HEMOGLOBINOPATHIES

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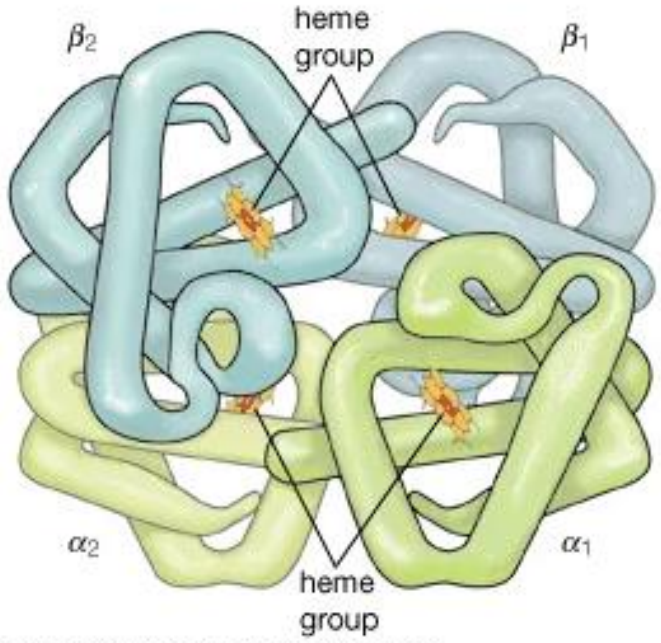
TODAY'S TOPICS

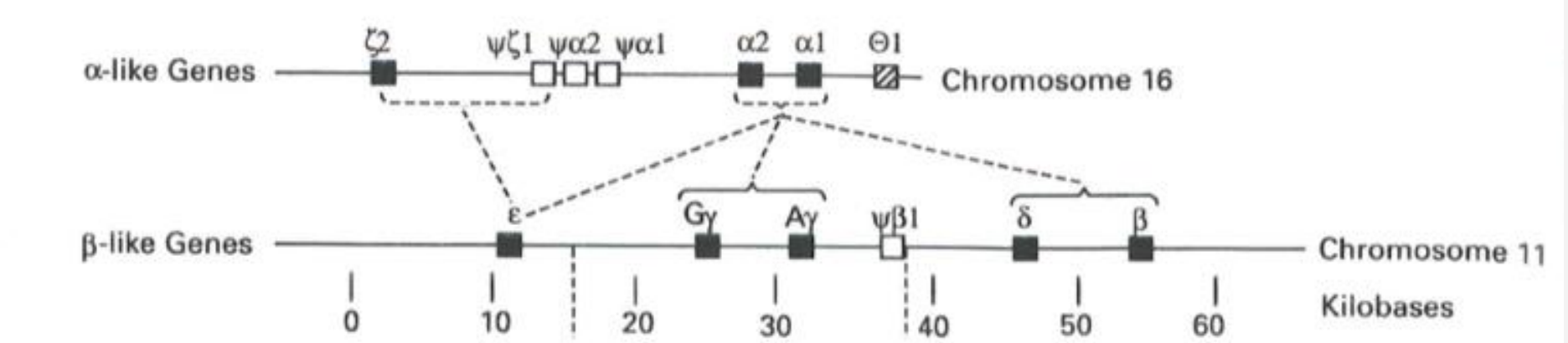
- Hemoglobin review
- Hemoglobinopathies
- Hemoglobin S
- Hemoglobin C
- Hemoglobin E
- Hemoglobin O-Arab
- Hemoglobin D and Hemoglobin G
- Hemoglobin M
- Compound Heterozygosity
- Unstable Hemoglobin Variants
- Concomitant CIS Mutations with HbS

HEMOGLOBIN MOLECULE

- 4 globin chains
  - 2 α-like- 141 amino acids
    - Alpha (α) and zeta (ζ)
  - 2 non-α like (β-like)- 146 amino acids
    - Beta (β) , gamma (γ) , delta (δ) , and epsilon (ε)
- 4 heme groups
  - 1 O_2 per heme and 4 per Hb



GLOBIN SYNTHESIS



Courtesy of B. Martien, Manual Hematology

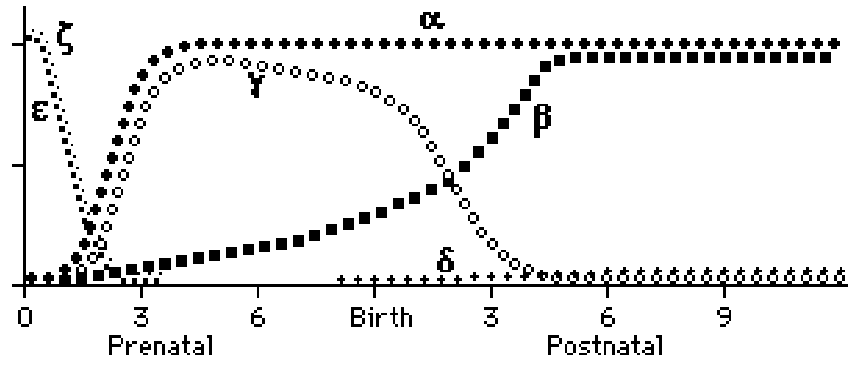
Chromosome 16 (α-like genes)

- Alpha and zeta

Chromosome 11 (β-like genes)

- Beta, gamma, delta, epsilon

GLOBIN SYNTHESIS



α alpha •••
β beta ▪▪▪
δ delta +++
ε epsilon . . .
γ gamma • • •
ζ zeta . . . .

Normal Hemoglobins

A	$\alpha_2\beta_2$	– ≈95 % adult	Gower 1	$\zeta_2\varepsilon_2$	embryonic Hgb; ↑ O_2 transport to embryo
A_{1c}	$\alpha_2\beta$-(glycosylation)$_2$	– ≈3 % adult (↑ in diabetes m)	Gower 2	$\alpha_2\varepsilon_2$	
A_2	$\alpha_2\delta_2$	– ≈2% adult	Portland	$\zeta_2\gamma_2$	
F	$\alpha_2\gamma_2$	– major fetal Hgb 3–9 th month; ↑ O_2 transport from placenta; <1 % adult	H	β_4	– non-functional
			Barts	γ_4	– trace at birth; non-functional

Courtesy of B. Martien, Manual Hematology

HEMOGLOBIN DEVELOPMENT

Fetal development

- 1st three months of embryo development
  - 1 alpha-like gene (zeta) and 1 β-like gene (epsilon) are activated
    - Gower-1 ($\zeta_2\varepsilon_2$)
- Alpha and gamma synthesis begin
  - Gower-2 ($\alpha_2\varepsilon_2$) and Portland ($\zeta_2\gamma_2$)
- Zeta and epsilon synthesis ceases
  - Leaves alpha and gamma chains
    - Pair to produce HbF ($\alpha_2\gamma_2$)

HEMOGLOBIN DEVELOPMENT

Birth through Adulthood

- 6 months after birth, gamma chain synthesis gradually decreases
  - Gamma chain gene silenced by transcriptional repressors
  - Replaced by beta chain synthesis
    - "γ-β switching"
  - HbA ($\alpha_2\beta_2$) is produced
- Delta globin gene is activated at birth and pairs with alpha globin
  - HbA_2 ($\alpha_2\delta_2$)

	Adult	Newborn
Hb A ($\alpha_2\beta_2$)	95%	10-40%
Hb A_2 ($\alpha_2\delta_2$)	<3.5%	0.2%
Hb F ($\alpha_2\gamma_2$)	<1-2%	60-90%

HEMOGLOBINOPATHY

- Hemoglobinopathy- disease state involving hemoglobin molecule
  - Result from a mutation in one or more genes that affect hemoglobin synthesis
  - Genes that are mutated either:
    - Code for proteins that make up hemoglobin molecule (globin or polypeptide chain)
    - Are involved in synthesizing or regulating synthesis of the globin chains
- Qualitative= **Hemoglobinopathies**
  - Synthesis is normal/near normal
  - Altered amino acid sequence within globin chain
    - Alter the structure and function
- Quantitative= **Thalassemia**
  - Reduction in hemoglobin synthesis
  - Reduction of specific hemoglobin can cause anemia
    - Stimulates production of other hemoglobins not affected to compensate for the anemia

HEMOGLOBINOPATHIES

HEMOGLOBINOPATHIES

Nomenclature

- Originally given a letter from the alphabet… too many variants
- Currently single capital letter used along with the place of discovery
  - Hb G- Philadelphia
  - Hb G- Copenhagen
  - Hb C- Harlem

Genetic Mutations

- More than 1200 hemoglobinopathies known to exist
- 1 or more genetic mutation that alter the amino acid sequence of polypeptide chain
- Types of mutations:
  - Point mutation
  - Deletion
  - Insertion
  - Fusion

TABLE 24.1 Genetic Abnormalities of Hemoglobin Variants

	NUMBER OF VARIANTS BY GLOBIN CHAIN*				
	α	β	δ	γ	Total
Point mutations with amino acid substitution	444	564	73	97	1178
Deletions	19	37	1	1	58
Insertions	12	16	0	0	28
Duplications	1	1	0	0	2
Total	476	618	74	98	1266
Fusions	—	—	—	—	9**

Rodak's Hematology, Clinical Principles and Applications 6th Edition

GENETIC MUTATION- POINT MUTATION

- Most common type of genetic mutation
- Replacement of one nucleotide in a normal gene with a different nucleotide
  - Substitution of 1 amino acid
    - Codon triplet intact, reading frame is unaltered
- 2 point mutations are possible
  - 2 mutations in same globin gene
    - 2 AA substitutions in same gene
  - Cause >35 mutations

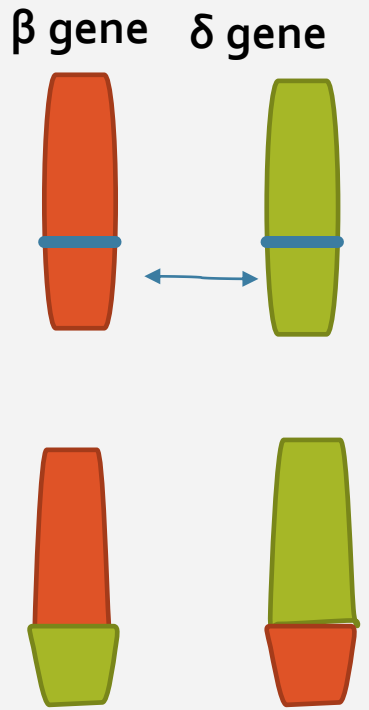
GENETIC MUTATION- DELETION AND INSERTION

- **Deletion**- removal of one or more nucleotides
- **Insertion**- addition of one or more nucleotides

- Varies whether or not the reading frame is affected
  - Disrupt the reading frame: void synthesis of corresponding globin chain
    - Quantitative thalassemia
  - Does not disrupt the reading frame
    - Hemoglobinopathies
    - Affects the structure/function
- Chain extension
  - Stop codon is mutated
  - Translation continues beyond typical last codon
  - Can cause quantitative and qualitative defects

GENETIC MUTATION- GENE FUSION

- 2 normal genes break between nucleotides, switch positions, and anneal to opposite gene
  - Head from 1 gene, tail from another gene
- Hybrid globin genes are able to be transcribed and translated if the reading frame is not disrupted and the globin chain lengths are similar
- Fusion genes will fold differently and affect hemoglobin function
- 9 fusion globin chains have been identified



ZYGOSITY

- **Zygosity**- association between # gene mutations and level of severity of the resultant genetic defect
  - Normal adult gene- 4 copies of α and γ, 2 copies of β and δ
    - 4 levels of severity of α and γ (4 copies)
    - 2 levels of severity for β and δ (2 copies) *

- Inheritance patterns (β chain variants) *
  - "**Trait**"- heterozygous
    - Only 1 β gene is mutated
    - Clinically more mild
  - "**Disease**"- homozygous
    - Both β genes are mutated
    - Clinically more severe

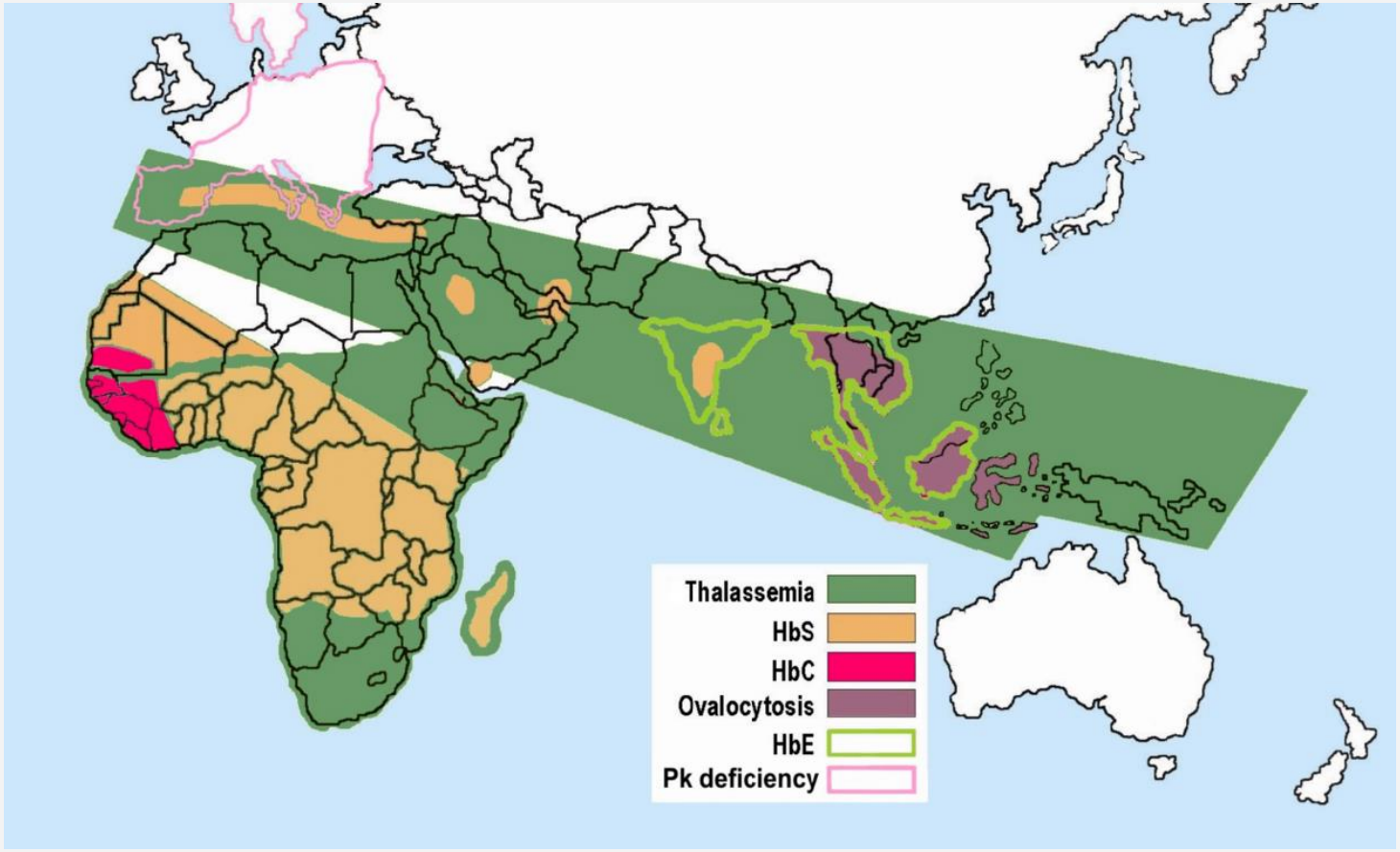
ZYGOSITYS AFFECT ON PATHOPHYSIOLOGY

- Can predict the severities of a disease
- β-hemoglobinopathies
  - 2 severities
    - Homozygous β-hemoglobinopathies
      - Both genes mutated
      - Variant hemoglobin dominant
    - Heterozygous β-hemoglobinopathies
      - One mutated β gene and one normal gene
      - 50/50 distribution
      - Variant Hb usually there in a lesser amount than Hb A

COMMON HEMOGLOBINOPATHIES FOUND IN THE UNITED STATES

Condition	Hb Types
Sickle Cell Trait	AS
Sickle Cell Anemia	SS
Hb C Trait	AC
Hb C Disease	CC
SC Disease	SC
Hb D Trait	AD
Hb D Disease	DD
Hb E Trait	AE
Sickle β Thalassemia	S-β- Thal
Thalassemia trait	Thal minor

HEMOGLOBINOPATHIES



Compendium of Hemoglobinopathies, SERBIA Education Library

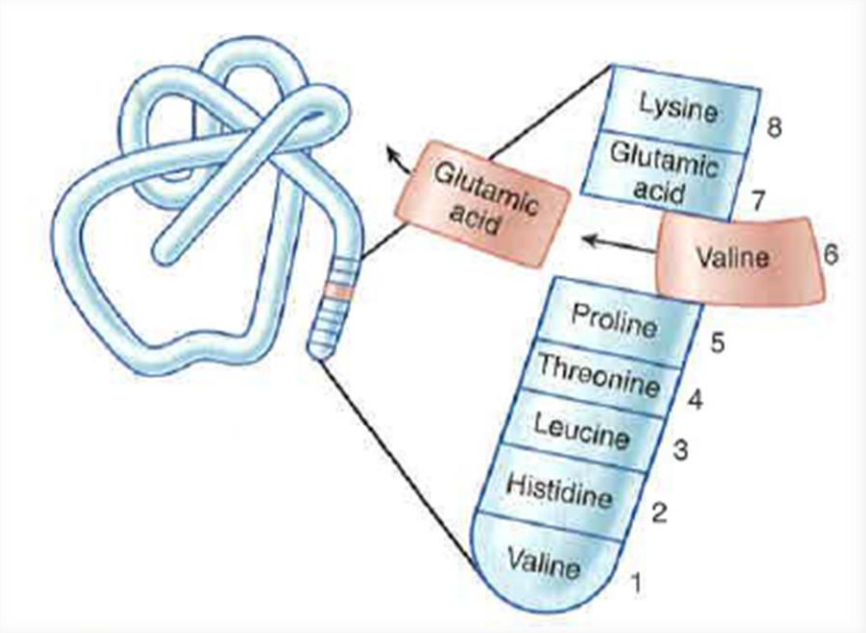
HEMOGLOBIN S

HB S DISEASE (SICKLE CELL DISEASE)

- Sickle Cell Disease- symptomatic hemoglobinopathies that have in common sickle cell formation and the associated crisis
  - Formations include
    - Homozygous Hb SS
    - Heterozygous Hb S with another β chain mutation
      - Hb S with Hb C or β thalassemia
- SCD most common hemoglobinopathy
  - Hb SS then variants Hb SC and Hb S- β thal
- Highest frequency found in the sub-Saharan Africa

SCD- ETIOLOGY AND PATHOPHYSIOLOGY

- Hb S structural formula $\alpha_2 \beta_2^{6\ Glu \rightarrow Val}$
  - Polar glutamic acid is replaced by nonpolar valine at the 6th position of the β chain
- Hb S structure
  - Valine (nonpolar) extends outward to bind hydrophobic niche
    - Hydrophobic niche is not present when fully oxygenated
    - Deoxygenation that occurs naturally creates hydrophobic niche for adjacent Hb S to bind
- Hemoglobin pairs (4 Hbs) polymerize and create elongated helical formation at the core
- Outer layer of 10 Hb molecules form around this, creating a long, slender Hb S polymer
  - Less soluble RBC
  - Formation tactoids or liquid crystals of Hb S polymers that grow in length beyond RBC diameter (cause sickling)



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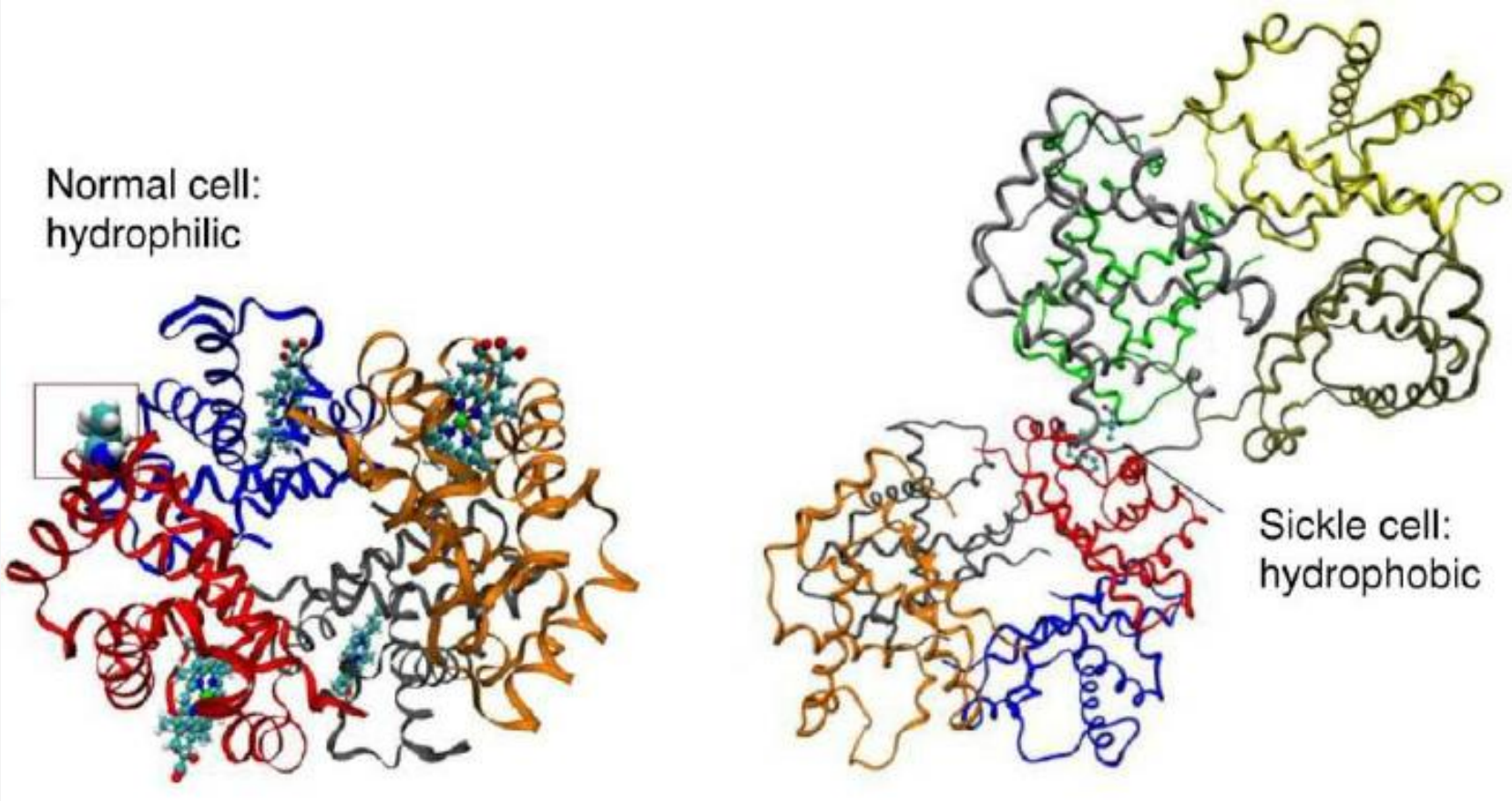
SCD- ETIOLOGY AND PATHOPHYSIOLOGY



SCD- ETIOLOGY AND PATHOPHYSIOLOGY

When does sickling occur?

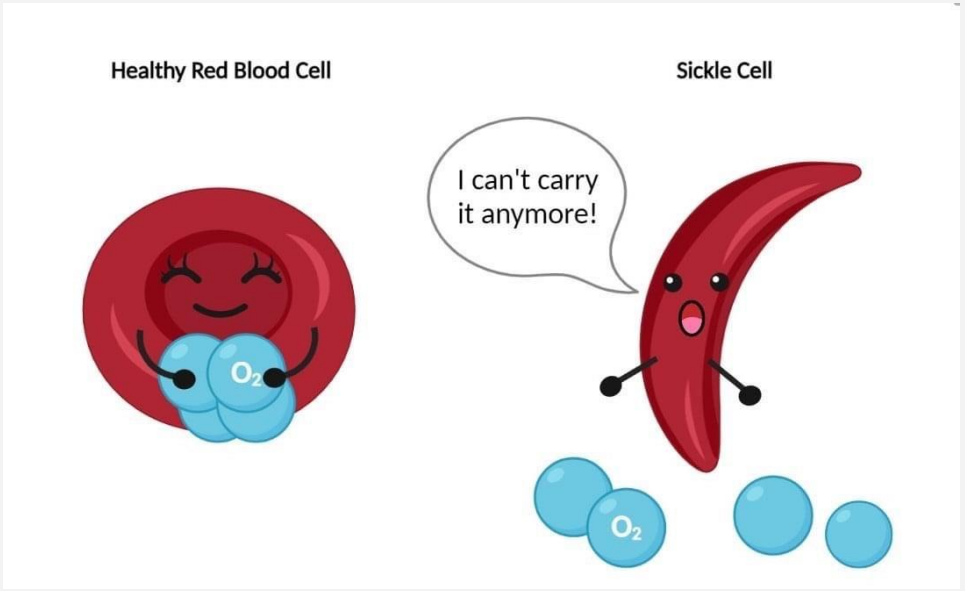
- Homozygotes- O_2 Saturation < 85%
- Heterozygotes- O_2 Saturation <40%

Reversible Sickle Cell

- Hb S containing RBCs that change shape due to oxygen tension
- Oxygenated- circulate as normal biconcave disc
- Deoxygenated- hemoglobin polymerize, ↑ viscosity, change shape

Irreversible Sickle Cell

- Do not change their shape regardless of change in oxygen tension or degree of Hb polymerization
- Seen on PB smear
- Recognized by the spleen as abnormal and removed from circulation



ARCH project MSCA

CLINICAL FEATURES OF SCD

- 8 genotypes cause severe disease
  - Hb SS, Hb S- β^0 thal, Severe Hb S- β^+-thal, Hb SD- Punjab, Hb SO-Arab, Hb-SC-Harlem, Hb-SC-Antilles, Hb S-Quebec-CHOR1
- Symptoms of variables depend on
  - Intracellular ratio of Hb S to Hb F
  - Factors that affect vessel tone and cellular activation
- Development of symptoms
  - Symptom free until 2nd half of 1st year of life
  - 1st 6 months- mutant β chains gradually replace normal γ chains → Hb S ↑, Hb F ↓
  - Causes hemolysis, hemolytic anemia, and splenomegaly

CRITICAL FEATURES OF SCD

Crises- Episodes of reoccurring pain occurred in patients with SCD

Vasoocclusive crisis (VOC)

- Hallmark of SCD
- SC blocks blood flow in the capillaries and post capillary venules
- Areas become oxygen deprived
- Pain occurs in the bones, lungs, liver, spleen, etc.
- Triggered by acidosis, hypoxia, dehydration, infection ,fever, and extreme cold

Splenic sequestration and infarcts

- Sudden trapping of blood in the spleen
  - ↓ in Hb
- Repeated splenic infarcts
  - Scarring
  - Diminished spleen tissue
  - Abnormal function
- Autosplenectomy
  - Evidenced by HJ and Pappenheimer bodies on PB

Acute chest syndrome

- Acute illness with fever and/or other respiratory symptoms that displays pulmonary infiltrates on a chest radiograph
- 2nd common cause of hospitalization

Avascular necrosis

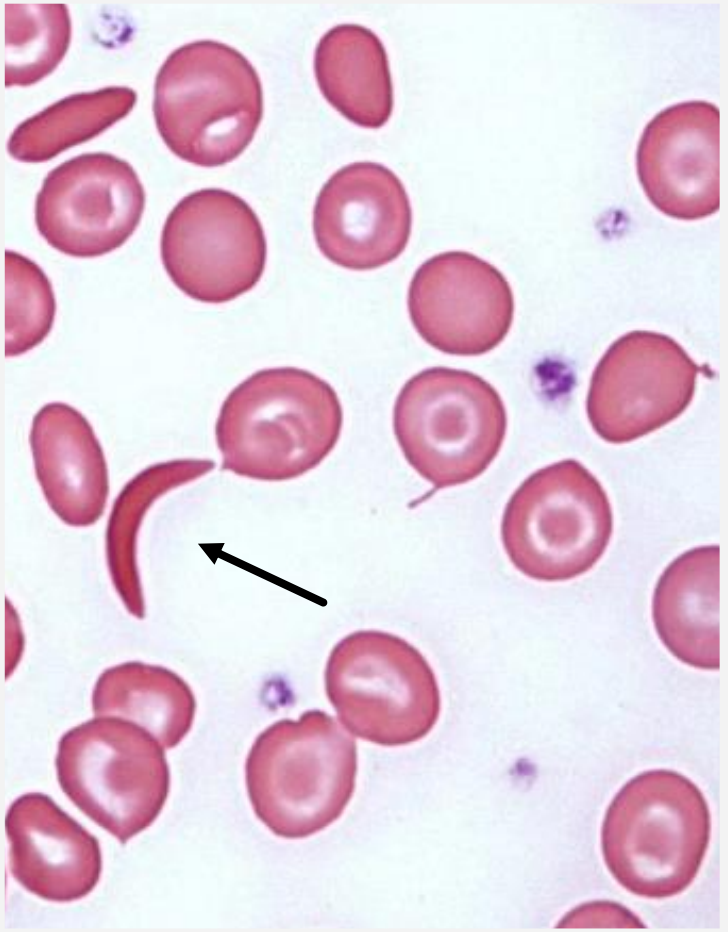
- Impaired blood supply to head of femur and hummerus
- 50% of SCD develop this
- Requires PT and surgery to relieve intramedullary pressure in the head of long bones
- Can cause skin ulcers

Decreased chances of malarial infection

- Offers protection against cerebral falciparum malaria in young patients
- Malarial parasites are living organisms within RBCs and use oxygen
  - Causes the cells to sickle and be removed
  - ↓ # malarial organisms, ↑ time for immunity to develop

DIAGNOSIS OF SICKLE CELL DISEASE

- Chronic, normocytic, normochromic hemolytic anemia
- Sickle cells and target cells seen on peripheral smear
- Poikilocytosis
- Increased polychromasia (Retic 10-25%)
- Diagnosis of SCD is generally a 2 step process
  - Insolubility of deoxygenated Hb S in solution
    - (+) sickle solubility
  - Confirmation of presence through Hb Electrophoresis, HPLC, or capillary electrophoresis



https://consultqd.clevelandclinic.org/what-are-the-latest-advances-in-treating-patients-with-sickle-cell-disease/

TREATMENT OF SCD

- Allogeneic bone marrow or hematopoietic stem cell transplantation is only curative therapy
- Supportive care mainstay of SCD
  - Adequate hydration
  - Prophylactic vitamin therapy
  - Avoidance of low oxygen environments
  - Analgesics for pain
  - Aggressive antibiotic therapy at the 1st signs of infection
  - Transfusions are used to decrease blood viscosity and percentage of circulating sickle cells
    - Usually 8 or more a year
    - Must watch for iron overload, transfusion reactions and related infections

SCD COURSE AND PROGNOSIS

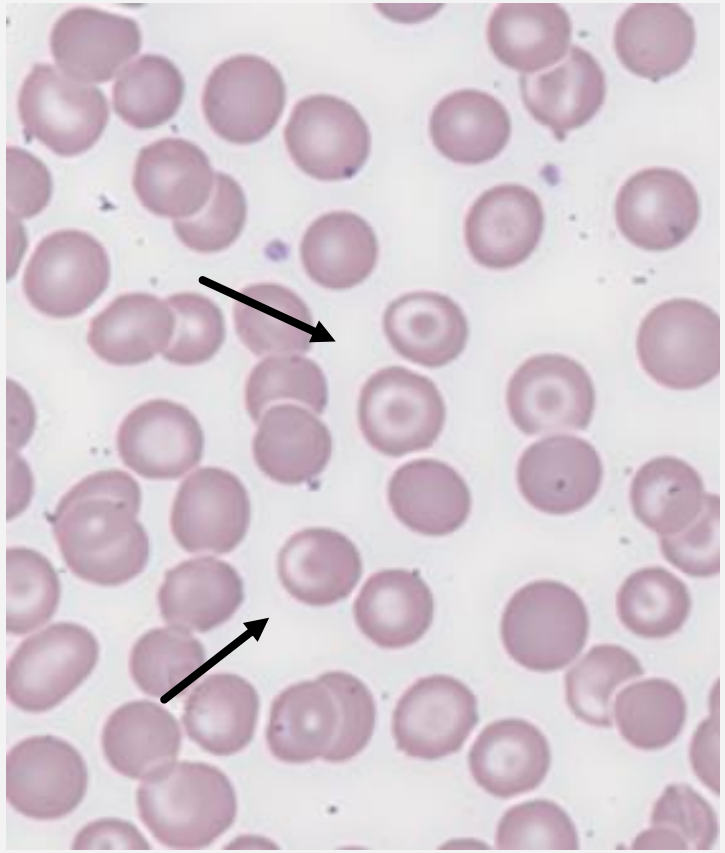
- Management has an increase in lifespan
- Able to pursue wide range of vocations
  - Discouraged from physical exertion, increased altitude, or temperature variations
- Newborn screenings have reduced mortality in children with SCD
- Monitoring during pregnancy
  - Requires intervention before conception, during, and after

SICKLE CELL TRAIT (HB S TRAIT, SCT)

- Heterozygous state (Hb AS)
- Hb A (60%) and Hb S (40%)
- Benign condition but will have symptoms under extreme conditions
  - Extreme hypoxia
    - Vascular occlusion with pooling sickles in spleen, focal necrosis in the brain, rhabdomyolysis and even death can occur
  - Severe respiratory infection, situations where oxygen levels are lower
    - Sickling can occur, patients may develop splenic infarcts
- Failure to concentrate urine is only consistent abnormality
  - Caused by diminished perfusion of the kidney vasa recta

TESTING FOR SCT

- PB has normal RBC morphology, few target cells
- (+) hemoglobin solubility
- Hb S and Hb A detected through electrophoresis or HPLC
- If Hb S levels less than 40% patient could also have α-thalassemia or iron or folate deficiency

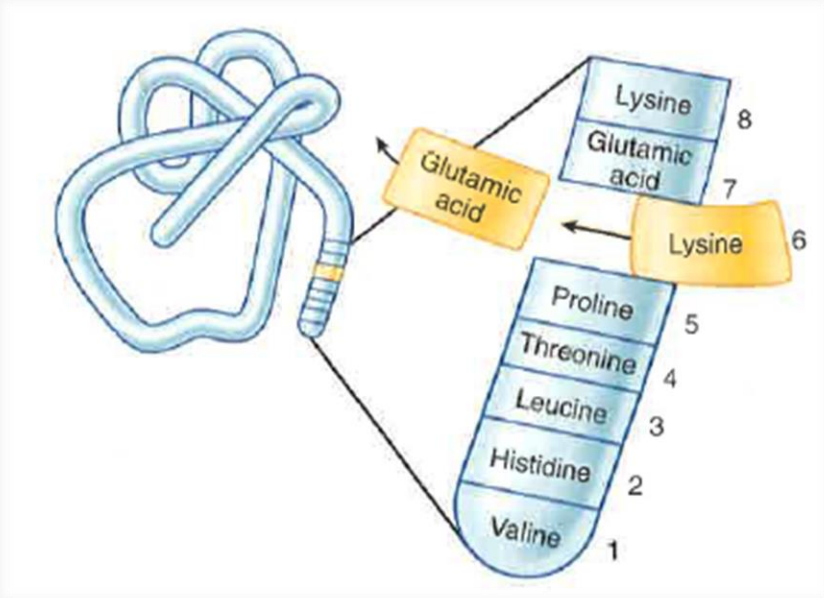


https://www.thebloodproject.com/target-cells/

HEMOGLOBIN C

HB C

- Hb C Disease
  - Most common nonsickling variant encountered in the US
    - Found almost exclusively in the African American population
  - 3rd most common in the world
  - Structural formula $\alpha_2\ \beta_2$ 6 Glu→ Lys
    - Results in a net charge of 2+
  - Forms polymers intracellularly
    - Forms crystals in an oxygenated state
    - Short, thick crystals in RBC occur in band 3 in RBC membrane nucleation center



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HB C- CLINICAL FEATURES

Hb C Disease (homozygous)

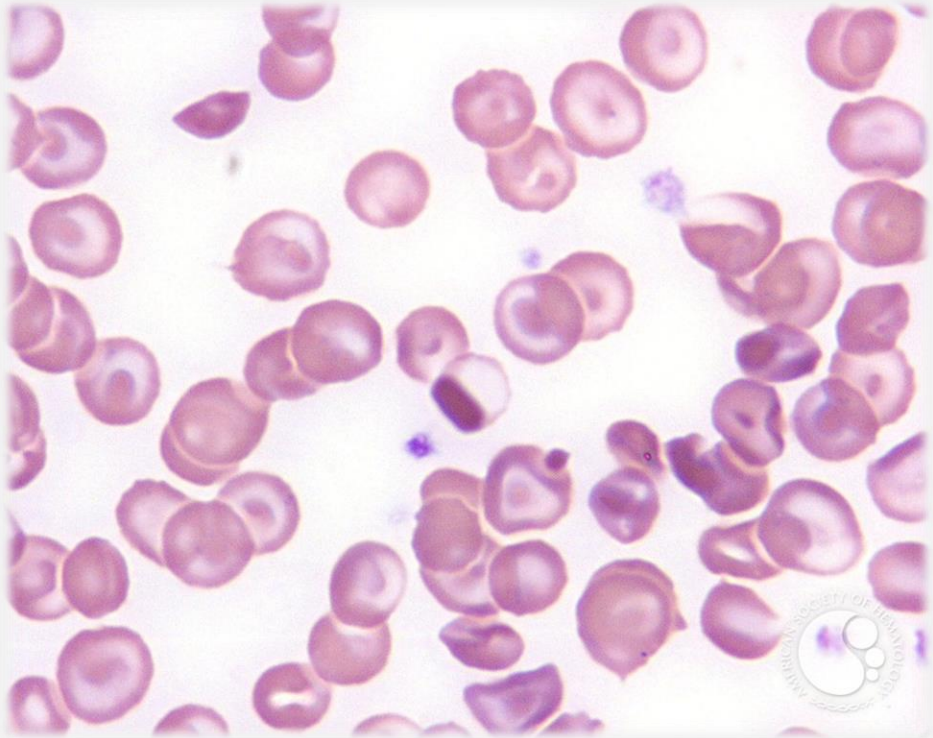
- Milder disease compared with SCD
- Mild splenomegaly and hemolysis may be present
- Vasoocclusive crisis does not occur

Hb C trait (heterozygous)

- Asymptomatic

HB C- LABORATORY DIAGNOSIS

- Mild to moderate, normochromic, normocytic anemia in HbCC
- ↑ Target cells and ↑ reticulocytes
- Hexagonal crystals
  - Short, thick crystal
  - Form within RBC
  - Maybe present on PB
  - Densely stained, pyramid shaped
- Occurs under high oxygen tension
- Does not alter RBC shape
  - Less splenic sequestration
  - Less hemolysis



http://imagebank.hematology.org/image/3137/homozygous-hemoglobin-c-disease--6

HB C- LABORATORY DIAGNOSIS

- (-) Hemoglobin solubility
- Diagnosed through electrophoresis, HPLC, and nucleic acid testing
  - Hb CC Disease: No Hb A and 90% Hb C
  - Hb AC Trait: 60% Hb A and 30% Hb C
- Electrophoresis
  - Alkaline electrophoresis
    - Hb C migrates with Hb A_2, Hb E, and Hb- O-Arab
  - Acid electrophoresis
    - Migrates separately

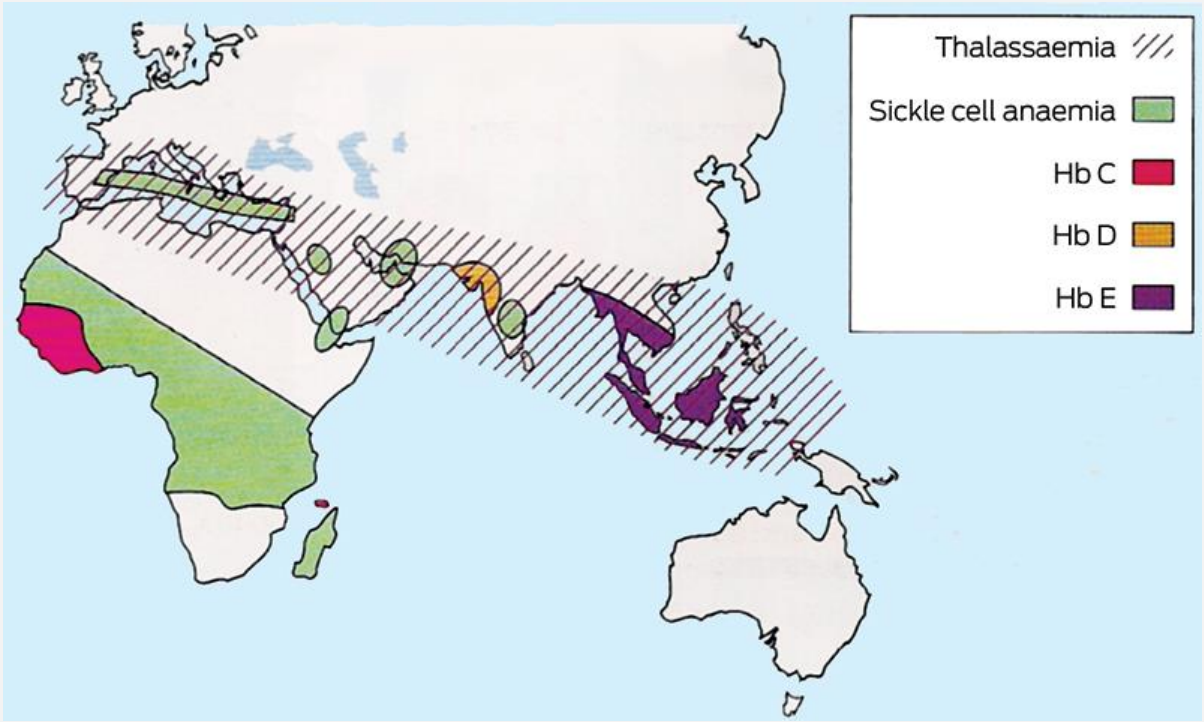
HB C-HARLEM (HEMOGLOBIN C-GEOREGTOWN)

- Double substitution on β chain
  - Valine for glutamic acid at 6
    - Identical to Hb S substitution
  - Aspartic acid for asparagine at 73
    - Same as Hb Korle Bu
- Laboratory Diagnosis
  - May have a (+) hemoglobin solubility
  - Migrates with Hb C on alkaline electrophoresis
  - Migrates with Hb S in acid electrophoresis
- Clinical features
  - Heterozygous are asymptomatic
  - Heterozygous for Hb S and Hb C- Harlem have symptoms similar to Hb SS Disease
- Rare

HEMOGLOBIN E

HB E

- Present in Asia population
  - Prevalence of 30% in SE Asia and can be as high as 50% in border areas of Cambodia, Laos, and Thailand



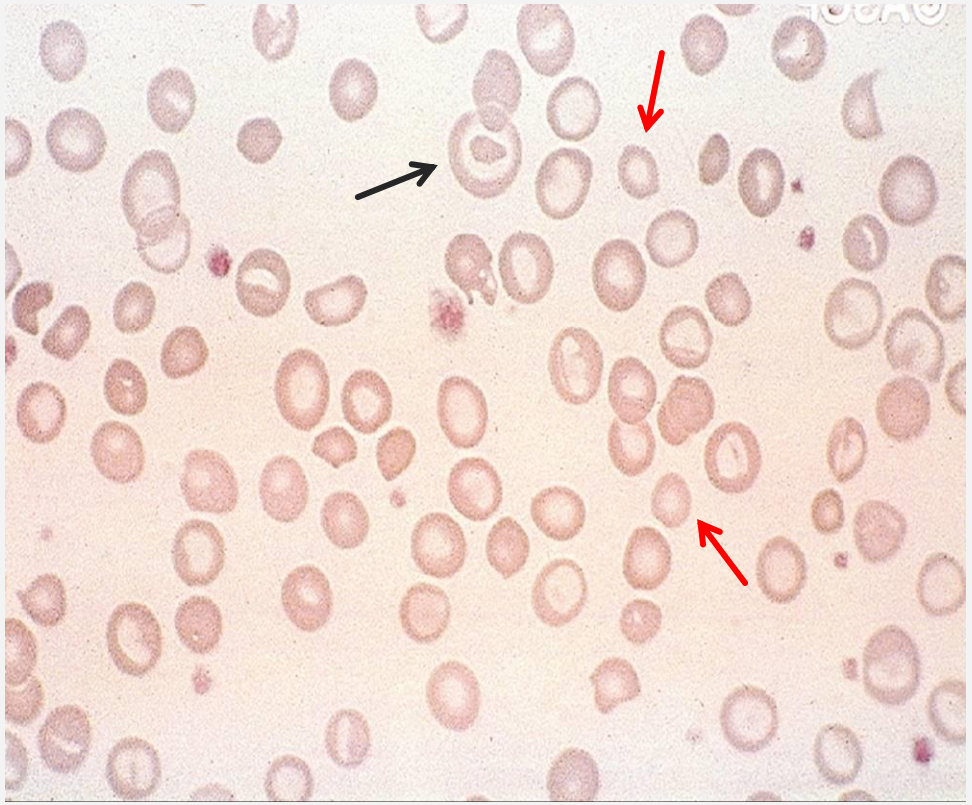
https://www.mja.com.au/journal/2016/204/6/antenatal-haemoglobinopathy-screening-australia

HB E

- β chain variant
  - $\alpha_2 \beta_2^{26\ Glu \rightarrow Lys}$
  - Insertion at 26 causes abnormal alternative splicing and decreased transcription of functional mRNA for Hb E globin chain
    - Reduce Hb E synthesis
- Mutation is both quantitative and qualitative
  - Qualitative- AA substitution
  - Quantitative- decrease production of globin chain
- Often coinherited with either α-thalaessemia, β-thalassemia or other hemoglobin variants
  - Hb E-β^0-thal is the most severe

HB E

- Laboratory Diagnosis
  - Mild anemia with microcytes and target cells
  - (-) solubility test
  - Electrophoresis
    - Alkaline agar: Hb E migrates with Hb C, O-Arab and A2
    - Acid agar: Separates from Hb C but migrates with A and O-Arab
  - Main concept is differentiating between iron deficiency, β-thal trait, and Hb E-β-thal
- Treatment and prognosis
  - No therapy is required for Hb E disease and trait
  - Hb E-β^0-thal is treated like β-thal major
    - Chronic transfusion therapy, iron chelation therapy, splenectomy with hypersplenism



https://doctorlib.info/hematology/rodak-hematology-clinical-principles-applications/28.html

HEMOGLOBIN O-ARAB

HB O-ARAB

- Rare disorder found in Kenya, Israel, Egypt, and Bulgaria
- Found in 0.4% of African American population
- β chain variant*
- $\alpha_2 \beta_2$ ^{121} Glu→ Lys
- Laboratory Diagnosis
  - (-) hemoglobin solubility
  - Alkaline agar: migrates with Hb A_2, Hb C and Hb E
  - Acid agar: differentiate from Hb C
- Clinically: minimal symptoms unless coinherited
  - Hb S-O-Arab: severe clinical symptoms similar to Hb SS
- No Treatment necessary

HEMOGLOBIN D AND HEMOGLOBIN G

HB D AND HB G

- Hb D and Hb G are a group of at least 16 β chain variants (Hb D) and 6 α chain variants (Hb G)
  - Do **not** sickle with reduced oxygen tension
  - Alkaline agar: Migrate at the same position as Hb S
  - Acid agar: migrate separately from Hb S

- Asymptomatic in heterozygous state
- Mild hemolytic anemia in homozygous state

- Hb D- Punjab and Hb-D-Los Angeles ($\alpha_2 \beta_2^{121\ Glu \rightarrow Gln}$)
  - Hb D- Punjab- 3% of population in NW India
  - Hb D- Los Angles- <2% African Americans

- Hb G-Philadelphia ($\alpha_2^{68\ Asn \rightarrow Lys} \beta_2$)
  - Most common Hb G variant encountered in African Americans

HEMOGLOBIN M

HB M

- Cause
  - Mutation in the α,β, and γ globin genes
  - All result in production of methemoglobin
- Genetic mutation result in a structural abnormality in the globin portion of the molecule
  - Usually a substitution of tyrosine amino acid for either the proximal (F8) or the distal (E7) histidine amino acid on the globin gene
- Carries iron in the ferric state (Fe^{3+})
  - Unable to carry oxygen
- Inherited as autosomal dominant disorders
- Affected individuals have 30%-50% methemoglobin
  - Less than 1% in healthy individuals
  - Will increase with ingestion of sulfonamides/oxidant drugs

HB M

- Characteristics
  - Brown blood
  - Heinz bodies on wet prep
- Diagnosis
  - Spectral absorption of hemolysate
  - Hemoglobin electrophoresis
    - Before testing, all Hb types converted to methemoglobin (addition of potassium cyanide)
    - Migration differences caused by amino acid substitutions not differences in Fe state
  - Confirmation with HPLC or DNA globin gene analysis
- Treatment
  - None
  - Diagnosis used to rule out/prevent inappropriate treatment for conditions like cyanotic heart disease

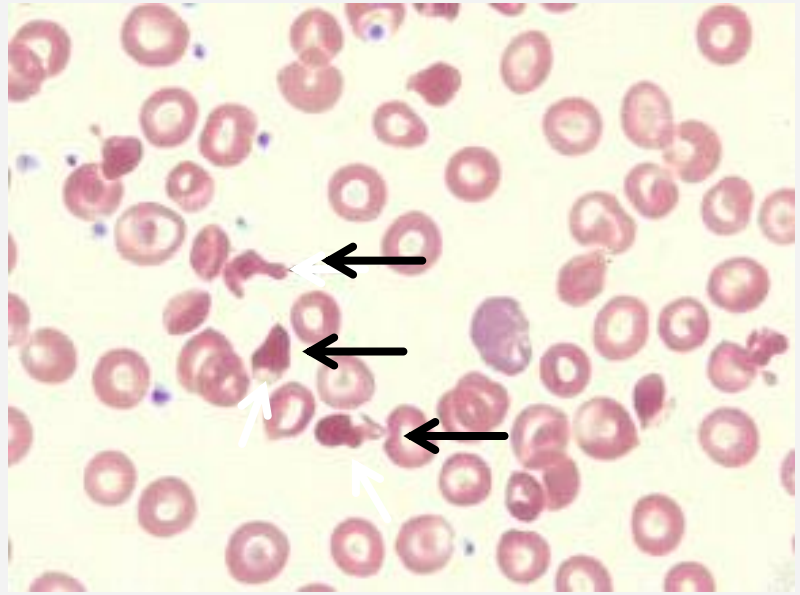
COMPOUND HETEROZYGOSITY

COMPOUND HETEROZYGOSITY

- Inheritance of 2 different β mutant genes
  - Hb S
  - β chain hemoglobinopathy or thalassemia
- Produce hemolytic anemia of vary severity
  - Hb S with Hb C, Hb D, Hb O, or β–thal
- Disorders of no consequence
  - Hb S with Hb E, Hb-G-Philadelphia, Hb Korle Bu

COMPOUND HETEROZYGOSITY

- **Hemoglobin SC**
  - Most common compound heterozygous syndrome that results from AA substitutions that are found on both of the two β –globin chains
  - Substitutions
    - Glutamic acid replaced by valine (Hb S) at position 6 on one β-globin chain
    - Glutamic acid replaced by lysine (Hb C) at position 6 on the other β-globin chain
  - Clinical features
    - Mild form of SCD but can be severe in some cases
    - Vasoocclusive episodes, moderate hemolytic anemia, and splenomegaly
    - Proliferative and severe retinopathy (more than sickle cell anemia)
  - Laboratory diagnosis
    - (+) Sickle Solubility
    - CBC – few sickle cells, target cells, crystal structures
    - Electrophoresis
  - Treatment
    - Same as SCD



https://imagebank.hematology.org/image/3967/hemoglobin-sc-crystals--2

COMPOUND HETEROZYGOSITY

- **Hb S-β –Thalassemia**
  - Most common sickle cell syndrome in the Mediterranean descent
  - 2nd most common heterozygous disorder
  - Symptoms
    - Mild to moderate sickle cell anemia
    - Severity depends on β chain production of β-thal
      - **Hb S-β^0 thal**
        - No β globin chain
        - Symptoms similar to Hb SS
      - **Hb S-β^+ thal**
        - Production of β globin chain at some level
        - Milder condition than patients with Hb SC

UNSTABLE HEMOGLOBIN VARIANTS

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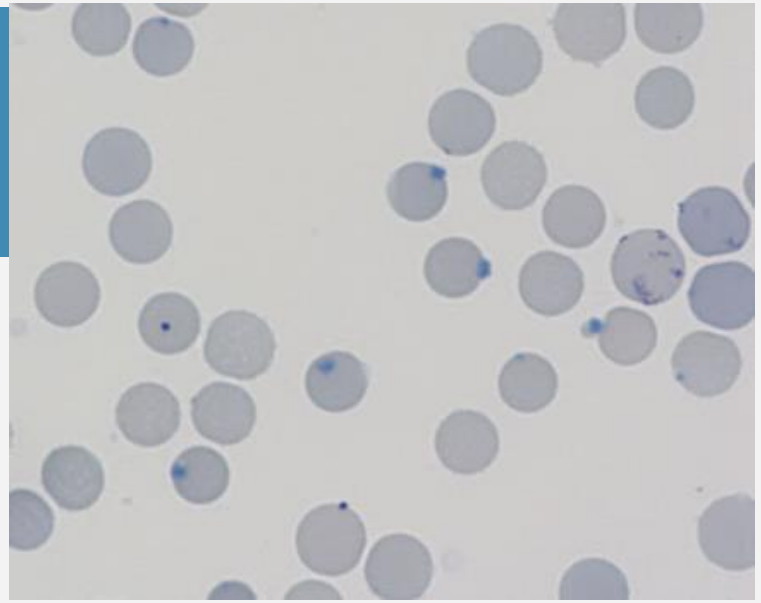
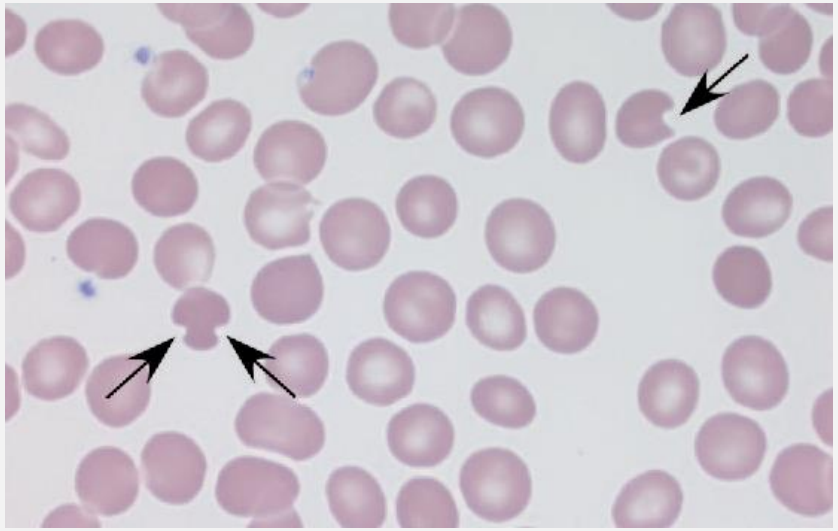
- Result from genetic mutations to globin genes
- Create Hb that precipitate in vivo
  - Produce Heinz bodies and cause hemolytic anemia
- No major clinical significance
- Increased oxygen affinity
- Unstable hemoglobin disease
  - "Congenital Heinz body anemia"
- Autosomal dominant; homozygous incompatible with life
- Mild hemolytic anemia in 25% of unstable Hb
- Examples:
  - Hb Köln
  - Hb Kurich
  - Hb Gun Hill



http://atlas.gechem.org/en/component/k2/item/1004-heinz-bodies



https://www.semanticscholar.org/topic/Bite-cell-(cell)/1867907

CONCOMITANT CIS MUTATIONS WITH HB S

CONCOMITANT CIS MUTATIONS WITH HB S

- Double substitution on the β chain
  - Seen in Hb C-Harlem, Hb S-Antilles and Hb S-Oman

- (+) sickle solubility may occur
- Alkaline agar: migrates with Hb C
- Acid agar: migrates in the Hb S position

- Clinically important when patients are compound heterozygotes for Hb S

REFERENCES

- Rodak's Hematology Clinical Principles and Applications 6th edition