بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

Biochemistry

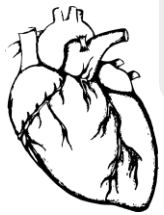
وَلَقَدْ خَلَقْنَا الْإِنسَانَ وَنَعْلَمُ مَا تُوَسْوِسُ بِهِ نَفْسُهُ ۖ وَنَحْنُ أَقْرَبُ إِلَيْهِ مِنْ حَبْلِ الْوَرِيدِ

Final | Lecture 8

Hemoglobinopathies

Written by : Shahd Thamer
Hala Marie
Jana Albrizat

Reviewed by : Shahd Thamer



Color coding used in the modified:

- *Black: the original slides*
- *Blue: the doctor's explanation/words*
- *Gray: additional information and explanation*
- *Red: important information*

Hemoglobinopathies

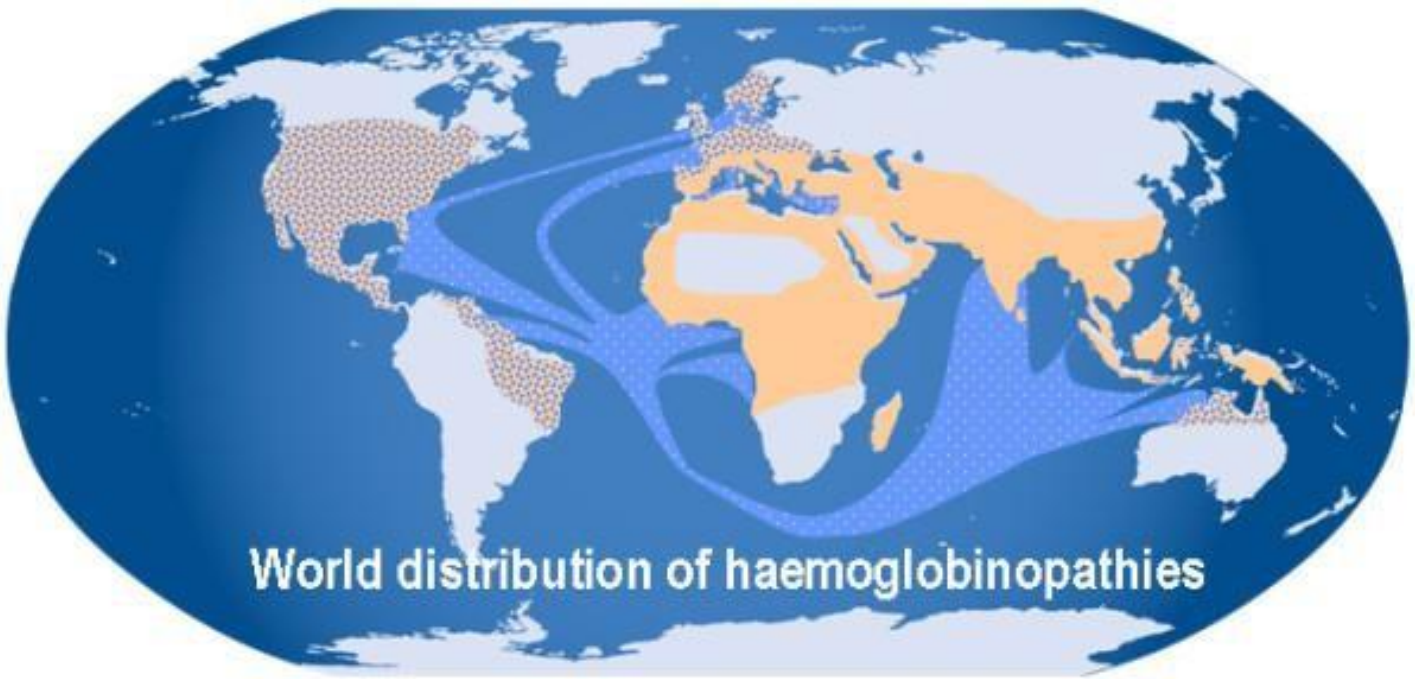
Prof. Mamoun Ahram

What are hemoglobinopathies?

- Hemoglobinopathies: Disorders of human hemoglobin.
- The most common genetic disease group in the world (5% of people are carriers) with substantial morbidity (about 300,000 born each year).
- Hemoglobin disorders account for 3.4% of deaths in children < 5 years.

Hemoglobin is the most studied protein because the diseases related to hemoglobin are globally spread and there are so many different diseases related to hemoglobin molecule, specially in the “old world” more specifically “Mediterranean region” Africa and Asia.

Notice the spread of hemoglobinopathies



But here Thalassemia is the most prevalent hemoglobinopathy; hence, mandatory premarital screening laws were enacted for prospective spouses, specifically if they were related

Hereditary hemoglobins disorders

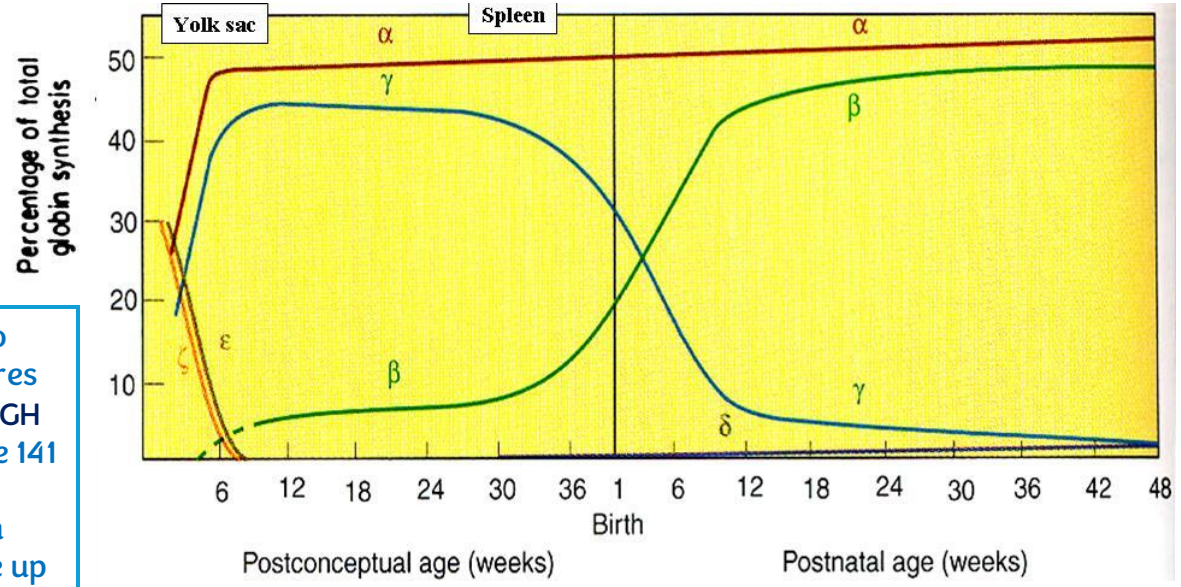
Hemoglobinopathies can be divided into three types

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

The amount of beta or alpha is not in the optimal level (alpha is more than beta or vice versa)

Meaning an adult can still have the fetal Hb

Recall, when we talk about hemoglobin we have multiple types of hemoglobin and they change in the human body as we develop.
Early on we have ε and ζ. Then we will start to have α and γ But After we will have α and β (As major forms) but we can have minor forms like γ, ε, ζ and so on.



In Qualitative abnormality: the quality of the chain is not in the optimal state Meaning its alpha2 beta 2 but the alpha or beta has a mutation , change In the amino acid which affected the protein structure which will eventually affect the protein function so the efficiency of oxygen binding is not up to standard

A single amino acid can change to many different abnormal structures Which gives variance EVEN THOUGH the amino acids in alpha chain are 141 and beta amino acids are 146 but because amino acid can mutate in many different ways we can have up to more than 800 diseases

Quantitative abnormalities (Thalassemias)

Thalassemias

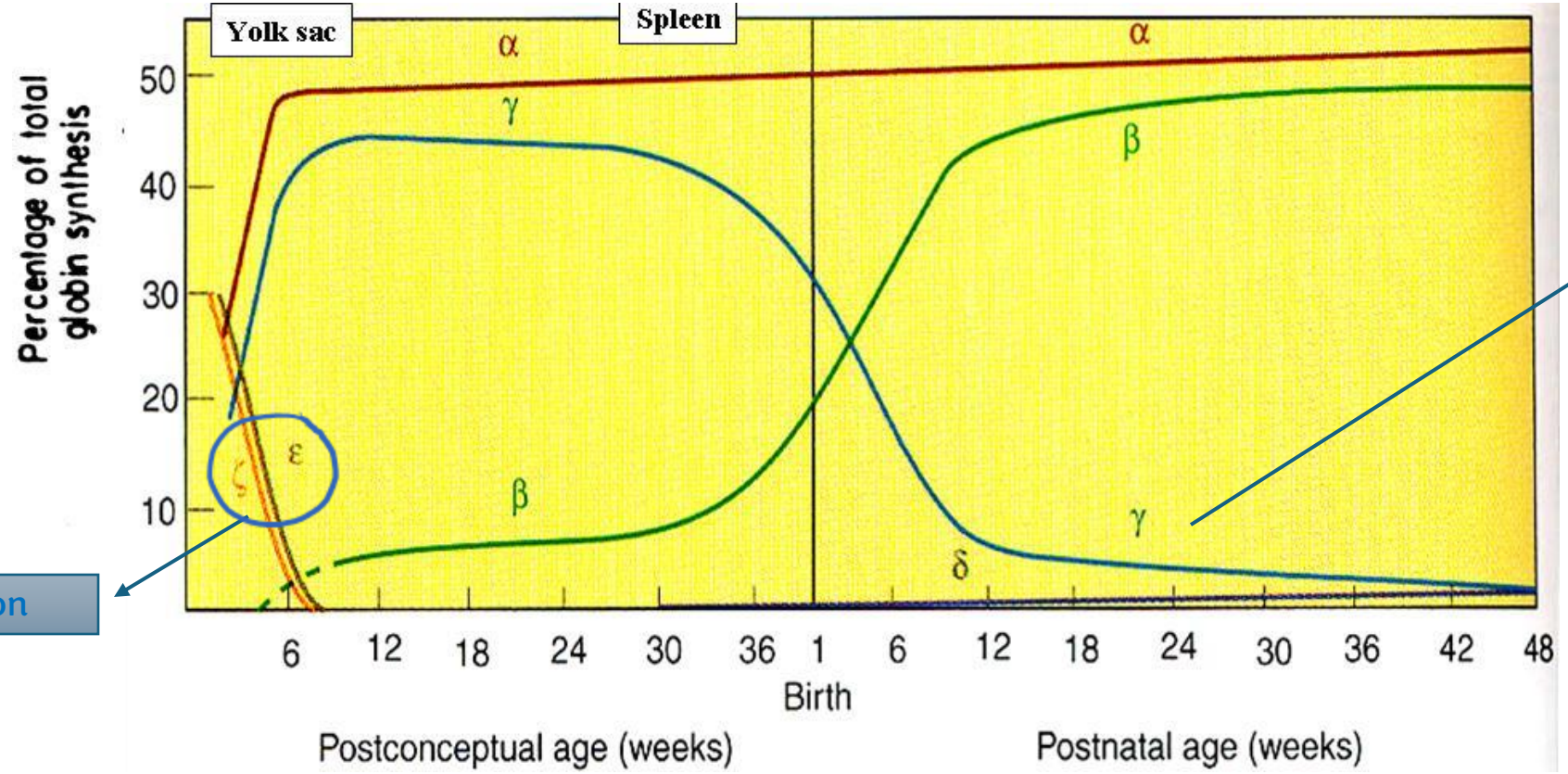
- Thalassemias: the most common human single-gene disorder.
- They are caused by a reduced amount of either the α or β protein, which alters the ratio of the α:β ratio. To give us alpha 2 beta 2

We notice two types Depending on the affected polypeptide alpha or beta
We notice again they are most spread in the Mediterranean region

If the balance is disrupted The probability of having alpha more than beta or beta more than alpha increases

The Alpha-Thalassemias

- Alpha-thalassemia: underproduction of the α-globin chains.
- HbA ($\alpha 2\beta 2$), HbF ($\alpha 2\gamma 2$), and HbA2 ($\alpha 2\delta 2$) are all affected in α-thalassemia.



Produce early on

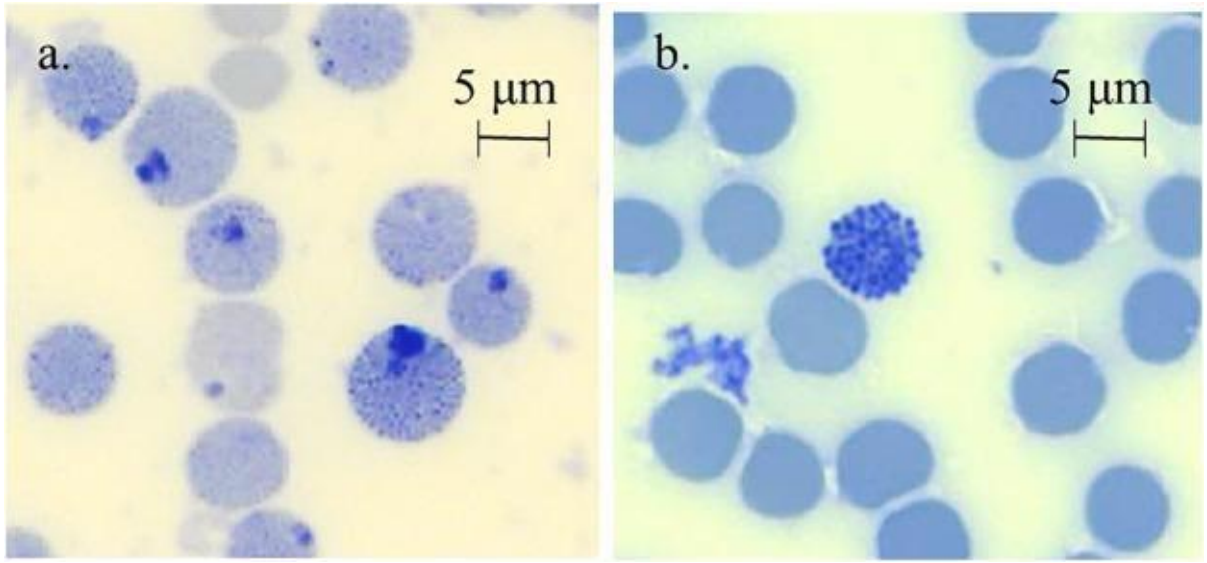
Exist in very low amounts we don't have many diseases related to them

HbH

Alpha thalassemia: Reduction of the production of the Alpha peptide so we will have more beta polypeptide and end up with a homotetramer of beta (beta4) instead of heterotetramer

- With the reduction of α chain production, and β-chain production is established, homotetramers of β (β4 or HbH) are formed.
- 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).
- The main type of mutation is gene deletion

They can be stained inside the RBCs



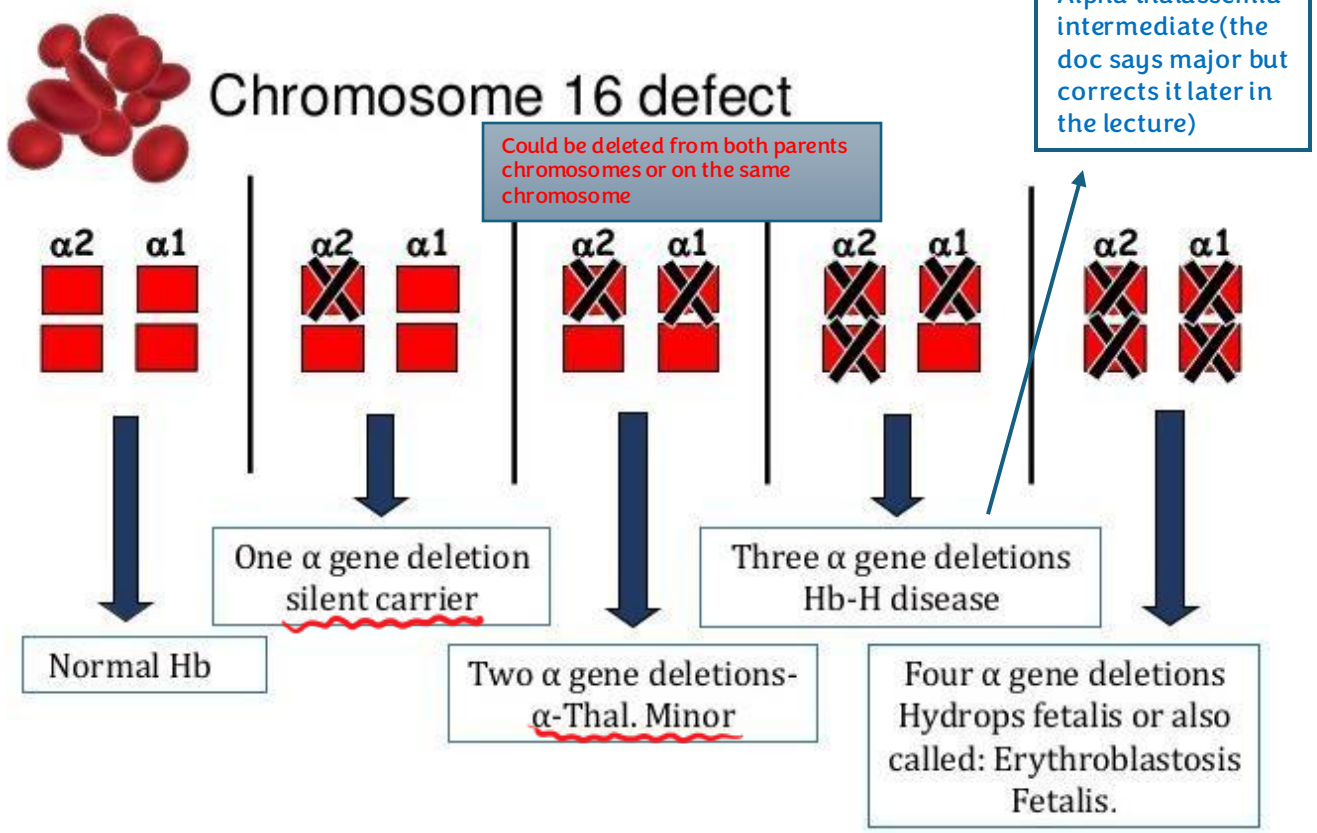
Alpha gene is gone, can be also point Mutation nucleotide changes in the dna leads to change in the amino acid (we will talk about it later). Deletion mesns the gene is gone so the potential protein of such gene is also gone

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.

Each chromosome has two genes for the alpha (2 from the mother and 2 from the father) giving a total of 4 alpha genes

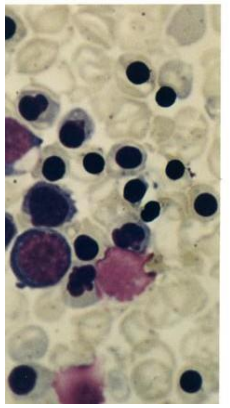
Each individual can have 4 copies of alpha gene(Diploid cells, 2 copies of chromosomes on from the mother and one from the father)

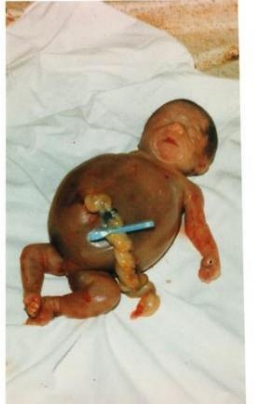


So if point mutation occurs no significant effect will happen because we have so many alternative genes that can do the work
So for a great apparent significant Obvious Clear Manifested gene deletion must happen (which point deletion won't do)

α-thalassemia major

Remember that after the embryonic stage we have the fetal stage in which we have the production of alpha and gamma

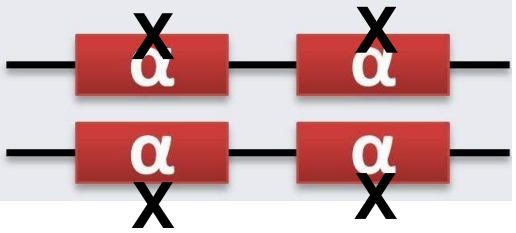




Hydrops fetalis Fatal

- 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 or death shortly after birth occurs. (The baby die shortly before or after birth)

Incompatible with Life
Hydrops Fetalis



So now all the alpha is gone, and we only have gamma

So this fetus, if there is no portion and the baby didn't get out by himself because he's dead the hemoglobin in his body will be composed of four gamma polypeptides

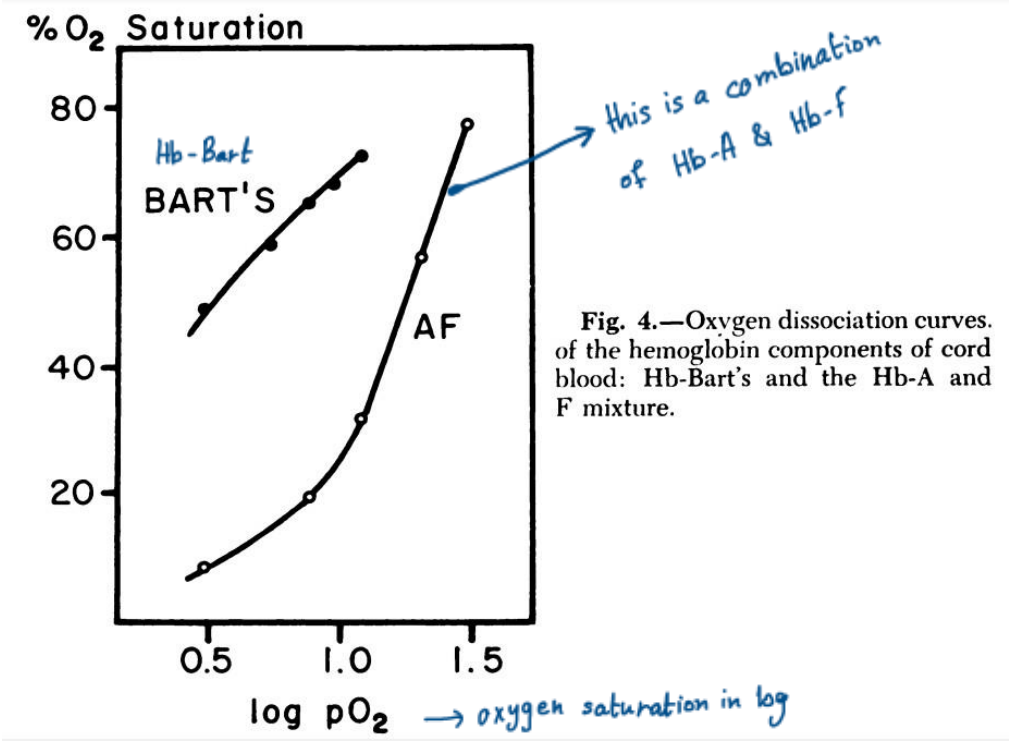


Fig. 4.—Oxygen dissociation curves. of the hemoglobin components of cord blood: Hb-Bart's and the Hb-A and F mixture.

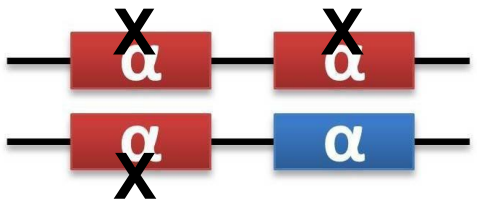
α-thalassemia intermedia

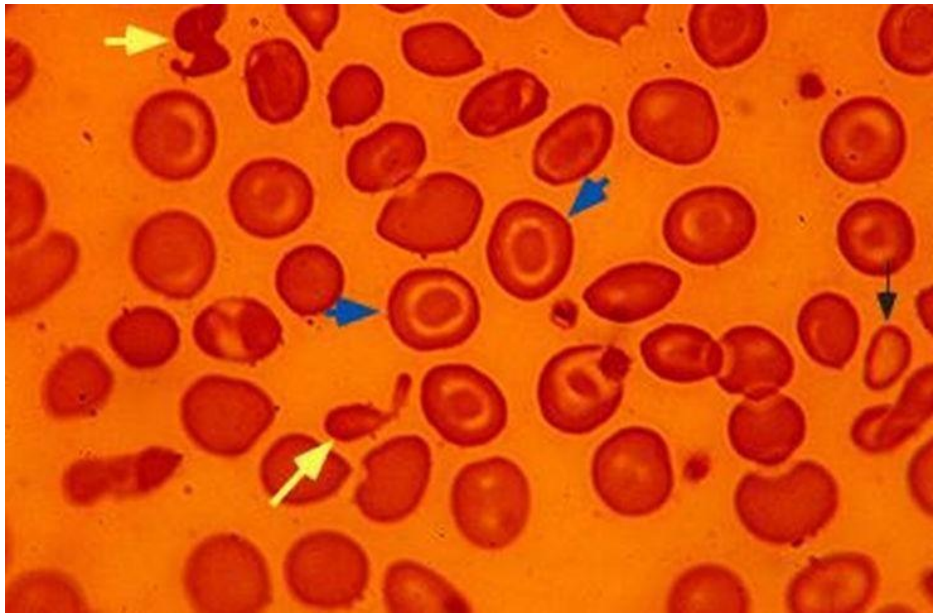
Hemoglobin H disease

- 3 of 4 genes deleted.
- Mild to moderate hemolytic anemia in adults. فقر دم
- A high level of β4 tetramer is present.
- Clinically, it is known as hemoglobin H disease.
- The disease is not fatal, but symptomatic.

It is not fatal, the baby still will be born, but he will have symptoms (for example, hemolysis which means that the RBCs will be broken easily + normally the RBCs have half life= 120 days, but in this case they only last for 10 days or something like that. Their half life is shorter than normal)

Hb H Disease: Symptomatic
Hemolytic and Microcytic anemia
Splenomegaly





‼ Do not mess up fetal (جنيني) with fatal (مميت)

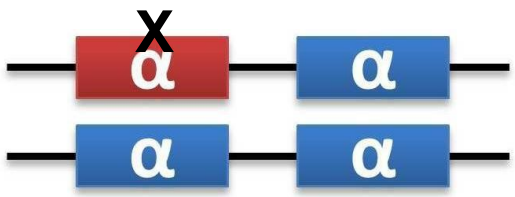
α-thalassemia minor and silent carrier

- α-Thalassemia trait: If 2 of the 4 genes are inactivated. (Deleted)
  - The individuals are generally asymptomatic.

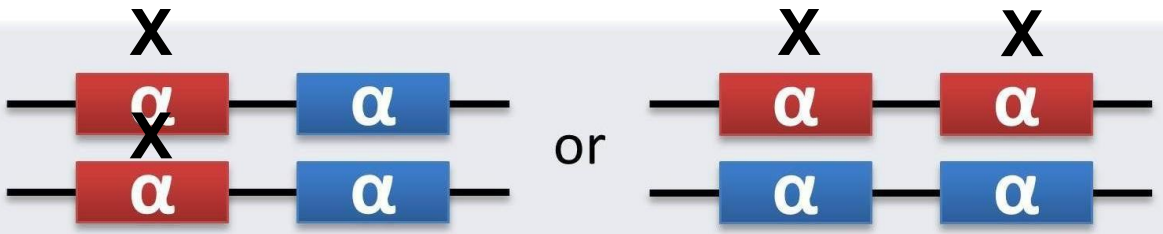
- Silent carrier: 1 of 4 genes deleted.
  - Individuals are completely asymptomatic.

This one is rare virtually because we have four genes so we have backup so it's a low probability that we will have a deletion for two or three or four genes

Carrier: Asymptomatic
No abnormalities



α-thal minor: Asymptomatic
Mild microcytic anemia



The beta-thalassemias More common

- β-globins are deficient and the α-globins are in excess and will form α-globin homotetramers.

- The main type of mutation is point mutations (a change in one nucleotide that changes the amino acid that is existed in better globin protein of the DNA sequence within the gene)
- The α-globin homotetramers are extremely insoluble, they tend to aggregate cluster together precipitate in RBCs , which leads to premature red cell destruction (the lifespan of the RBCs is shorter than normal (hemolysis)) in the bone marrow and spleen.

β-thalassemia major and minor depending on the point mutation

- **β-thalassemia major**

- A complete lack of a normal HbA gene **(The beta globin gene)** is denoted as β^0-thalassemia or β-thalassemia major.

- Affected individuals suffer from severe anemia **and they have pain as well in their bodies** beginning in the first year of life and need blood transfusions.

  - Long-term transfusions (periodically / very frequently) lead to the accumulation (precipitation) of iron in the organs, particularly the heart, liver and pancreas and, finally, death in the teens to early twenties. And because of the blood transfusion hemolysis will also occur.
  - The iron is a very precious metal in our body so the body has to maintain it for two reason: 1-what is important for the hemoglobin function, 2- it's toxic so when it's accumulate in the tissue, it will affect the tissue and the cells it would cause destruction for the organs for example the spleen, bone marrow , heart ,liver, kidneys, and so on.

β-thalassemia minor

- Individuals with one normal β-globin gene and a mutated gene are termed β-thalassemia minor. (they have one good gene and one bad gene)
- Individuals with beta-thalassemia minor are generally asymptomatic.
- One good gene is sufficient. A lot of diseases are recessive diseases which means that the two genes have to be gone. If one is existed, the person will be fine.

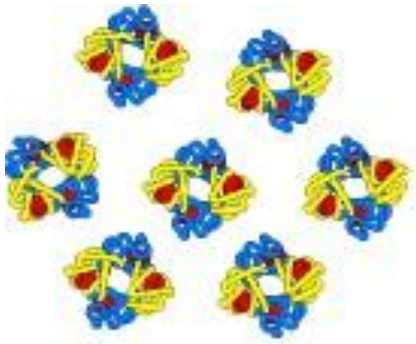
Qualitative abnormalities

There's too many types of it because any amino acid can be changed to anything so we have genetic variance

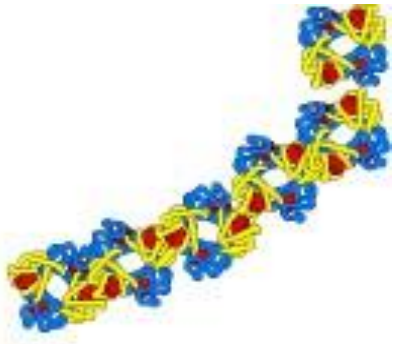
Sickle cell hemoglobin (HbS)

In this disease, one litter in the DNA of the beta globin changed through the amino acid changed

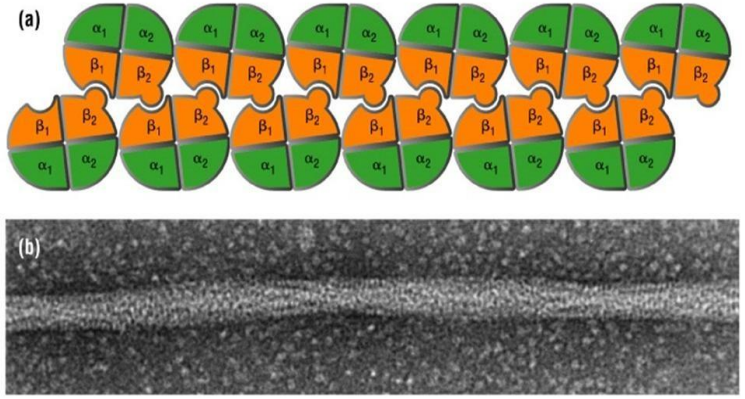
- It is caused by a change of amino acids in the 6th position of β globin (Glu to Val).
- The hemoglobin is designated α2βs2 (Bs2 cuz beta is sickled) or HbS. S for sickle
- The hemoglobin tetramers aggregate instead of being individually separate than the others they cluster together into arrays upon deoxygenation in the tissues.
- 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).

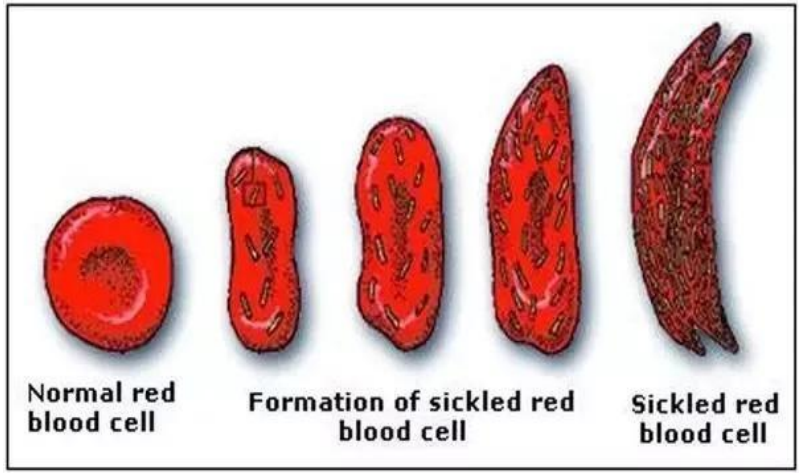


NORMAL HEMOGLOBIN



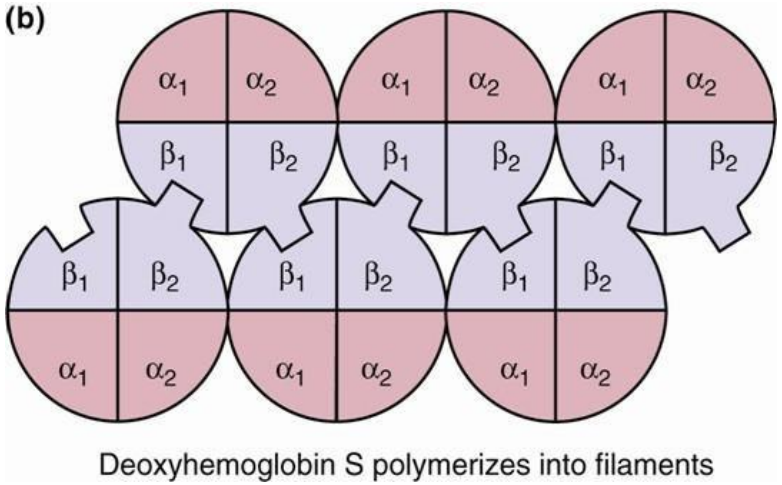
CLUMPED HEMOGLOBIN

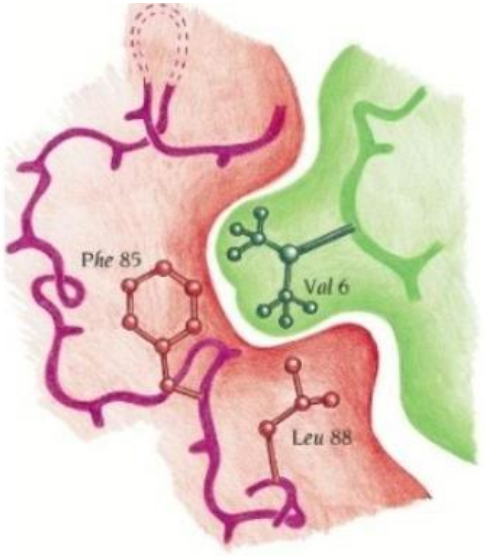




How does the fiber form?

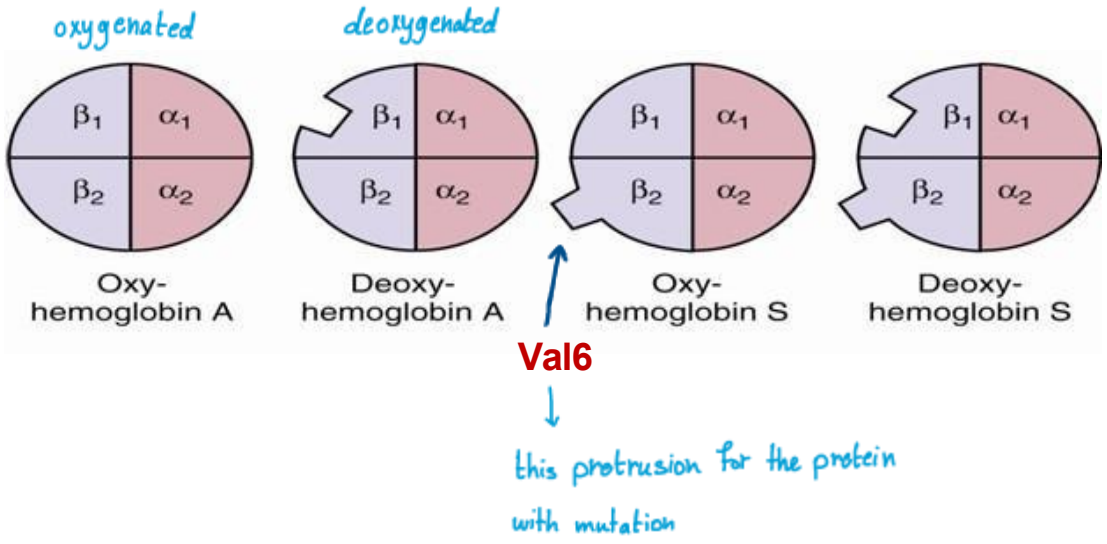
- Fiber formation only occurs in the deoxy- or T-state.
- The mutated valine of the β2 chain is protruded (the protein structure changed so there is a protrusion (,طعجة and inserted into a hydrophobic pocket on the surface of β1 chain.
- Notice in the pictures below the difference between the oxygenated and deoxygenated protein structure when it's the oxygenated, the beta chain will have a small space and it swings between these two structures





Recall that the glutamate is negatively charged amino acid , valine is a non-polar amino acid.

Which means that we changed something that likes to be on the cell surface to something that likes to hide. So the structure of the protein will be changed a little. The mutations could happen to an amino acid that likes to be on the surface or inside or near to the heme. Usually these point mutations (changes in amino acids) specially if they are on the surface are usually asymptomatic they do not usually have a great effect except for sickle cell anemia it is the only symptomatic one.



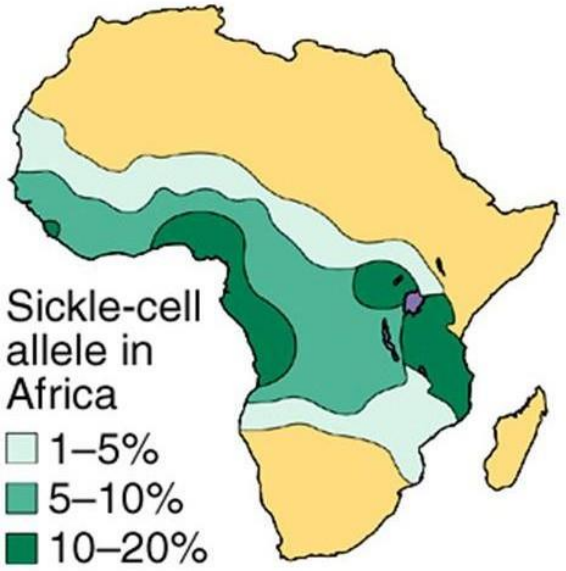
So when it is deoxygenated and abnormal (doc mistaken it accidently by saying “normal”), the protrusion will fill the gaps, and this causes the aggregation.

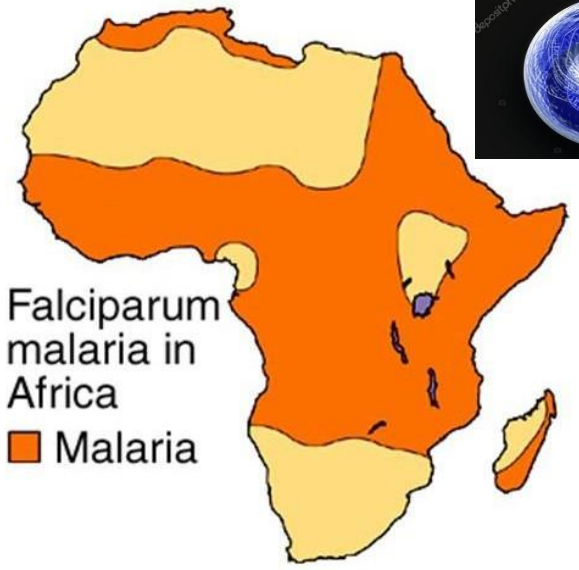
So the person in order to reduce the symptoms, they should not get their hemoglobin deoxygenated. Their bodies should be oxygenated as much as possible, so anything that increases deoxygenation of hemoglobin will increase the symptoms and clustering because of the oxygenated will have the gap.

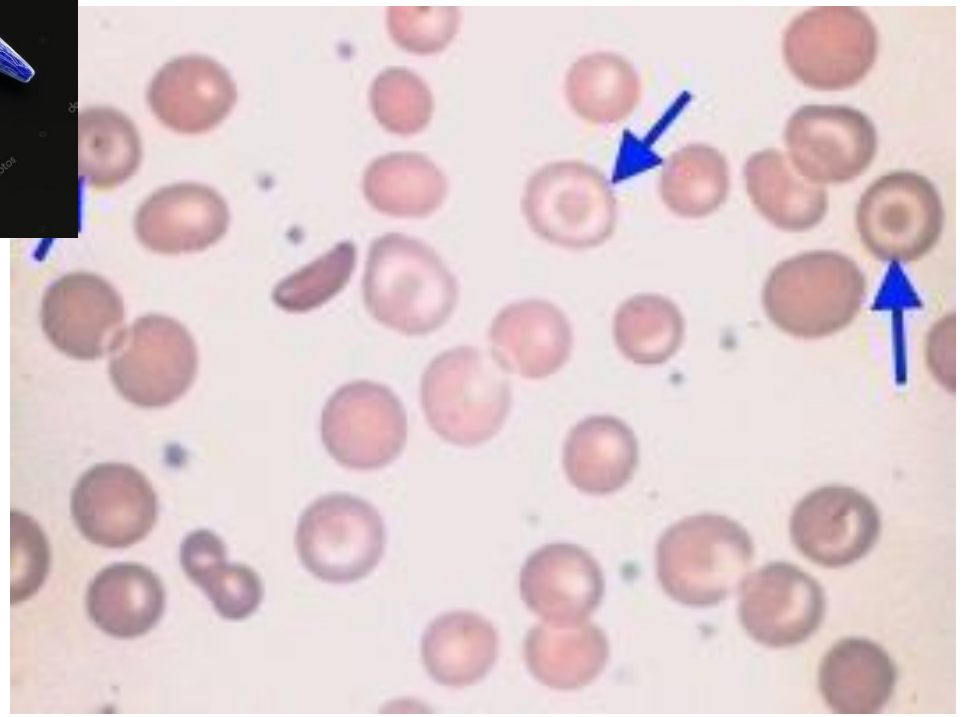
Sickle cell trait

- Advantage: selective advantage from plasmodium falciparum that causes malaria. Why?

- It occurs in heterozygotes (individuals with both HbA and HbS), who are clinically normal, but their cells sickle when subjected to low oxygen.





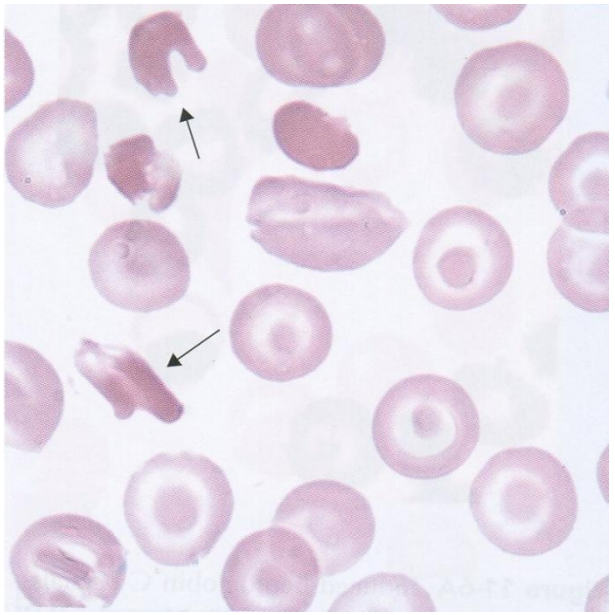


- Sickle cell trait is when an individual has one good beta globin and one bad beta globin , this individual is called a carrier or a heterozygote , a person who is a heterozygote has 2 types of genes (for e.g. HbA /HbC).
- Normally, an individual has 2 genes or more precisely, 2 alleles, that determine a characteristic, such as eye or hair color .One allele might be dominant over the other. An individual who has 2 different alleles for a particular gene is called heterozygote.
- Carriers of sickle cell trait (most commonly from central Africa) have an advantage because they are resistant against malaria which is very common in central Africa .
- The malaria parasite invades the RBCs in order to survive and reproduce, this leads to death among individuals with malaria, for people with sickle cell trait that's not the case as their RBCs life span is shorter than the parasite's life cycle , so their RBCs may be removed or destroyed before the parasite can complete its life cycle .

Hemoglobin C (HbC)

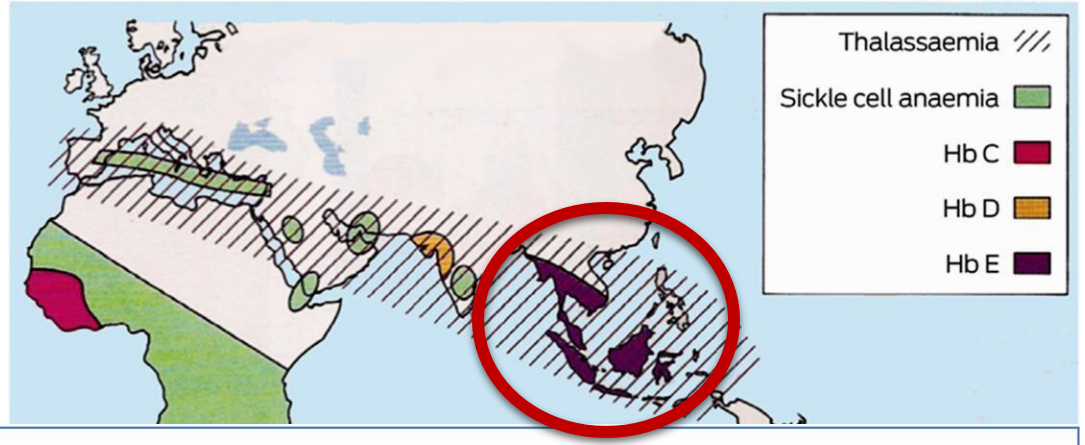
- (HbC) is also due to a change at the 6th position of β globin replacing the glutamate with lysine (designated as βc).
- This hemoglobin is less soluble than HbA so it crystallizes in RBCs reducing their deformability in capillaries.
- HbC also leads to water loss from cells leading to higher hemoglobin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

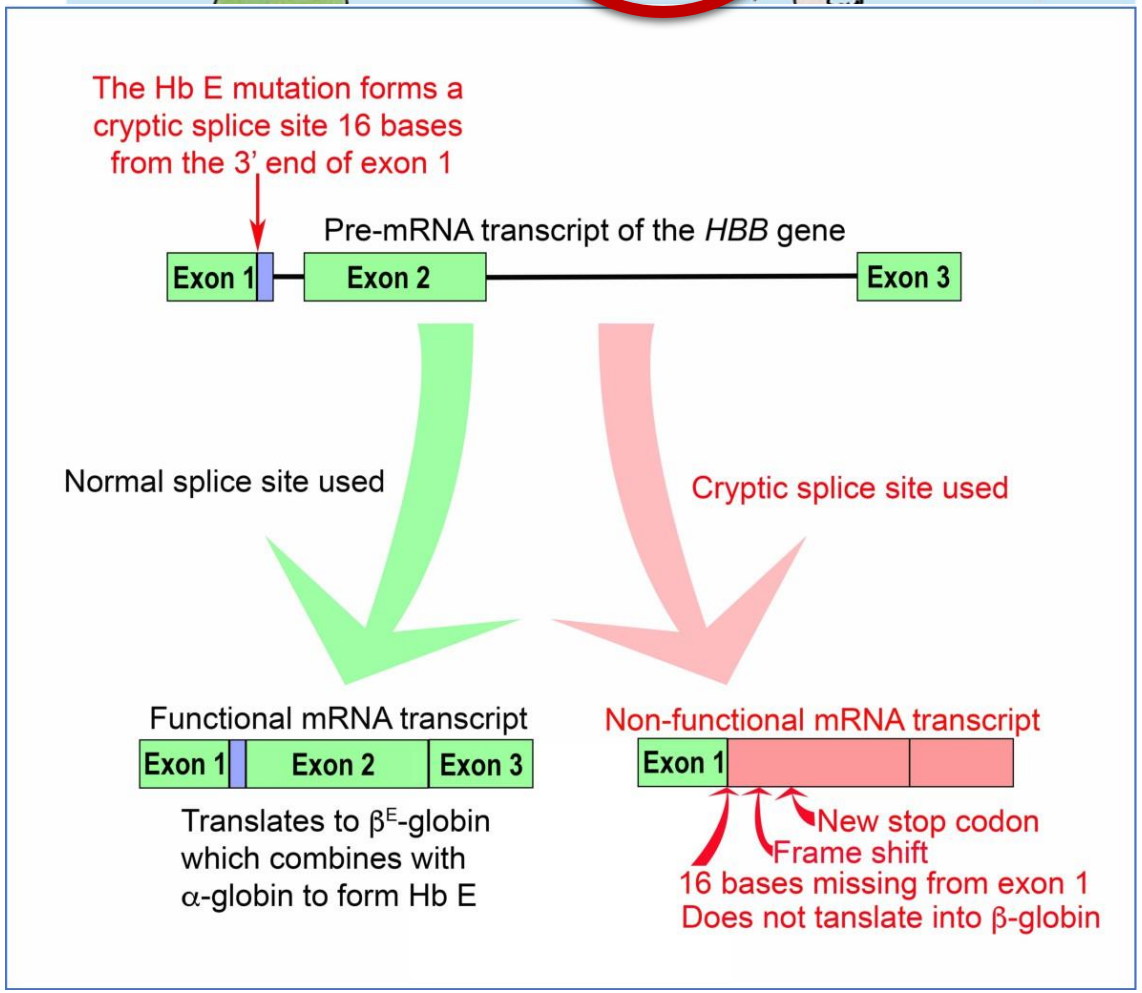
In HbC there is a change in the sixth amino acid of beta globin , glutamate to lysine. (notice that in the sickle cell disease we change the same amino acid position but to valine).
In HBC we changed a negatively charged amino acids (glutamate) to a positively charged one (lysine) , the protein structure isn't greatly altered but there will be some symptoms thus the name 'minor hemolytic disorder'.
For some reason there will be more water loss from the cells and as a result the concentration of hemoglobin increases and it tends to aggregate



Hemoglobin E

- It is common in Southeast Asia
- It has both quantitative and qualitative characteristics.
- It is caused by a mutation that changes glutamic acid to lysine (at carbon position 26)producing either a less efficient hemoglobin or a shorter-than-normal protein.
- Mild disease





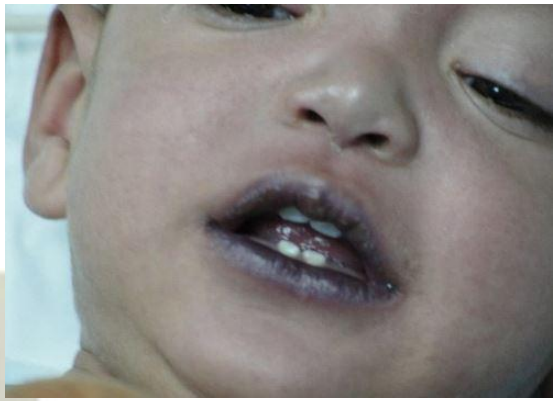
Hemoglobin E or HbE is both quantitative and qualitative, the quantity of hemoglobin is less and the quality isn't up to standard , however its symptoms are mild.
This disease is most common in Southeast Asia like malaysia , Indonesia and so on.
The doctor said not to look into details about how this disease occur.

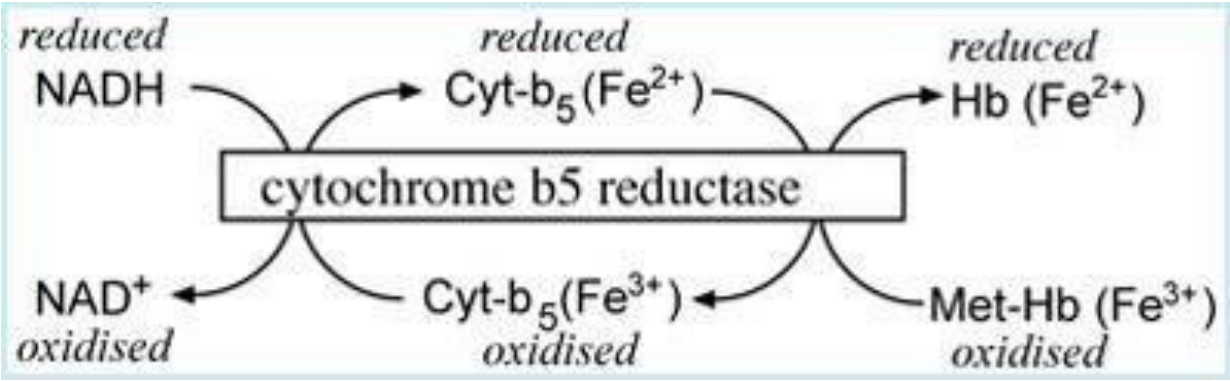
Altered Oxygen Transport

Defects in oxygen transport

Methemoglobin (HbM)

- Hemoglobin can be reversibly oxygenated because iron remains in the reduced (ferrous, Fe^{+2}) state. Why?
- Oxygen release may cause the oxidation of Fe^{+2} to Fe^{+3}, forming methemoglobin (HbM), except that the enzyme methemoglobin reductase reduces iron back.
- If not, a condition known as methemoglobinemia develops.





Methemoglobin reductase AKA
NADH-Cytochrome b5 reductase

Normal Blood

Chocolate Brown coloured Blood

- In the hemoglobin molecule, the iron must be in the ferrous state (Fe+2) in order to bind oxygen.
- When iron releases oxygen, it undergoes oxidation and it's converted to the ferric state (Fe+3) , as a result it won't be able to bind to oxygen effectively.
- Hemoglobin containing iron in the ferric state is called methemoglobin (HbM). The condition in which an individual has an abnormally high level of HbM is called methemoglobinemia
- Individuals with methemoglobinemia may develop bluish discoloration of skin because iron in its ferric state cannot bind to oxygen the blood turns blue thus the bluish discoloration of the skin.
- We have already mentioned that the hydrophobic environment surrounding the heme group helps prevent oxidation of iron and maintains it in the ferrous state. Another mechanism that helps maintain iron in its ferrous state is an enzyme called methemoglobin reductase.
- This enzyme reduces methemoglobin to hemoglobin , by converting the iron from the ferric state to ferrous state ,thereby restoring functional hemoglobin.

Hereditary persistence of fetal hemoglobin (HPFH)

Fetal hemoglobin (HfA)is composed of alpha and gamma chains, right before birth the beta chains increases and the gamma decreases promoting formation of adult hemoglobin (HbA)

- 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.
- There is no deletion of the fetal globin genes.
- Think: treatment for β-thalassemia!!!!

By increasing the production of fetal hemoglobin (HbF) since the beta globin gene is defective, we can try to reactivate the gamma globin that produce HbF which can then replace some of the function of the HbA and help to improve oxygen transport.

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.

- The embryonic and fetal globin genes including ε (epsilon), γ (gamma), and β (beta) are located close to one another on the same chromosome.
- During development, these genes are expressed sequentially: one gene becomes active while another is switched off. In individuals with hereditary persistence of fetal hemoglobin, this switch does not occur properly. As a result, the γ-globin gene remains active, and these individuals continue to produce HbF into adulthood, often without any symptoms.
- The condition may only be discovered through a blood test.

Quiz!

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	27	Forgot to mention the carbon position where the mutation occurs	Added it
V1 → V2			

Additional Resources:

Reference Used:

Prof. Mamoun Ahram recorded lecture

Extra References for the Reader to Use:

Marks' Basic Medical Biochemistry (6th edition)

رسالة من الفريق العلمي:

وَأَن لَّيْسَ لِلْإِنسَانِ إِلَّا مَا سَعَىٰ * وَأَنَّ سَعْيَهُ سَوْفَ يُرَىٰ

تذكّر دائماً أن كل دقيقة تقضيها في التركيز والفهم هي خطوة ثابتة نحو هدفك. لا تقلق بشأن النتيجة بقدر ما تهتم بإعطاء السعي حقه، فالمجهود لا يضيع، والتوفيق قرين الإخلاص والعمل.

" اللهم إنّي أسألك فهم النبيين، وحفظ المرسلين، وإلهام الملائكة المقربين. اللهم اجعل ألسنتنا عامرة بذكرك، وقلوبنا بخشيتك، وأسرارنا بطاعتك، إنك على كل شيء قدير."