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TITLE: Genetics of Sickle Cell Disease

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Slide 1:

Hello, my name is Fang Zhao. I am a molecular genetic pathology fellow at the Cleveland Clinic. Welcome to this Pearl of Laboratory Medicine on "Genetics of Sickle Cell Disease."

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Here is the outline of this presentation. First, I will give a brief overview of normal hemoglobins and the globin genes; second, I will talk about the molecular genetics of hemoglobin S and sickle cell disease; third, I will discuss the clinical genetic aspects of sickle cell disease.

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As we know, hemoglobin is the oxygen carrier in vertebrate red blood cells.

Each hemoglobin molecule consists of four subunits: two α-globin chains and two β- (or β-like) globin chains. The image on the top of the right side illustrates the structure of adult human hemoglobin, HbA, has a α2β2 structure in which the four chains are folded and fitted together to form a globular tetramer.

Each subunit is composed of a polypeptide chain, globin, and a prosthetic group, heme, which is an iron-containing pigment that combines with oxygen to give the molecule its oxygen-transporting ability. As illustrated in the image at the bottom of the right side, each globin chain is made of 8 segments, from A to H; and heme is inserted between E and F.

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During human development from the embryo to adult, there are changes in the predominant normal hemoglobins. As shown in this table, hemoglobin Gower 1, Gower 2, and Portland I are present in the embryonic period; hemoglobin F, consisting of two α chains and two γ chains, is the predominant hemoglobin in the fetal period; hemoglobin A (α2β2) and hemoglobin A2 (α2δ2) are normal hemoglobins in adult, with hemoglobin A being the predominant component.

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The synthesis of hemoglobins are directed by the globin genes, which are organized into two clusters, the alpha-like gene cluster and the beta-like gene cluster.

The alpha-like gene cluster is located on the short arm of chromosome 16. It contains three functional genes (α1, α2, and ζ2), three pseudogenes, which are DNA sequences that closely resemble known genes but are nonfunctional, and one gene of undetermined function. It is noted that the α1 and α2 genes have close nucleotide sequences and an identical coding sequence.

The beta-like gene cluster is located on the short arm of chromosome 11, and contains five functional genes (β, δ, Gγ, Aγ, and ε) and one pseudogene.

Within each complex, the genes are all in the same 5'-3' orientation and are arranged in the order in which they are expressed during development.

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Hemoglobin S results from a single nucleotide substitution in the beta-globin gene, an adenine-to-thymine substitution in the six codon replaces glutamic acid with valine in the sixth amino acid position of the beta-globin chain.

In oxygenated blood, Hb S are normal in their ability to perform their principal function of binding oxygen;

But in deoxygenated blood, Hb S are only one fifth as soluble as normal hemoglobin.

- This relative insolubility of deoxyhemoglobins S causes the sickle hemoglobin molecules to aggregate in the form of rod-shaped polymers or fibers.
- These molecular rods distort the erythrocytes to a sickle shape that prevents them from squeezing single file through capillaries, thereby blocking blood flow and causing local ischemia.
- They may also cause disruption of the red cell membrane (hemolysis) and release of free hemoglobin, which can have deleterious effects on the availability of vasodialtors, such as nitric oxide, thereby exacerbating the ischemia.

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Sickle cell disease refers to a group of disorders characterized by the presence of at least one Hb S and a second β-globin chain pathogenic variant resulting in abnormal hemoglobin polymerization.

Sickle cell disease (Hb S/S) is caused by the homozygous beta-globin gene variant p.Glu6Val; it is the most common cause of SCD in the US, and accounts for 60-70% of SCD;

Other forms of SCD result from coinheritance of Hb S with other abnormal beta-globin chain variants, the most common forms being sickle-hemoglobin C disease (Hb S/C) and two types of sickle beta-thalassemia; rarer forms result from coinheritance of other Hb variants such as D-Punjab, O-Arab, and E.

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Although all patients with homozygous SCD have exactly the same molecular defect, there is considerable clinical variation, ranging from death in early childhood to the normal life span from few complications.

It is known that there are some genetic modifiers of sickle cell disease, including alpha-thalassemia, types of the second beta-globin pathogenic variant, and the genetic factors that affect levels of hemoglobin F.

Alpha thalassemia results from impaired production of alpha globin chains, which leads to a relative excess of beta globin chains. It is noted that the concurrence of sickle cell anemia and alpha-thalassemia results in less severe hemolytic anemia apparently as a result of reduced intraerythrocytic concentration of hemoglobin S and its retarded polymerization.

In addition, individuals with HbS/S and S/β0-thalassemia are generally more severely affected than individuals with Hb S/C or S/β+-thalassemia.

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Furthermore, it has been known for many years that patients with increased levels of HbF often tend to have a relatively mild clinical course because HbF reduces the tendency of HbS to polymerize within the red cell. Several genetic factors have been shown to affect the levels of hemoglobin F.

For example, rare deletions within the beta-globin gene cluster can increase the level of hemoglobin F.

Studies show that five SNPs at three quantitative trait loci (QTL) may act directly on the expression of the gamma-globin genes or affect the process of erythropoiesis to increase the level of Hb F or the proportion of F cell production.

The rs7482144 SNP lies in the promoter of the γ-globin gene on chromosome 11 (could be explained by a direct effect on γ-globin gene expression); the rs4671393 SNP Lies in the intron of an oncogene, BCL11A, that is expressed in erythroid precursors; there are three SNPs (rs28384513, rs9399137, rs4895441) lying in the intergenic region between HBS1L and MYB have independent effects on HbF variance in SCD. Although the role of *HBS1L* is unknown, *MYB* is known to play an important role in normal erythropoiesis.

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Now, move on to discuss the clinical genetic aspects of sickle cell disease. First, the prevalence of SCD. The Hb S allele is common in persons of African, Mediterranean, Middle Eastern, and Indian ancestry and in persons from the Caribbean and parts of Central and South America, but can be found in individuals of any ethnic background.

Among African Americans, the prevalence of sickle cell trait (Hb A/S) is about 10%.

Approximately one in every 300-500 African Americans born in the US has SCD (Hb S/S).

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Sickle cell disease is inherited in an autosomal recessive manner, which means if one parent is a carrier of the HBB HbS pathogenic variant and the other is a carrier of any of the HBB pathogenic variants (eg, HbS, HbC, β-thalassemia), each child has a 25% chance of being affected, a 50% chance of being unaffected and a carrier, and a 25% chance of being unaffected and not a carrier.

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The diagnosis of SCD is established by identification of significant quantities of HbS with or without an additional abnormal β-globin chain variant by hemoglobin analysis by gel or capillary electrophoresis or HPLC or by identification of biallelic HBB pathogenic variants where at least one allele is the p.Glu6Val pathogenic variant on molecular genetic testing.

As the diagnosis of SCD can be typically confirmed by detecting HbS, DNA-based genetic testing is commonly reserved for prenatal diagnosis. Molecular genetic testing approaches can be single-gene testing or use of a multigene panel. For the single-gene testing approach, sequence analysis of HBB is performed first, and followed by gene-targeted deletion/duplication analysis if only one or no pathogenic variant is found. A multigene panel that includes HBB and other genes of interest may also be considered.

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The management of sickle cell disease include multiple components. First, prevention of complications, including use of penicillin prophylaxis started in the newborn period, appropriate immunizations, blood transfusions for those at risk for stroke, and hydroxyurea and pharmaceutical-grade L-glutamine to prevent pain episodes. Second, treatment of complications, including pain medications for vaso-occlusive events and antibiotics for infection. Third, potential management for cure. Currently, a life-long cure for SCD is available only through hematopoietic stem cell transplantation.

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As SCD arises from a defined single nucleotide substitution in the β-globin gene whose expression is restricted to erythroid cells, gene therapy has long been proposed as a potential cure for sickle cell disease.

Currently, there are three strategies for gene therapy for sickle cell disease: first, gene addition: integrating lentiviral vector carrying a β-globin, γ-globin, or antisickling β-globin cassette; the second strategy is to induce the expression of γ-globin gene by using shRNA-mediated knockdown of BCL11A; disruption of BCL11A enhancer; or forced chromatin looping to promote association of the β-globin locus control region with the γ-globin genes; the third approach is Gene correction: direct correction of the sickle mutation by using targeted genome engineering methods.

Currently, several clinical trials for SCD gene therapies are open. The most updated information can be accessed via searching the ClinicalTrials.gov website.

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In summary, the hemoglobin molecule is a tetramer consisting of two alpha globin chains and two beta (or beta-like) globin chains. The synthesis of hemoglobins are directed by the alpha-like gene cluster on the chromosome 16 and the beta-like gene cluster on the chromosome 11.

SCD results from a single nucleotide substitution that changes the codon 6 of β-globin from glutamic acid to valine (p.Glu6Val). Several genetic modifiers may determine the clinical severity of SCD, including α-thalassemia, rare deletions within the beta-globin gene cluster, and five SNPs that may act directly on the expression of the γ-globin genes.

SCD is an autosomal recessive disorder. The current clinical approach to SCD is reliant upon supportive and hydroxyurea. Three strategies for gene therapy for SCD have been studied, including gene addition, Hb F induction and gene correction. Several clinical trials for SCD gene therapies are now open.

Slide 16: References

Here is the list of references I have used to prepare for this presentation.

Slide 17: Disclosures

Slide 18: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on “Genetics of Sickle Cell Disease.”