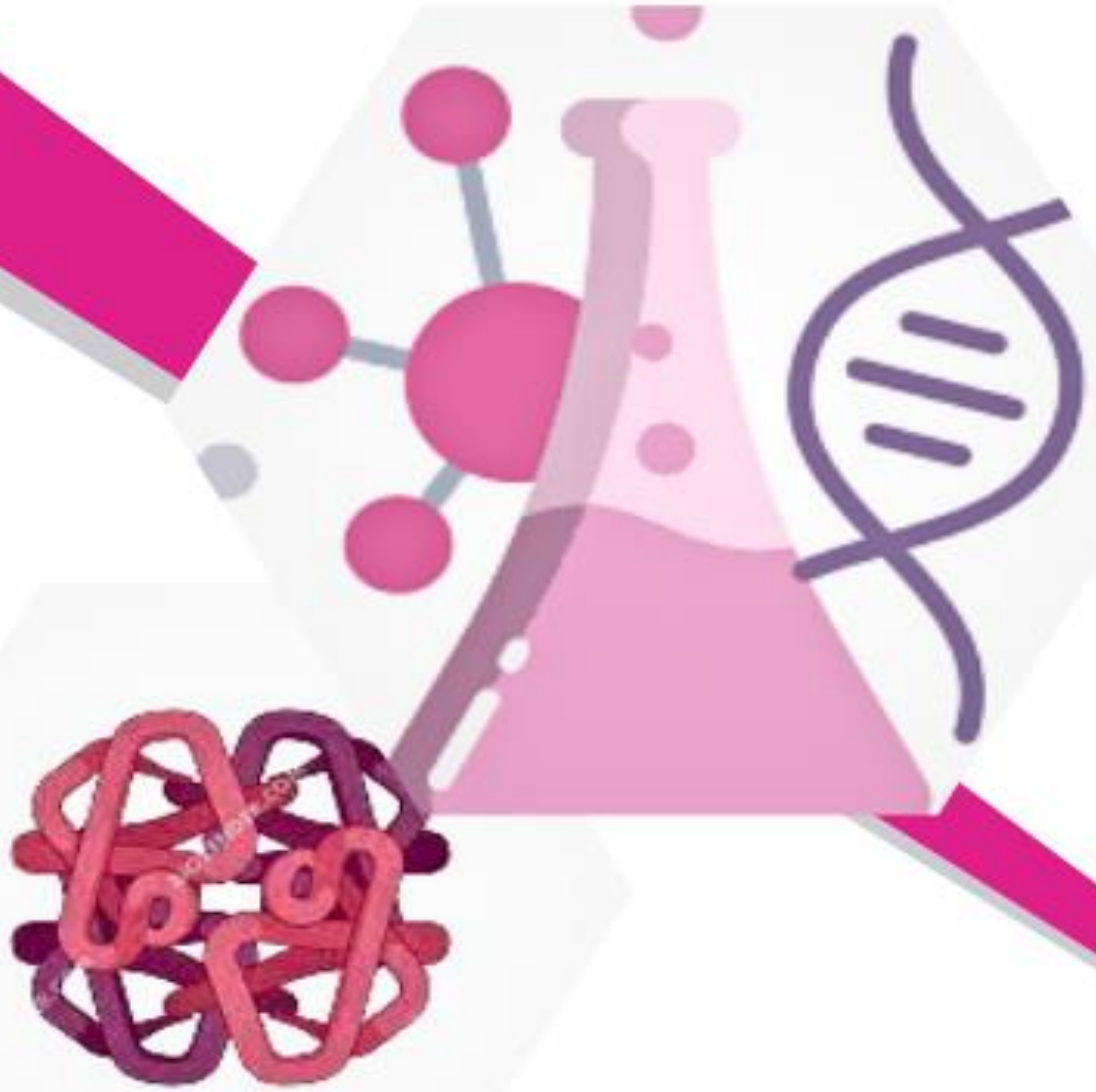
Doctor.021

no. 3

HLS

BIOCHEMISTRY



Writer: Faten AlDraawi & Dania Abu Samha

Corrector: Faten AlDraawi & Dania Abu Samha

Doctor: Mamoun Ahram

HEMOGLOBINOPATHIES

WHAT ARE HEMOGLOBINOPATHIES?

- **Hemoglobinopathies: Disorders of human hemoglobin, so we have deficiency in oxygen transport system.**
- **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.**

As shown on the map, these disorders are concentrated in the old world (Africa, Asia, Middle East, ..)

HEREDITARY HEMOGLOBINS DISORDERS

1. **Quantitative abnormalities are abnormalities in the relative amounts (number) of α and β subunits (thalassemias), the problem's in Hb levels (quantity) in the RBCs.**
2. **Qualitative abnormalities: mutations resulting in structural variants, the quality of Hb molecule is compromised (negatively affected). Here we've normal amount of Hb, but with low efficiency.**
   - ✓ **Over 800 variants have been identified, each A.A in Hb has the chance to be mutated and results in defective Hb.**
3. **Hereditary persistence of fetal hemoglobin (HPFH): impairment of the perinatal switch from γ to β globin, they have fetal Hb (α2, γ2) throughout their lives, and they're asymptomatic.**

QUANTITATIVE ABNORMALITIES (THALASSEMIAS)

- **Thalassemias: the most common human single-gene disorder.**

***Jordan has high prevalence of thalassemia.**

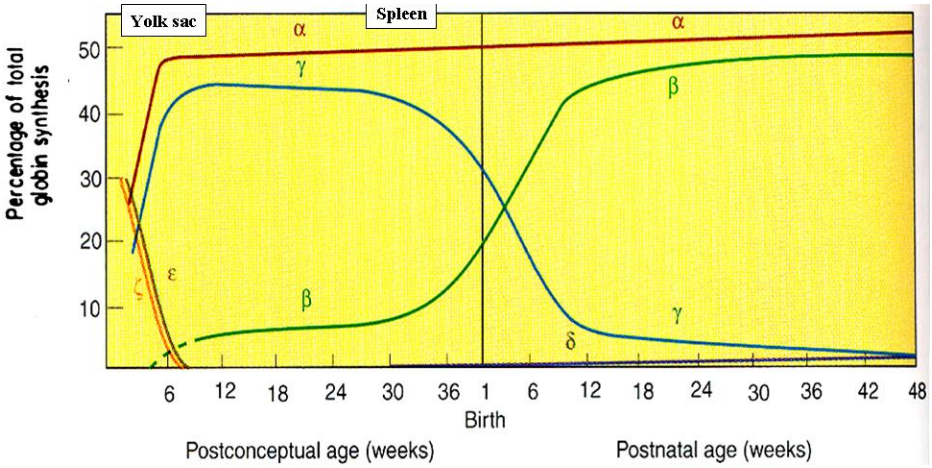
- **They are caused by a reduced amount of either the α or β protein, which alters the ratio of the α:β ratio, in normal RBCs we've equal**

amount of α and β subunits, while in thalassemias we would have either reduced quantity of α (α thalassemias) or reduced quantity of β (β thalassemias).

THE ALPHA-THALASSEMIAS

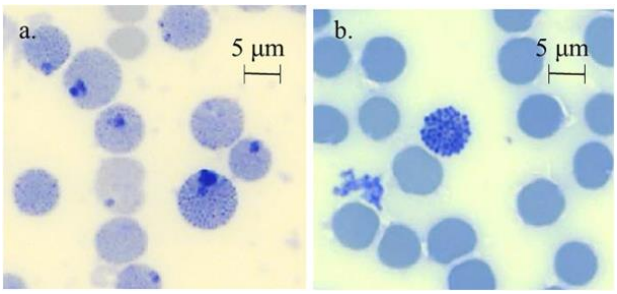
- Alpha-thalassemia (get it between the 6th and 8th weeks of gestation): underproduction of the α-globin chains.
- It's caused mainly by a deletion (rarely by pointed mutations).
- Patients are affected from fetal age throughout their lives.
- HbA (α2β2), HbF (α2γ2), and HbA2 (α2δ2) are all affected in α-thalassemia.
- ⬇α,⬆β,⬆γ.

Remember :
Alpha chain is produced early on and it continuos 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.
*people with alpha thalassemia will be affected faster than beta thalassemia.



HBH

- With the reduction of α chain production, and β-chain production is established, homotetramers of β (β4 or HbH) are formed, the four subunits are β instead of α2β2.



- The HbH tetramers have a high affinity towards oxygen (reduced oxygen carrying capacity, it doesn't release oxygen into tissues easily) and are highly unstable (meaning that they denature,

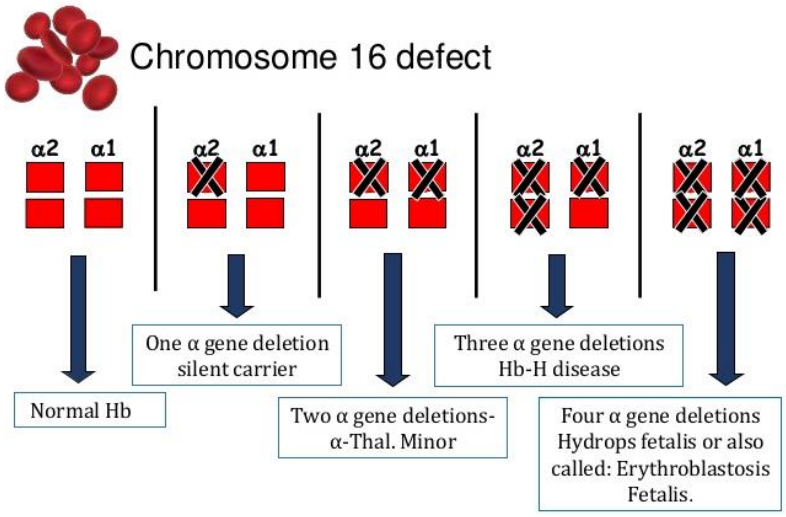
aggregate and precipitate resulting in the formation of Heinz bodies (which are clusters of HbH) especially near the plasma membrane, so as a result there will be hemolysis of RBCs).

- The main type of mutation is deletion (rarely point mutations).

VARIABLE SEVERITY

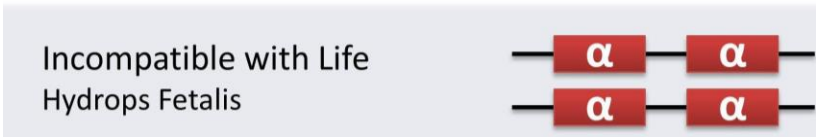
- With α-thalassemias, the level of α-globin production can range from none to very nearly normal levels.
- This is due in part to the fact that normally each individual has 4 genes for α-globin, 2 genes on each chromosome :

✓ Deletion of 1 gene is asymptomatic and called silent carrier.
✓ Deletion of 2 genes is called minor α-thalassemia.
✓ Deletion of 3 genes is called α-thalassemia intermedia (HBH disease).
✓ Deletion of all the 4 genes is called major α-thalassemia (hydrops fetalis).



α-THALASSEMIA MAJOR HYDROPS FETALIS

- 4 of 4 genes are deleted.
- The predominant fetal hemoglobin is a tetramer of γ-chains.
- γ4 or Hb Bart: a homotetramer of γ.



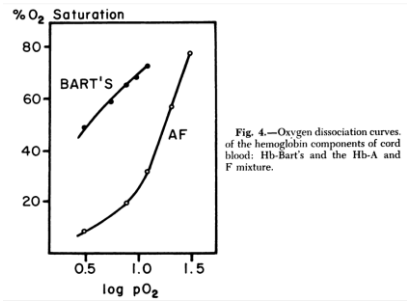


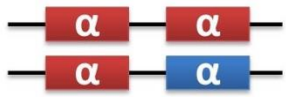
Fig. 4.—Oxygen dissociation curves of the hemoglobin components of cord blood: Hb-Bart's and the Hb-A and F mixture.

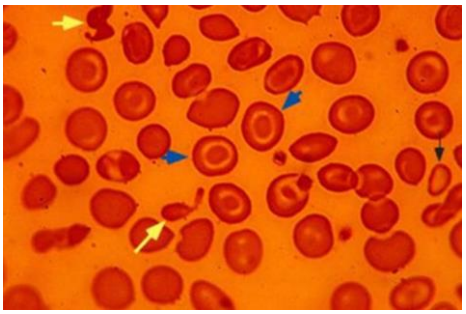
- **Hb Bart has a high affinity towards oxygen, resulting in oxygen starvation in fetal tissues.**
- **This situation is called hydrops fetalis.**
- **Stillbirth or death shortly after birth occurs.**

α-THALASSEMIA INTERMEDIA AND HEMOGLOBIN H DISEASE

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

Hb H Disease: Symptomatic
Hemolytic and Microcytic anemia
Splenomegaly

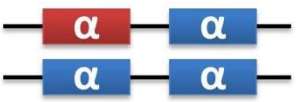




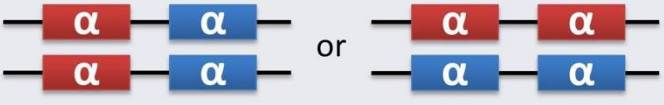
α-THALASSEMIA MINOR AND SILENT CARRIER

- **α-Thalassemia trait: If 2 of the 4 genes are inactivated, could be on different chromosomes or on the same chromosome, and we've homotetramer of β.**
  - **The individuals are generally asymptomatic.**
- **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
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

THE BETA-THALASSEMIAS

- **we've 2 genes for β, 1 on each chromosome.**
- **β-globins are deficient and the α-globins are in excess and will form α-globin homotetramers, α:β ratio is increased.**
- **Main type of mutation is point mutations, mutations within the promoter or LCR, translation initiation codon, splicing positions, poly-adenylation termination signal or stop codon, etc.**
- **The α-globin homotetramers are extremely insoluble, which leads to premature red cell destruction in the bone marrow and spleen.**

β-THALASSEMIA MAJOR AND MINOR

*β-THALASSEMIA MAJOR:

- **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 (tissue damage), particularly the heart, liver and pancreas and, finally, death in the teens to early twenties.**

*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.

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 Here, maybe both genes are affected, or one of them isn't functioning at all, and the other one is affected.	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**

QUALITATIVE ABNORMALITIES

*protein is there, but isn't functioning 100%.

Classification of molecular mutations

- We're basically talking about point mutations which can take place anywhere in Hb molecule, and these abnormalities are divided depending on the site of mutation into :
  - **Mutations in surface residues :**
    - **Usually asymptomatic (e.g. sickle cell hemoglobulin (HbE)); an exception is HbS which has dramatic effects.**

- **Mutations in internal residues :**
    - **Hb is a globular protein with hydrophilic AA's outside and hydrophobic AA's inside, so any change in the environment will produce unstable hemoglobin and Heinz bodies and cause hemolytic anemia (e.g. Hb Hammersmith, Hb Constant Spring (Hb CS)).**
- **Mutations stabilizing methemoglobin :**
    - **In which heme is bound to ferric iron [Stabilizing heme-Fe^{+3}]; decreasing its capacity to bind oxygen resulting in hypoxia and cyanosis (which's a bluish-purple of the skin due to shortage of oxygen in the blood).**
        - Normally, Hb should be in ferrous form (Fe^{+2}) in order to be functional, and oxidation of iron (when oxygen is released from Hb) is prevented by the hydrophobic AA's that surrounds the heme and enzymaticaly.

*For more clarification: https://youtu.be/2RFcH_nlR84?si=jbDTYRoMMKGnqlBD

- **Mutations at α1-β2 or α2-β1 contacts :**
    - **At contact regions between α&β subunits.**
    - **Altered oxygen affinity by changing the equilibrium of T-state and R-state (mainly higher; a condition known as polycythemia).**

Let's start with some examples of qualitative hemoglobinopathies!

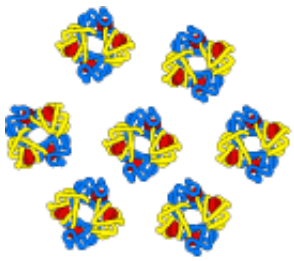
very important to know the changed amino acids

SICKLE CELL HEMOGLOBIN (HBS)

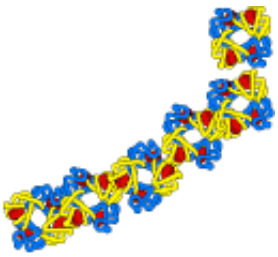
- **It is caused by a change of amino acids (nucleotide substitution) in the 6th position of β globin (Glu "negative and polar AA" to Val "nonpolar and aliphatic AA").**
- **The hemoglobin is designated α2βs2 or HbS.**
- **The hemoglobin tetramers aggregate into arrays upon deoxygenation in the tissues (structure of Hb will be affected, especially T-Hb "deoxygenated", leading to clustering and aggregation of Hb molecules).**

- **This aggregation leads to deformation of the red blood cell (from biconcave to sickled shape).**
- **It can also cause hemolytic anemia (life span of RBCs is reduced from 120 days to <20 days).**

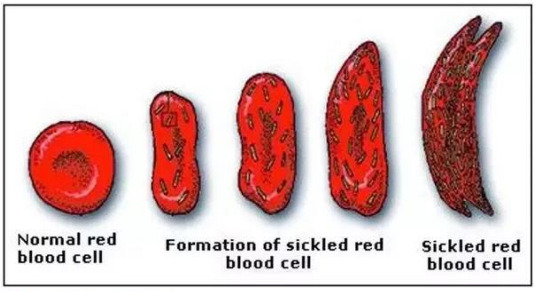
Repeated cycles of oxygenation and deoxygenation lead to irreversible sickling so cells cannot squeeze though capillaries in a single file and therefore block blood flow causing local hypoxia. Long-term recurrent clogging of the capillary beds leads to damage to internal organs, in particular the kidneys, heart and lungs.

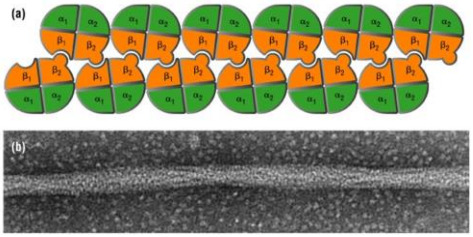


NORMAL HEMOGLOBIN



CLUMPED HEMOGLOBIN

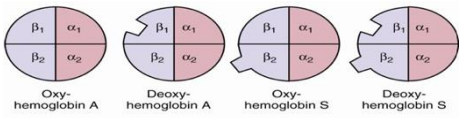


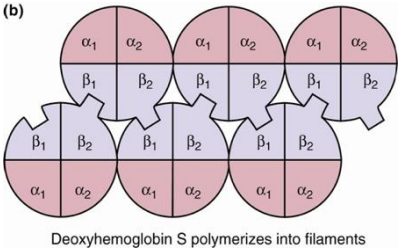


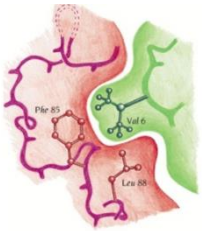
HOW DOES THE FIBER FORM?

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

- 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 in β1 chain. Secondly, in HbS, the mutated valine (Val6) of the β-2 chain forms a hydrophobic protrusion on the surface. Consequently, the protrusion of one Hb will fit in a pocket of another Hb. *Remember: valine is hydrophobic.







IMPORTANT

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

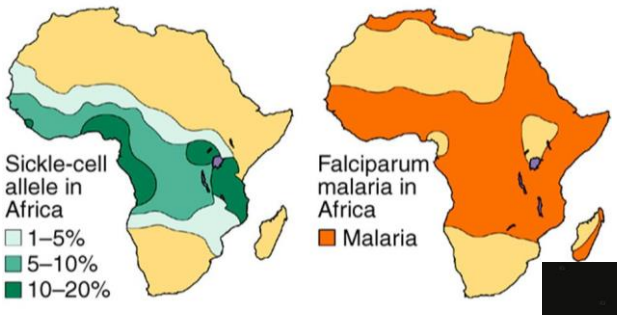
Variables that increase sickling

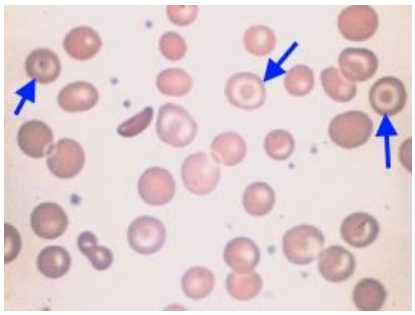
- **Decreased oxygen pressure (high altitudes)**
- **Increased pCO_2**
- **Decreased pH**
- **Increased 2,3-BPG**
- **Dehydration (why?)**

SICKLE CELL TRAIT

*Is common in areas where high rate of malaria such as central Africa.

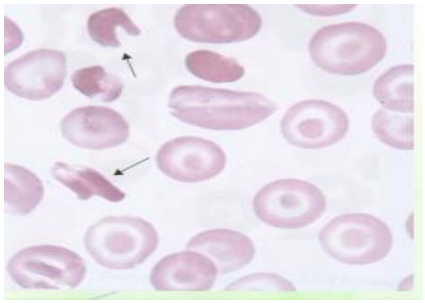
- **It occurs in heterozygotes (individuals with both HbA and HbS "have a normal gene and a sickle cell one"), who are clinically normal, but their cells sickle when subjected to low oxygen.**
- They experience more hemolysis than normal, 'cause the half life of RBCs will be reduced.
- **Advantage: selective advantage from plasmodium falciparum that causes malaria (they'll be immune against malaria). Why? Since their RBC's life spam will be shorter than malaria life cycle.**



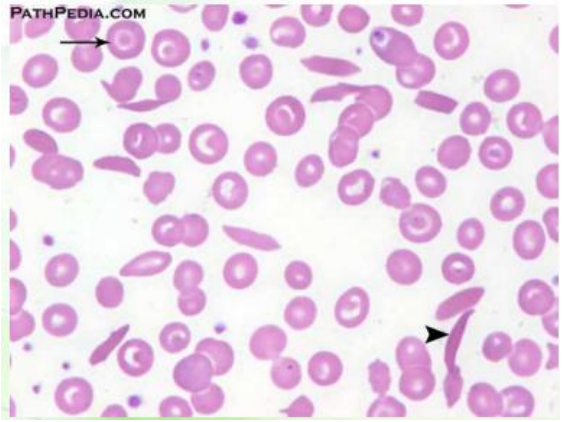


HEMOGLOBIN C (HBC)

- (HbC) is also due to a change at the 6th position of β globin replacing the glutamate (-ve AA) with lysine (+ve AA) (designated as βc).
- This hemoglobin is less soluble than HbA so it crystallizes in RBCs (so it's not dynamic) 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.

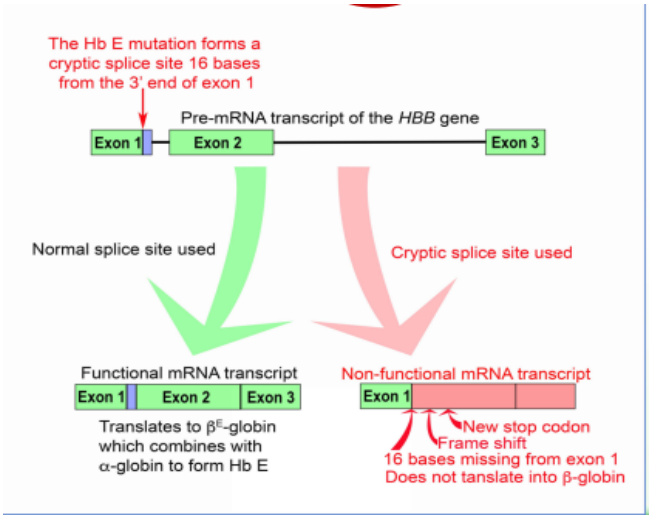


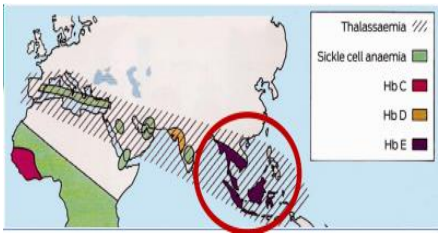
- Individuals with both βc and βs mutations have HbSC disease, a mild hemolytic disorder that may have no clinical consequences but is clinically variable. This patient has one β gene with mutation of sickle cell anemia and the other gene with c mutation and we call this HBSC disease, it's a likely moderate disorder.



HEMOGLOBIN E (HBE)

- **It is common in Southeast Asia.**
- **It has both quantitative and qualitative characteristics, so this mutation makes the protein quality compromised (unstable protein / reduced function), in addition the quantity of the protein is reduced.**
- **It is caused by a single point mutation in codon 26 that changes glutamic acid (GAG) to lysine (AAG) creating an alternative RNA splice site and a defective protein.**
- We talked before in molecular biology about **RNA splicing** (a process that removes the intervening, non-coding sequences of genes **(introns)** from pre-mRNA and joins the protein-coding sequences **(exons)** together in order to enable translation of mRNA into a protein), at the end of the exon there is a **splicing site** where one exon ends and the other starts.
- This mutation folds the cell, telling it that you can do **splicing on position 26** producing a truncated exon 1, so as a result of that, portion of the protein will be **deleted**, so the transcript will be non-functional producing a dysfunctional protein, so the amount of β chain will be reduced.
- **Individuals with this mutation make only around 60% of the normal amount of - globin protein.**
- **Mild disease but can be severe if coinherited with beta-thalassemia.**



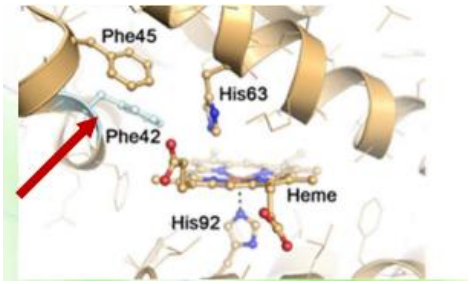


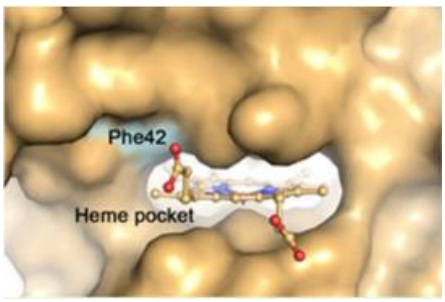
HB HAMMERSMITH

- Heme molecule is hydrophobic, and it's surrounded with hydrophobic AAs, and this hydrophobic environment around protein **(hydrophobic pocket)**, stabilizes the interaction between heme and protein. In this mutation, the hydrophobic **phenylalanine** changes intro the hydrophilic **serine** which affects the **hydrophobic pocket** and as a result the heme molecule becomes **unstable** and its affinity towards oxygen is reduced.

- **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 with a serine within the globin, reducing the hydrophobicity of the heme-binding pocket, heme positioning, and oxygen binding affinity causing cyanosis.**

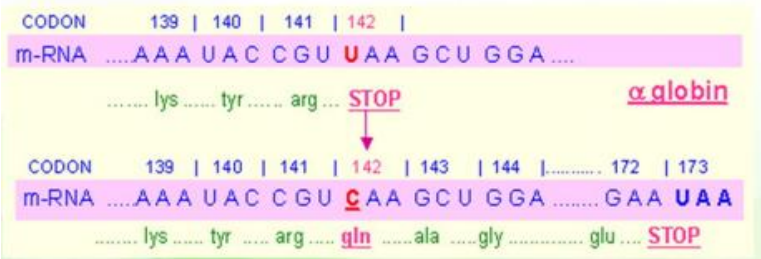




HB CONSTANT SPRING (HBCS)

- A mutation that leads to creation of codon of an AA instead of a stop codon, so when translation happens, instead of stopping in the stop codon rather translation will be continued until the next stop codon, creating a larger unstable protein than normal.
- **Hemoglobin Constant Spring (Hb CS) is an abnormal Hb caused by a mutation at the termination codon of the α2-globin gene leading to the production of unstable mRNA and protein products.**
  – The anemia is usually moderate.
- **Heterozygotes have the genotype (αα/ααCS) and have α-thalassemia trait phenotype.**
- **It is commonly found among Southeast Asian and Chinese people.**

- **If co-inherited with α-thalassemia, it leads to an α-thalassemia intermedia syndrome. Since that thalassemia is common in old world, the patient could have a combination of (α/β) thalassemia on one of the chromosomes and the other gene has a point mutation like Hb CS for example which makes the severity of the condition higher.**

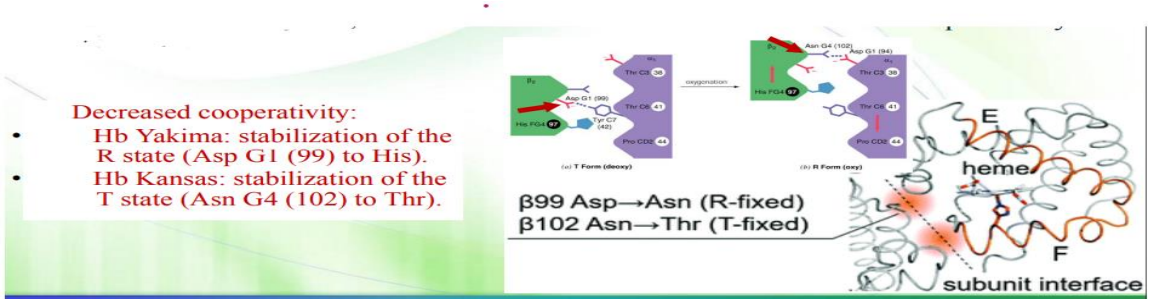


MUTATIONS AT α1-β2 CONTACTS

- Remember from the 1st lecture that we have an interaction between Asp 99 and Tyr 42 which stabilizes the T-state, and when oxygen binds to iron, α chain slides and an interaction happens between Asn 102 and Asp 94 which stabilizes the R-state. If Asp was deleted, T-state will not be stabilized anymore and the equilibrium shifts to R-state. And If a mutation is in Asn equilibrium shifts to T-state.

- **Hb Cowtown: Substitution of His146 (responsible for the Bohr Effect & sensing Ph around Hb) to Leucine produces more hemoglobin in the R state (increased affinity). Here His146 (last AA in β chain) is changed into leucine/any other AA, Hb will be mainly in R-state with high affinity to oxygen and it will not be able to release oxygen in tissues.**

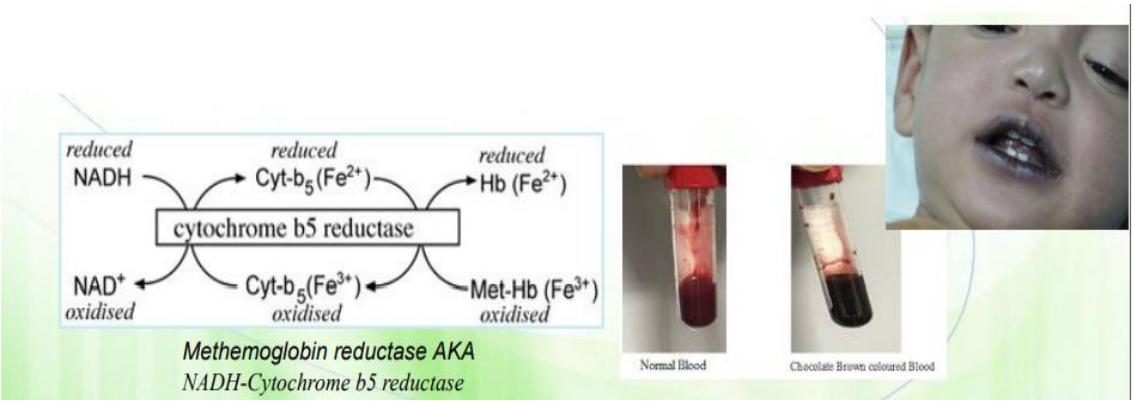
- **Elimination of hydrogen bonds between the chains can also alter the quaternary structure:**



ALTERED OXYGEN TRANSPORT

METHMOGLOBIN (HBM)

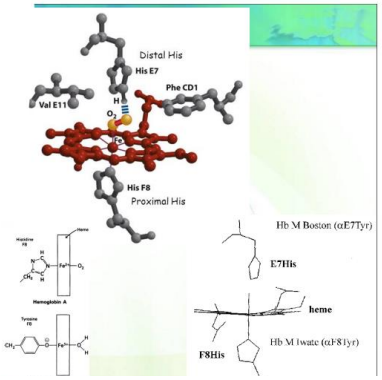
- For Hb to be functional, iron should be in **ferrous state (fe2+)**. Sometimes when oxygen is released from Hb, the iron could be **oxidated naturally to ferric state (fe3+)**, and as a result, this heme molecule will not be able to associate to oxygen. Normally oxidation of iron is **prevented** by hydrophobic AAs surrounding heme molecule. In case it happens, there is an enzyme called **Methemoglobin HBM (reductase)**, it reduces iron. HBM needs NADH **(from glycolysis)** to be functional.
- Patients with this condition are **bluish**, because Hb molecule is not fully bound to oxygen.
- **Oxyhemoglobin can undergo reversible oxygenation because its heme iron is in the reduced (ferrous, Fe+2) state.**
- **During oxygen release from heme, Fe+2 is oxidized to Fe+3, forming methemoglobin (HbM), except that the enzyme methemoglobin reductase reduces iron back.**
- **If not, a condition known as methemoglobinemia develops.**



➢ WHY HBM?

- **Some mutant globins (α and β) bond with heme in such a way as to resist the reductase.**
  - **Hb Boston: distal histidine is mutated into a tyrosine resulting in oxidation of ferrous iron by tyrosine's oxygen.**
  - **HbM Iwate: proximal histidine is replaced by a tyrosine.**

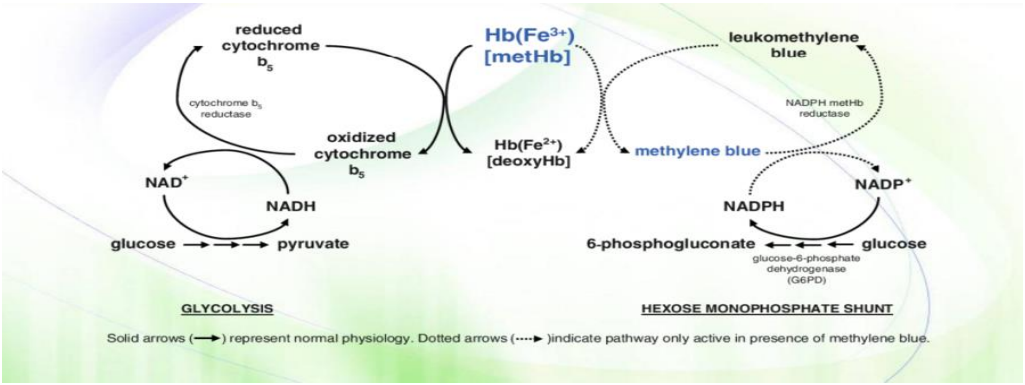
- A deficiency of the reductase enzyme.
- Certain drugs or drinking water containing nitrate.



➢ TREATMENT (methylene blue):

For the enzyme to be functional, it should be reduced, and reduce iron from ferric to ferrous, NADH which comes from glycolysis is required to keep enzyme active.

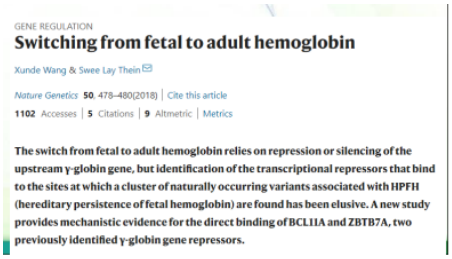
As treatment methylene blue is used, which is converted into active form and reduces iron. To regenerate the active molecule we need NADPH, which comes from glucose-phosphate pathway.



HERIDITARY PERSISTANCE OF FETAL HEMOGLOBIN (HPFH)

- Persons with HPFH continue to make HbF as adults. **No switch to HbA. Scientists are trying to study this mutation to treat thalassemia by induction of HbF in patients instead of destructed β globin chain.**
- Because the syndrome is benign most individuals do not even know they carry a hemoglobin abnormality.

- Many HPFH individuals harbor large deletions of the δ- and β -coding region of the cluster.
- There is no deletion of the fetal globin genes.
- 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.

HEMOGLOBIN ELECTROPHORESIS

- It's used for hemoglobinopathies diagnosis.
- Electrophoresis is basically separation of proteins based on size/ charge or both.
- In case of Hb, separation is done based on charge.
- Remember some of mutations we studied:

  - HbS (Glu to Val) >> -ve polar to non-polar
  - HbC (Glu to Lys) >> -ve to +ve

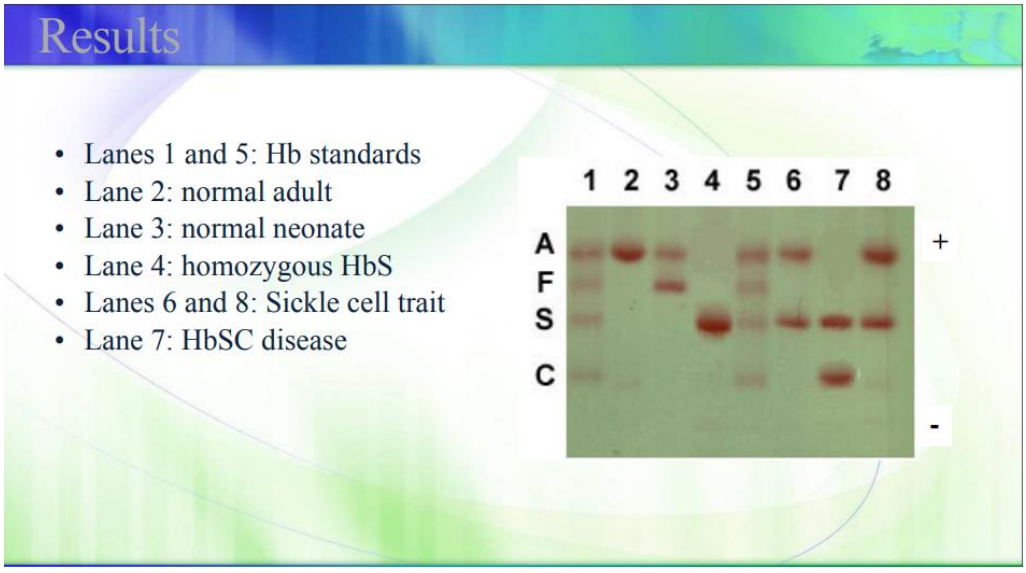
MUTATION AND MIGRATION

- 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.

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.



" ينبغي على شباب أمّة الإسلام أن يستيقظوا من غفلتهم، وأن يعلموا أنهم وإن كانوا غافلين، وإن كانوا نائمين، وإن كانوا لاهين لاعبين، فإنّ أعداءهم ليسوا كذلك، وأعداؤهم يمكرون بالليل والنهار"
-الشيخ أحمد السيد -

V2, page 7 , table and after the table