HEMATO
LYMPHATIC
SYSTEM

BIOCHEMISTRY

3

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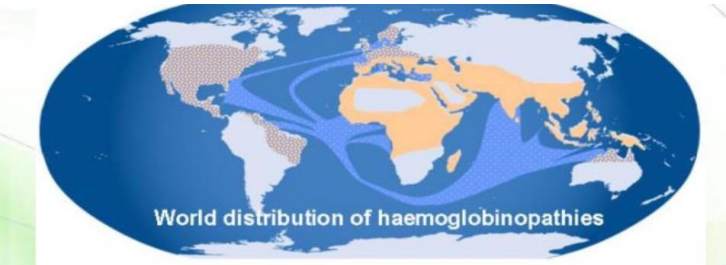
Hey, we are pretty sure that you'll enjoy this easy long lecture, have fun ❤☺

Hemoglobinopathies

Hemoglobinopathies are basically disorders of human hemoglobin so we have deficiency in oxygen transport system, these are really serious diseases for 2 reasons:

- They are the most common genetic disease in the world {5% of people are carriers} with morbidity 300,000 each year.
- They account for 3.4% deaths in children younger than 5.

Notice the map below, the distribution of Hemoglobinopathies around the world and it is concentrated in the old world (the Middle East has significant share including Jordan) so it's a quite serious problem.



This group of diseases is classified into:

1. **Qualitative abnormalities:** mutations resulting in structural variants (every single AA in hemoglobin has a chance to be mutated and as a result it will form abnormal hemoglobin), over 800 variants have been identified.

2. **Quantitative abnormalities:** when the molecule is fine in structure but there are less quantities in alpha & beta chains like (Thalassemia), Jordan has a high prevalence of thalassemia.

3. **Hereditary persistence of fetal hemoglobin (HPFH):** impairment of the perinatal switch from gamma to beta globin

Now let's talk about each one in further details

Qualitative abnormalities; we are basically talking about point mutations which can take place anywhere in the Hb molecule, and these abnormalities (mutations) are divided into:

1. Mutations in surface residues: Usually asymptomatic (e.g. HbE), an exception is HbS which is produced in people with sickle cell disease.

2. Mutations in internal residues: Often producing unstable hemoglobin, Heinz bodies and causing hemolytic anemia (e.g. Hb Hammersmith), note: Hb is a globular protein with hydrophilic AA's outside & hydrophobic AA's inside so the Hb will be unstable if this environment is changed.

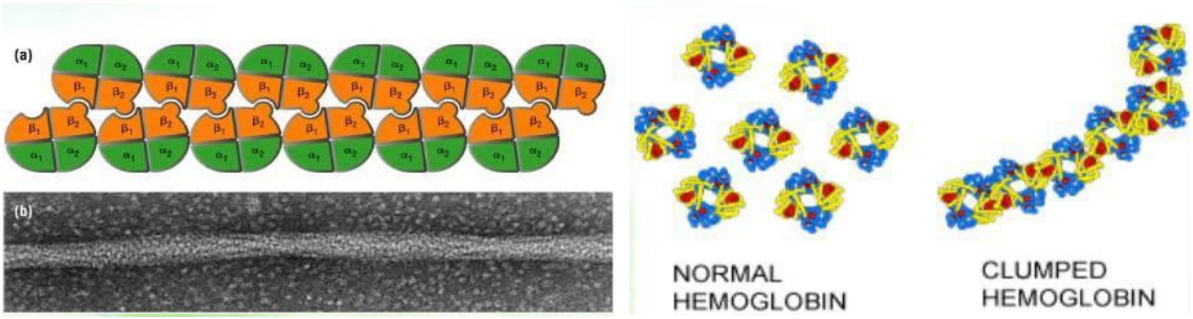
3. Mutations stabilizing methemoglobin: In which heme is bound to ferric iron [hemeFe +3] decreasing its capacity to bind oxygen and resulting in cyanosis, cyanosis is (a bluish-purple hue to the skin).

4. Mutations at α1-β2 contacts: Which alter the equilibrium of the T-state and R-state so this affects oxygen affinity (mainly becomes higher, a condition known as polycythemia).

Let's dig deep in each disease, ready?

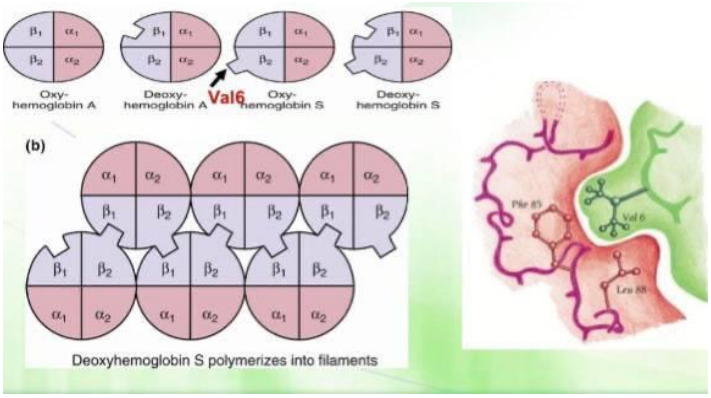
1-Sickle cell hemoglobin (HbS): the most common of the RBC sickling diseases, is a genetic disorder of the blood caused by a single nucleotide substitution (a point mutation) in the gene for β-globin, resulting in a change of the amino acid in the 6th position (Glu which is a negative AA to Val "nonpolar").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

So what happens after that? Instead of getting Hb protein as individual protein we will have Hb as aggregates! 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 day. **Note:** Fiber formation (aggregation) only occurs at low O2 tension / T state.



Repeated cycles of oxygenation and deoxygenation lead to irreversible sickling so cells cannot squeeze though capillaries in a 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.



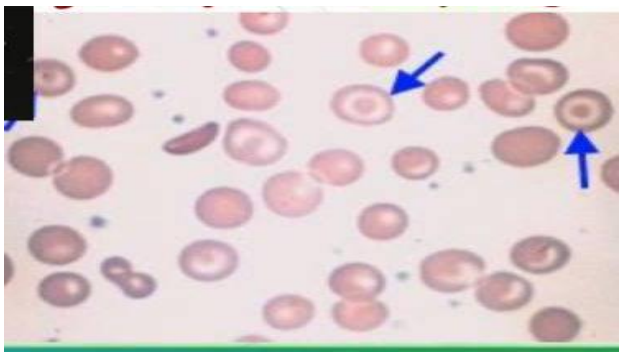
ببساطة : انا كحبة هيموغلوبين سواء نورمال ولا مريضة بالأنيميا المنجلية لما اكون بدون اوكسجين بكون عندي زي الحفرة الصغيرة بشكلي طيب ؟ بيجي عندي هاد المرض بعمل بالبروتين تاعي زي الفتق الصغير بسبب تغير الحمض الاميني لفالين وبصير الفتق يقعد بالحفرة وبعملوا كلمبنغ هيموغلوبين

Variables that increase sickling: • Decreased oxygen pressure (high altitudes)

• Increased pCO2 • Decreased pH • Increased 2, 3-BPG • Dehydration

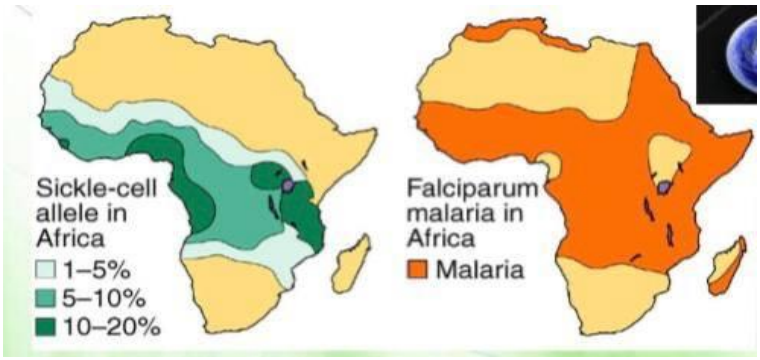
Note: these variables increases the proportion of HbS in the deoxy state (that is, reduces the affinity of HbS for O2 or stabilizes the T-state) so increases the extent of sickling.

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. Advantage: selective advantage from plasmodium falciparum that causes malaria, this organism spends a part of its life cycle in the RBC, and since these cells in individuals heterozygous for HbS -like those

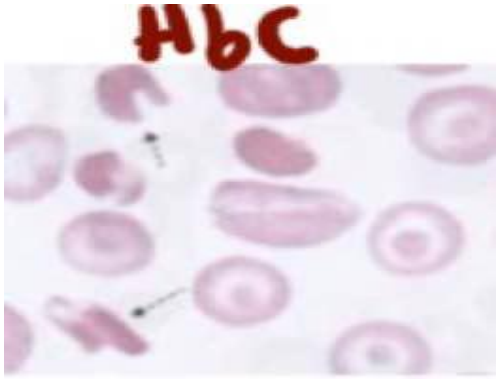
in homozygotes- have a shorter life span than normal, the parasite cannot complete that specific intracellular stage of its life cycle.

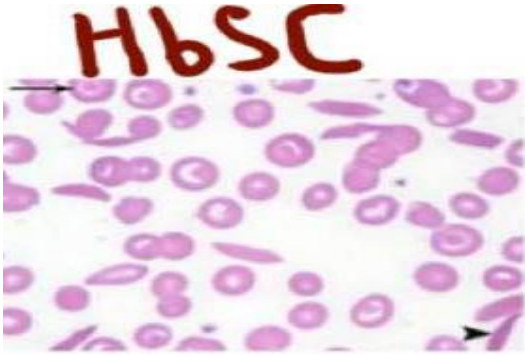


In Africa, the geographic distribution of sickle cell anemia is similar to that of malaria.

2-HbC: 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 beta globin (from Glu to lysine) 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.

But if it is combined with HbS gene (HbSC) which will cause mild not minor hemolytic anemia: 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.





A quick revision: homozygotes sickle cell = major hemolytic disorder. Heterozygotes = mild hemolytic disorder. HbSC = mild hemolytic disorder. HbC = minor hemolytic disorder.

3-HbE: this problem is common in Southeast Asia so if someone have a housemaid coming from there, there will be a good chance that she has HbE.

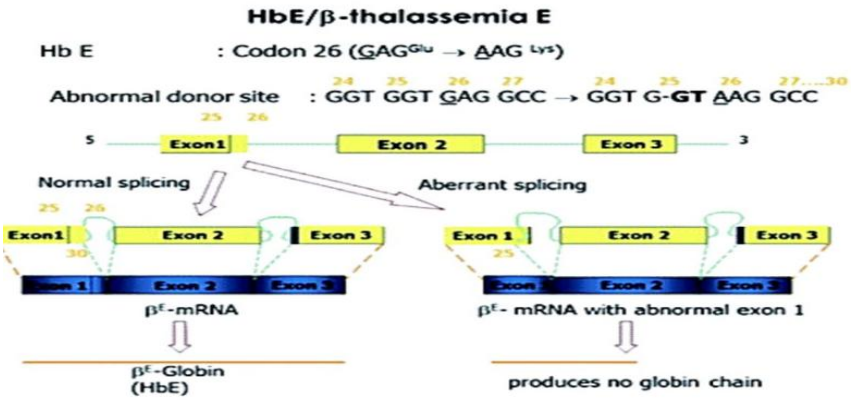
It is caused by a point mutation in codon 26 that changes glutamic acid (GAG) to lysine (AAG) creating an alternative RNA splice site and a defective protein.

Individuals with this mutation make only around 60% of the normal amount of β-globin protein.

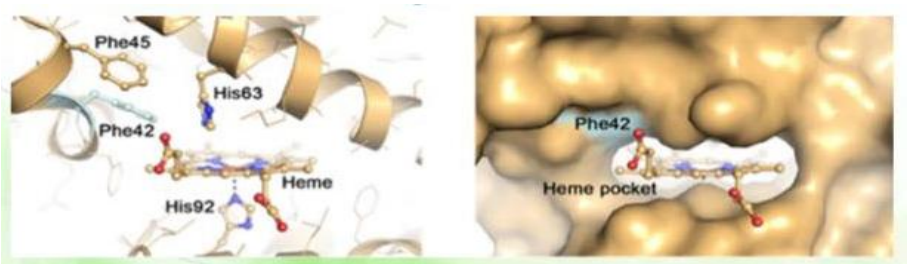
It has both quantitative and qualitative characteristics. Howwww??

Amino acid substitution caused a genetic change (structural) which will create alternative RNA splicing site → defective protein (qualitative)

This protein once formed it's not as stable as the normal protein leading it to be degraded → less amount of protein (quantitative)



4-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 beta globin, reducing the hydrophobicity of the heme-binding pocket, heme positioning, and oxygen binding affinity causing cyanosis. **More explanation :** because the phenylalanine exists in the heme pocket so it interacts the hydrophobic interactions with the heme molecule stabilizing the positioning of the heme into the pocket now if this phenylalanine changes to serine that will cause destabilization interaction between heme & hemoglobin protein.



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

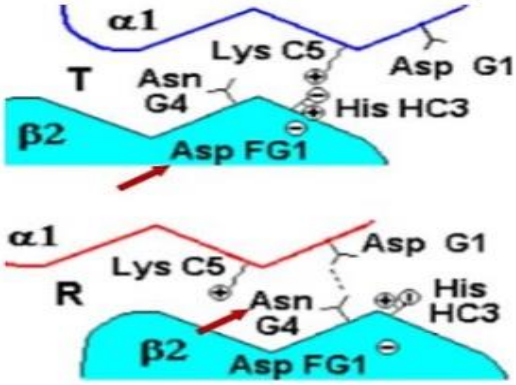
1. Hb Cowtown: Is a mutant with increased oxygen affinity. It results from the substitution of His146 (responsible for the Bohr Effect) to Leucine which, in turn, produces more hemoglobin in the R state

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)

Note: The reason for the name is the names of the cities in which these mutations were found.



Methemoglobinemia

Methemoglobinemia: Methemoglobin is a hemoع iron in the ferric state rather than the ferrous so it will become unable to bind O2

Do normal people have it? Yesss but in very low quantity so when presents in excess, a condition known as Methemoglobinemia develops, for 2 important reasons we have it in low amount, **first one is**: hydrophobic pocket surrounding the heme molecule decreases the probability of oxidizing heme iron from Fe2+ to Fe3+ due to electron rearrangement between amino acid residues and **the second reason**: there are protective reductase systems that convert HbM to HbA. **Why HbM?** • Some mutant globins (alpha and beta) bond heme in such a way as to resist the reductase:

البوابة تاعتي اللي كانت بين الحديد جوا والاوكسجين من برا بطلت موجودة !! صار الجزيء عرضة بنسبة اعلى للتأكسد

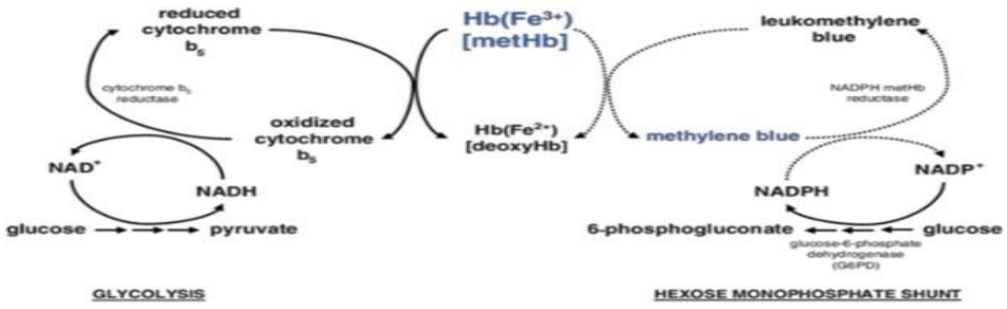
– **Hb Boston**: distal histidine is mutated into a tyrosine resulting in oxidation of ferrous iron by tyrosine's oxygen. It also attracts H2O into the pocket.

– **HbM Iwate**: proximal histidine is replaced by a tyrosine.

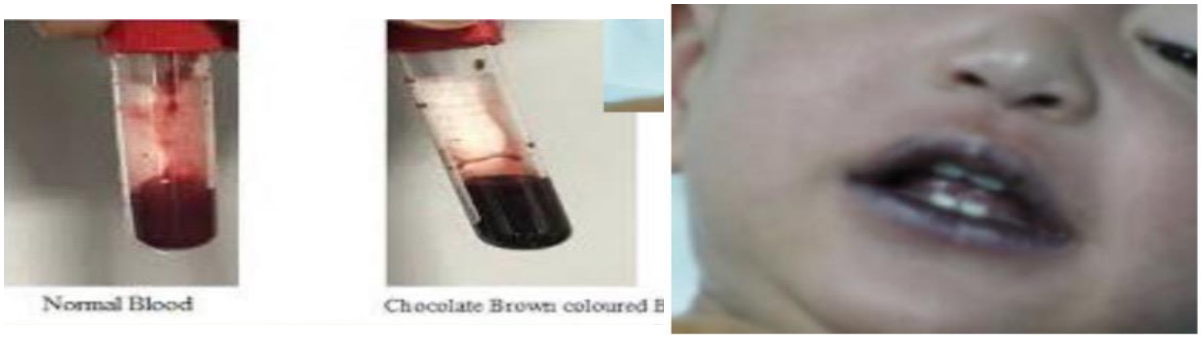
• A deficiency of the reductase enzyme.

• Certain drugs or drinking water containing nitrates, nitrates increase the probability that iron is oxidized.

Now let's talk more about the protective reductase system: 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.



And let's see how a patient and his blood would look like



Let's discuss now quantitative Hemoglobinopathies or what is known as thalassemia.

Thalassemias are the most common human single gene disorder they are prevalent in the old world including the Middle East.

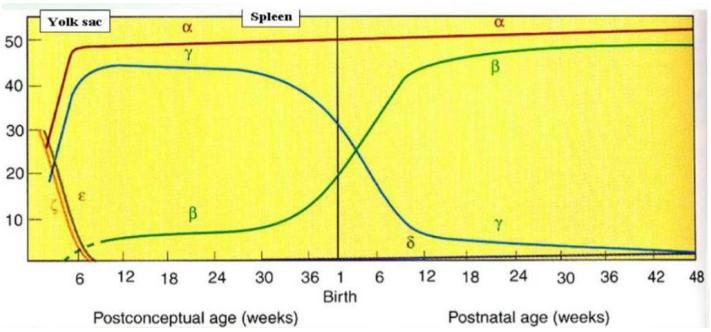
What is thalassemia exactly??

Basically it's a disease caused by a destruction in the α:β ratio. In normal RBC what we have is equal amount of alpha and beta chains but in thalassemia we would have either more alpha or more beta.

Alpha thalassemia

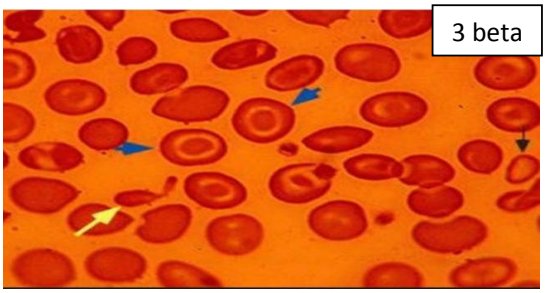
It is caused mainly by a deletion mutation remember that we have 4 alpha genes on chromosome 16 (2 on each chromosome), there's underproduction of the α-globin chains that means we have more β chains. As a result, HbA (α2β2), HbF (α2γ2), and HbA2 (α2δ2) are all affected.

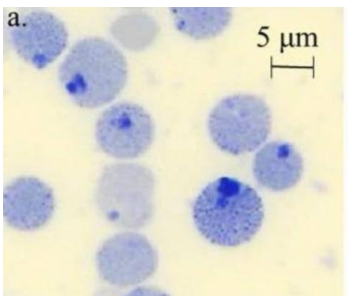
Remember that alpha chain is produced early on and it continues throughout life on the other hand beta chains start to be produced slowly in the early fetal stage but there is a big jump right before birth and it continues throughout life in equal quantities.

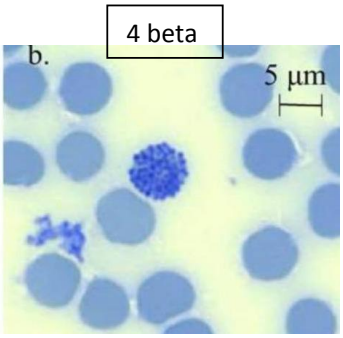


That was the scenario in normal individuals, what about abnormal ones?

If there is low production of alpha chains this means that there is high probability that you have a hemoglobin molecule that is composed of 4 β chains such molecule is known as a homotetramers of β (β4 or HbH)

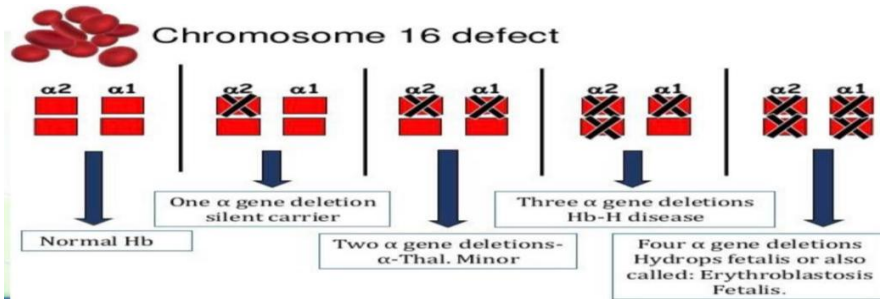






This HbH tetramers have a markedly reduced oxygen carrying capacity.

With α-thalassemia, the level of α-globin production can range from none to very nearly normal levels. This is due in part to the fact that each individual has 4 genes. So one can have all 4 genes active and the other could have 2, 3 or all 4 defective and that is what cause **variable severity**



genotype	# of normal alpha genes	Name	phenotype
αα/αα	4	Normal	none
αα/α-	3	Silent carrier	completely asymptomatic (Hb and MCV may be near the lower limit of normal range)
--/αα α-/α-	2	Thalassemia trait (minor)	Generally asymptomatic. Mild microcytic anemia
--/α-	1	Thalassemia intermedia (HbH disease)	Mild to moderate microcytic anemia
--/--	0	Thalassemia major	predominant fetal hemoglobin is a homotetramers of γ-chains (called Hb Bart or γ4) which will cause hydrops fetalis (the baby dies as still birth or after few days)

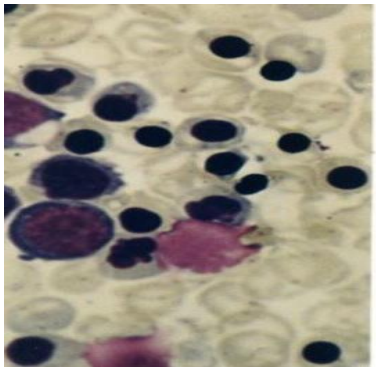
What is hydrops fetalis??

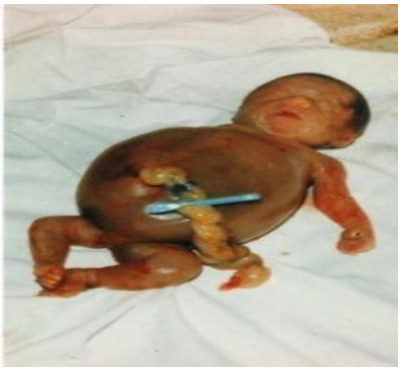
It is a case where all four alpha genes are deleted which will results into high quantities of homotetramers of gamma

Chains such hemoglobin is known as Hb Bart or γ4

Hb Bart has no oxygen carrying capacity resulting in oxygen starvation in the fetal tissues. Resulting in a fetus

That is dead so you would have still birth or death shortly after birth.





Beta thalassemia

Beta → bbboint mutation

Here we have the opposite; we have underproduction of beta chains due to single point mutation.

Beta thalassemia results from a defective beta gene, now remember normal individuals have two genes one on each chromosome (chromosome 11), so mutations in this disease are point mutations, you can have different mutations within the gene itself; it could be a mutations within the promoter, translation initiation codon, splicing positions, or poly-adenylation termination signal.

That would result in the formation of α-globin homotetramers which are extremely insoluble, leads to premature red cell destruction in the bone marrow and spleen.

The same idea in alpha thalassemia applies here **(variable severity)**; there are different types of beta thalassemia depending on the severity of the mutation (number of genes affected) if the patient is heterozygous for the thalassemia then he is termed as thalassemia minor and he would be asymptomatic and if he is homozygous for the disease then he would have severe symptoms and so on.

genotype	name	phenotype
β/ β	Normal	None
β/ β + β/ β 0	Thalassemia trait (minor)	-Asymptomatic -Mild microcytic hypochromic anemia
β +/ β + β 0/β + β E/β + β E/β 0	Thalassemia intermedia	-Variable severity Mild to moderate anemia -Possible extramedullary hematopoiesis -Iron overload
β 0/β 0	Thalassemia major	-severe anemia beginning in the first year of life and need blood transfusions (iron overload) -extramedullary hematopoiesis

It might come to your mind (What causes the iron overload?)

The answer is simply 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.

Now the final condition is known as hereditary persistence of fetal hemoglobin these individuals have abnormality in not transitioning from γ to β during development that would result in people producing HbF throughout their life.

Because the syndrome is benign most individuals do not even know they carry a hemoglobin abnormality.

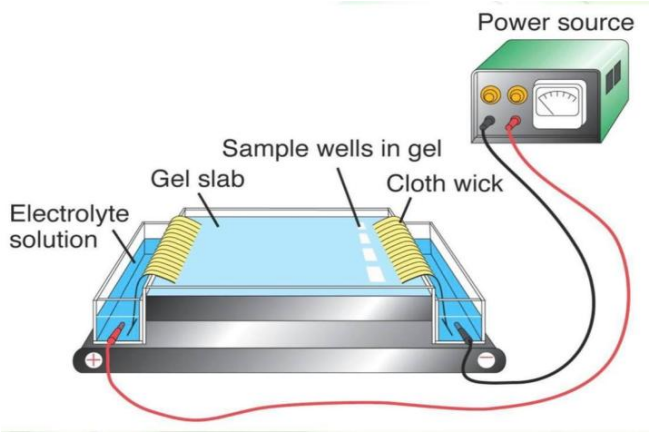
Don't we said that HbF has higher affinity towards O2, so those people would benefit from this mutation??

Yes, it has higher affinity but it doesn't really give any advantage to those people

Now the beauty of this remember when we talked about genetics of globin synthesis? Where individuals who don't produce any β globin why don't we let them produce γ globin? In this way we can switch on HbF for those people who need it.

Yaaaay we have finished talking about Hemoglobinopathies now let's see how we detect the defective hemoglobin by the method that we have taken thousands of times;

Our lovely **ELECTROPHORESIS.**



It's a technique where molecules such as proteins can be separated according to their size, charge and so on

The same thing can be done with hemoglobin especially those who have Amino acid substitution in abnormal Hbs results in an overall change in the charge of the molecule.so it depends not on the size rather it depends on the charge.

Glu (-) → Val (non-polar) reduction in negativity so it will travel away from the anode

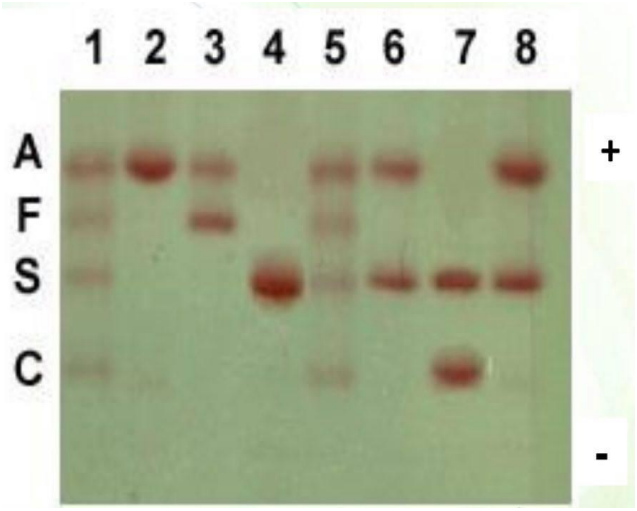
Glu (-) → Lys (+) more positive which means migrating closer to the cathode

HbF molecules as a whole has a more positive charge than HbA so it would migrate away from the anode

And if the individual is heterozygous then the Hb will appear as 2 bands each representing its charge.

As we can see from the test results the migrating pattern is different, why is it? Because we have different charges

- 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



Because we love to make things easier for you, here is a mindmap for the mutations mentioned in today's lecture ♥ goooood luck