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 with the disorder).
- Hemoglobin disorders account for 3.4% of deaths in children < 5 years

Quantitative abnormalities in the hemoglobin (Thalassemia)

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

The α-Thalassemias

- Alpha-thalassemia: **underproduction** of the α-globin chains.
- Mainly a **deletion mutation** that causes the gene to be non-functional
- 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, 2 on each chromosome**

Variable severity

from the most severe to the least

α-thalassemia major (Hydrops fetalis) or [Hb Bart] (most severe)

- 4 of 4 genes are deleted.
- The predominant fetal hemoglobin is a **tetramer of γ-chains**.
- γ4 or Hb Bart: a hometetramer of γ.
- Hb Bart has a high affinity towards oxygen.
- This condition is called hydrops fetalis.
- Stillbirth (death in the womb) or death shortly after birth occurs.
- This condition causes ***severe anemia and fluid accumulation under the skin*** (hydrops fetalis), often resulting in fetal death.
- In this case, the loss of all alpha chains leads to the formation of hemoglobin composed of four gamma chains (Hb Bart's), which **cannot release oxygen effectively**. Death usually occurs before or shortly after birth

α-thalassemia intermedia or Hemoglobin H (HbH) disease

- 3 of 4 genes deleted.
- Clinically, it is known as hemoglobin H disease.
- With the reduction of a chain production, and b-chain production is established, there is a chance that **hometetramers of b (b4 or HbH)** can form, with low normal hemoglobin.
- 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**).
- Mild to moderate, not severe, ***hemolytic anemia in adults,*** **may need blood transfusions**. May shift to the severe side.
- The disease is not fatal.
- The main type of mutation is deletion

α-thalassemia minor and silent carrier

α-thalassemia trait

If 2 of the 4 genes are inactivated.

- The individuals have **mild microcytic anemia**

α-thal minor: Asymptomatic
Mild microcytic anemia

Silent carrier

1 of 4 genes deleted.

- Individuals are completely asymptomatic.

Carrier: Asymptomatic
No abnormalities

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

*Number of normal alpha globin genes

Note microcytic anemia has to do with the shape of the RBC while hemolytic has to do with lysis of the RBCs.

The β-Thalassemias

- β-globins are deficient and the **α**-globins are in excess and will form **α- globin hometetramers.**
- The main types of mutation are **point mutations** that lead to non- functional protein.
- The mutations occur within the promoter or LCR (enhancer), the exon, translation initiation codon, splicing positions, or poly-adenylation termination signal.
- The **α**-globin hometetramers are extremely insoluble, which leads to premature red cell destruction in the bone marrow and spleen.
- Delta and gamma are common globin become apparent

Beta thalassemia has **two types** because there is one beta gene on each chromosome. Either there is no beta chain production at all, or there is partial production.

severity

from the most severe to the least

β-thalassemia major (Cooley's anemia)

- A complete lack of HbA is denoted as b0 thalassemia or b-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, particularly the heart, liver and pancreas and, finally, death in the teens to early twenties. Can be treated with ***iron chelators*** which improve the prognosis.
- This condition is survivable but causes extremely severe anemia. This may cause the body to **continue producing fetal hemoglobin since beta chains** are produced later while alpha chains are produced from the start. And patient can live

β-thalassemia minor

- Individuals heterozygous for β- thalassemia with one normal β-globin gene and a mutated gene are termed b- thalassemia minor.
- Individuals with beta- thalassemia minor are generally asymptomatic with ***mild anemia***

Common genotypes	Name	Phenotype
β/β	Normal	None
β/β+	Beta thalassemia trait	Thalassemia minor: asymptomatic, mild microcytic hypochromic anemia
β/β0	Beta thalassemia intermedia	Variable severity Mild to moderate anemia Possible extramedullary hematopoiesis Iron overload
β+/β+ β+/β0 β0/β0 β+/β0	Beta thalassemia major (Cooley's Anemia)	Severe anemia Transfusion dependence Extramedullary hematopoiesis Iron overload

β+: complete lack of β chain
β0: some expression of β chain
β: normal expression of β chain
β*: point mutation that is characterized by substitution of a.a. 26 from Glu to Lys on the surface of the b molecule that causes lower production of B chain.

that in the 2nd raw the thalasemia is minor when half the beta is functional.

In the third raw there is some partial functional b thalesemia produced B+, however βε βo is more on the severe end

homozygous βε βε leads to mild microcytic anemia as it causes the production of B chain to be reduced

Hereditary persistence of fetal hemoglobin

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

- Fetal hemoglobin has a higher affinity for oxygen than adult hemoglobin. In conditions where fetal hemoglobin persists after birth, symptoms are usually mild or absent.
- 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!!

Qualitative abnormalities in hemoglobin

mutations resulting in structural variants.

- **Over 800 variants have been identified.**

caused by **point mutations**, and most of them occur in the **beta** globin chain.

Mutations in surface residues

Sickle cell hemoglobin (HbS)

- It is caused by a change of amino acids in the 6th position of ***β*** globin (Glu negatively charged to Val hydrophobic).
- This change causes the hemoglobin molecules to stick together and form long fibers. Therefore, during electrophoresis at alkaline pH, because of the less negative charge of Hb S, it ***migrates more slowly toward the anode*** (positive electrode) than does Hb A. The hemoglobin is designated **α2*β*s2** or HbS
- The hemoglobin tetramers ***aggregate into arrays upon deoxygenation*** in the tissues and increase their sickle trait upon conditions that increase the deoxygenation such as high altitudes. When hemoglobin binds to CO_2 or 2,3- bisphosphoglycerate, all these interactions increase sickling.
- 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).
- Sickle cell anemia is characterized by lifelong ***episodes of pain ("crises")***, the pain in sickle cell anemia occurs due to occlusion of small arteries and veins, which can also lead to stroke, and increased susceptibility to infections, usually beginning in early childhood. Other symptoms include acute chest syndrome, stroke, splenic and renal dysfunction, and bone changes due to marrow hyperplasia

(HbS) control

- adequate hydration
  Hydration is an important management measure, as dehydration increases blood viscosity and the likelihood of red blood cell sickling (clumping).
- analgesics
- aggressive antibiotic therapy
  if infection is present or transfusion is done
- Intermittent transfusions
  reduce the risk of stroke, but the benefits must be weighed against the complications of transfusion, which include iron overload (**hemosiderosis**), **bloodborne infections**, and **immunologic complications**.
- Hydroxyurea
  antitumor drug, is therapeutically useful because it increases circulating levels of Hb F, which decreases RBC sickling

How does the fiber form?

- Fiber formation only occurs in the deoxy- or T-state.
- The mutated valine of the ***β*2** chain is protruded and inserted into a hydrophobic pocket on the surface of ***β*1** chain

Variables that increase sickling

(simply anything that leads to **deoxygenation**)

- Decreased oxygen pressure (high altitudes)
- Increased pCO2
- Decreased pH
- Increased 2,3-BPG
- Dehydration (why?)
- Because dehydration increases the concentration of the hemoglobin

Sickle cell trait

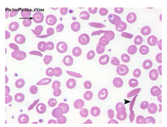
- It occurs in **heterozygotes** (individuals with both HbA and HbS), who are clinically normal or show less severe symptoms relative to those with **homozygous trait**, but they have few cells that sickle when subjected to low oxygen. They have lower potential to sickle than the homozygote trait.
- Generally, sickle cells have ***selective advantage against malaria*** because they have a shorter life span, so the parasite, **plasmodium falciparum** will not have enough time to complete the intracellular stage of its development

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 dehydration occurs because the HbC mutation in the beta- globin chain changes the charge and solubility of hemoglobin inside red blood cells, this activates a potassium-chloride cotransporter (KCC) leading to **increased cellular loss of potassium and water**.
- This problem causes only a **minor hemolytic disorder**

HbSC disease

- Individuals with both ***β***c and ***β***s mutations have HbSC disease, a ***mild to severe hemolytic disorder*** that may have no clinical consequences but is clinically variable



Hemoglobin E

Mild disease but can be severe if co-inherited with beta-thalassemia, where the genotype is fE/β0, leading to low/mutated production and no production respectively.

quantitative characteristics

(lead to less HB production)

Individuals with this mutation make only around 60% of the normal amount of b-globin protein making it some sort of thalassemia.

qualitative characteristics

(the point mutation affects the functionality; however, it is towards the milder end)

It can cause alternative RNA splice site and an **earlier stop codon** which causes lower production of hemoglobin leading to ***microcytic anemia***

It can cause a point mutation in codon 26 that changes glutamic acid (GAG) to lysine (AAG) creating a slightly different hemoglobin

Mutations in internal residues

Hb Hammersmith

- Hb Hammersmith results from a **point mutation** that leads to formation of unstable hemoglobin and denaturation of the globin protein.
- The most common point mutation of Hb Hammersmith **substitutes** an internal **phenylalanine (hydrophobic A.A)** with a **serine (positively charged)** within the ***β*** **globin**, reducing the hydrophobicity of the heme-binding pocket, heme positioning, and lower oxygen binding affinity causing ***cyanosis***.

Hb ConstantSpring (Hb CS) Not HbSC

- 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 longer than normal unstable mRNA and protein products.
- The **anemia is usually moderate.**
- Heterozygotes have the genotype (αα/ααCS) and have **α°-thalassemia trait phenotype**, like if it was (αα/α°α°).
- It is commonly found among Southeast Asian and Chinese people.
- If **co-inherited** with α-thalassemia, it leads to an ***α°-thalassemia intermedia syndrome*** like if it was (αα°/α°α°)

Mutations at α1-β2 contacts

Eliminating hydrogen bonds between the chains can also alter the quaternary structure and affects cooperativity

Hb Cowtown

Substitution of **His146** (responsible for the Bohr Effect) to **leucine** produces more hemoglobin in *the R state* (increased affinity) **reducing O2 supply to the tissue**.

Hb Yakima

stabilization of *the R state* (**Asp G1 (99) to His**) **reducing O2 supply to the tissue**

Hb Kansas

stabilization of *the T state* (**Asn G4 (102) to Thr**) **less binding of O2in the lungs**.

Mutations stabilizing methemoglobin

HbM

- Hemoglobin can be reversibly oxygenated because iron remains in the reduced (ferrous, Fe+2) state.
- **Oxygen binding** to Fe+2 may cause the **oxidation of Fe+2 to Fe+3**, forming methemoglobin (HbM), except that *the enzyme methemoglobin reductase* reduces iron back.
- Symptoms are related to the degree of tissue hypoxia, and include anxiety, headache, and dyspnea.

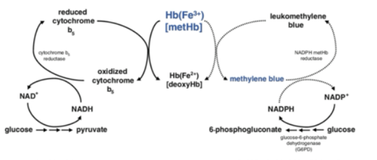
In rare cases, coma and death can occur. Treatment is with **methylene blue**, which is **oxidized** as **Fe+3 is reduced**

- The erythrocytes of newborns have approximately half the capacity of those of adults to reduce methemoglobin.

non-reversible

Some mutant globins (***α-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.***

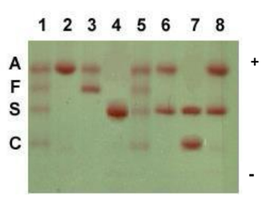


Solid arrows (⟶) represent normal physiology. Dotted arrows (⇢) indicate pathway only active in presence of methylene blue.

NADPH reduces methylene blues to leukomethylene blue which reduced Fe+3 to Fe+2, NADPH comes from the Hexose monophosphate shunt

reversible

such as those caused by: Certain drugs or drinking water containing nitrates.

Results

- Lanes **1** and **5**: **Hb standards**
- Lane 2: normal adult
- Lane 3: normal neonate
- Lane 4: homozygous HbS
- Lanes 6 and 8: Sickle cell trait
- Lane 7: HbSC disease