THE THALASSEMIAS

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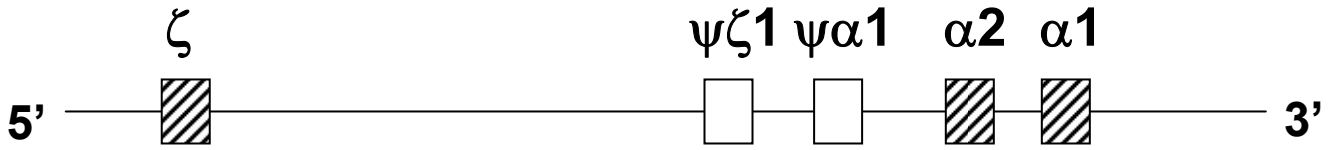
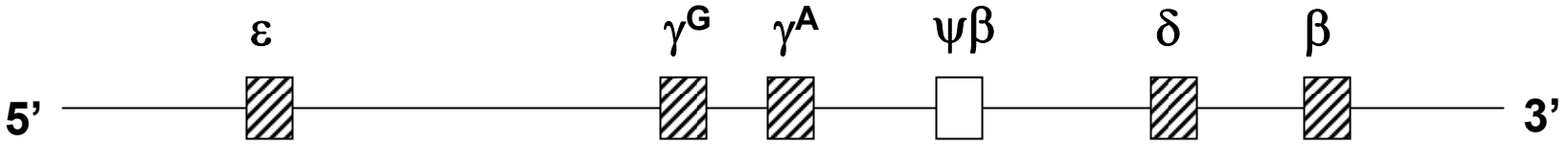
OVERVIEW

- Review of human globin genes
- α-thalassemias
- β-thalassemias
- β-thalassemia mutations affecting fetal hemoglobin
- Thalassemic hemoglobinopathies

GLOBIN GENE CLUSTERS



α Globin gene cluster: Chromosome 16

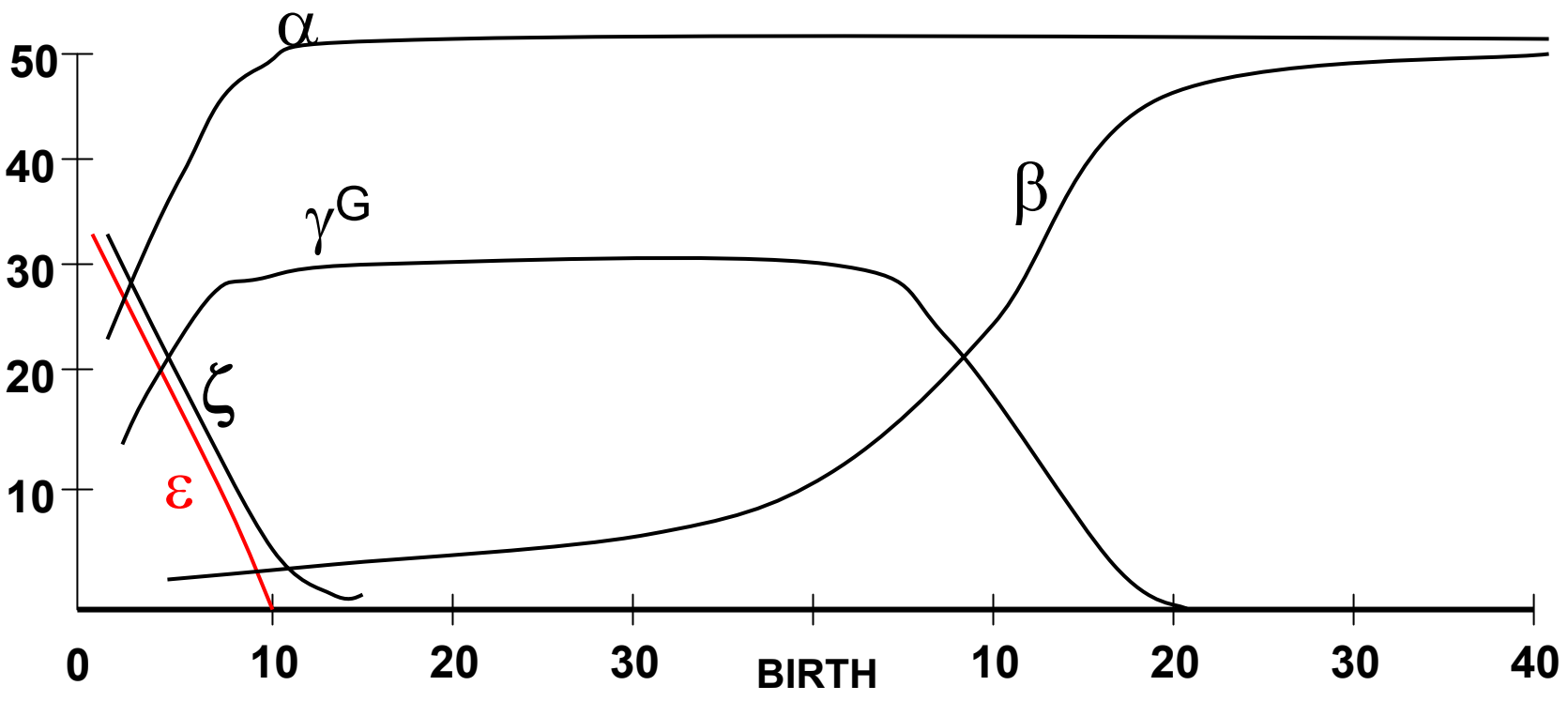


β Globin gene cluster: Chromosome 11

HUMAN HEMOGLOBINS

	Hb Species			
Embryonic – 1st two trimesters	Hb Gower 1	$\zeta_2\varepsilon_2$	Zeta	Epsilon
	Hb Gower 2	$\alpha_2\varepsilon_2$	Alpha	Epsilon
	Hb Portland	$\zeta_2\gamma_2$	Zeta	Gamma
Post-natal	Hb A	$\alpha_2\beta_2$	Alpha	Beta
	Hb F	$\alpha_2\gamma_2$	Alpha	Gamma
	Hb A_2	$\alpha_2\delta_2$	Alpha	Delta
α-thalassemia	Hb H	β_4	-	Beta
	Hb Bart's	γ_4	-	Gamma

GLOBIN GENE EXPRESSION DURING HUMAN DEVELOPMENT



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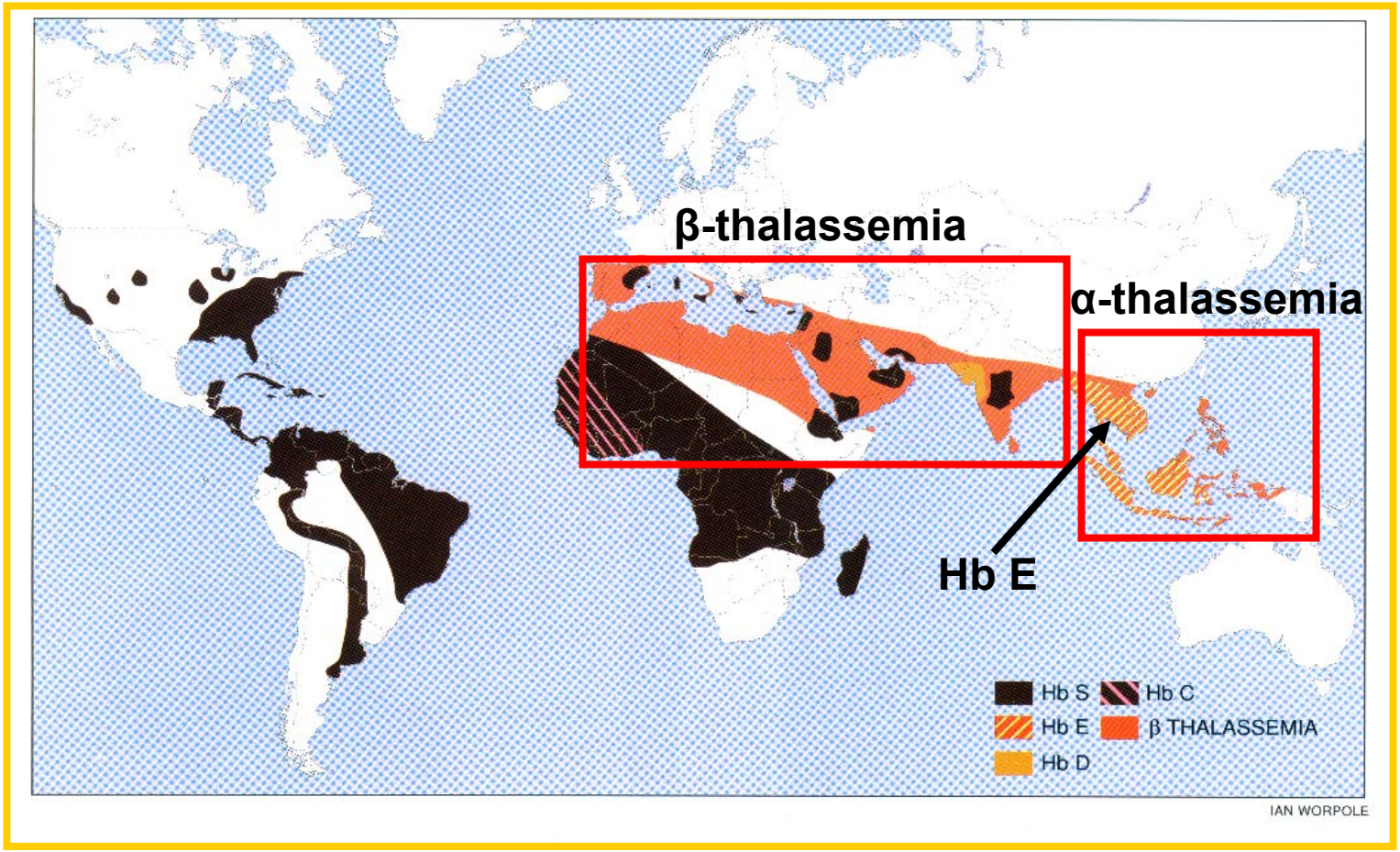
THALASSEMIA

- Thalatta - Greek for the “sea” in Xenophon’s Anabasis
- Historical ties to Mediterranean populations in Greece and Italy
- Quantitative disorders of hemoglobin synthesis
  - α thalassemia
  - β thalassemia
- Abnormal α:β globin ratio

THALASSEMIA

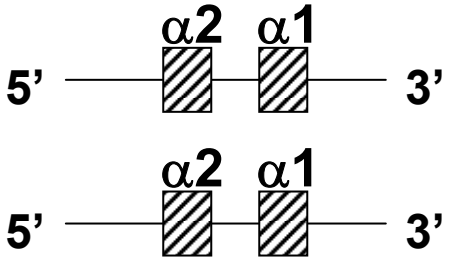


α-THALASSEMIA

- **α thalassemia - most common single gene disease syndrome in the world**
- **α^+ thal prevalence:**
  - 5-10% Mediterranean
  - 20-30% West African
  - Up to 68% in Asia/Pacific Rim
- **Increased prevalence of malaria in α^+ thal: ? A natural vaccination**

α-THALASSEMIA

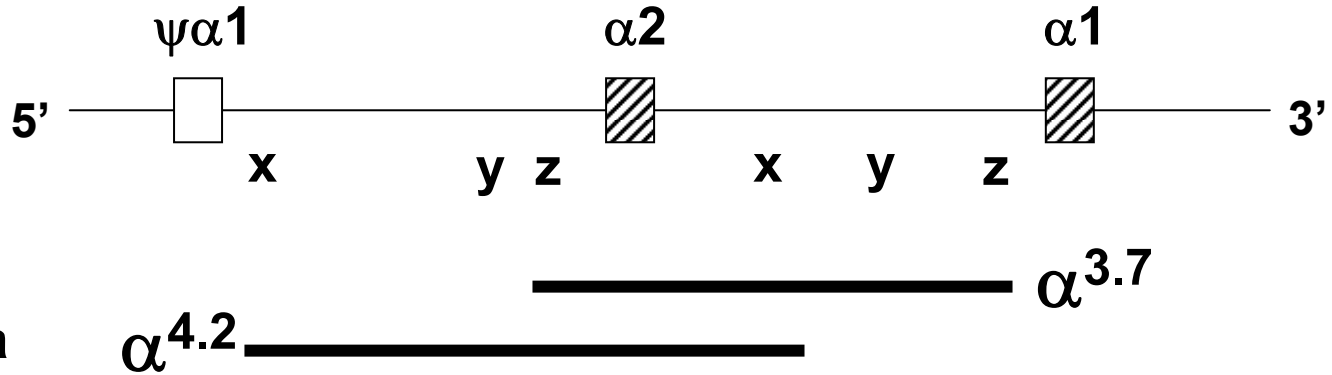
- **Normal:**
  - 4 α globin genes



- **Abnormal:**
  - 0-3 alpha globin genes
  - Small DNA deletions (meiotic crossover events)
  - Large DNA deletions (Southeast Asian)
  - Less common point mutations (Hb Constant Spring)

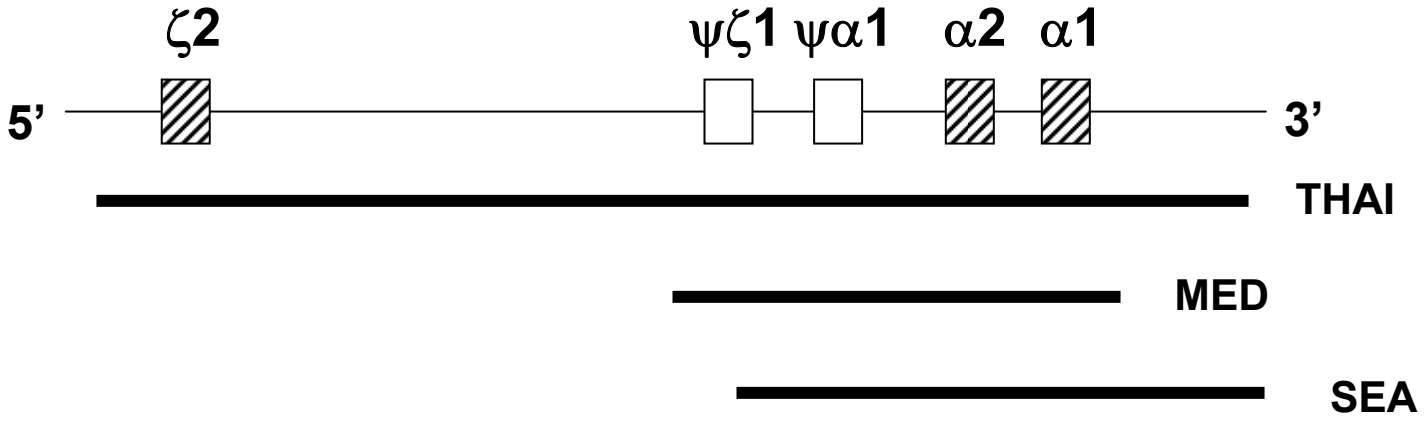
α-THALASSEMIA DNA DELETIONS

- Small DNA deletions: Remove 1 α gene
  - 3.7 kb deletion, rightward (z box); (- $\alpha^{3.7}$)
  - 4.2 kb deletion, leftward (x box); (- $\alpha^{4.2}$)
  - Nonreciprocal homologous recombination event: -α / αα and ααα / αα



α-THALASSEMIA DNA DELETIONS

- Large DNA deletions:
  - Remove 2 α genes, gives rise to α^0 phenotype
  - Southeast Asian variant (SEA; Δ20 kb)
  - Mediterranean variant (MED)



HB CONSTANT SPRING

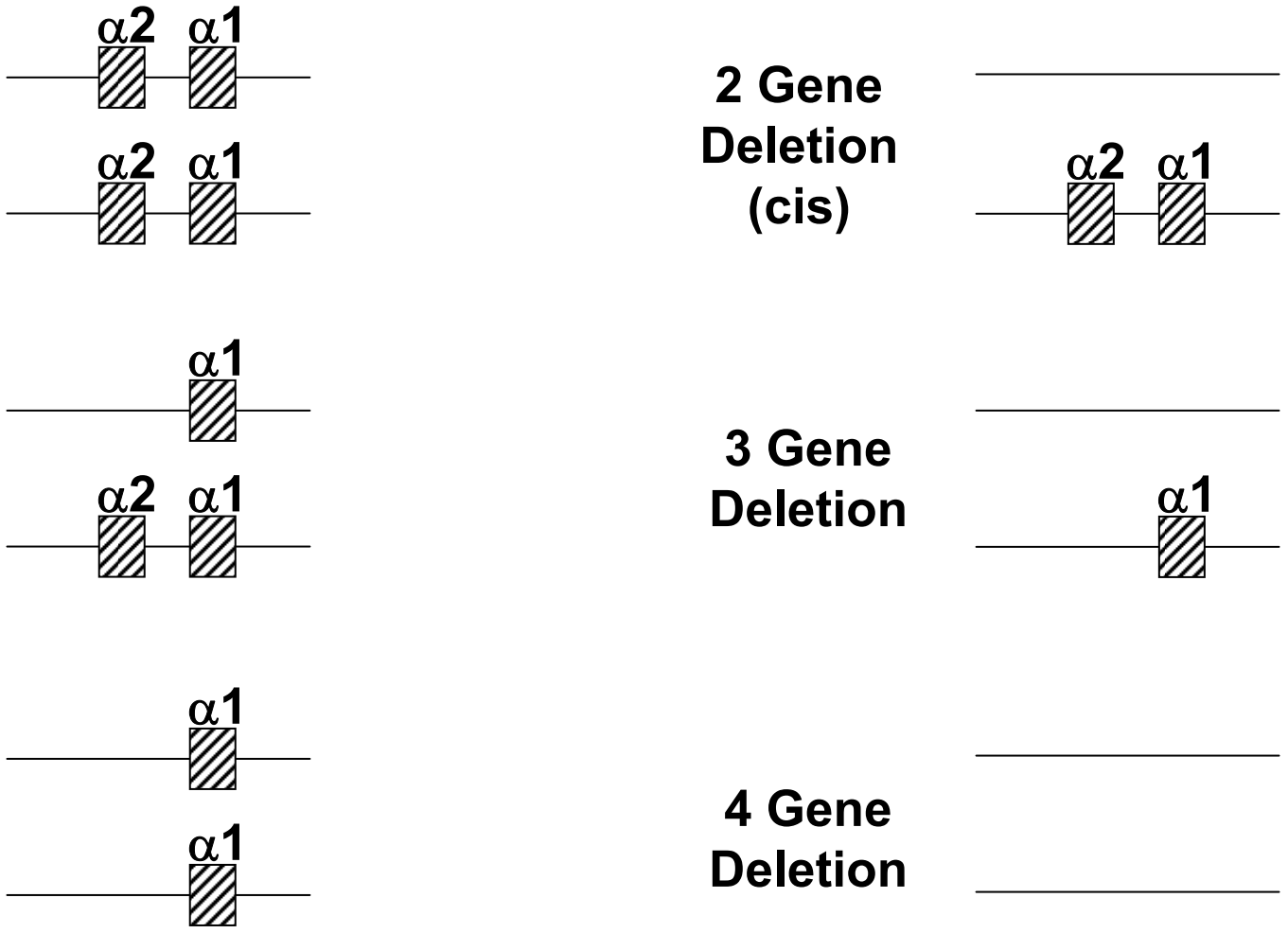
Nondeletional α Globin Variant ($\alpha^{cs}\alpha$)

- Common in Asia (Thailand, Cambodia)
- Slow migration on electrophoresis due to increased size of α globin protein

Mutation at Stop Codon

- TAA (stop) $\rightarrow$ CAA (glutamine)
- Longer globin chain, 141 AA $\rightarrow$ 172 AA
- Thalassemic phenotype (1%) as elongated globin is unstable and denatures in cell

α-THALASSEMIA



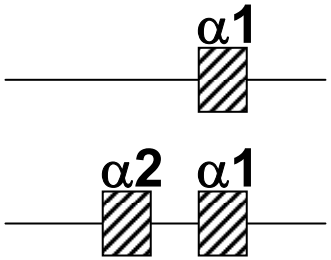
α-THALASSEMIA

Clinical Disease	Deletion	Genotype	α:β ratio
Silent Carrier	1 gene	α α/- α	0.75:1
α-Thalassemia Trait	2 genes	- α/- α α α/- -	0.5:1
Hb H Disease	3 genes	- α/- -	0.25:1
Hydrops Fetalis	4 genes	- -/- -	0:1

- 30% of African Americans are silent carriers
- 1-2 % of African Americans have α-thal trait

α-THAL: SILENT CARRIER

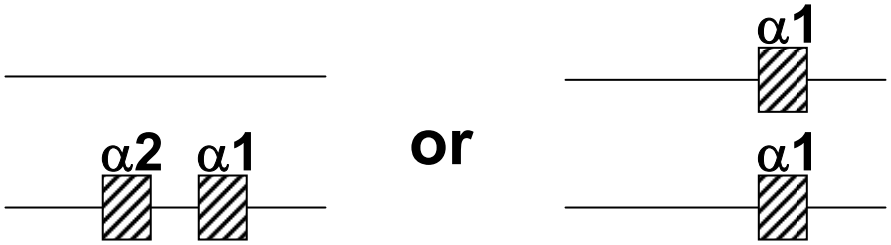
- Silent carrier = 1 gene deletion



- Birth: 1-2% Hb Bart's (γ_4)
- No anemia and normal morphology of rbcs

α-THAL TRAIT

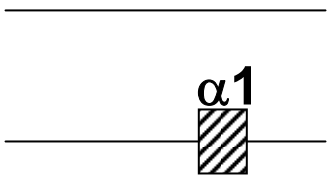
- 2 gene deletion: either -α/-α or αα/--



- Birth: Excess γ-globin production; 3-8% Hb Bart's
- Adult: Minimal HbH (β_4; excess β chains)
- Anemia: hypochromic, microcytic
  - Hb 9-11 gm/dL
  - MCV 65-75 fL

HB H DISEASE

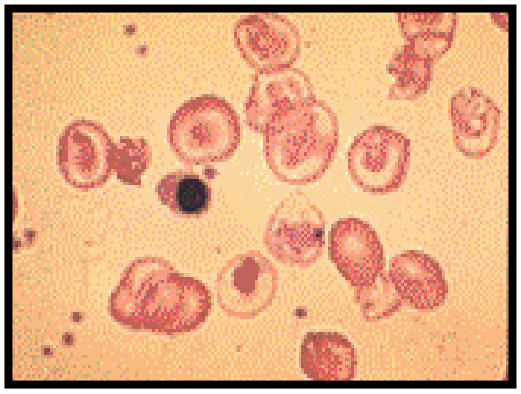
- 3 gene deletion

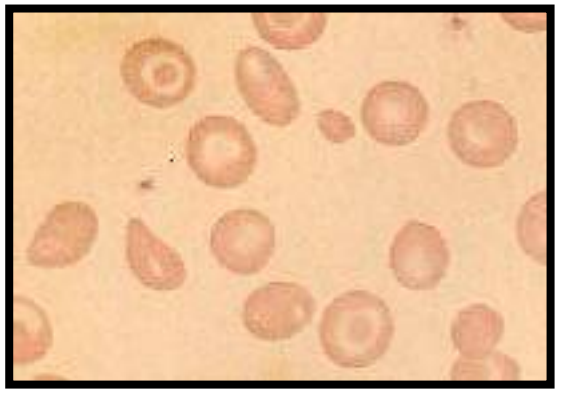


- Birth: 20-50% Hb Bart's
  MCV < 100 fL
- Adult: excess β chains = 5-30% HbH (β_4)
  Heinz body positive (denatured Hb)
- Anemia: hypochromic, microcytic

HB H DISEASE

- Anemia: hypochromic, microcytic (MCV 60 fL)
  - Moderate anemia
    - Hb 9.5 + 1.5 gm/dL
    - Hct 25-32%
  - Smear: rbc fragments, tear drops, targets





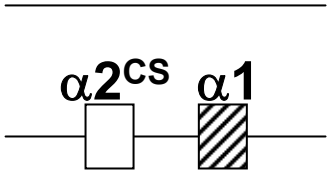
HB H DISEASE

Clinical Features

- Mild to moderate hemolytic anemia
  - Heinz body positive- Hb Bart's and Hb H both precipitate and cause rbc lysis
- Most often non-transfusion dependent
  - Symptomatic transfusion
- +/- icterus, gallstones, leg ulcers, splenomegaly
  - Splenectomy possible if transfusion dependent
  - Supportive care
- Increased Fe^{2+} absorption - iron overload common in pts. > 35 years

NONDELETIONAL HB H DISEASE

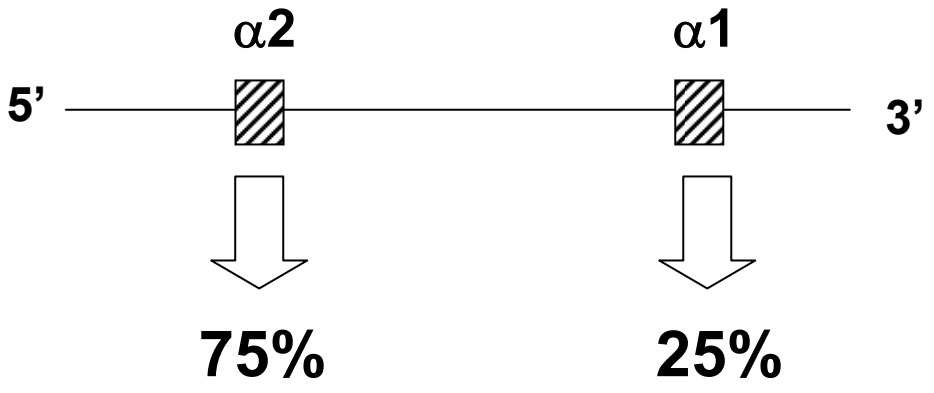
- 2 gene deletion and unstable structural variant



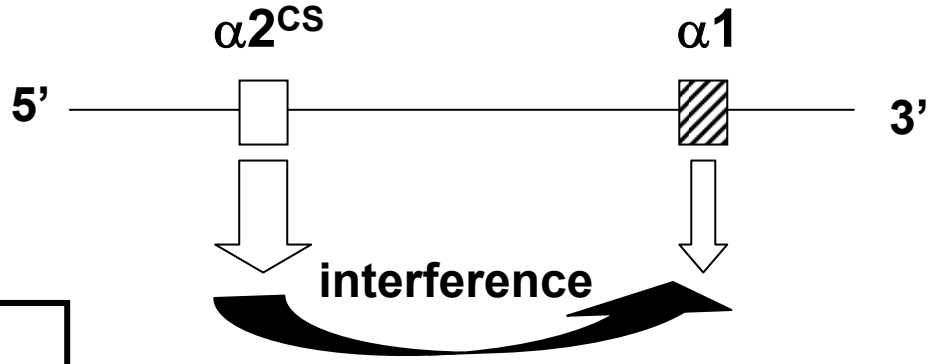
- A more severe <u>hemolytic anemia</u>
  - Diagnosis: younger age due to symptoms
  - Larger spleen
  - More red cell transfusions
  - Higher Hb H: average 16%
  - Higher Hb Bart's: 3%
  - Maybe 30% require splenectomy

HB CONSTANT SPRING

Normal α-globin transcription



Constant Spring α-globin transcription



- Explains more severe phenotype of nondeletional Hb H disease

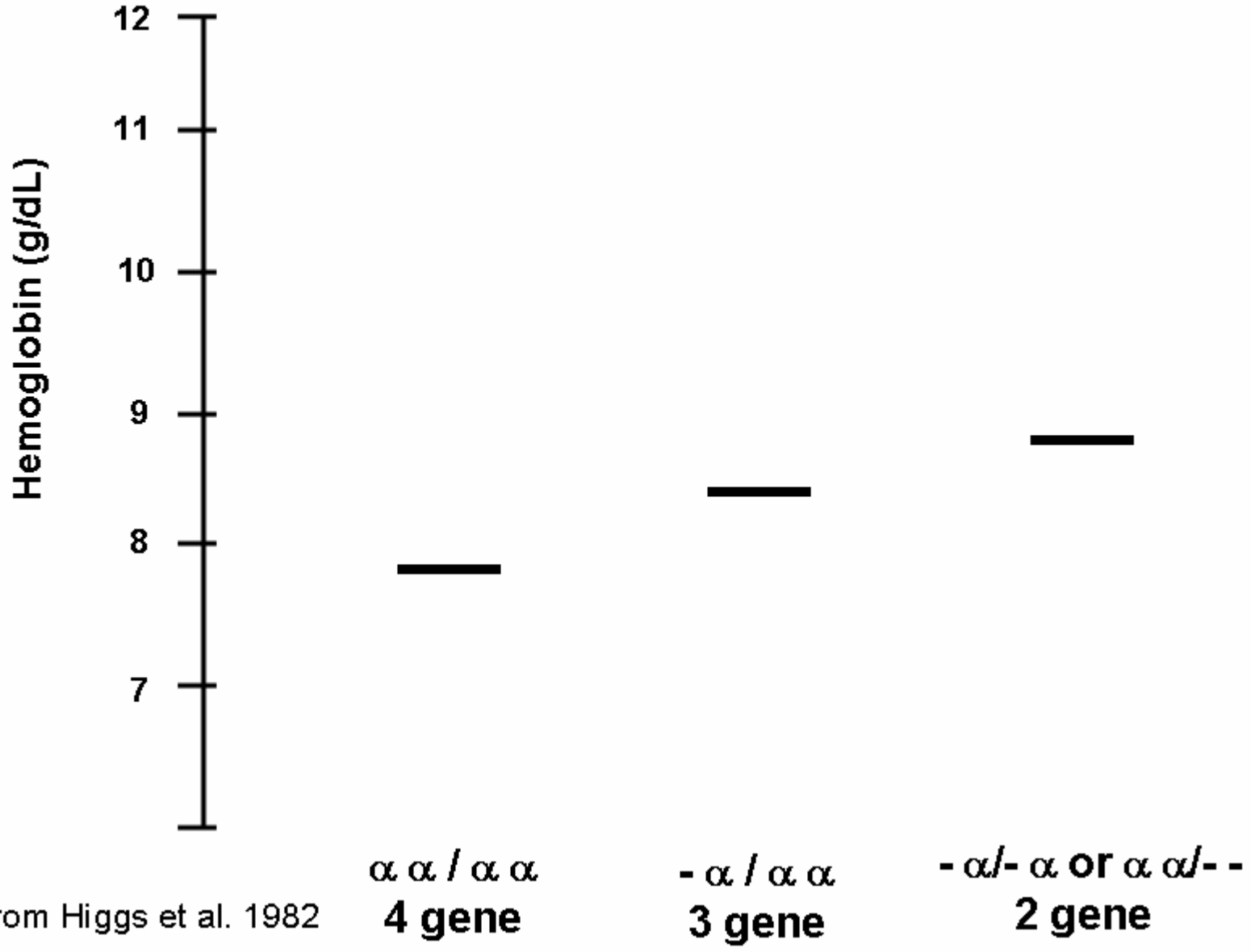
↓ normal α1 transcript

HYDROPS FETALIS

- **4 gene deletion - no α-globin**

- **A nonimmune hydrops**
- **Birth:**
  - 100% Hb Bart's and Hb H
  - Embryonic hemoglobins
  - Fetal demise from severe anemia
- **Rx: Transfusion support *in utero* and after birth; stem cell transplantation**

EFFECT OF α-THALASSEMIA IN SS



Adapted from Higgs et al. 1982

SS MOUSE: *HBA* IS A MODIFIER

Variable	 Transgenic Mouse	 Transgenic Mouse	 Transgenic Mouse	 Knockout—Transgenic Mouse
Human globin genes expressed	β^S, α	β^{SAD}, α	$\beta^S, \beta^{S\ Antilles}, \alpha$	β^S, α
Mouse globin genes expressed	β, α	β, α	β, α	None
Expression of sickle or sickle-related genes	β^S, 75%	β^{SAD}, 26%	β^S, 42% $\beta^{S\ Antilles}$, 38%	β^S, 100%
Hemolytic anemia	No	Compensated	Compensated	Severe
Micro-occlusive disease	Mild	Moderate	Moderate	Severe

Nagel. NEJM : (1999)

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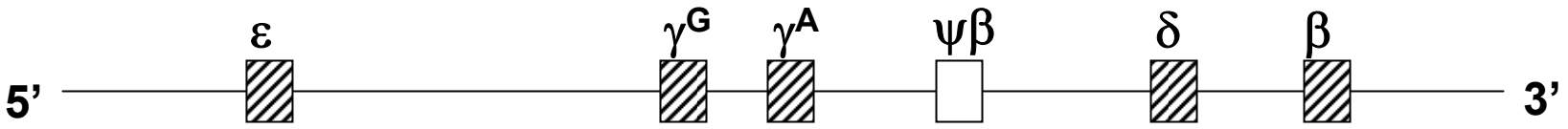
β-THALASSEMIA

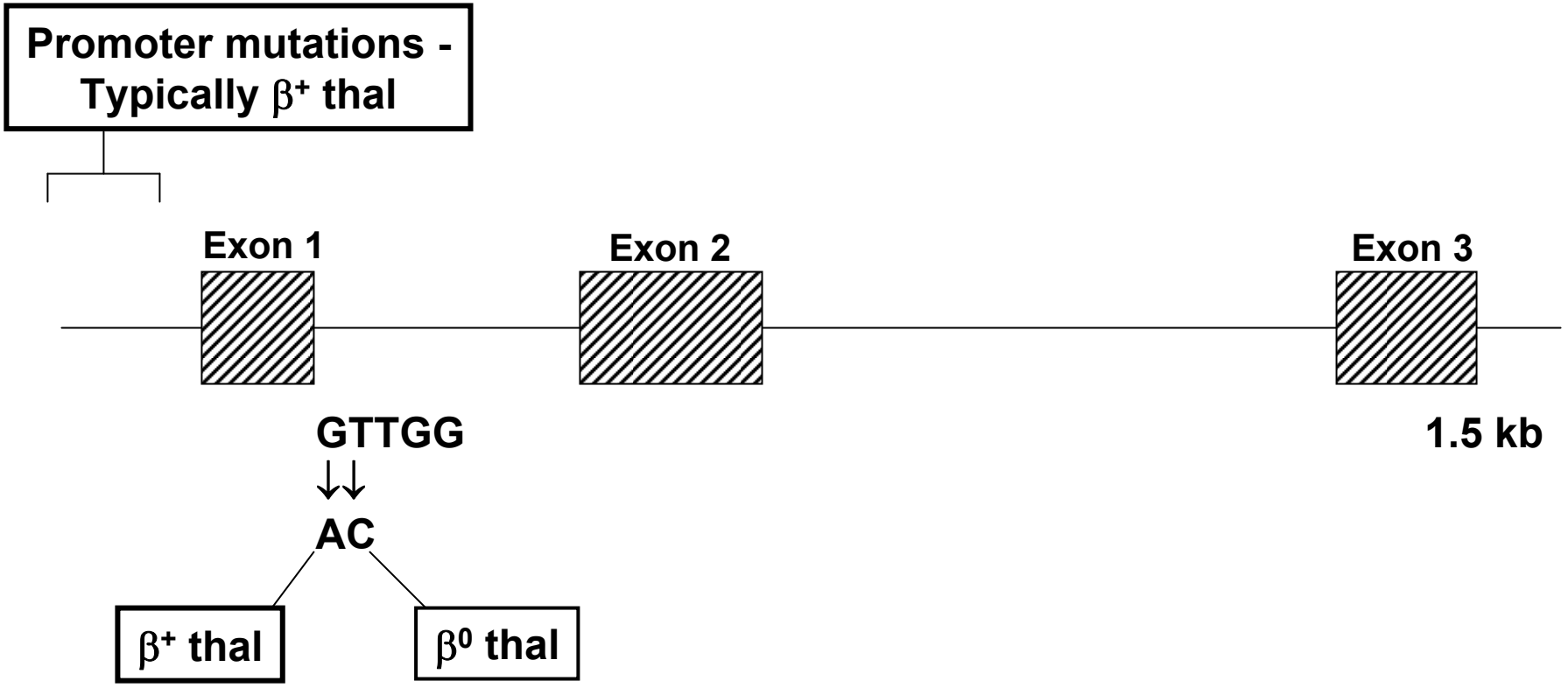
- Normal
  - **2 β globin genes**
  - **Large gene cluster on chromosome 11**



- Abnormal
  - **0 or 1 β gene**
  - **Majority are point mutations: β^+ or β^0 thal**
  - **Rare DNA deletions**
    - **δβ-thalassemia versus HPFH (hereditary persistence of fetal hemoglobin)**

β-THALASSEMIA MUTATIONS

Hundreds of single nucleotide mutations giving rise to absent or decreased beta globin message



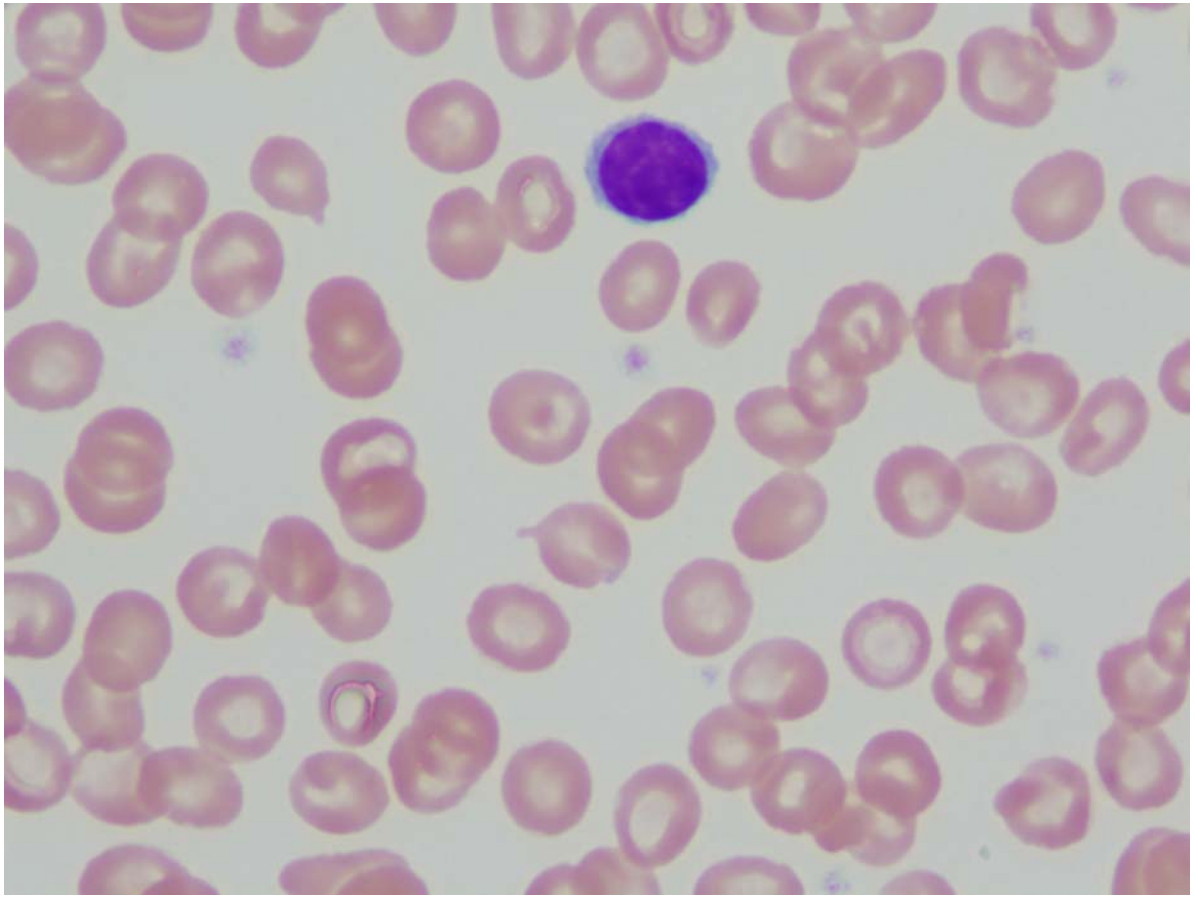
β-THALASSEMIA TRAIT

- **A β^0 or β^+ mutation on 1 allele**
- **Imbalance of α:β chain synthesis ratio**
- **<u>Hypochromic, microcytic anemia</u>**
  - Hb 9-11 gm/dL
  - Low MCV (65 fL)
  - Normal RDW (nl range 11.5-14.5)
  - Must differentiate from Iron Deficiency
    - MCV/RBC < 13 = β thal trait
- **Elevated Hb A_2 (>3.5%) and Hb F**

β-THAL TRAIT WORKUP

- **Family History**
- **CBC with differential**
  - **Look at MCV, RDW, RBC**
- **Smear**
- **Hemoglobin electrophoresis**
  - Cellulose acetate
  - Citrate agar
  - HPLC for quantitative Hb A_2 and F

β-THAL TRAIT WORKUP



Hb 12.3
MCV 67.5
ARC 56,600

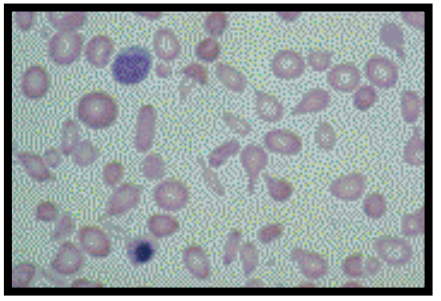
HPLC:
HbA 95.8%
HbA_2 4.2%

β-THALASSEMIA INTERMEDIA

- **Moderate disease severity**
- **By definition, non-transfusion dependent**
- **Wide allelic spectrum of disease**
  - **β^0 / β^+ thalassemia**
  - **β^0 / β^0 thal plus α-thalassemia**
  - **Elevated Hb F production**
- **Increased absorption of iron**

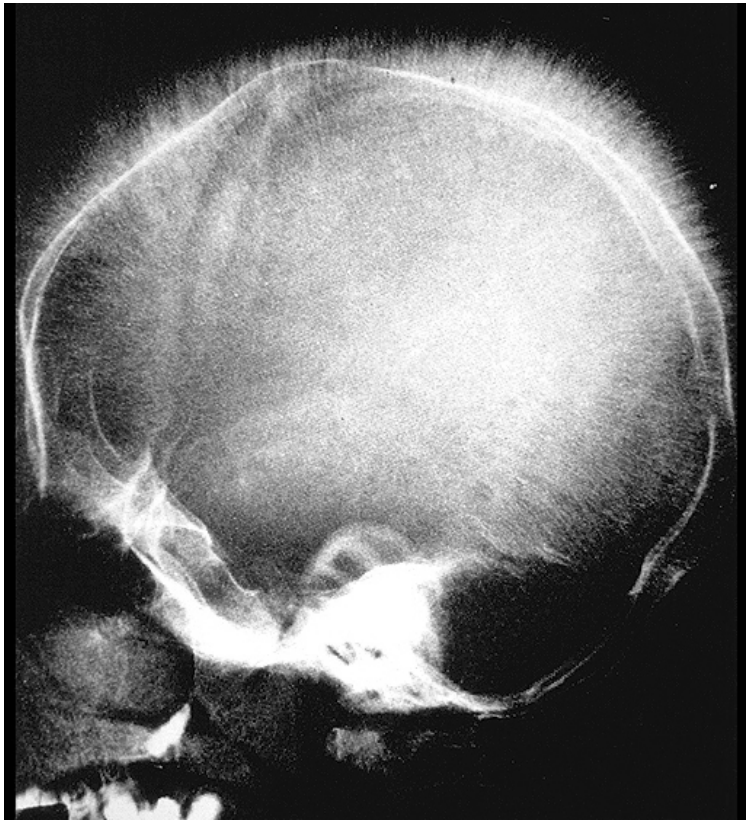
β-THALASSEMIA MAJOR

- **β^0 alleles on both chromosomes**
- **By definition, transfusion dependent**
- **Imbalance of α:β chains**
- **Severe hypochromic, microcytic smear with severe poikylocytosis**
- **Hb Electrophoresis**
  - Birth: F
  - Later: FA_2



β-THALASSEMIA MAJOR

- By definition, transfusion dependent
- Untreated = massive HSM, hypersplenism, skeletal dysplasia, life threatening infections, premature death
- Hb 2.0 – 4.0: mostly fetal Hb
- Transfusion therapy to turn off patient's own blood generation!



β-THALASSEMIA MAJOR

- By definition, transfusion dependent
- Monthly transfusion replacement therapy
- Long term complications: Too much iron
  - Liver
  - Heart
  - Endocrine organs

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β-THAL MUTATIONS AFFECTING HB F

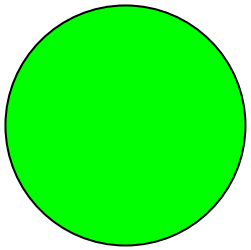
- β-thalassemia ordinarily has elevation of Hb A_2 (>3% with slight ↑Hb F)
- Deletional mutations significantly increasing Hb F production classically grouped into 2 broad categories:
  - $^{G}\gamma^{A}\gamma$ Hereditary Persistence of Fetal Hemoglobin (HPFH)
  - $^{G}\gamma^{A}\gamma$ $(\delta\beta)^0$ Thalassemia

HPFH AND δβ-THALASSEMIA

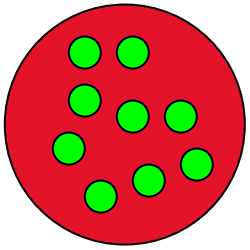
- **Clinical Phenotypes in Heterozygotes**

	HPFH	δβ-Thalassemia
Red cell morphology	Normal	Abnormal
MCH	Nearly normal	Decreased
Hematocrit	Normal	Slightly decreased
Hb F (%)	15-30	1-15
HB F distribution in RBCs	Pancellular	Heterocellular

PANCELLULAR VS. HETEROCELLULAR HB F



Pancellular HbF in HPFH (an F cell)



Heterocellular HbF In $^{G}\gamma^{A}\gamma\ (\delta\beta)^{0}$ Thalassemia

- A rare and extreme example of effect of HbF
- Molecular basis - <u>large β-globin gene cluster deletions</u>
  - Deletional HPFH
  - δβ-thalassemia
- Increased total HbF and F cells in HPFH

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THALASSEMIC HEMOGLOBINOPATHIES

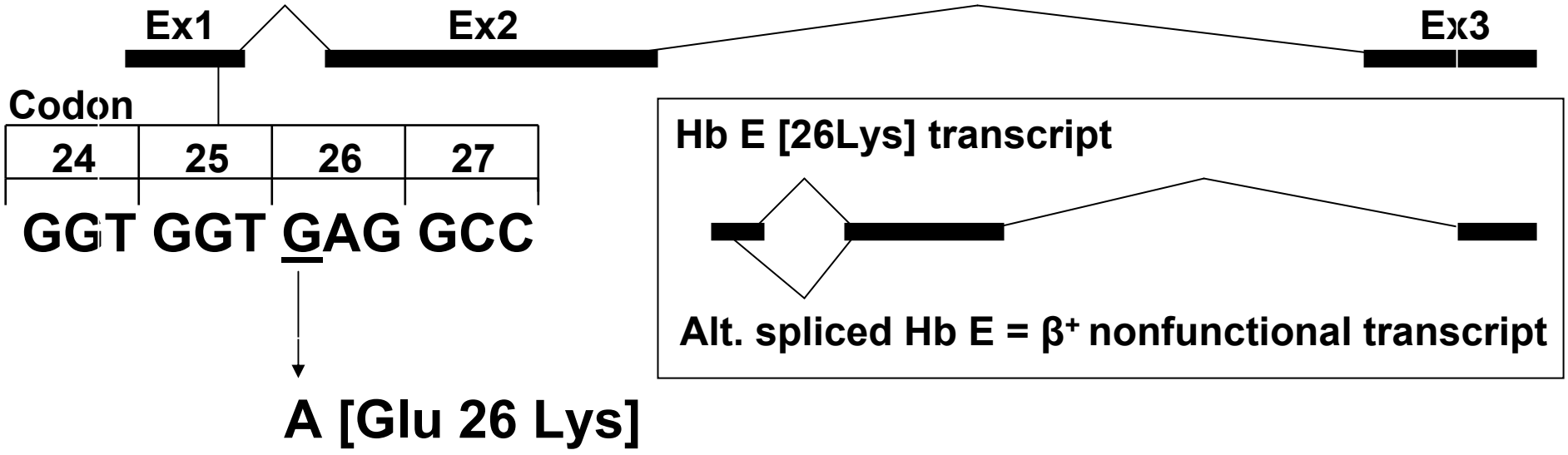
- Defined:
  - Structural change in the hemoglobin molecule

  AND
  - Thalassemia phenotype (↓ globin expression)

- Examples
  - Hb E (most common)
  - Hb Kenya
  - Hb Knossos
  - Hb Lepore
  - Hb Malay
  - Hb Constant Spring

HEMOGLOBIN E

- Creates an alternate splice donor site in exon 1 which decreases β expression
- Only a % of Hb E is aberrantly spliced (nonfunctional + creating β^+ transcript)



HEMOGLOBIN E TRAIT

- **Asymptomatic**
- **Mild hypochromic, microcytic anemia**
  - Hb >12 gm/dL
  - Low MCV (74 fL)
  - Smear – no nucleated red cells, occasional target cells
- **Hb A_2 comigrates with HbE on cellulose acetate: 19-34% total Hb**

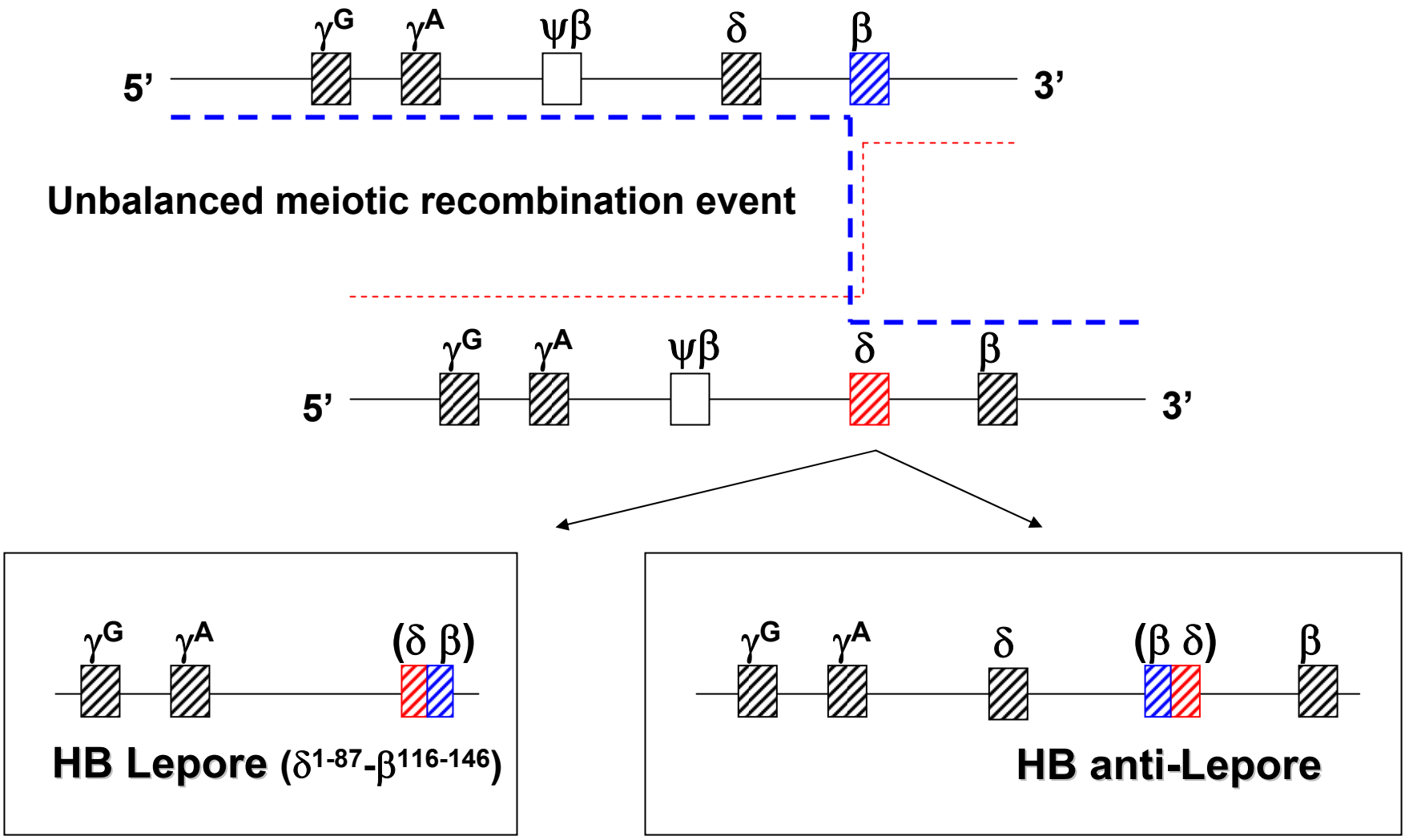
HOMOZYGOUS HB E

- **Usually asymptomatic**
- **Mild hypochromic, very microcytic anemia**
  - Hb >10 gm/dL
  - Very Low MCV (50 to 66 fL)
  - Smear – usually no nRBCs, frequent target cells
- **Hb A_2 + HbE accounts for >90% of total hemoglobin**
- **Occasionally require splenectomy**

HB E β-THALASSEMIA

- **A common, severe thalassemia in Southeast Asia**
- **Transfusion dependent (ie. Thalassemia major phenotype)**
- **Compound heterozygosity**
  - Untransfused Hb 2.3 – 7.0 gm/dL
  - Nucleated RBCs on smear (absent in homozygous Hb E)
  - Untreated = thal major with massive HSM, hypersplenism, skeletal dysplasia, life threatening infections, premature death

HEMOGLOBIN LEPORE



HOMOZYGOUS HB LEPORE

- **At least three Lepore variants depending on site of meiotic recombination**

  Washington, Hollandia, and Baltimore

- **Clinical and laboratory findings are identical to β thal major (except electrophoresis)**

  Electorphoresis

  Cellulose acetate: co-migrates with Hb S (experienced lab :Lepore migrates slightly faster as a faint band)

  Citrate agar: Lepore co-migrates with Hb A

  Laboratory diagnosis of homozygous Hb Lepore

  Hb F 80 - 90%

  Hb A absent

  Hb A_2 absent

  Hb Lepore 10% (co-migrates with Hb S)

HB LEPORE TRAIT

- **Clinical and laboratory findings are identical to β thal trait (except electrophoresis)**

  Slight anemia with slight reticulocytosis (eg. Hb 12.0 gm/dL and absolute reticulocyte count 109,000)

  Microcytic (typical MCV 65)

  Electorphoresis results for Hb Lepore trait

  - Hb A decreased (eg. 75%)
  - Hb F slight elevation (2-3%)
  - Hb A_2 decreased or normal
  - Hb Lepore 5-15%

HEMOGLOBIN KENYA