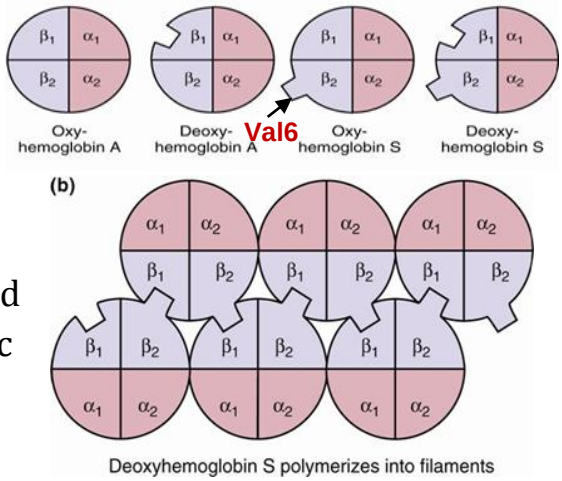


- Repeated cycles of oxygenation and deoxygenation lead to **irreversible sickling** (irreversible misshapen RBCs). Such sickled cells (crescent-shaped cells) cannot squeeze through capillaries in single file and therefore block blood flow causing local hypoxia.
- Long-term recurrent clogging of the capillary beds leads to damage to internal organs, in particular the **kidneys**, **heart** and **lungs**.



How does the fiber form?

Fiber formation (aggregation) only occurs in the deoxy or T-state. There are two things that make this possible. First, in any deoxygenated hemoglobin molecule (whether normal or mutated), a region of the protein creates a hydrophobic pocket. Secondly, in HbS, the mutated valine (Val6) of the β-2 chain forms a hydrophobic protrusion on the surface.



Variables that increase sickling

The extent of sickling (the severity of disease) is **enhanced** by any variable that **increases** the proportion of HbS in the **deoxy state** (that is, **reduces** the affinity of HbS for O_2 or stabilizes the **T-state**). These variables include (1) decreased oxygen pressure [pO_2] (as in high altitudes), (2) increased pCO_2, (3) decreased pH, (4) dehydration, and (5) an increased concentration of 2,3-BPG in RBC.

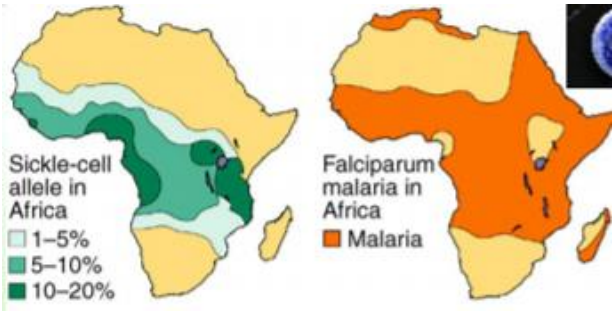
Sickle cell trait

Heterozygotes have one normal and one sickle cell gene. The blood cells of such heterozygotes contain both HbS and HbA. These individuals are said to have sickle cell trait. They usually do not show clinical symptoms, but their cells sickle when subjected to low oxygen.

The high frequency of the mutation among Africans suggests that a selective advantage exists for **heterozygous** individuals. For example, **heterozygotes** for the sickle cell gene are **less susceptible** to the severe **malaria** caused by the parasite **Plasmodium falciparum.** This organism spends a part of its life cycle in the RBC, and since these cells in individuals **heterozygous** for HbS -like those in homozygotes- have a **shorter life span** than normal, the parasite cannot complete that specific intracellular stage of its life cycle. This fact may provide a selective **advantage** to **heterozygotes** living in regions where malaria is a major cause of death.

In Africa, the geographic distribution of **sickle cell anemia** is similar to that of **malaria**.

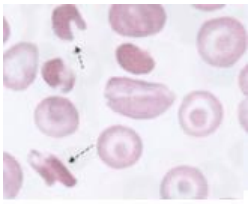
[I.e. heterozygotes become protected against malaria and don't die]



B. Hemoglobin C (HbC) disease

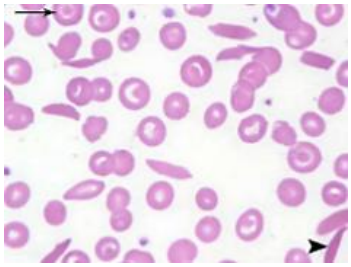
Like HbS, HbC is a hemoglobin variant that has a single amino acid substitution in the **sixth position** of the β-globin chain. In HbC, however, a **lysine** "+" (lysine ends with "C-ine") substituted for the glutamate "- ".

This hemoglobin (HbC) is less soluble than HbA, so it **crystallizes** in RBCs, **reducing** their deformability in capillaries (i.e. reducing their ability to squeeze through). It also leads to **water loss** from cells leading to **higher** hemoglobin concentration. This problem causes only a **minor hemolytic** disorder.



C. Hemoglobin SC (HbSC) disease

In HbSC disease, the individual has both of their β-globin alleles mutated: one has the sickle cell βS mutation whereas the other carries the βC mutation (both of their β-globin genes are mutated differently). It's a mild hemolytic disorder which may have no clinical consequences, but it is clinically **variable**.

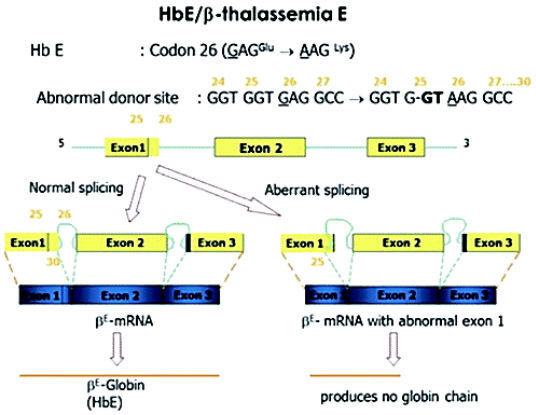


D. Hemoglobin E (HbE disease)

It is a mild, inherited blood disorder characterized by an abnormal form of hemoglobin, called hemoglobin E. It's common in Southeast Asia.

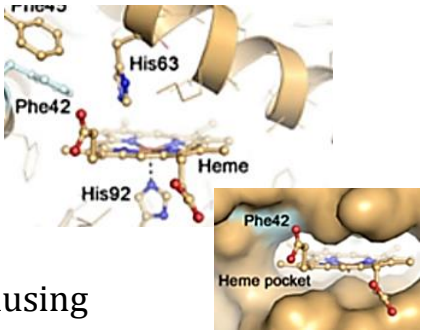
The disorder has both quantitative and qualitative characteristics. It is caused by a point mutation in codon **<u>26</u>** that changes **glutamic acid** (GAG) to **lysine** (AAG).

This mutation affects β-gene expression by creating an alternative **splicing site** in the mRNA of the β-globin gene. Through this, there is production of a defective protein (HbE) that's not stable and tends to be degraded. Individuals with this mutation make only around 60% of the **normal amount** of β- globin protein, and therefore have deficient O_2 carrying capacity.



E. Hb Hammersmith

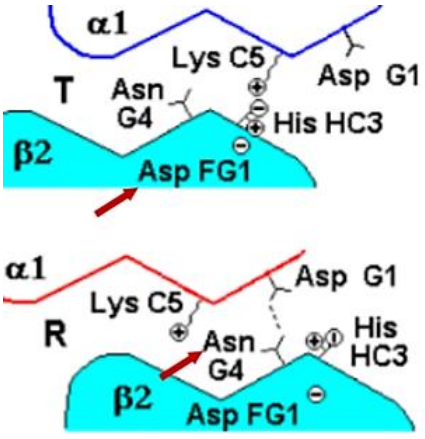
Hb Hammersmith results from a point mutation that leads to formation of unstable hemoglobin and denaturation of the globin protein.



The most common point mutation of Hb Hammersmith substitutes an **internal phenylalanine** (hydrophobic) with a **serine** (hydrophilic) within the **β globin**, **reducing** the hydrophobicity of the heme-binding pocket, **heme positioning,** and oxygen binding affinity causing **cyanosis** (low oxygen levels in RBCs).

F. Mutations that alter the equilibrium of the T-state and R-state

1. Hb Cowtown: Is a mutant with **<u>increased</u> oxygen affinity**. It results from the substitution of **His146** (responsible for the Bohr Effect) to **Leucine** which, in turn, produces more hemoglobin in the **R state.**



2. Elimination of **hydrogen bonds** between the chains can also alter the quaternary structure and thus the T/R equilibrium.

I) Hb Kansas: stabilization of the **T state** (Asn to Thr).
II) Hb Yakima: stabilization of the **R state** (Asp to His).

G. Methemoglobinemias

Normal hemoglobin can undergo **reversible oxygenation** because its heme **iron** is in the **reduced** (ferrous,) state *(i.e. it can **carry** oxygen to the cells because its iron is in the ferrous (Fe^{+2}) state)*.

During oxygen release from heme, Fe^{+2} is **oxidized** to Fe^{+3}, forming **methemoglobin** (**HbM**) *(HbM **cannot** carry oxygen to the cells because its iron is in the ferric (Fe^{+3}) state)*. Therefore, in human blood, a trace amount of methemoglobin is normally produced spontaneously, but when present in excess, a condition known as methemoglobenemia develops.
Spontaneously formed methemoglobin is normally **reduced** (regenerating normal hemoglobin) by two ways. First, there are protective reductase systems that convert HbM to HbA. Secondly, the hydrophobic pocket surrounding the heme molecule decreases the probability of oxidizing heme iron from Fe^{2+} to Fe^{3+} due to electron rearrangement between amino acid residues. Disruptions with these enzyme systems lead to methemoglobinemia.

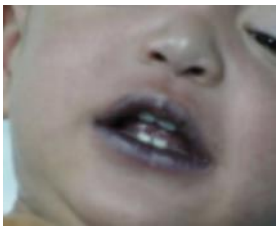
Causes of elevated methemoglobin (methemoglobinemia):

1. Methemoglobinemia may result from inherited defects. For example, certain mutant α- or β-globin chain bond heme in such a way as to resist the reductase.

- Hb Boston: The **distal** histidine is mutated into a **tyrosine** resulting in oxidation of the ferrous iron by **tyrosine's oxygen**. It also attracts H_2O into the pocket.
- HbM Iwate: The **proximal** histidine is replaced by a **tyrosine**.

1. A deficiency of the reductase enzyme (which converts methemoglobin to hemoglobin).
2. The action of certain drugs or drinking water containing nitrates (nitrates increase the probability that iron is oxidized).

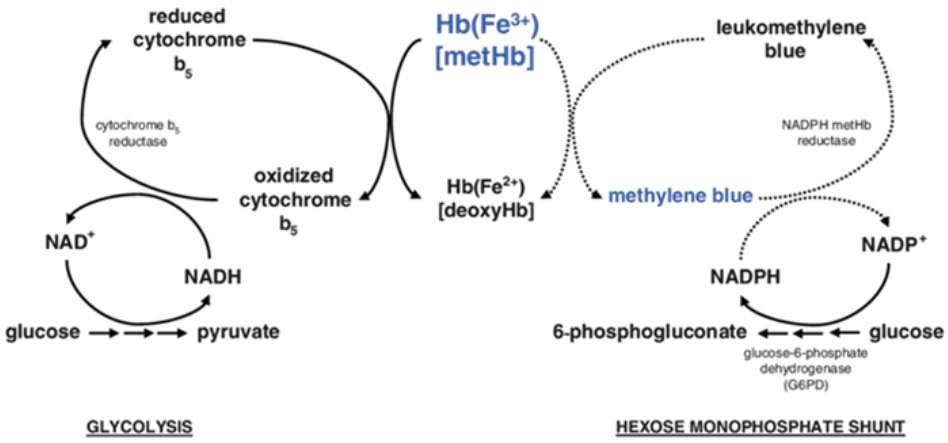
Methemoglobinemias are characterized by "chocolate cyanosis" (a brownish blue coloration of the skin and mucous membranes and brown-colored blood) caused by the dark-colored HbM.



The major enzyme for methemoglobin reduction is **cytochrome b5 reductase (NADH-methemoglobin reductase).** It uses **cytochrome b5** as an electron acceptor and reduces it by giving it hydrogen atoms from NADH. Then, the reduced **Cyt $_{b5}$** is used to convert methemoglobin to hemoglobin.

However, there's an **alternative** enzyme called **NADPH-methemoglobin reductase**, which requires an exogenous electron acceptor (like **methylene blue**) and reduces it using NADPH. This reaction produces a compound called **leukomethylene blue**, which is then oxidized to reduce methemoglobin to hemoglobin.

That's why **methylene blue** can be used to treat the disease.



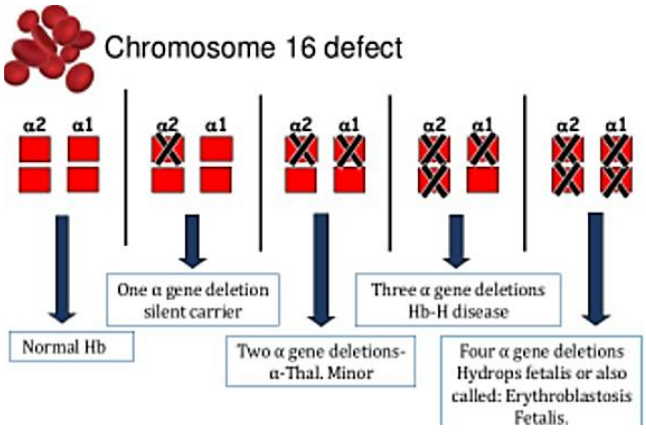
Quantitative Abnormalities (Thalassemias)

The thalassemias are the most common single gene disorders in humans. They are caused by a **reduced** amount of either the α or β globin which alters the α:β ratio.

[Normally, synthesis of the α- and β-globin chains is coordinated, so that each α-globin chain has a β-globin chain partner. This leads to the formation of α2β2 (HbA). In thalassemias, the synthesis of either the α- or the β-globin chain is defective.]

I) The Alpha-Thalassemias

In alpha thalassemia, there's underproduction of the α-globin chains. As a result, HbA (α2β2), HbF (α2γ2), and HbA2 (α2δ2) are all affected.



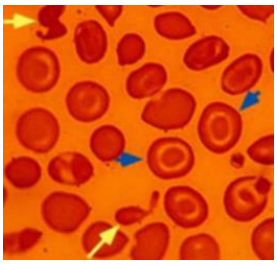
It's typically a result of deletion mutations (rarely point mutations), and because each individual's genome contains four copies of the α-globin gene (two on each chromosome 16), there are several levels of α-globin chain deficiencies.

Levels of α-globin chain deficiencies

1. If one of the four genes is defective, the individual is termed **a silent carrier**. The individuals are **completely** asymptomatic

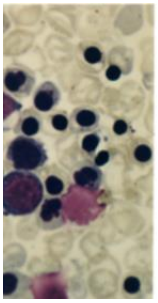
2. If two α-globin genes are inactivated, the individual is designated as having α-thalassemia trait, and the condition is called **alpha-thalassemia minor.** The individuals are **generally** asymptomatic.

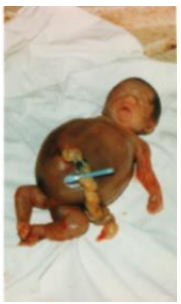
3. If three α-globin genes are defective, the individual has mild to moderate hemolytic anemia in adulthood and a high level of β- chains homotetramer (called **Hemoglobin H** or β4) is present. The HbH tetramers have a markedly reduced oxygen carrying capacity.



Clinically, it is known as **hemoglobin H disease** or **alpha-thalassemia intermedia**. The disease is not fatal, but it is symptomatic.

4. If all four α-globin genes are defective, the predominant fetal hemoglobin is a homotetramer of γ-chains (called **Hb Bart** or γ4).





It's clinically called **alpha-thalassemia major.** Hb Bart has **no** oxygen carrying capacity resulting in oxygen starvation in the fetal tissues; a situation called **hydrops fetalis.** Stillbirth or death shortly after birth occurs.

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

I) The Beta-Thalassemias

In these disorders, β-globins are deficient and the α-globins are in excess and will form α-globin homotetramers. They typically result from **point mutations** within the promoter, translation initiation codon, splicing positions, or polyadenylation termination signal.

The α-globin homotetramers are extremely **insoluble**, which leads to premature red cell destruction in the **bone marrow** and **spleen**.

There are only two copies of the β-globin gene in each cell (one on each chromosome 11). Therefore, individuals with β-globin gene defects have either one or two defective β-globin genes.

Accordingly, beta thalassemia can be classified into:

1) **β -Thalassemia minor**, where individuals are heterozygous for β-thalassemia (i.e. they carry one normal β-globin gene and a mutated gene). These people are **generally** asymptomatic.

2) **β-Thalassemia major**, where both genes are defective and there's a complete lack of HbA (**<u>no</u> β globin** chains are produced). It's denoted as **βo-thalassemia.** Individuals suffer from severe anemia beginning in the first year of life and need blood transfusions.
   Because the β-globin gene is not expressed until late in fetal gestation, infants born with β-thalassemia major are seemingly healthy **at birth** but become severely anemic, usually during the first year of life.
   These patients require **regular transfusions of blood.** 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.

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 : HbE

Hereditary Persistence of Fetal Hemoglobin (HPFH)

The idea is that people with HPFH continue to make HbF as adults. This is due to the fact, that many of these individuals harbor large **deletions** of the **δ-** and **β-**coding region of the cluster. On the other hand, they don't have deletions of the fetal globin genes.

Because the syndrome is **benign**, most individuals do not even know they carry a hemoglobin abnormality. [That's why there's an idea of **switching on** the fetal globin gene in individuals with beta thalassemia to treat their condition.]

*[We mentioned before that HbF has a stronger oxygen affinity than adult hemoglobin, however, this **doesn't** give an advantage to HPFH individuals].*

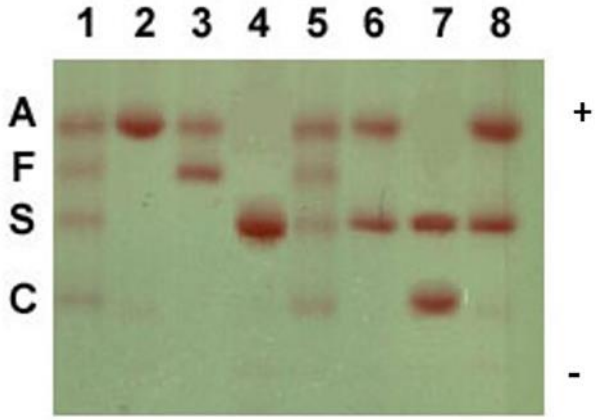
Hemoglobin Electrophoresis

Electrophoresis of hemoglobin proteins from individuals is an effective diagnostic tool in determining if an individual has defective hemoglobin and the relative ratios of the patient's hemoglobin pattern.

Identification and separation of different hemoglobins on the gel is dependent on the **charge** of the hemoglobin molecule. A **change** in the **amino acid composition** of the globin chains results in alteration of the overall **charge** of the hemoglobin molecule, thus resulting in a change in the **speed of migration** in a voltage gradient. Therefore, in gel electrophoresis, different hemoglobins (each representing a different disorder) migrate at different speeds, depending on their charges.

- 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. Therefore, HbS moves faster toward the cathode $^{(-)}$ than HbA does.
- In **homozygous** individuals, the HbA tetramer electrophoreses as a single band (normal individual), and the HbS tetramer as another single band (HbS homozygous). On the other hand, hemoglobin from a **heterozygous** individual (with both alleles) appears as **two** bands.
- Since HbC contains a **lysine** $^{(+)}$ instead of the normal glutamate, HbC travels even faster to the **cathode** $^{(-)}$.
- Fetal Hb as a whole molecule is more positively charged than adult Hb.
- A standard Hb sample is a sample that contains all forms of hemoglobin.

- Lanes 1 and 5 are hemoglobin standards
- Lane 2 is a normal adult
- Lane 3 is a normal neonate
- Lane 4 is a homozygous HbS individual
- Lanes 6 and 8 are heterozygous sickle individuals
- Lane 7 is a SC disease individual



Good Luck