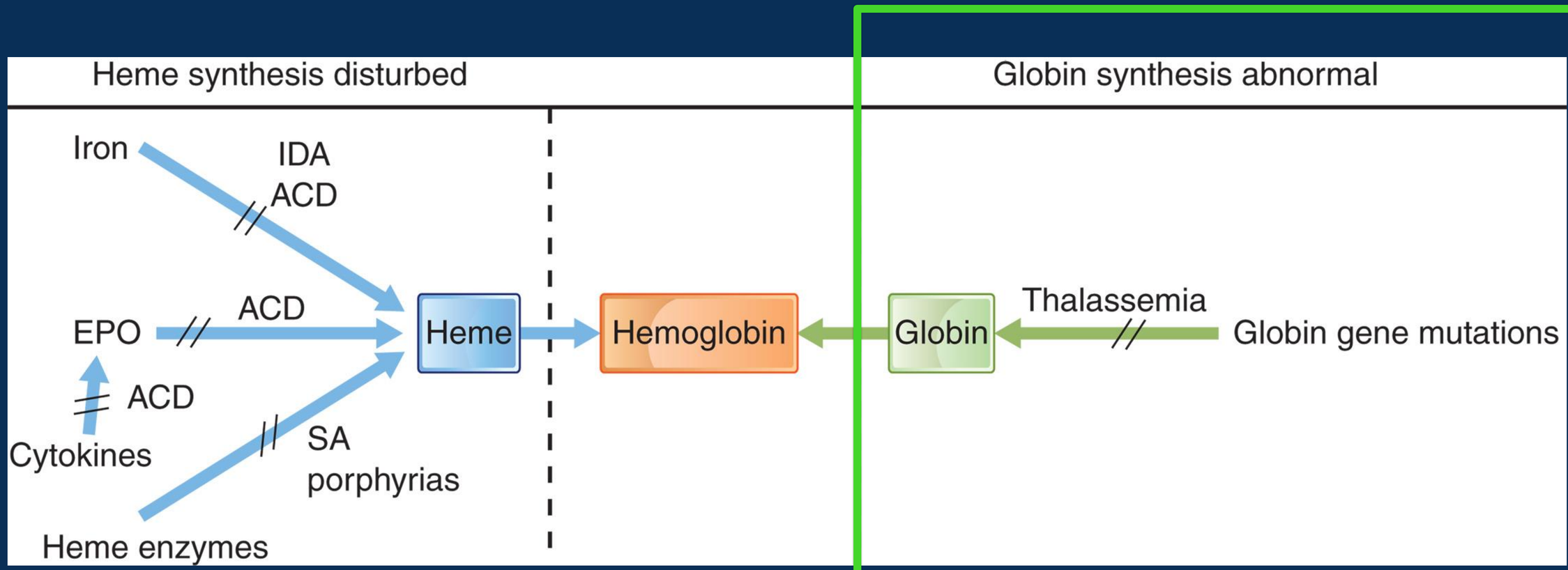
Hemoglobinopathies

Objectives

- Define hemoglobinopathy
  - Differentiate QUALitative vs QUANTitative
- Laboratory assessment of hemoglobin variants
- Structural variant nomenclature
- Pathophysiology, clinical, and laboratory findings for:
  - Qualitative: Sickle Cell Anemia, Sickle Cell Trait, Hemoglobin E, Hemoglobin C, Hemoglobin D

Hemoglobin Production



Hemoglobin Production

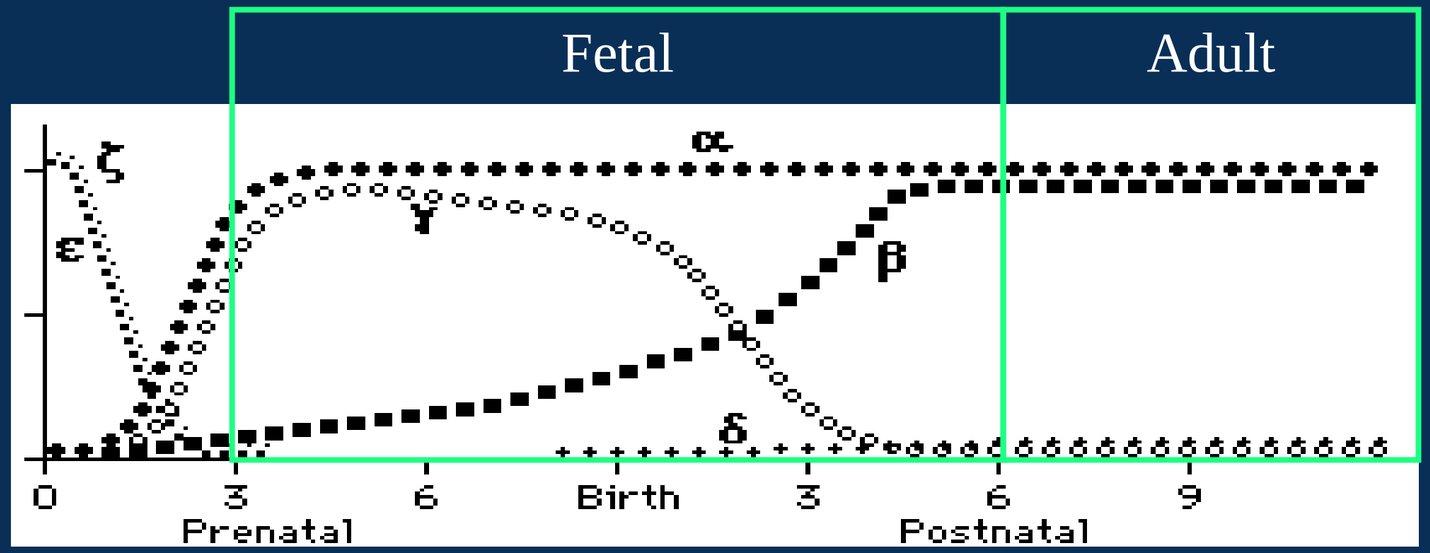
- Disruption related to:
  - Globin genes
    - => Hemoglobinopathies

Hemoglobinopathy

- Genetic abnormality affecting structure or synthesis of HGB
  - Specifically, globin chains (heme normal)
- Worldwide, highest prevalence:
  - African blacks, ethnic groups from Mediterranean basin and Southeast Asia
- Qualitative (structural) defects [hemoglobinopathy]
- Quantitative (synthesis) defects [thalassemias]
- Combinations possible

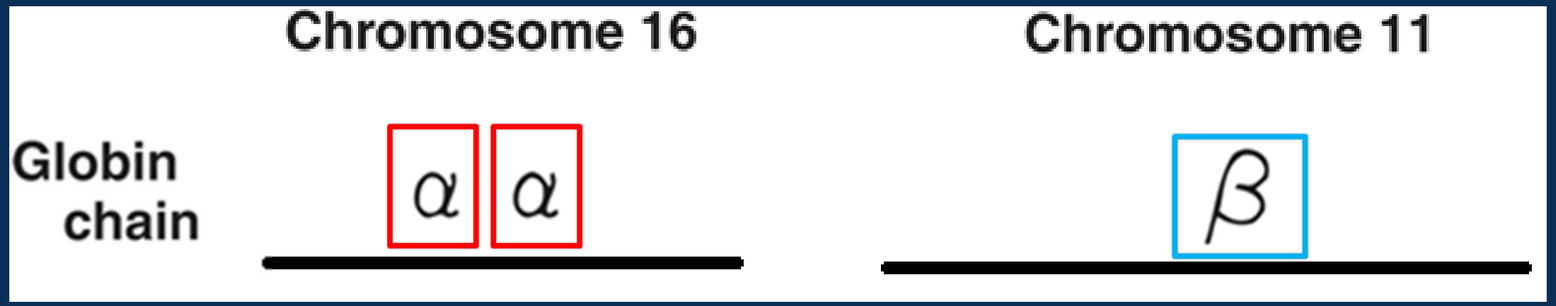
Qualitative (Structural) Variants

- Clinical presentation depends on class of globin chain involved (α, β, δ, or γ)
  - Most mutations seen in the β-chain
- Fetal
  - HbF ($\alpha_2\gamma_2$)
- Adult
  - > 95% HbA ($\alpha_2\beta_2$)
  - < 2% HbF ($\alpha_2\gamma_2$)
  - 1.5 – 3.5% HbA_2 ($\alpha_2\delta_2$)



Qualitative (Structural) Variants

- α-chain gene (4 copies)
  - Mutation of a single gene locus leads to a small amount of abnormal HGB
- β-chain gene (2 copies)
  - Associated with clinical phenotype when mutated



Variant Identification Algorithm

Screen

- Patient demographics, history
- CBC (RBC, RDW)

Reflex

- Smear review (Morphology, Heinz bodies)
- Solubility test, heat precipitation test
- HGB A_2 and F quantitation
  - High-performance liquid chromatography (HPLC)
  - Capillary and traditional electrophoresis
  - Isoelectric focusing (IEF)

Confirmatory

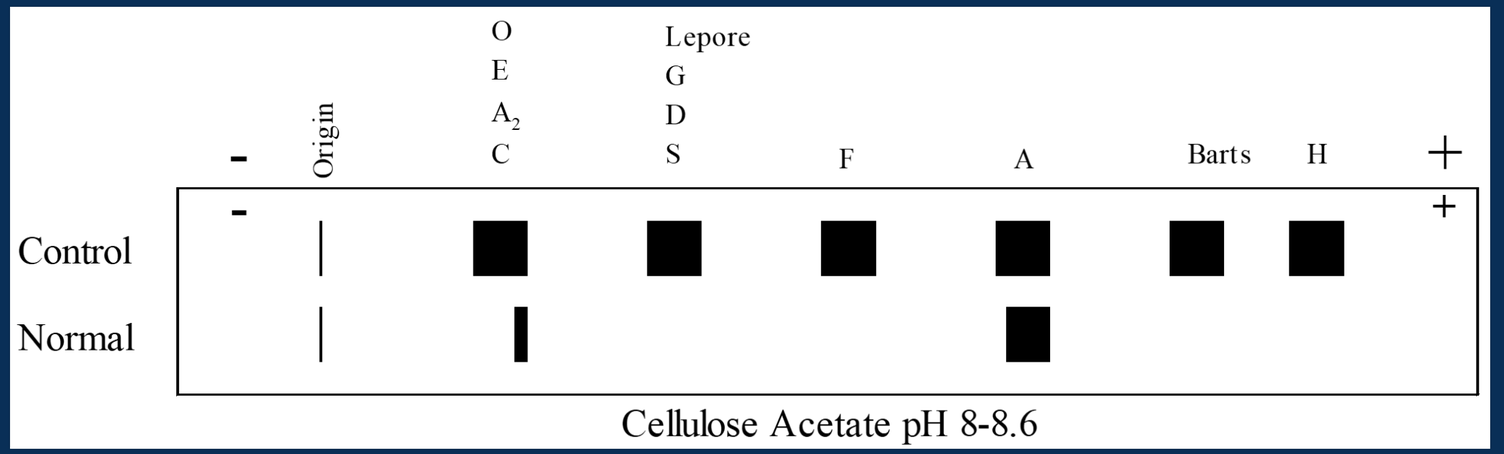
- Polymerase chain reaction (PCR)

Variant Identifications

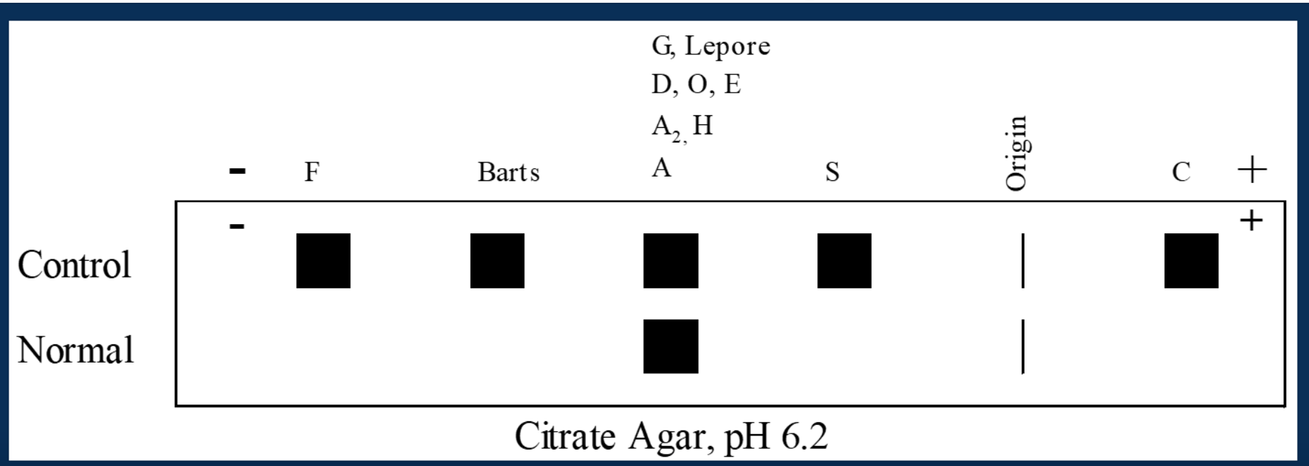
- Solubility test
  - Rapid test for heterozygous and homozygous HbS
  - May be negative (severe anemia)
  - Should not be used for screening in newborns
  - False positives
    - Unstable Hgb’s and rare Hgb variants
    - Elevated plasma proteins and lipids

Variant Identifications

- HGB variant carries an electrical charge (net +, net -)
  - Depends on amino acid sequence of globins
  - pH of medium (acid + alkaline mediums)



Cellulose Acetate pH 8-8.6



Citrate Agar, pH 6.2

Variant Nomenclature

- Disease/anemia = homozygous (both β-chain genes affected)
- Trait = heterozygous (one β-chain gene affected)
- Common names
  - HgS : 1st discovered
  - Other Hgb's = successive letters
    - Except: HbA = Adult, HbF = Fetal
  - Added geographic area where Hgb's discovered
    - e.g. HbG Honolulu, HbJ Capetown, Hb Ft. Worth

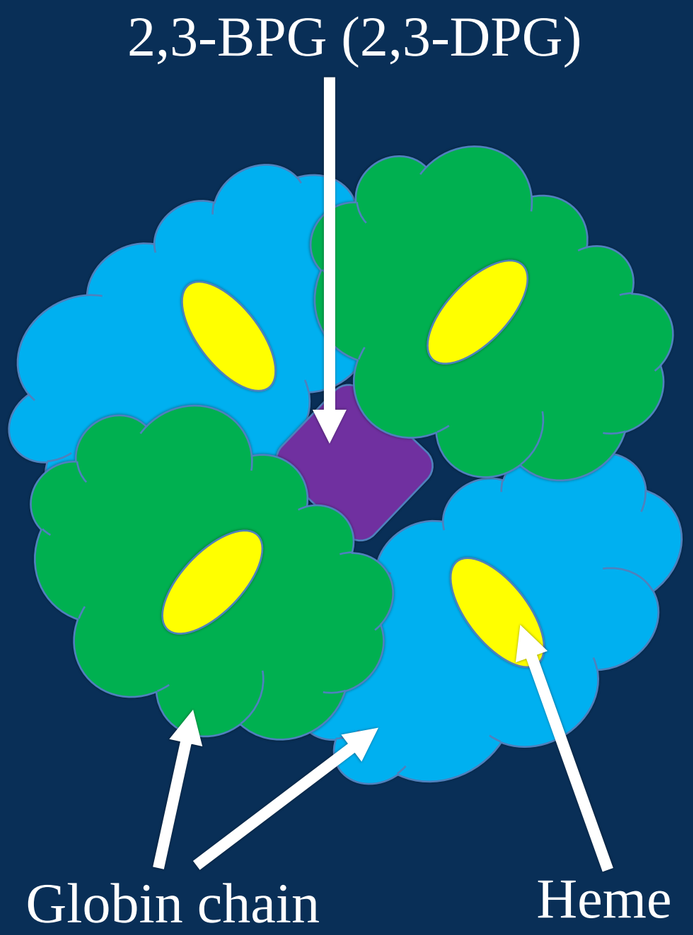
Variant Nomenclature

- Scientific names
  - Mutated globin chain
  - Position of the affected amino acid
  - Helical position of the mutation
  - Amino acid substitution

- HbS: β6 (A3) Glu→Val
  - β-chain
  - Amino acid in the 6th position
  - A3 helix position
  - Valine substituted for glutamic acid

Pathophysiology

- Amino acid substitutions = symptoms
  - Mutations affect solubility
    - Nonpolar amino acid substituted for a polar
      - e.g. HgbS, HgbC
  - Mutations affect function
    - Heme-iron stability, methemoglobin (Fe^{+++})
    - Allosteric properties, O_2 affinity (↑ or ↓)
  - Stability of hemoglobin (Heinz bodies)
    - Weakening of the binding of heme to globin

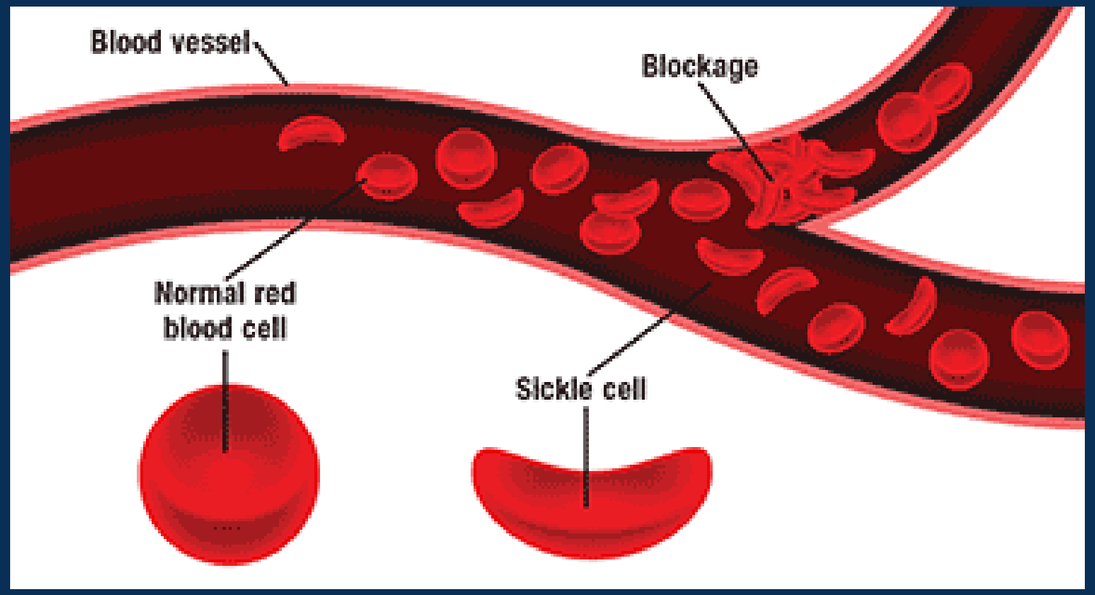


Sickle Cell Anemia (SCA)

- Most common symptomatic hemoglobinopathy
  - Tropical Africa, Mediterranean, Middle East, India, Nepal, North/Central/South America
- African Americans
  - 0.3–1.3% sickle cell disease (homozygous)
  - 8–10% sickle cell trait (heterozygous)

SCA – Pathophysiology

- β6 (A3) Glu→Val
  - Homozygous: $\beta^S\beta^S$
  - Produces net decrease in negative charge
- Solubility in the deoxygenated state is markedly ↓
  - Polymerization of molecules forms rigid aggregates
    - Vaso-occlusive crisis
  - Deoxygenation environments
    - Hypoxia, acidosis, hypertonicity, temperatures > 37°C
- 2,3-BPG level of homozygotes is ↑ (why?)

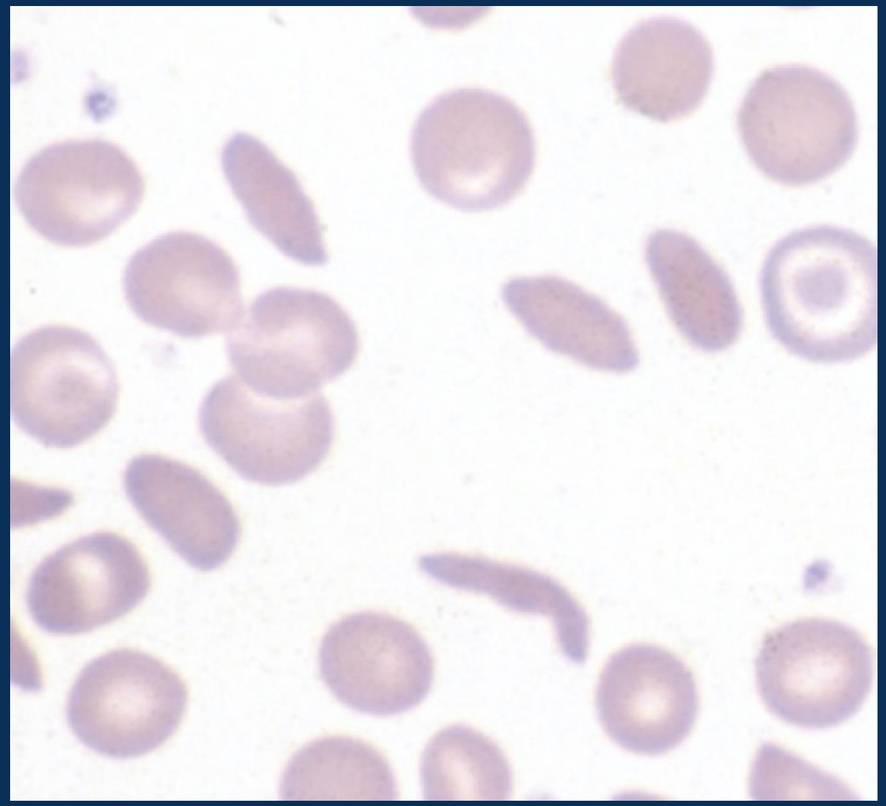


SCA – Clinical Findings

- First clinical signs at ~ 6 months (why?)
- Vaso-occlusive crisis
  - Episodes of blocked capillaries
  - Splenomegaly in early childhood
    - Acute splenic sequestration
- Dactylitis – painful symmetrical hands and feet swelling
- Slow blood flow (thrombosis, stroke)
- Acute chest syndrome
  - Most common cause of death in children

SCA – Laboratory Findings

- CBC, peripheral blood smear
  - Normocytic, normochromic anemia
    - Retic = 10–20 %
    - HGB = 6–10 g/dL
    - HCT = 18-30 %
    - ↑ RDW
  - Sickle cells, target cells, basophilic stippling, HJ bodies
- Solubility test +



SCA – Laboratory Findings

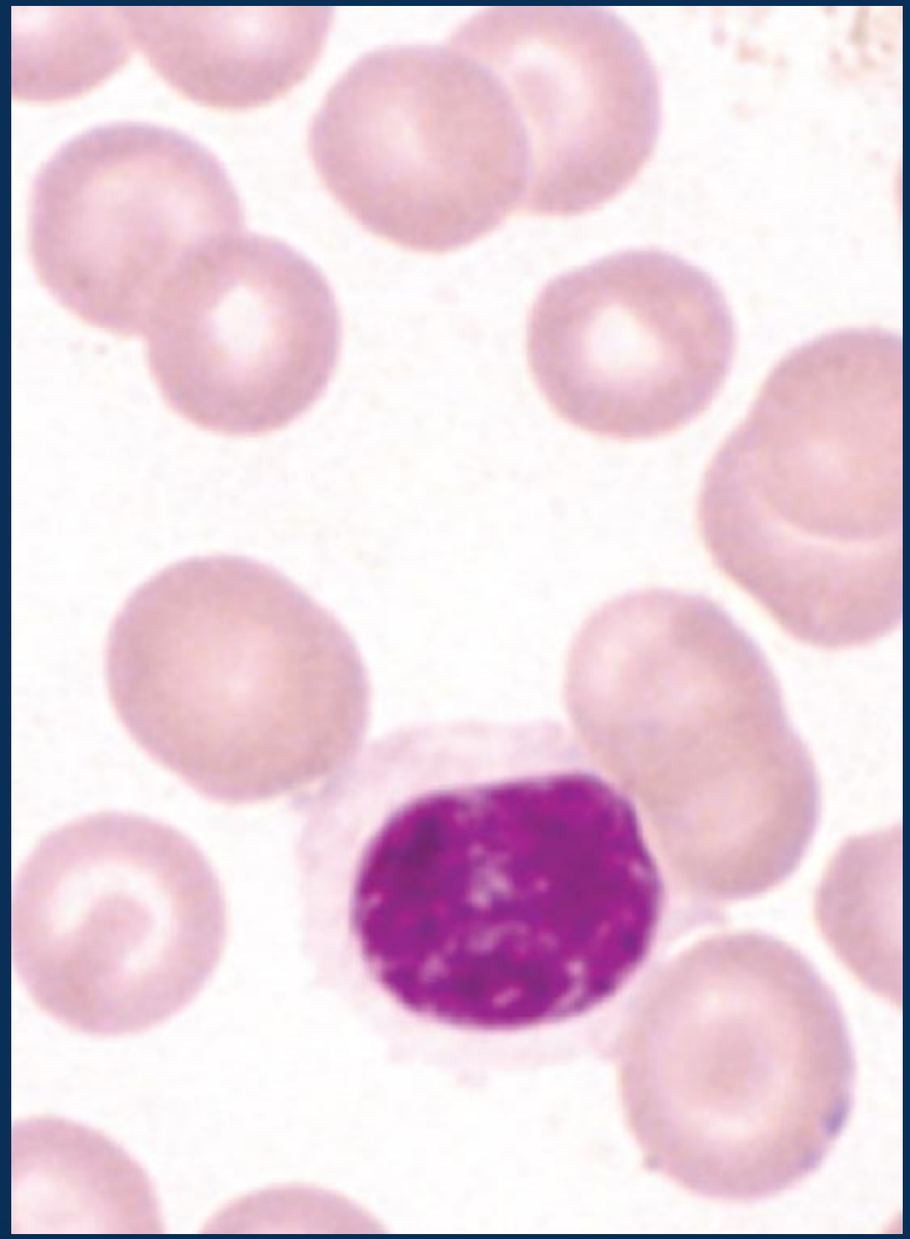
- Bone marrow
  - Erythroid hyperplasia (M:E?)
- Hgb electrophoresis (adult vs newborn?)
  - 80–95% HbS
  - 5–20% HbF
- HPLC, IEF, PCR
- Therapy
  - Hydroxyurea, blood transfusions

Sickle Cell Trait

- Heterozygous: $\beta^A\beta^S$
- No clinical symptoms
- Normal CBC, no anemia or sickle cells
- Solubility test +
- Hb electrophoresis
  - HbA 50–65%
  - HbS 35–45%
  - HbF normal

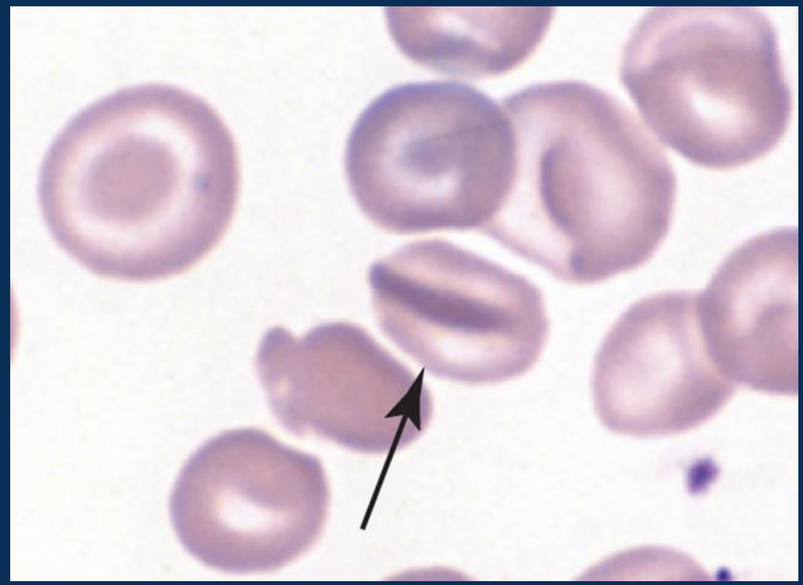
Hemoglobin E

- 2nd most common hemoglobinopathy
  - Southeast Asia
    - Trait as high as 50% in areas of Thailand
    - Homozygous HbE (HbE ≥ 90%) $\beta^E\beta^E$
  - Mild asymptomatic, microcytic hypochromic anemia
    - Target cells
- Heterozygous HbE (HbE 35–45%) $\beta^A\beta^E$
  - Slight microcytosis



Hemoglobin C

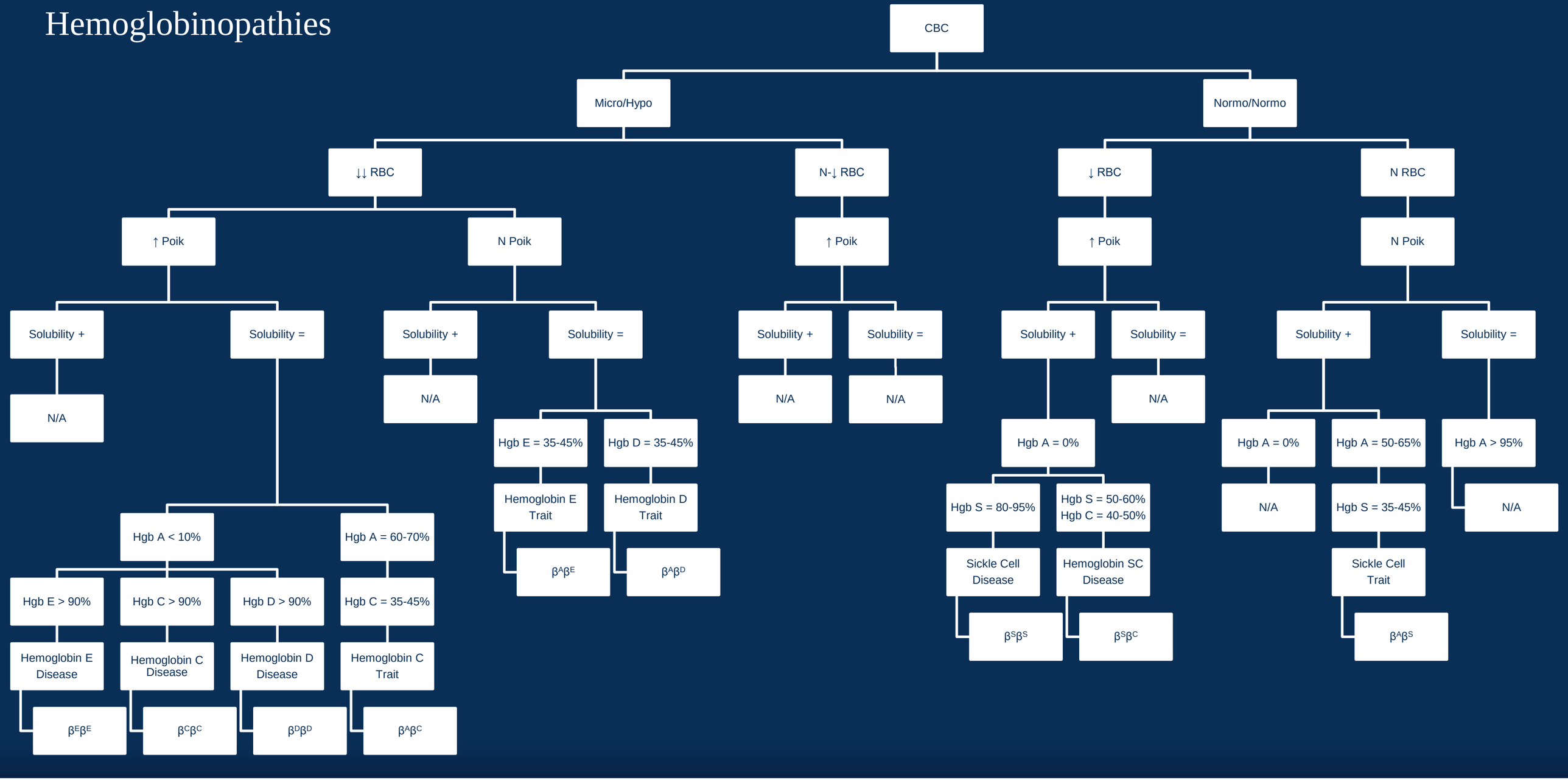
- 3rd most common Hb variant
  - West African blacks (Trait = 17–28%)
  - African Americans (Trait = 2–3%; Disease = 0.02%)
- Homozygous (90% HbC) $\beta^C\beta^C$
  - Asymptomatic microcytic hypochromic anemia
  - target cells, rare HbC crystal
- Heterozygous (30–40% HbC) $\beta^A\beta^C$
  - Mild hyochromia; target cells
- HgSC ($\beta^S\beta^C$): HbA absent, %HbS > %HbC



Hemoglobin D

- Several molecular variants
  - Most common in African Americans, Indians
- Solubility =
- Homozygous (95% HbD)
  - Mild hemolytic anemia with target cells
- Heterozygous
  - Asymptomatic

Hemoglobinopathies



Thalassemias

Objectives

- Define thalassemia (quantitative)
- General pathophysiology, clinical, and laboratory findings
- Genotypes, deomgraphics, and laboratory findings for
  - α-Thalassemias
  - β-Thalassemias
- Mention other thalassemias

Thalassemia

- One of the most common genetic disorders
- Decreased or absent synthesis of globin chain(s)
  - Produce reduced amounts of normal Hgb
- Two major types of classical thalassemia
  - α-thalassemia: impaired α-chain synthesis
  - β-thalassemia: impaired β-chain synthesis

Thalassemia - Pathophysiology

- Normal α-chain:β-chain synthesis = 1:1
- Excess α-chains
  - Highly insoluble, precipitate in the cell, bind to cell membrane causing damage
  - ↓ RBC deformability
- Excess β-chains
  - Combine to form HbH (β4) = high O_2 affinity
  - Unstable

Thalassemia – Clinical Findings

- Chronic hemolysis
  - Splenomegaly; functional hyposplenism
  - Gallstones – large amounts of bilirubin excreted by liver
- BM expansion from ↑ erythropoiesis
  - Extramedullary erythropoiesis
- Pregnant women with thalassemia
  - Developing infants impacted more than mother

Thalassemia – Laboratory Findings

- Peripheral blood
  - Microcytic hypochromic anemia
  - RBC count normal or ↓, but ↑ relative to hemoglobin and hematocrit levels
  - Target cells, basophilic stippling, nRBCs
  - Precipitated excess chains (supravital)
  - ↑ Retic

α-Thalassemia

- ↓ synthesis of α-chains
- Affected individuals
  - Found primarily in people of Mediterranean, Asian, African ancestry
    - Commonly seen in blacks, Indians, Chinese, Middle Eastern people
  - Patients from African descent
    - Milder version of α-thalassemia

α-Thalassemias

α-chain genes (4 copies)

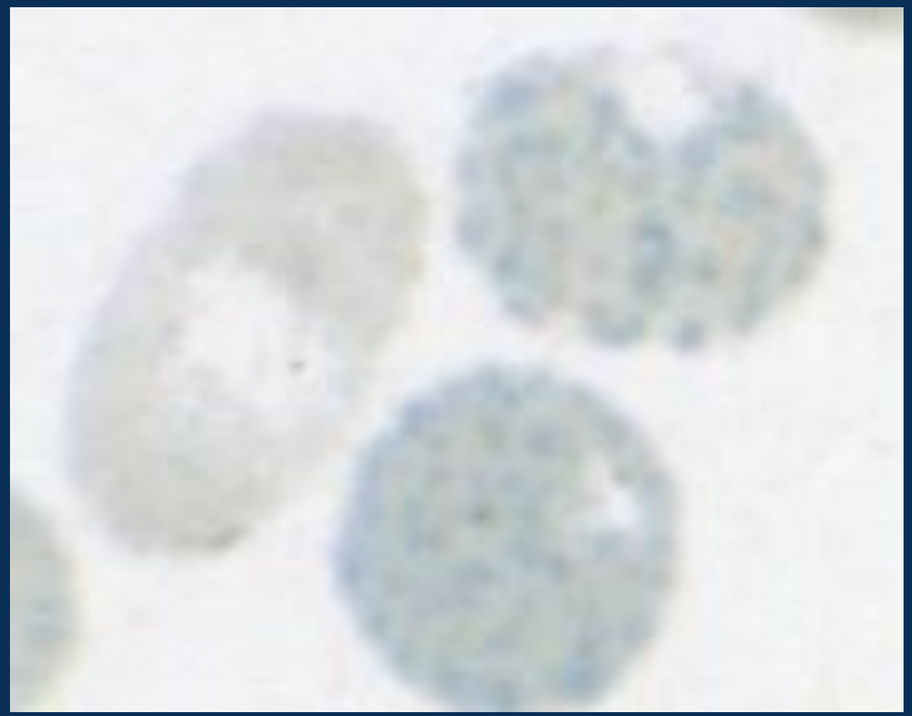
Genotype	Phenotype
- - / - - or α^0 / α^0	Hydrops fetalis
- - / - α or α^0 / α^+	Hemoglobin H Disease
- - / α α, - α / - α or $\alpha^0 / \alpha, \alpha^+ / \alpha^+$	α-thalassemia minor
- α / α α or α^+ / α	Silent carrier

Hydrops fetalis

- - - / - - = α^0 / α^0
  - Incompatible with life
  - Severe microcytic hypochromic anemia
    - Hgb = 3-10 g/dL
    - Marked anisopoikilocytosis, nRBCs
  - Hb electrophoresis
    - 80–90% Hb Bart's (γ4)
    - 10–20% Hb Portland (embryonic Hb)

HbH disease

- - - / - $\alpha = \alpha^0 / \alpha^+$
- Chronic, moderately severe hemolytic anemia
  - Microcytic hypochromic
  - Poikilocytosis, target cells, ↑ retics, nRBCs
  - HbH inclusions (brilliant cresyl blue), “golf ball”
- Hb electrophoresis
  - Hb Bart's ($\gamma 4$) at birth; HbH ($\beta 4$) at adult
    - ↑ O_2 affinity



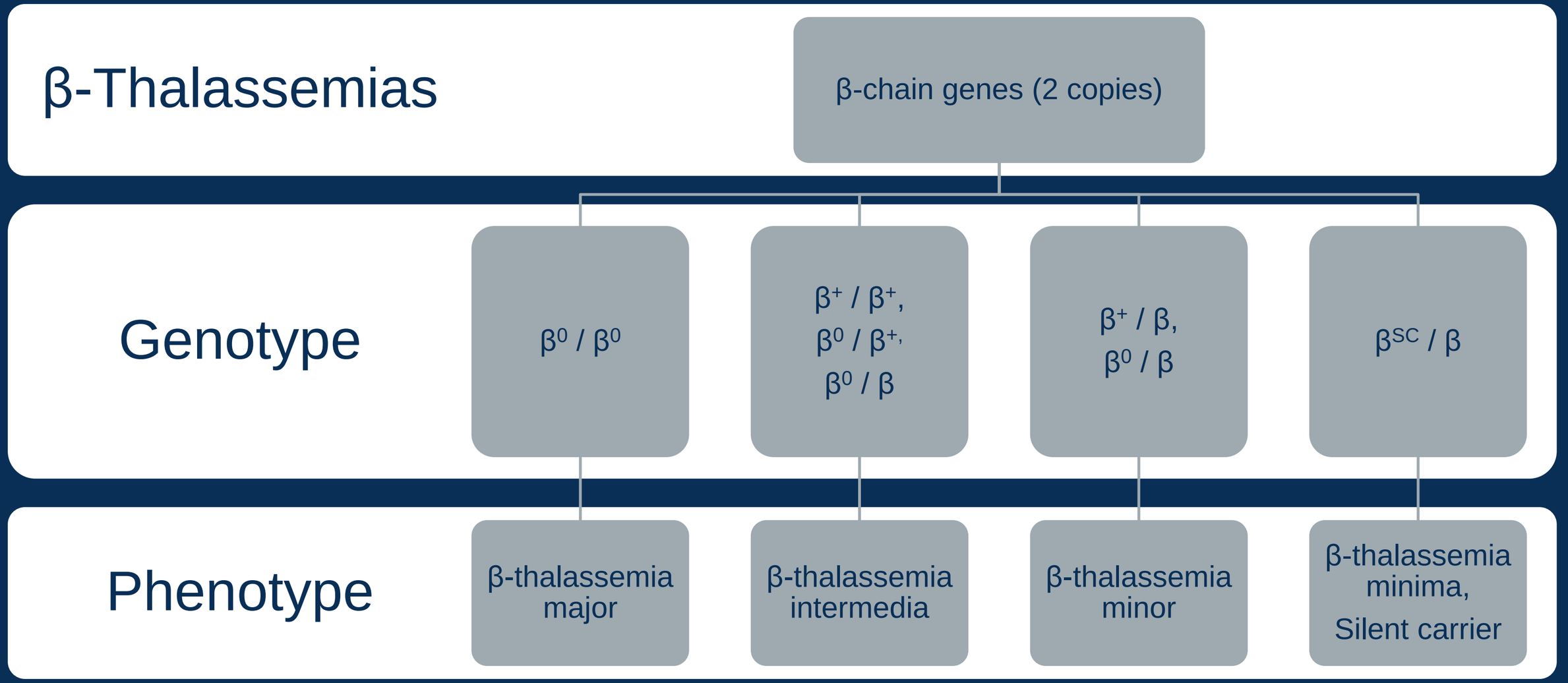
α-thalassemia minor

- - - / α α = α^0 / α = - α / - α = α^+ / α^+
- Asymptomatic to mild anemia
- Mild microcytic hypochromic anemia
  - MCV 60-70 fL
  - Few target cells
- Newborn 5–6% Hb Bart's (0% at > three months)

Silent carrier

- - α / α α = α^{+} / α
- Normal Hb synthesis
- Asymptomatic
- Borderline microcytosis
- Diagnosis requires gene analysis

β-Thalassemias



β-Thalassemia

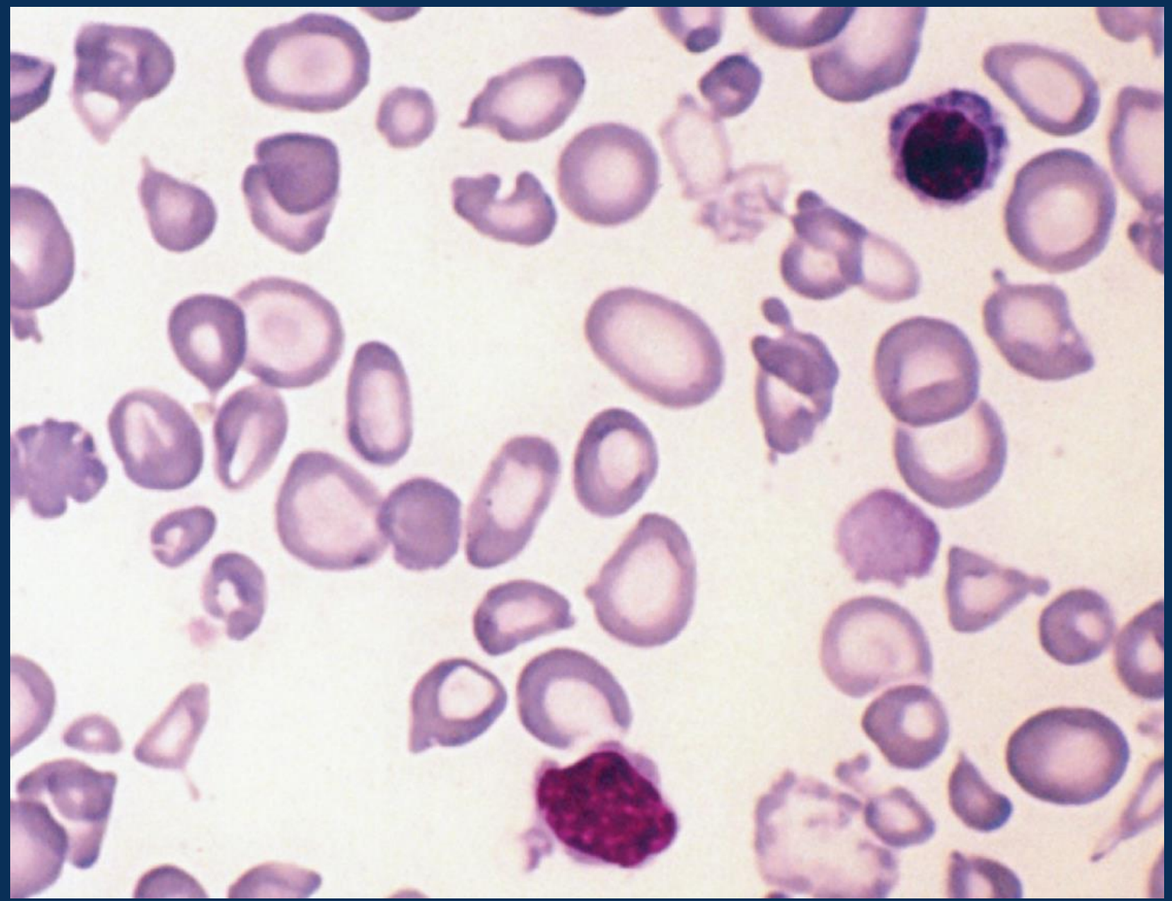
- Affected individuals
  - β^0 - most severe mutation
    - Mediterranean regions
      - Northern Italy, Greece, Algeria, Saudi Arabia and Southeast Asia
  - β^+ - two severities
    - Mediterranean region, Middle East, Indian subcontinent, Southeast Asia (more severe)
    - African descent (milder version)

β-thalassemia major

- β^0 / β^0 (no HbA) = β^+ / β^+ (some HbA)
  - β^0 / β^+ (double heterozygous)
- Cooley's anemia
- Infants (occur ~ 6 months of age)
  - Irritability, pallor, failure to thrive
  - Diarrhea, fever, enlarged abdomen
- Ineffective erythropoiesis
  - BM expansion, thinning of calcified bone
  - Extramedullary hematopoiesis

β-thalassemia major

- Hb can be as low as 2–3 g/dL
- MCV < 67 fL, ↓ MCH and MCHC
- Peripheral blood smear
  - Anisocytosis and poikilocytosis
  - Basophilic stippling, polychromasia
  - nRBCs
  - Normal or ↑ RDW
- BM erythroid hyperplasia

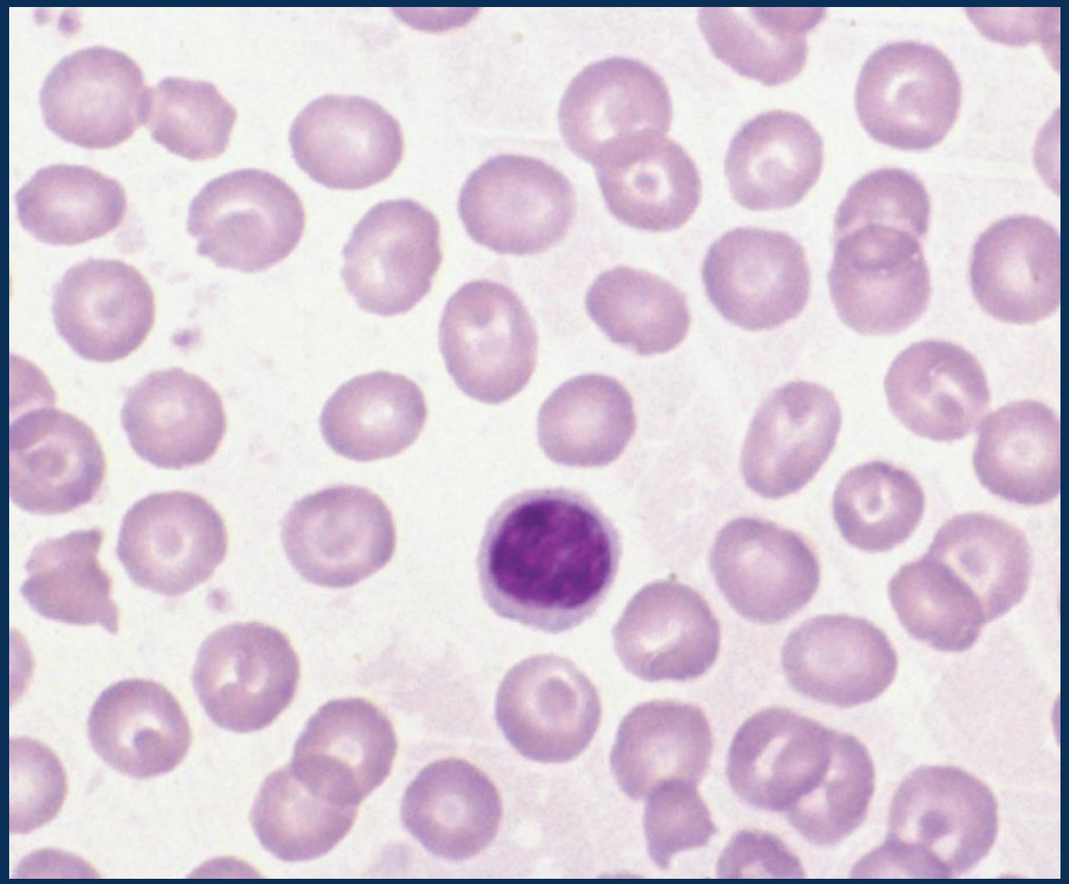


β-thalassemia intermedia

- $\beta^+ / \beta^+ = \beta^0 / \beta^+ = \beta^0 / \beta$ (some HbA)
- Severe: HbA_2 5–10%, HbF 30–75%; milder forms
- Symptoms of intermediate clinical severity
  - Intensify with physiologic stress
- Microcytic hypochromic anemia
- Hb 7–10 g/dL
- RBC count is disproportionately higher than Hb
- Target cells, basophilic stippling, nRBCs
- BM erythroid hyperplasia

β-thalassemia minor

- $\beta^+ / \beta = \beta^0 / \beta =$ (sufficient amounts of HbA)
- Asymptomatic, symptoms when stressed
- Hb 9–14 g/dL => RBC count ↑ > 5×10^{12}/L
- HbA_2 3.5–7%
- MCV 55–70 fL, MCHC 29–33 g/dL
- Variable anisocytosis and poikilocytosis
  - Target cells, basophilic stippling
- Slightly ↑ reticulocytes



β-thalassemia minima

- β^{SC} / β = (silent carrier)
- Asymptomatic β-thalassemia
- No major lab abnormalities
- Mildly imbalanced α- to non α-globin chain synthesis ratio

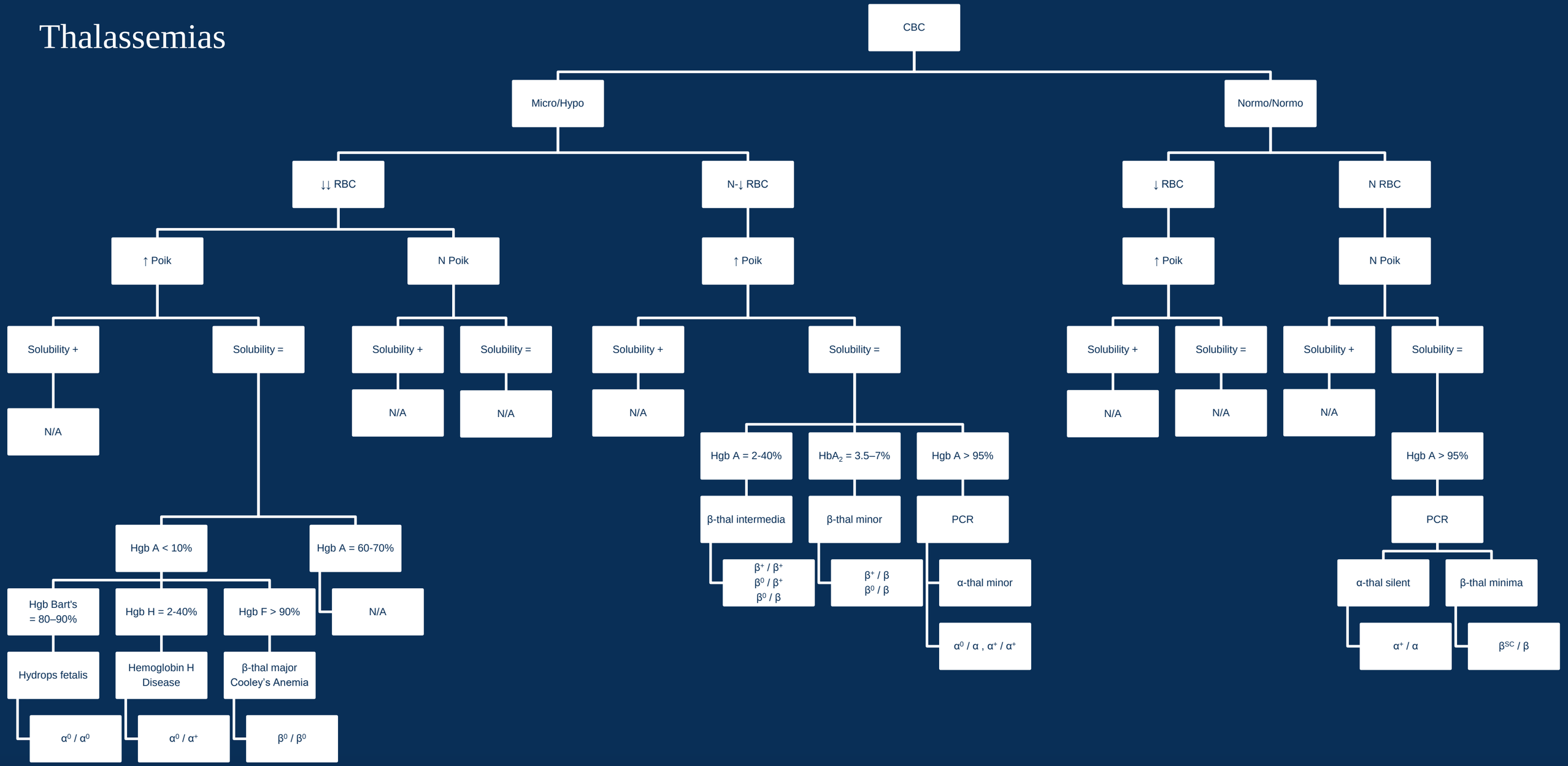
Other Thalassemias

- δβ-Thalassemia
  - Greek, African, Italian, Arabian ancestry
  - Production of β- and δ-chains is affected
  - Clinically classified as thalassemia intermedia
- Hb Constant Spring
  - Common in Thailand
  - α-chains elongated, normal β-chains

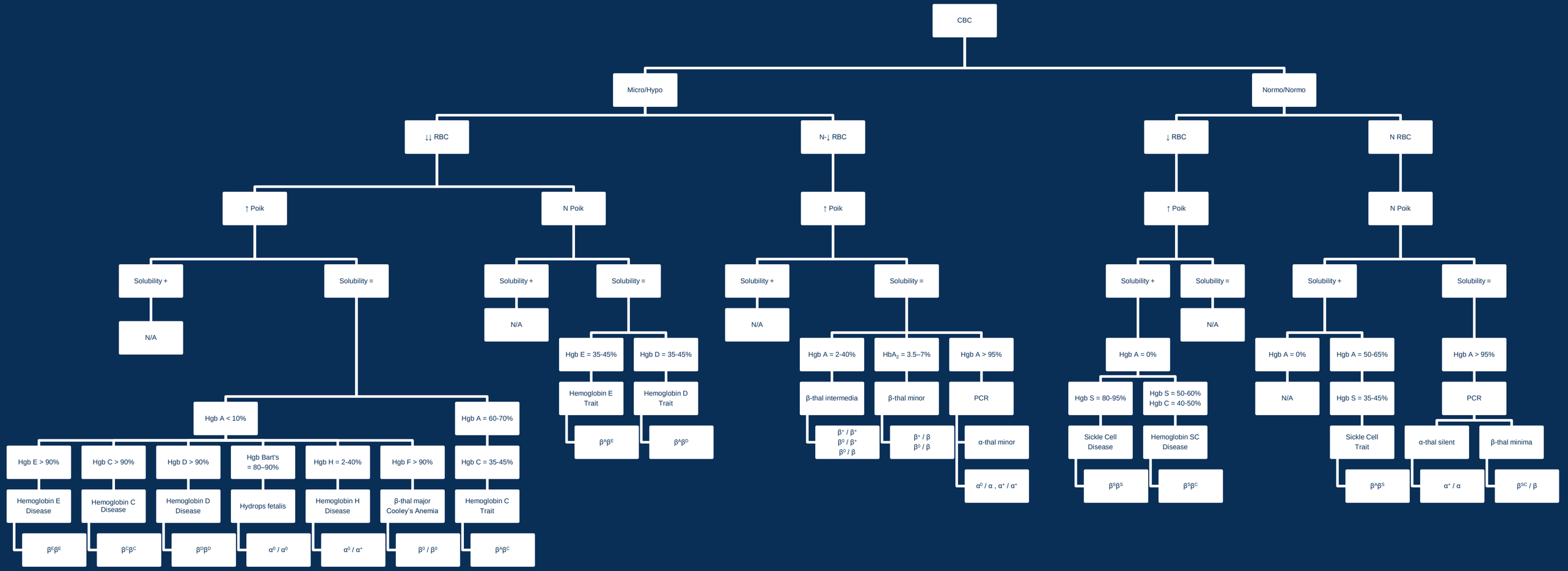
Other Thalassemias

- Hereditary persistence of fetal Hb
  - Absence of δ- and β-chain synthesis
  - Compensated by ↑ γ-chain synthesis
  - Homozygotes (100% HbF = $\alpha_2 + \gamma_2$)
    - Asymptomatic Microcytic hypochromic RBCs
    - RBCs 6–7 x10^{12}/L, Hb 14.8–18.2 g/dL
  - Kleihauer-Betke acid elution stain
    - Pancellular (Black, Greek)
    - Heterocellular (Swiss)

Thalassemias



Hemoglobinopathies & Thalassemias



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