THE HEMOGLOBIN GENES AND THEIR DISFUNCTIONS

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The hemoglobin molecule

Mammalian hemoglobins (molecular weights of about 64,500) are composed of four peptide chains called **globins**, each of which is bound to a **heme**. Normal human hemoglobin of the adult is composed of a pair of two identical chains (α and β).

Iron is coordinated to four pyrrole nitrogens of protoporphyrin IX, and to an imidazole nitrogen of a histidine residue from the globin side of the porphyrin. The sixth coordination position is available for binding with **oxygen** and other small molecules.

A model of hemoglobin at low resolution. The α chains in this model are yellow, the β chains are blue, and the heme groups red.
A problem of development

- The mammalian fetus obtain oxygen from maternal blood (in the placenta), not from air. How can fetus’s blood accomplish this?
- The solution involves the development of a **fetal hemoglobin**. Two of the four peptides of the fetal and adult hemoglobin chains are identical, the alpha (α) chains, but adult hemoglobin has two beta (β) chains, while the fetus has two gamma (γ) chains. As a consequence, fetal hemoglobin can bind oxygen more efficiently than can adult hemoglobin. This small difference in oxygen affinity mediates the transfer of oxygen from the mother to the fetus. Within the fetus, the myoglobin of the fetal muscles has an even higher affinity for oxygen, so oxygen molecules pass from fetal hemoglobin for storage and use in the fetal muscles.

In the placenta, there is a net flow (arrow) of oxygen from the mother's blood (which gives up oxygen to the tissues at the lower oxygen pressure) to the fetal blood, which is still picking it up.
Fetal hemoglobins

In human fetuses, until birth, about 80 percent of β chains are substituted by a related γ chain. These two polypeptide chains are 75 percent identical, and the gene for the γ chain is close to the b-chain gene on chromosome 11 and has an identical intron-exon structure. This developmental change in globin synthesis is part of a larger set of developmental changes that are shown in Figure below. The early embryo begins with α, γ, ε, and ζ chains and, after about 10 weeks, the ε and ζ are replaced by α, β, and γ. Near birth, β replaces γ and a small amount of yet a sixth globin, δ, is produced. The normal adult hemoglobin profile is 97% α₂β₂, 2-3% α₂δ₂, and 1% α₂γ₂.

Developmental changes in the synthesis of the α-like and β-like globins that make up human hemoglobin.
Organization of globin gene family in human

- The β, δ, γ, and ε chains all belong to a "β-like" group; they have very similar amino acid sequences and are encoded by genes of identical intron-exon structure that are all contained in a 60-kb stretch of DNA on chromosome 11.

- The α and ζ chains belong to an "α-like" group and are encoded by genes contained in a 40-kb region on chromosome 16. Two slightly different forms of the a chain are encoded by neighboring genes with identical intron-exon structure, as are two forms of the ζ chain.

- In addition, both chromosome 11 and chromosome 16 carry pseudogenes, labeled Ψα and Ψβ. These pseudogenes are duplicate copies of the genes that did not acquire new functions but accumulated random mutations that render them nonfunctional.

- At every moment in development, hemoglobin molecules consist of two chains from the "α-like" group and two from the "β-like" group, but the specific members of the groups change in embryonic, fetal, and newborn life. What is even more remarkable is that the order of genes on each chromosome is the same as the temporal order of appearance of the globin chains in the course of development.
Chromosomal locations of globin genes

Chromosomal distribution of the genes for the $\alpha$ family of globins on chromosome 16 and the $\beta$ family of globins on chromosome 11 in humans.

Gene structure is shown by black bars (exons) and colored bars (introns).
Globin genes and hemoglobin molecules

- The various forms of hemoglobin molecules and the genes from which they are coded
Summary of hemoglobin types

There are hundreds of hemoglobin variants that involve genes both from the alpha and beta gene clusters. The list that follows touches on some of the more common normal and abnormal hemoglobin variants.

Normal Hemoglobins

- **Hemoglobin A.** This is the designation for the normal hemoglobin that exists after birth. Hemoglobin A is a tetramer with two alpha chains and two beta chains ($\alpha_2\beta_2$).

- **Hemoglobin A2.** This is a minor component of the hemoglobin found in red cells after birth and consists of two alpha chains and two delta chains ($\alpha_2\delta_2$). Hemoglobin A2 generally comprises less than 3% of the total red cell hemoglobin.

- **Hemoglobin F.** Hemoglobin F is the predominant hemoglobin during fetal development. The molecule is a tetramer of two alpha chains and two gamma chains ($\alpha_2\gamma_2$).
The role of hemoglobinopathies

Hemoglobinopathies occupy a special place in human genetics for many reasons:

- They are by far the most common serious Mendelian diseases on a worldwide scale
- Globins illuminate important aspects of evolution of the genome and of diseases in populations
- Developmental controls are probably better understood for globins than for any other human genes
- More mutations and more diseases are described for hemoglobins than for any other gene family
- Clinical symptoms follow very directly from malfunction of the protein, which at 15 g per 100 ml of blood is easy to study, so that the relationship between molecular and clinical events is clearer for the hemoglobinopathies than for most other diseases
Some clinically significant variant hemoglobins

- **Hemoglobin S** ($\alpha_2\beta^S_2$, severe). This the predominant hemoglobin in people with sickle cell disease.

- **Hemoglobin C** ($\alpha_2\beta^C_2$, relatively benign). This results from a mutation in the beta globin gene and is the predominant hemoglobin found in people with hemoglobin C disease.

- **Hemoglobin E** ($\alpha_2\beta^E_2$, benign). This variant results from a mutation in the hemoglobin beta chain. People with hemoglobin E disease have a mild hemolytic anemia and mild splenomegaly. Hemoglobin E is common in S.E. Asia.

- **Hemoglobin Constant Spring** (named after isolation in a Chinese family from the Constant Spring district of Jamaica). (severe). In this variant, a mutation in the alpha globin gene produces an alpha globin chain that is abnormally long. Both the mRNA and the alpha chain protein are unstable.

- **Hemoglobin H**. ($\beta_4$, mild). This is a tetramer composed of four beta globin chains: it occurs only with extreme limitation of alpha chain availability. Hemoglobin H forms in people with three-gene alpha thalassemia as well as in people with the combination of two-gene deletion alpha thalassemia and hemoglobin Constant Spring.

- **Hemoglobin Barts** ($\gamma_4$, lethal). With four-gene deletion alpha thalassemia no alpha chain is produced. The gamma chains produced during fetal development combine to form gamma chain tetramers. Individuals with four-gene deletion thalassemia and consequent hemoglobin Barts die in utero (hydrops fetalis).
Two groups of hemoglobinopathies

Hemoglobinopathies are classified into two main groups:

- The **thalassemias** are generally caused by **inadequate quantities** of the polypeptide chains that form hemoglobin.
  - The most frequent forms of thalassemia are therefore the **α- and β-thalassemias**
  - Alleles are classified into those producing no product (α₀, β₀) and those producing reduced amounts of product (α⁺, β⁺).

- Abnormal hemoglobins with **amino acid changes** cause a variety of problems, of which **sickle cell disease** is the best known.
  - In sickle cell disease, a missense mutation (glutammmic acid to valine at codon 6) replaces a polar by a neutral amino acid on the outer surface of the β-globin molecule.
  - Other amino acid changes can cause anemia, cyanosis, polycythemia (excessive numbers of red cells), methemoglobinemia (conversion of the iron from the ferrous to the ferric state), etc.
Sickle cell anemia

The **E6V** (glutamic acid to valine at codon 6) mutation replaces a polar by a neutral amino acid on the outer surface of the β-globin molecule. The red blood cells of people with sickle cell disease contain an abnormal type of hemoglobin, called **hemoglobin S**. The deficiency of oxygen in the blood causes hemoglobin S to crystallize, distorting the red blood cells into a sickle shape, making them fragile and easily destroyed, leading to anemia. Sickled red cells have decreased survival time (leading to anemia) and tend to occlude capillaries, leading to ischemia and infarction of organs downstream of the blockage.

Electrophoresis of hemoglobin from an individual with sickle-cell anemia, a heterozygote (called sickle-cell trait), and a normal individual. The smudges show the positions to which the hemoglobins migrate on the starch gel.
A single amino acid substitution

Incorrect base in DNA

Valine in number 6 position in β polypeptide

Abnormal hemoglobin

Sickling of red blood cells

Rapid destruction of sickle cells

Clumping of cells, causing interference with blood circulation

Accumulation of red blood cells in spleen

Anemia

Local failures in blood supply

Overactivity of bone marrow

Tiredness

Dilation of heart

Muscle joint damage

Damage to gastrointestinal tract

Enlargement of spleen

“Tower skull”

Poor physique

Rheumatism

Abdominal pain

Heart damage

Kidney damage

Brain damage

Lung damage

Heart failure

Kidney failure

Paralysis

Pneumonia

Fibrosis of spleen

The compounded consequences of one amino acid substitution in hemoglobin to produce sickle-cell anemia.
Major and minor thalassemia

- In 1925, Thomas Cooley, a US pediatrician, described a severe type of anemia in children of Italian origin.
  - He noted abundant **nucleated** red blood cells in the peripheral blood and initially thought that he was dealing with erythroblastic anemia, described earlier. Before long, Cooley realized that erythroblastemia is neither specific nor essential in this disorder. He noted a number of infants who became seriously anemic and developed splenomegaly (enlargement of the spleen) during their first years of life. The disease was deadly, usually before age 10. Very soon, the disease was named after him, Cooley's anemia.

- In the same years, in Europe, Riette described Italian children with unexplained **mild hypochromic and microcytic anemia**, and other authors in the United States reported a mild anemia in **both parents** of a child with Cooley anemia; this anemia was similar to that described by Riette in Italy.

- In 1936, it was realized that all disorders designated diversely as von Jaksch's anemia, splenic anemia, Cooley's anemia, erythroblastosis, and Mediterranean anemia, were in fact a single entity, mostly seen in patients who came from the Mediterranean area, hence to name the disease they proposed 'thalassemia' derived from the Greek word θαλασσα, meaning 'the sea'. It was also recognized that Cooley severe anemia was the **homozygous form** of the **mild anemia** described by Riette and Wintrobe. The severe form then was labeled as **thalassemia major** and the mild form as **thalassemia minor**.
Complexity of thalassemias

- The fundamental abnormality in thalassemia is impaired production of either the α or β hemoglobin chain. Thalassemia is a difficult subject to explain, since the condition is not a single disorder, but a group of defects with similar clinical effects. More confusion comes from the fact that the clinical descriptions of thalassemia were coined before the molecular basis of the thalassemias were uncovered.

- The initial patients with Cooley’s disease are now recognized to have been afflicted with β-thalassemia. In the following few years, different types of thalassemia involving polypeptide chains other than beta chains were recognized and described in detail.

- In recent years, the molecular biology and genetics of the thalassemia syndromes have been described in detail, revealing the wide range of mutations encountered in each type of thalassemia. Beta thalassemia alone can arise from any of more than 150 mutations.
The two chromosomes #11 have one beta globin gene each (for a total of two genes). The two chromosomes #16 have two alpha globin genes each (for a total of four genes). Hemoglobin protein has two alpha subunits and two beta subunits. Each alpha globin gene produces only about half the quantity of protein of a single beta globin gene. This keeps the production of protein subunits equal. Thalassemia occurs when a globin gene fails, and the production of globin protein subunits is thrown out of balance.

If only one beta globin gene is defective, the other gene supply almost enough protein, though people may show mild anemia symptoms (thalassemia minor); the severe b-thalassemia disease (thalassemia major) arise when both homologous genes are defective.
Summary of genetic defect in β-thalassemia

- $\beta^+ : \text{reduced beta-globin chain synthesis}$
- $\beta^0 : \text{no beta-globin chain synthesis}$
- More than 100 point mutations and several deletional mutations have been identified within and around the beta-globin chain gene all affecting the expression of the beta-globin chain gene resulting in defects in activation, initiation, transcription, processing, splicing, cleavage, translation, and/or termination
- Genetic defect:
  - abnormal or no synthesis of the beta-globin chain $\rightarrow$ bone marrow fails to produce adequate erythrocytes and increased hemolysis of circulating erythrocytes $\rightarrow$ anemia $\rightarrow$ medullary hematopoiesis and extramedullary hematopoiesis (hepatosplenomegaly, lymphadenopathy)
α-thalassemia

- In α-thalassemia, there is deficient synthesis of α-chains. The resulting excess of β-chains bind oxygen poorly, leading to a low concentration of oxygen in tissues (hypoxemia).

- Deletions of HBA1 and/or HBA2 tend to underlie most cases of α-thalassemia. The severity of symptoms depends on how many of these genes are lost.

- Reduced copy numbers of α-globin genes produce successively more severe effects. Most people have four copies of the α-globin gene (αα/αα). People with three copies (αα/α-) are healthy; those with two (whether the phase is α-/α- or αα/--) suffer mild α-thalassemia; those with only one gene (α-/--) have severe disease, while lack of all four α genes (--/--) causes lethal hydrops fetalis.
Mechanism of $\alpha$-globin gene deletion

- Deletions of $\alpha$-globin genes in $\alpha$-thalassemia. Normal copies of chromosome 16 carry two active $\alpha$-globin genes and an inactive pseudogene arranged in tandem. Repeat blocks (labeled X and Z) may misalign, allowing unequal crossover. The diagram shows unequal crossover between mis-aligned Z repeats producing a chromosome carrying only one active $\alpha$ gene. Unequal crossovers between X repeats have a similar effect. Unequal crossovers between other repeats (not shown) can produce chromosomes carrying no functional $\alpha$ gene. Individuals may thus have any number from 0 to 4 or more $\alpha$-globin genes. The consequences become more severe as the number of $\alpha$ genes diminishes.