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Pearl Title Genetics of Sickle Cell Disease

Name of Presenter Fang Zhao, MD, PhD

Affiliation Molecular Genetic Pathology Fellow
Cleveland Clinic

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Outline

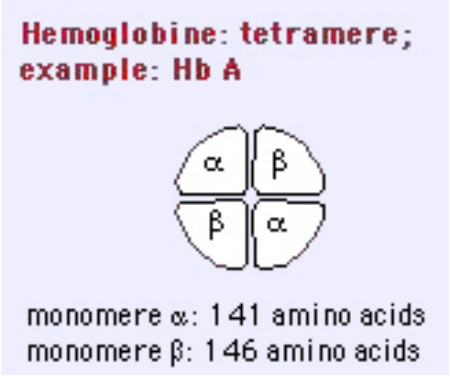
- Overview of normal hemoglobins and the globin genes
- Molecular genetics of hemoglobin S and sickle cell disease
- Clinical genetic aspects of sickle cell disease

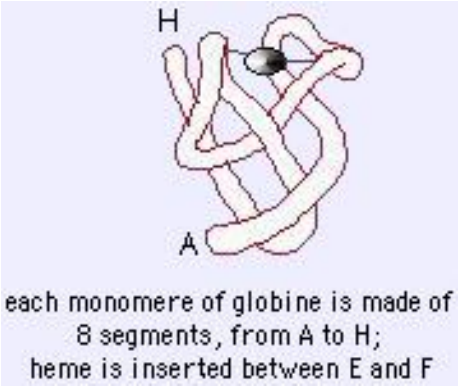
Normal Hemoglobins

Structure of hemoglobin

- Each hemoglobin molecule consists of four subunits
  - Two α-globin chains
  - Two β- (or β-like) globin chains

- Each subunit is composed of two components
  - A polypeptide chain, globin
  - A prosthetic group, heme





http://atlasgeneticsoncology.org/Educ/GenHemoglobID30014ES.html

Normal Hemoglobins

Developmental period	Types of hemoglobin	Chains composition
Embryonic	Hemoglobin Gower 1	ζ2ε2
	Hemoglobin Gower 2	α2ε2
	Hemoglobin Portland I	ζ2γ2
Fetal	Hemoglobin F	α2γ2
Adult	Hemoglobin A	α2β2
	Hemoglobin A2	α2δ2

The Globin Genes

α-like genes

- Location: chromosome 16p
- Three functional genes (α1, α2, and ζ2)
- Three pseudogenes (ψα1, ψα2, and ψζ1)
- One gene of undetermined function (θ1)

β-like genes

- Location: chromosome 11p
- Five functional genes (β, δ, Gγ, Aγ, and ε)
- One pseudogene (ψβ1)

Hemoglobin S

Beta-globin (HBB) gene, c.20A>T, p.Glu6Val

(reference sequences NM_000518.4, NP_000509.1)

Oxygenated Hb S

↓

Soluble

↓

Performs normal function of binding oxygen

Deoxygenated Hb S

↓

1/5 as soluble as normal Hb

↓

Aggregate in the form of rod-shaped polymers or fibers

↙ ↘

Distort the RBCs to a sickle shape

↓

Prevent them from squeezing single file through capillaries

↓

Block blood flow & Cause local ischemia

Disruption of the RBC membrane

↓

Hemolysis

Sickle Cell Disease (SCD)

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.

Include:

- SCD (Hb S/S)
- Sickle-hemoglobin C disease (Hb S/C)
- Sickle β-thalassemia
  - Hb S/β+ thalassemia
  - Hb S/β0 thalassemia
- Sickle-hemoglobin D, O, and E disease (or other β-globin chain variants)

Genetic complexity in SCD

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 with few complications.

Genetic modifiers of SCD

- α-thalassemia
  - The concurrence of sickle cell anemia and alpha-thalassemia results in less severe hemolytic anemia

- Types of the second β-globin pathogenic variant
  - Individuals with HbS/S and S/β0-thalassemia are generally more severely affected than individuals with Hb S/C or S/β+-thalassemia.

Genetic complexity in SCD

Genetic modifiers of SCD (continued)

- Genetic factors that affect levels of HbF
  - It has been known that patients with increased levels of HbF often tend to have a relatively mild clinical course
  - Rare deletions within the β-globin gene cluster
    → Increase HbF
  - Five SNPs at three quantitative trait loci (QTL)
    - rs7482144: Lies in the promoter of the γ-globin gene on chr11
    - rs4671393: Lies in the intron of an oncogene, *BCL11A*
    - rs28384513, rs9399137, rs4895441: Lie in the intergenic region between *HBS1L* and *MYB*

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).

Inheritance pattern of SCD

- Autosomal recessive inheritance
  - 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
→ a 25% chance of being unaffected and not a carrier

Diagnosis of SCD

- 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 high-performance liquid chromatography (HPLC) or by identification of biallelic *HBB* pathogenic variants where at least one allele is the p.Glu6Val pathogenic variant on molecular genetic testing.

- Molecular genetic testing approaches
  - Single-gene testing: 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.

Management of SCD

- Prevention of complications
  - Use of penicillin prophylaxis started in the newborn period
  - Appropriate immunizations
  - Blood transfusions for those at risk for stroke
  - Hydroxyurea and pharmaceutical-grade L-glutamine to prevent pain episodes
- Treatment of complications
  - Pain medications for vaso-occlusive events
  - Antibiotics for infection
- Potential management for cure
  - Hematopoietic stem cell transplantation

Gene therapy of SCD

- Strategies
  - Gene addition: integrating lentiviral vector carrying a β-globin, γ-globin, or antisickling β-globin cassette
  - Induction of γ-globin expression: shRNA-mediated knockdown of *BCL11A*, disruption of *BCL11A* enhancer; forced chromatin looping to promote association of the β-globin locus control region with the γ-globin genes
  - Gene correction: targeted genome engineering leads to correction of the sickle mutation such that β^S is repaired as β^A.

- The most updated information on clinical studies can be accessed via searching ClinicalTrials.gov

Summary

- The hemoglobin molecule is a tetramer consisting of two α-globin chains and two β- (or β-like) globin chains. The synthesis of hemoglobins are directed by the α-like gene cluster on the chromosome 16 and the β-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 act directly on the expression of the γ-globin genes.

- SCD is an autosomal recessive disorder. The current clinical management is largely 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.

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Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

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