

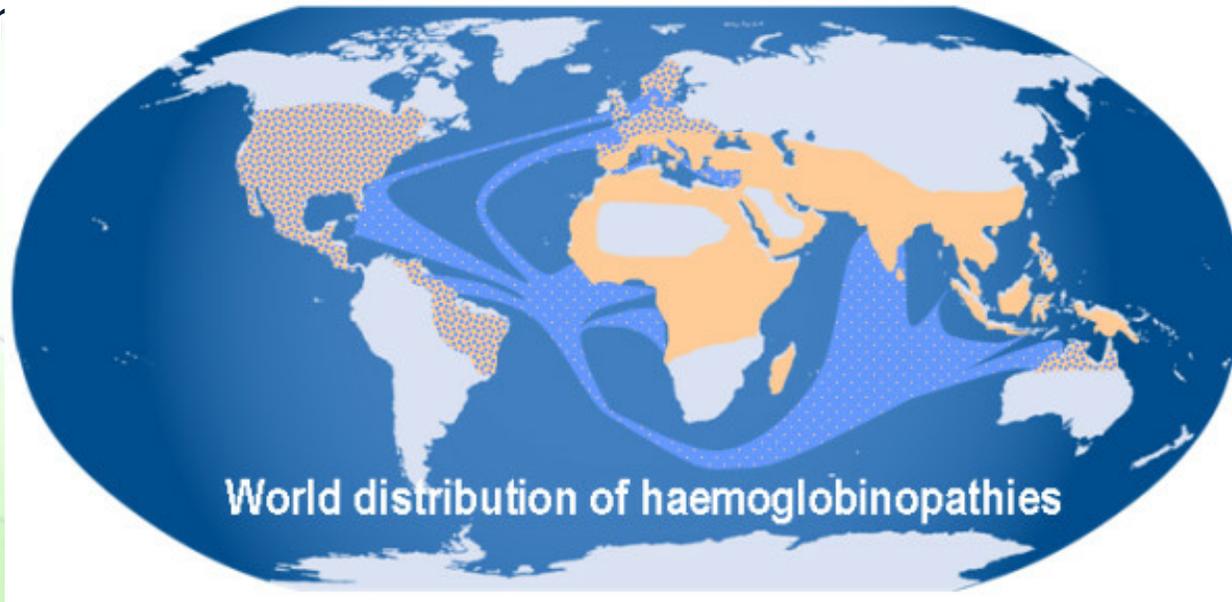


Hemoglobinopathies

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Hematopoietic-lymphatic system
2020

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).
- Hemoglobinopathies are a leading cause of death in children < 5 years.



Hereditary hemoglobins disorders

- Qualitative abnormalities: mutations resulting in structural variants.
 - Over 700 variants have been identified.
- Quantitative abnormalities are abnormalities in the relative amounts of α and β subunits (thalassemias).
- Hereditary persistence of fetal hemoglobin (HPFH): impairment of the perinatal switch from γ to β globin.

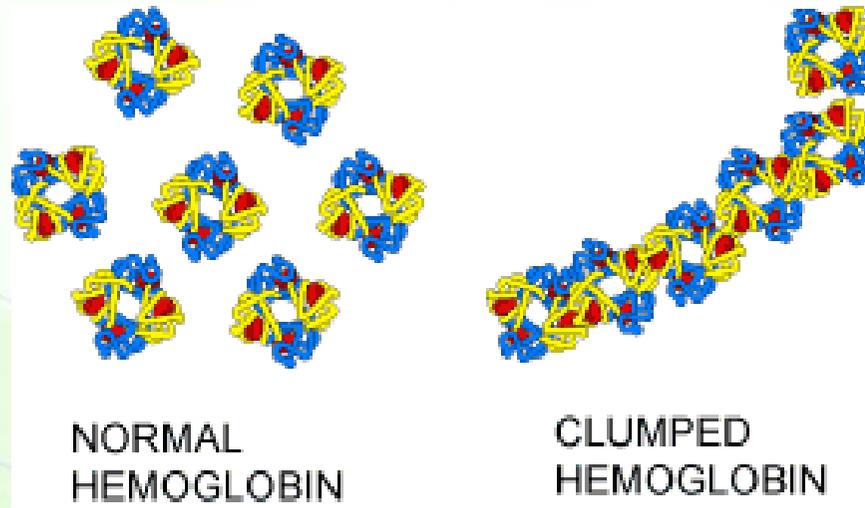
Qualitative abnormalities

Classification of molecular mutations

- Mutations in surface residues
 - Usually asymptomatic (HbE); an exception is HbS
- Mutations in internal residues
 - Often producing unstable hemoglobin, producing Heinz bodies and causing hemolytic anemia (Hb Hammersmith)
- Mutations stabilizing methemoglobin
 - Stabilizing heme-Fe⁺³; resulting in cyanosis
- Mutations at $\alpha 1$ - $\beta 2$ contacts
 - Altered oxygen affinity (mainly higher; a condition known as polycythemia)

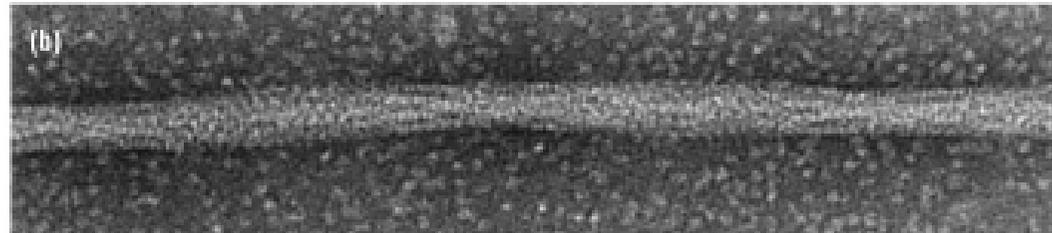
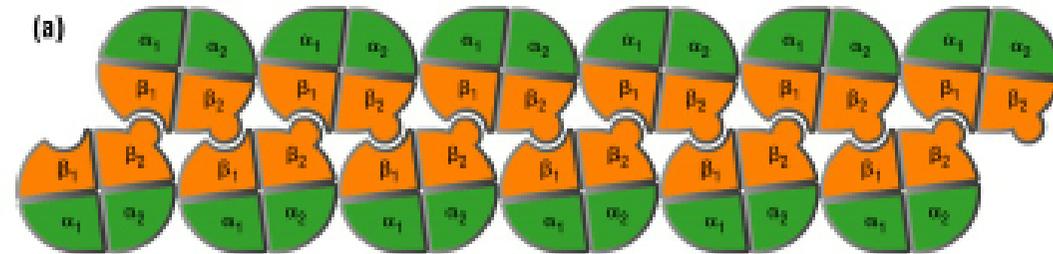
Sickle cell hemoglobin (HbS)

- It is caused by a change of amino acids in the 6th position of β globin (Glu to Val).
- The hemoglobin is designated $\alpha_2\beta_s_2$ or HbS.
- The hemoglobin tetramers aggregate into arrays upon deoxygenation in the tissues.
- This aggregation leads to deformation of the red blood cell.
- It can also cause hemolytic anemia (half-life is reduced from 120 days to <20 days)



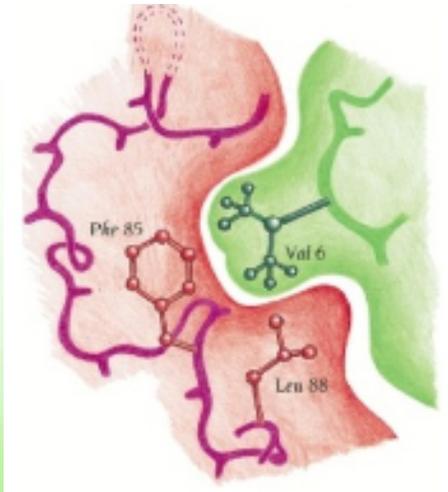
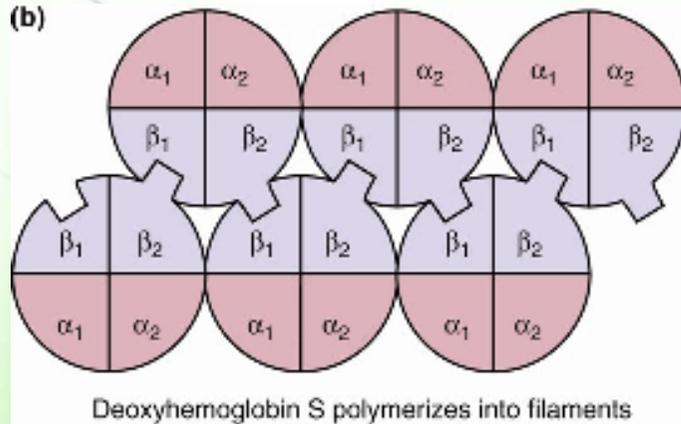
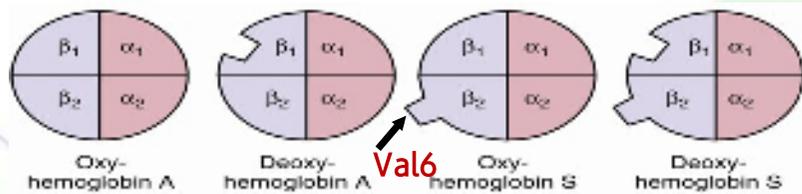
Cellular effect on system

- Repeated cycles of oxygenation and deoxygenation lead to irreversible sickling.
- 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 the internal organs, in particular the kidneys, heart and lungs.



How does the fiber form?

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

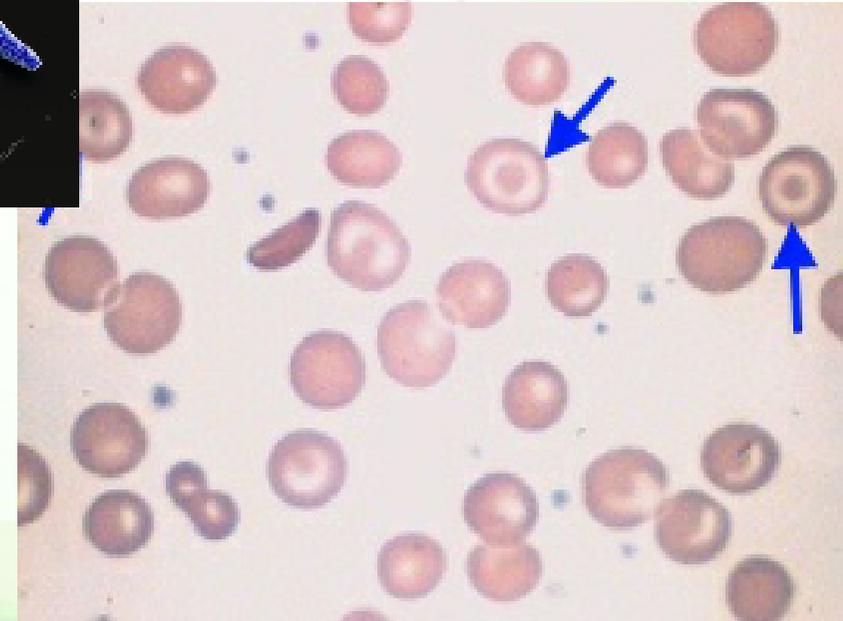
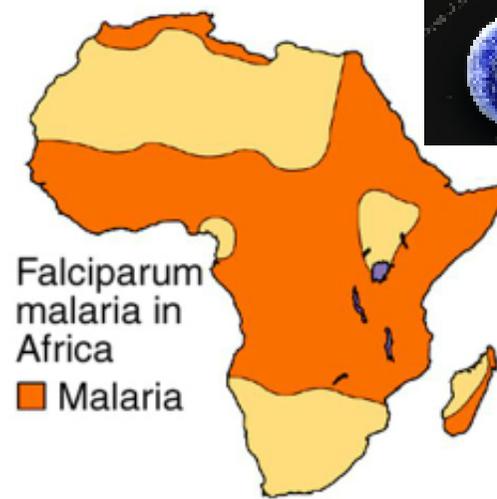
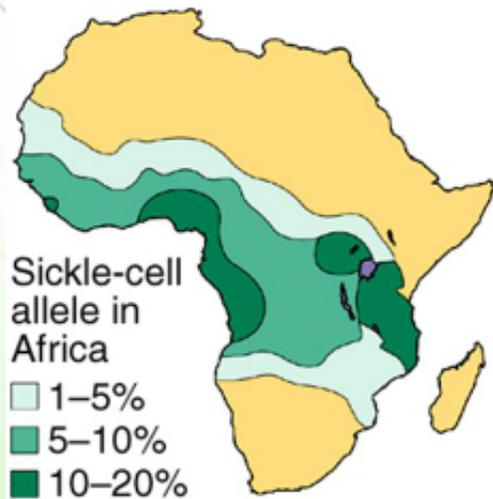


Variables that increase sickling

- Decreased oxygen pressure (high altitudes)
- Increased $p\text{CO}_2$
- Decreased pH
- Increased 2,3-BPG
- Dehydration

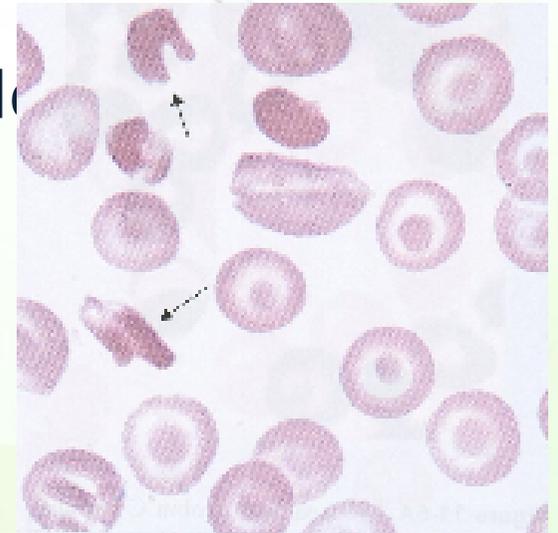
Sickle cell trait

- It occurs in heterozygotes (individuals with both HbA and HbS), who are clinically normal, but their cells sickle when subjected to low oxygen.
- Advantage: selective advantage from plasmodium falciparum that causes malaria. Why?



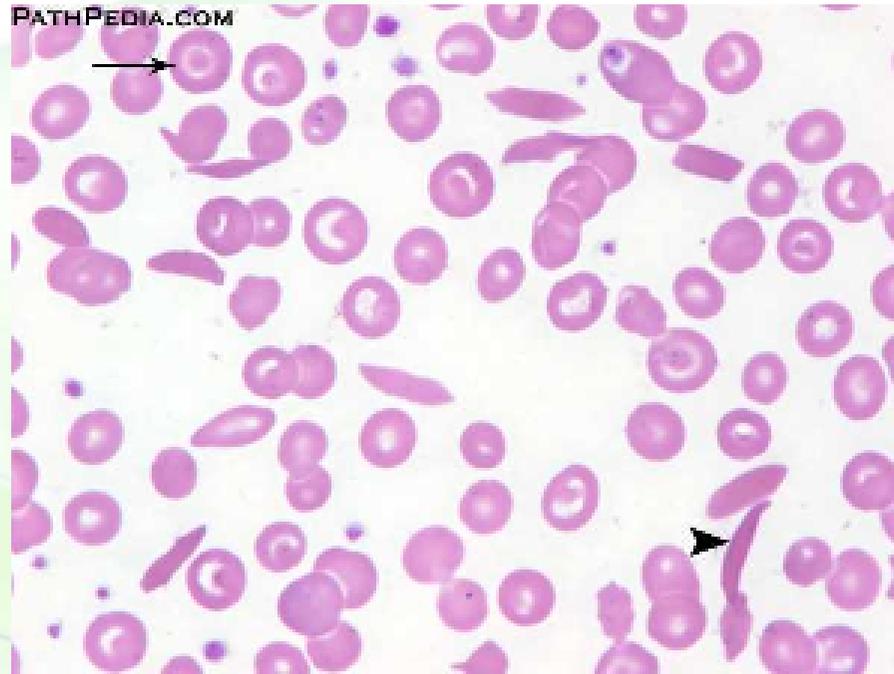
Hemoglobin C (HbC)

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



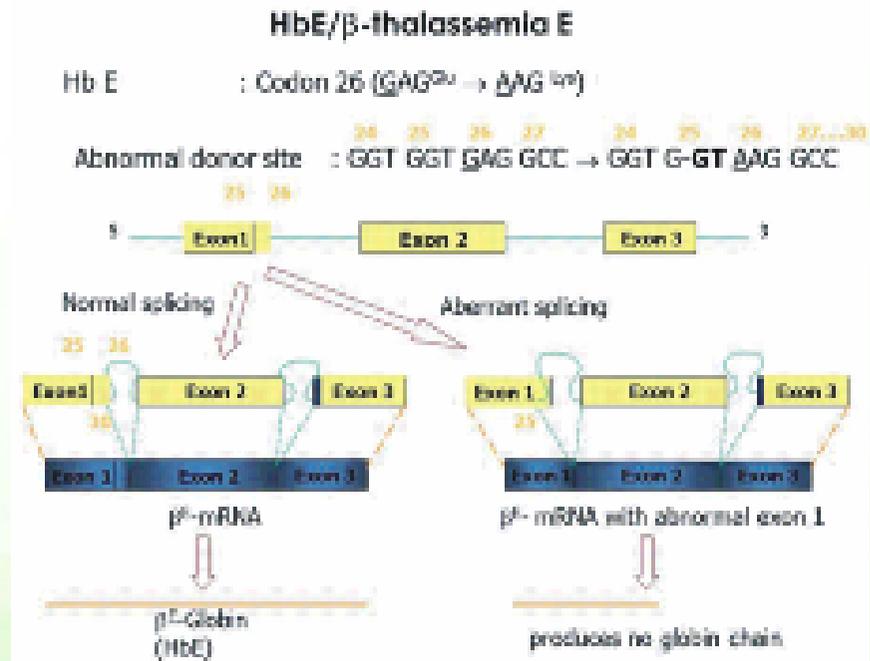
HbSC disease

- Individuals with both β^c and β^s mutations have HbSC disease, a mild hemolytic disorder which may have no clinical consequences, but it is clinically variable.



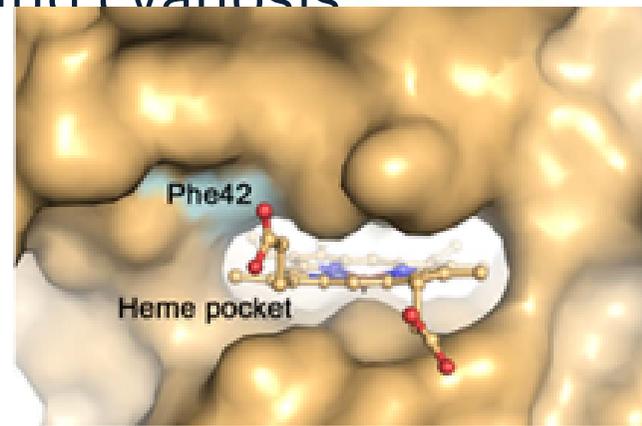
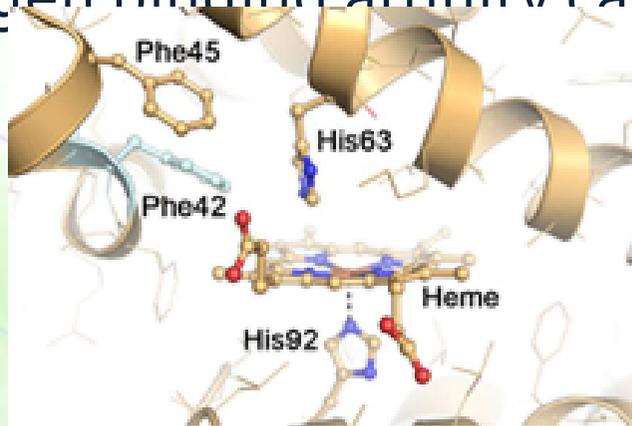
Hemoglobin E

- It is common in Southeast Asia
- It has both quantitative and qualitative characteristics.
- 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.



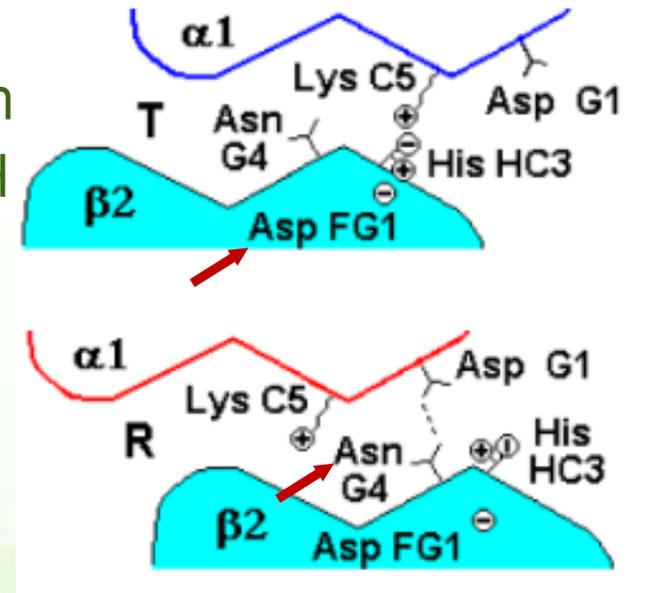
Hb Hammersmith

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



Stabilization of T/R state

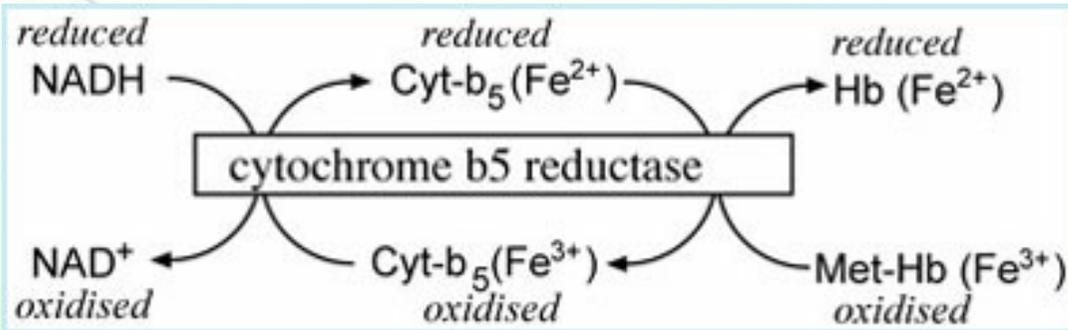
- Hb Cowtown: Substitution of His146 (responsible for the Bohr Effect) to Leucine produces more hemoglobin in the R state (increase affinity).
- Elimination of hydrogen bonds between the chains can also alter the quaternary structure:
 - Hb Kansas: stabilization of the T state (Asn to th
 - Hb Yakima: stabilization of the R state (Asp to H



Altered Oxygen Transport

Methemoglobin (HbM)

- 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.



*Methemoglobin reductase AKA
NADH-Cytochrome b5 reductase*



Normal Blood



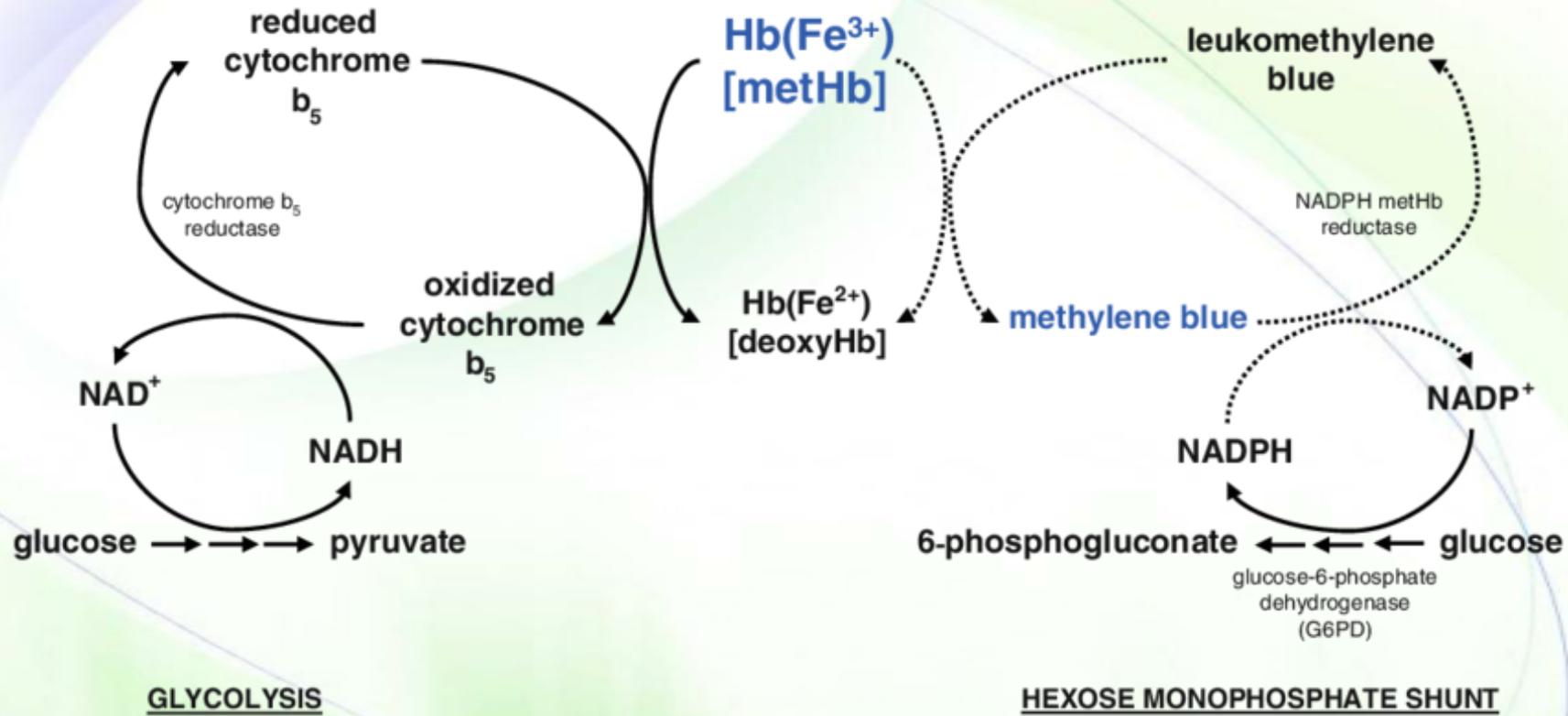
Chocolate Brown coloured Blood



Why HbM?

- Some mutant globins (α and β) bond 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. 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.

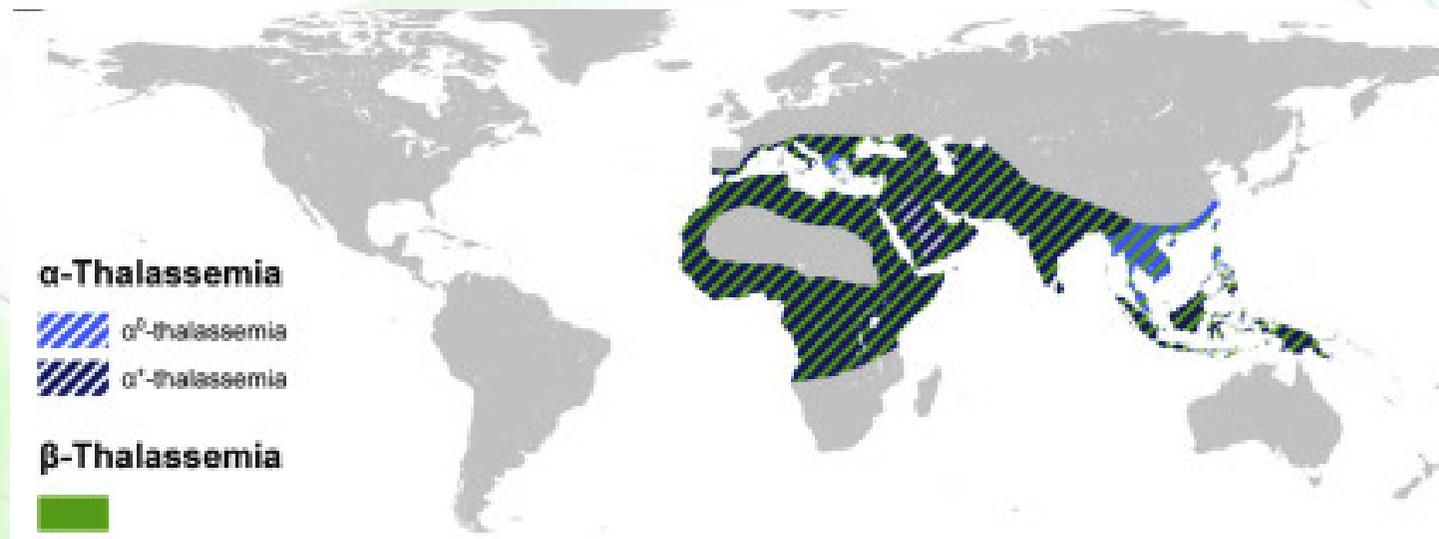
Treatment (methylene blue)



Quantitative abnormalities (thalassemias)

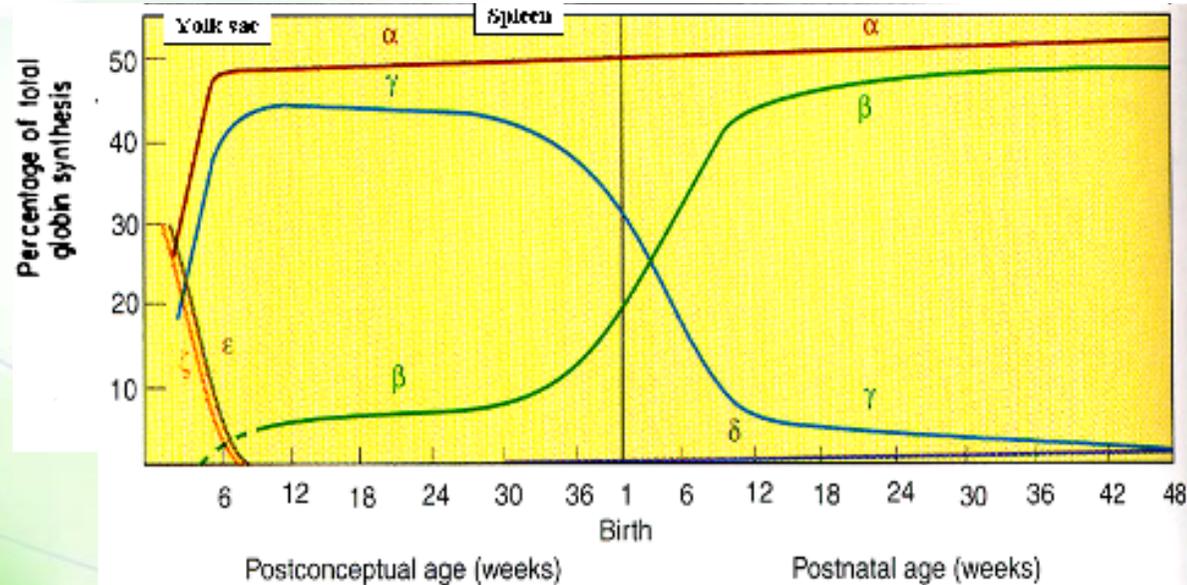
Thalassemias

- 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.



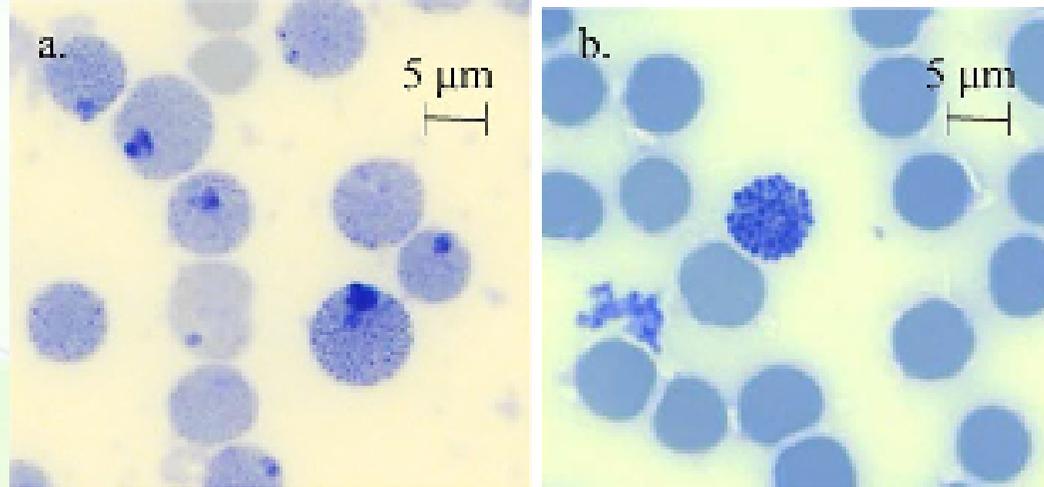
The Alpha-Thalasseмииs

- 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.



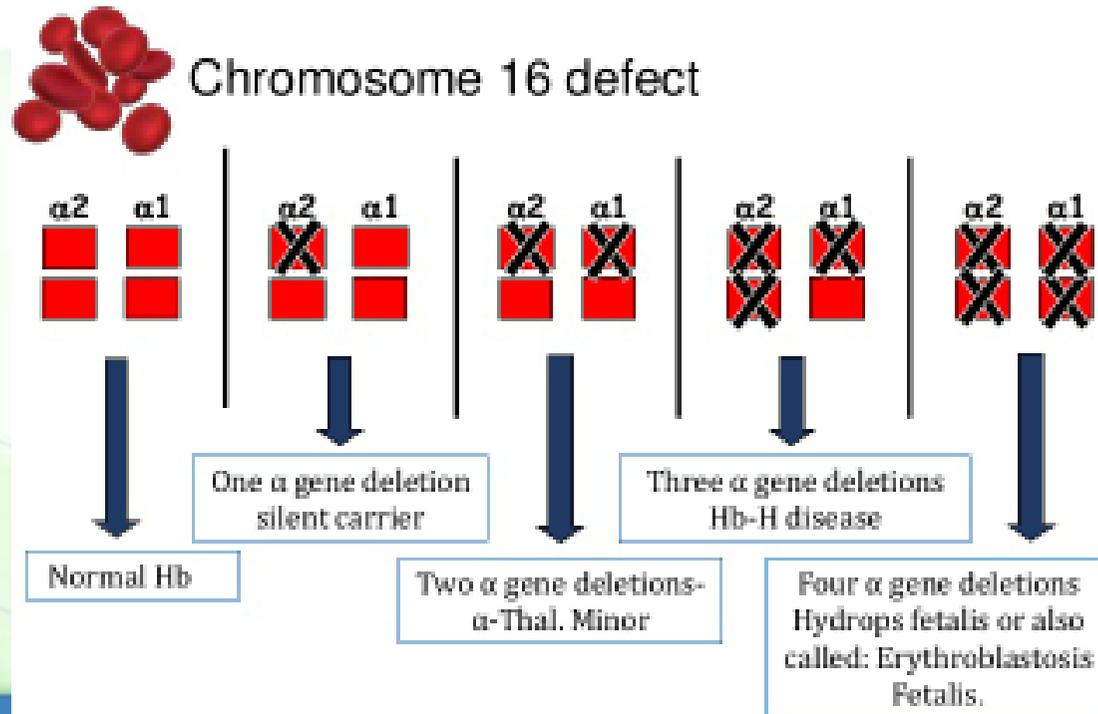
HbH

- With reduction of α chain production, and β -chain production is established, homotetramers of β (β_4 or HbH) are formed.
- The HbH tetramers have a markedly reduced oxygen carrying capacity.
- Main type of mutation is deletion (rarely point mutations)



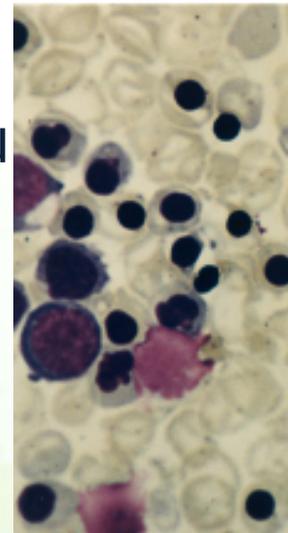
Variable severity

- With the 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 each individual has 4 genes.



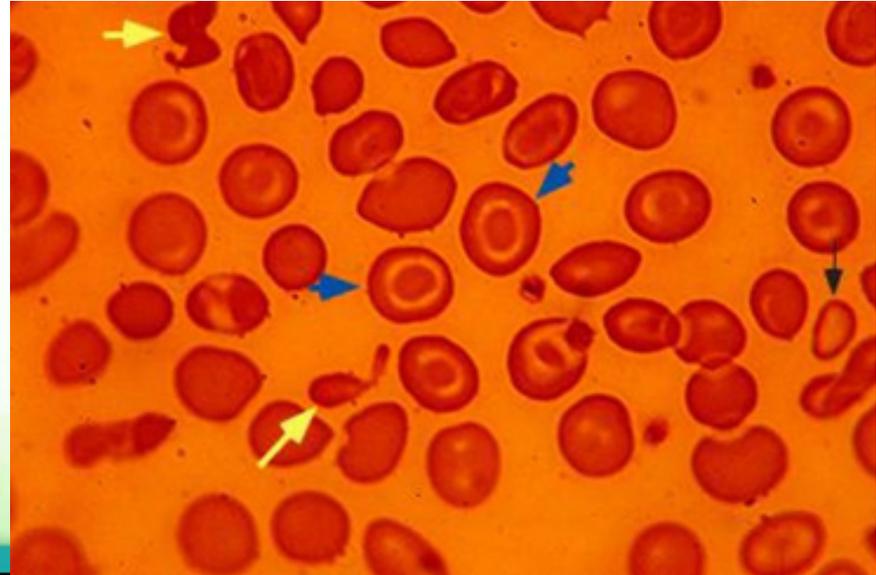
Hydrops fetalis

- 4 of 4 genes are deleted.
- The predominant fetal hemoglobin is a tetramer of γ -chains.
- γ_4 or Hb Bart: a homotetramer of γ .
- Hb Bart has no oxygen carrying capacity resulting in oxygen starvation in the fetal tissues.
- This situation is called hydrops fetalis.
- Stillbirth or death shortly after birth occurs.



Hemoglobin H disease

- 3 of 4 genes deleted.
- Mild to moderate hemolytic anemia in adults.
- A high level of β_4 tetramer is present.
- Clinically, it is known as hemoglobin H disease.
- The disease is not fatal.



Minor α -thalassemia and silent carrier

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

Summary of α -thalassemias

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

^aNumber of normal alpha globin genes

The beta-thalasseмииs

- β -globins are deficient and the α -globins are in excess and will form α -globin homotetramers.
- Main type of mutation is point mutations, 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.

β -thalassemia major

- A complete lack of HbA is denoted as β^0 -thalassemia or β -thalassemia major.
- Afflicted individuals suffer from severe anemia beginning in the first year of life and need blood transfusions.
 - Long-term transfusions lead to the accumulation of iron in the organs, particularly the heart, liver and pancreas and , finally, death in the teens to early twenties.

β -Thalassemia minor

- Individuals heterozygous for β -thalassemia is termed β - thalassemia minor.
- Afflicted individuals carry one normal β -globin gene and a mutated gene.
- Thalassemia minor individuals 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	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)

- 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.
- 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

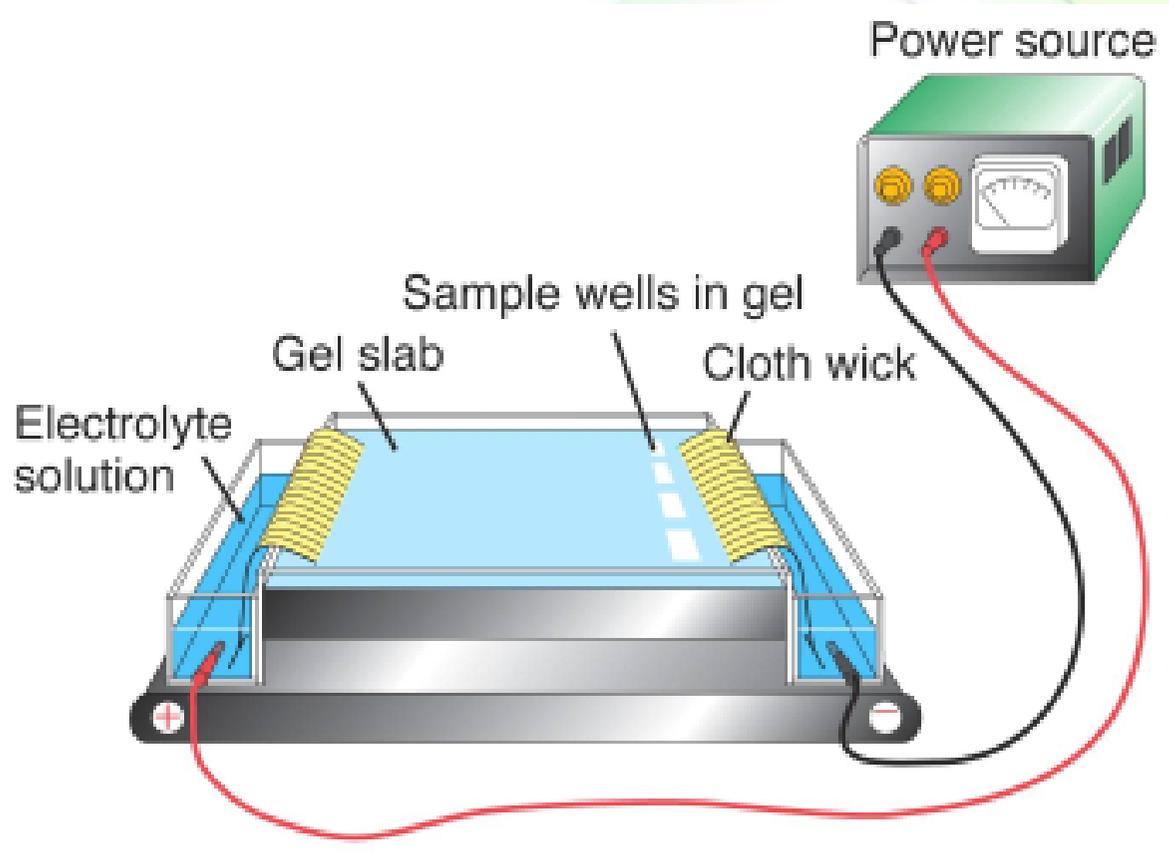
Xunde Wang & Swee Lay Thein 

Nature Genetics 50, 179–180(2018) | [Cite this article](#)

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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



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.

