Hemoglobinopathies: Clinical & Hematologic Features and Molecular Basis

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Types of Normal Human Hemoglobins

	ADULT	**FETAL**
Hb A ($\alpha_2\beta_2$)	96-98%	15-20%
Hb A_2 ($\alpha_2\delta_2$)	2.5-3.5%	undetectable
Hb F ($\alpha_2\gamma_2$)	< 1.0%	80-85%

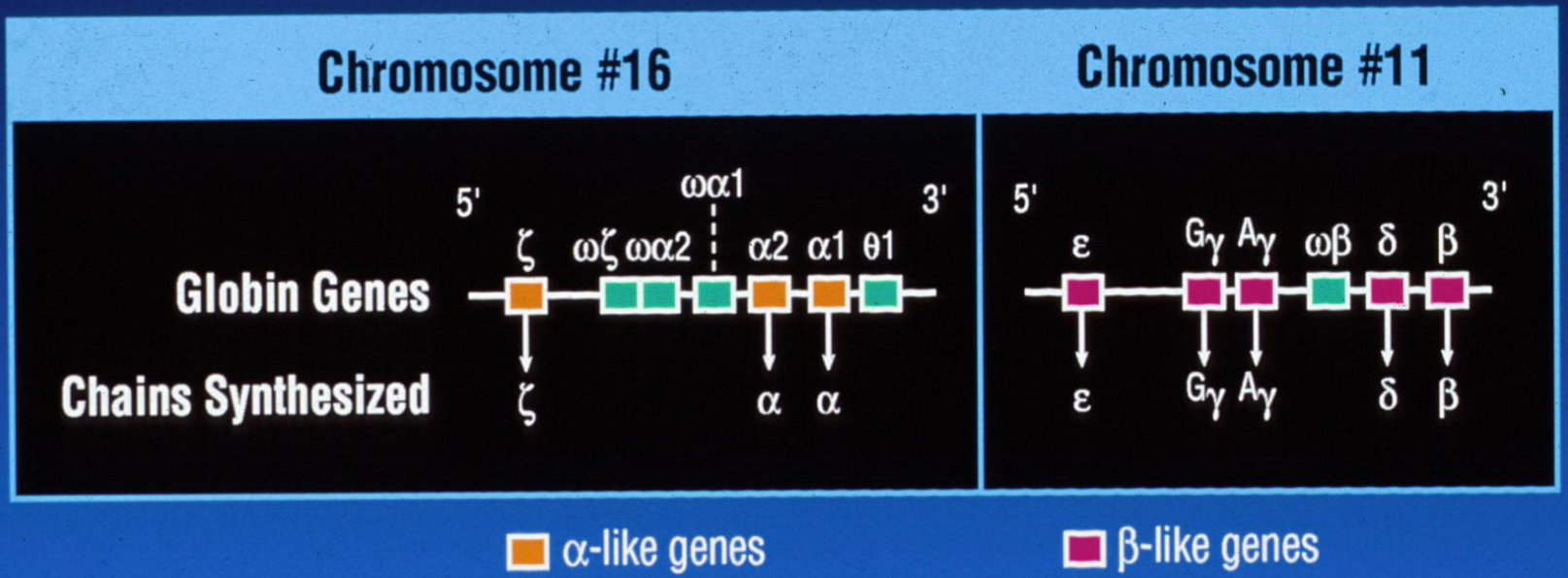
Embryonic Hbs:

Hb Gower-1 ($\xi_2\varepsilon_2$)

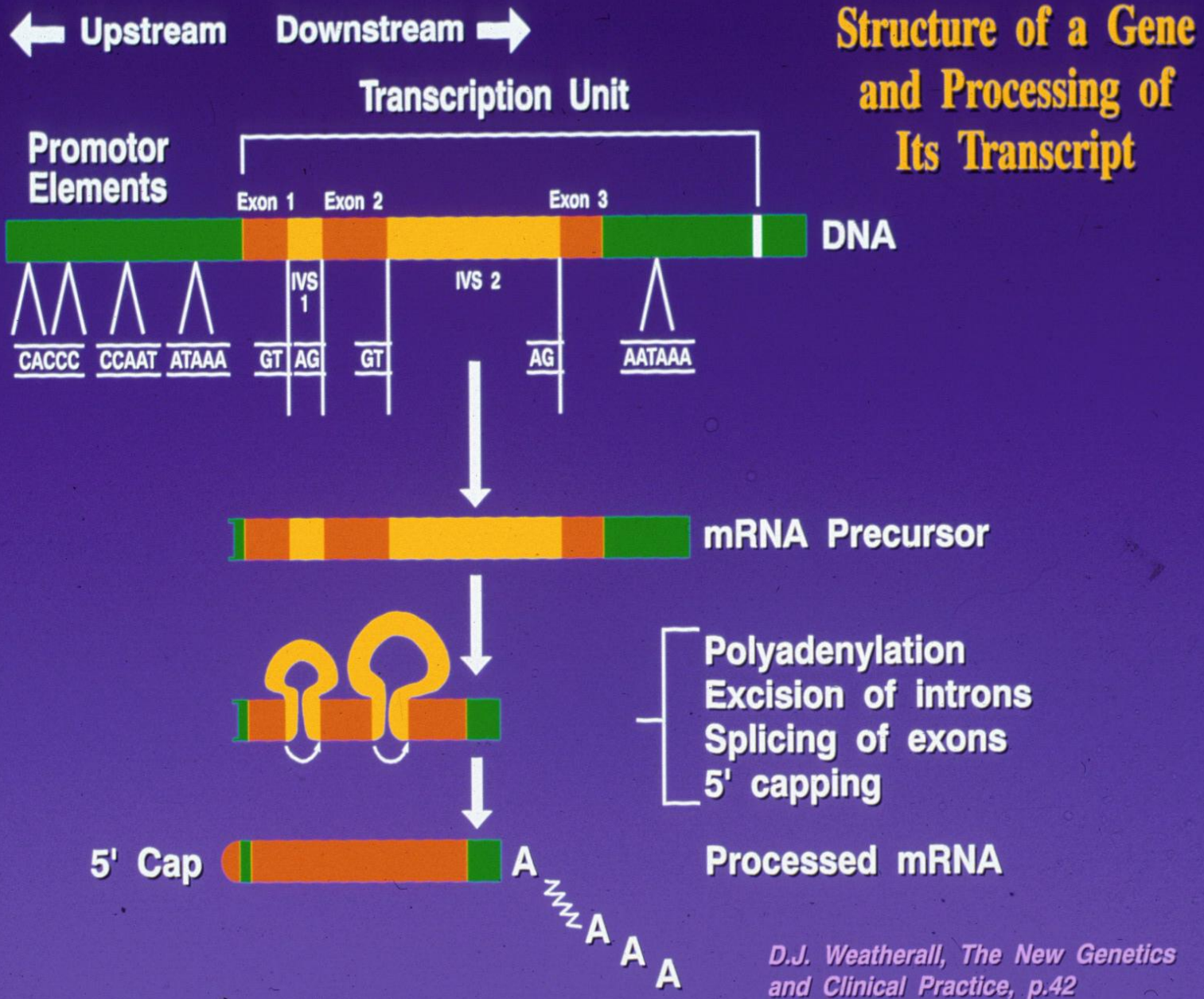
Hb Gower-2 ($\alpha_2\varepsilon_2$)

Hb Portland-1($\xi_2\gamma_2$)

Chromosomal Organization of the Human Globin Genes

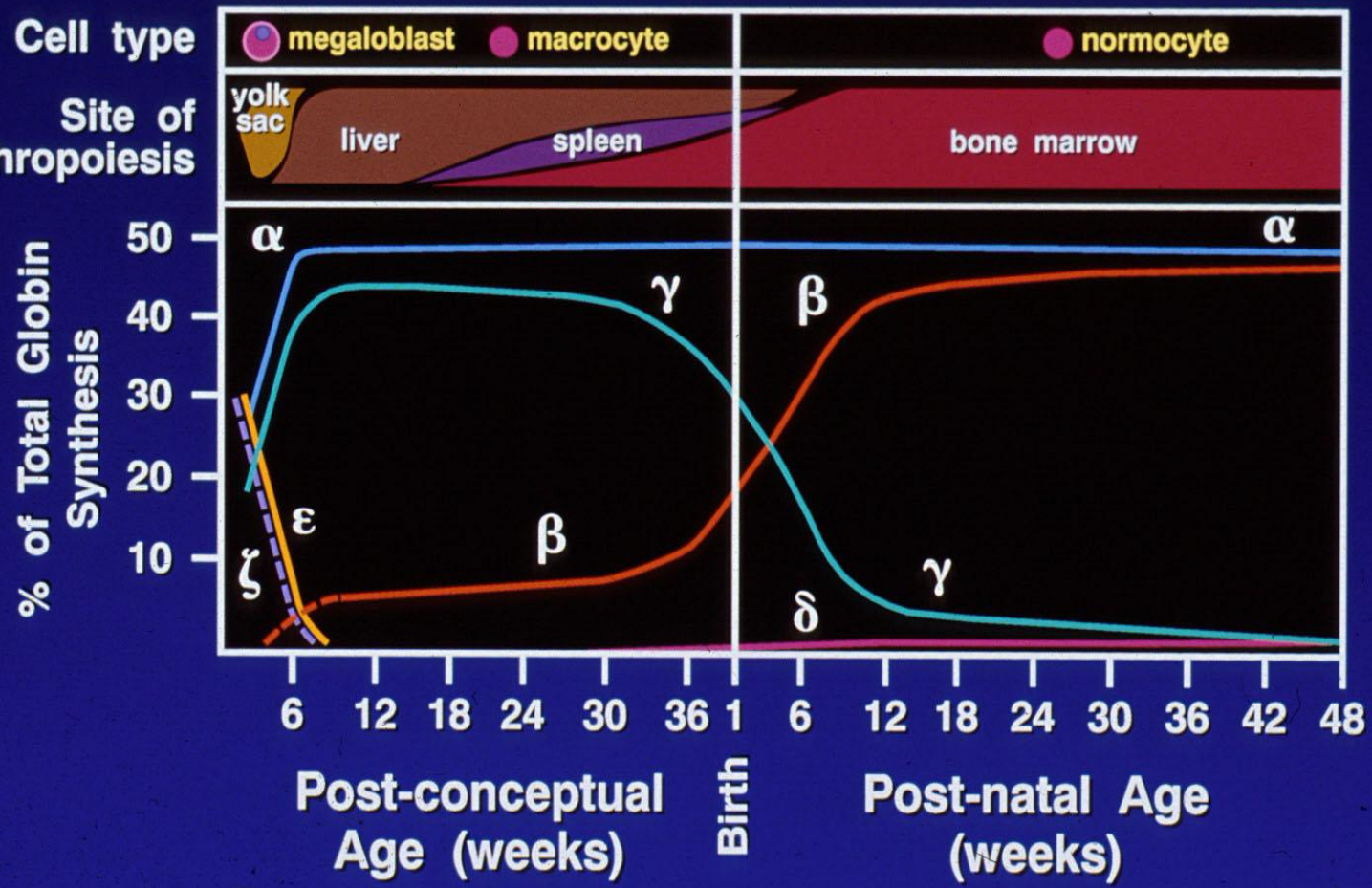


Structure of a Gene and Processing of Its Transcript



D.J. Weatherall, The New Genetics and Clinical Practice, p.42

Hemoglobin Switching: Changes in Globin Chain Production and Sites of Hematopoiesis During Development



Hemoglobinopathies

- Qualitative – Hb Variants
  (missense mutations) Hb S, C, E, others

- Quantitative – Thalassemias
  Decrease or absence of production of one or more globin chains

Functional Properties of Hemoglobin Variants

- Increased O_2 affinity
- Decreased O_2 affinity
- Unstable variants
- Methemoglobinemia

Clinical Outcomes of Substitutions at Particular Sites on the Hb Molecule

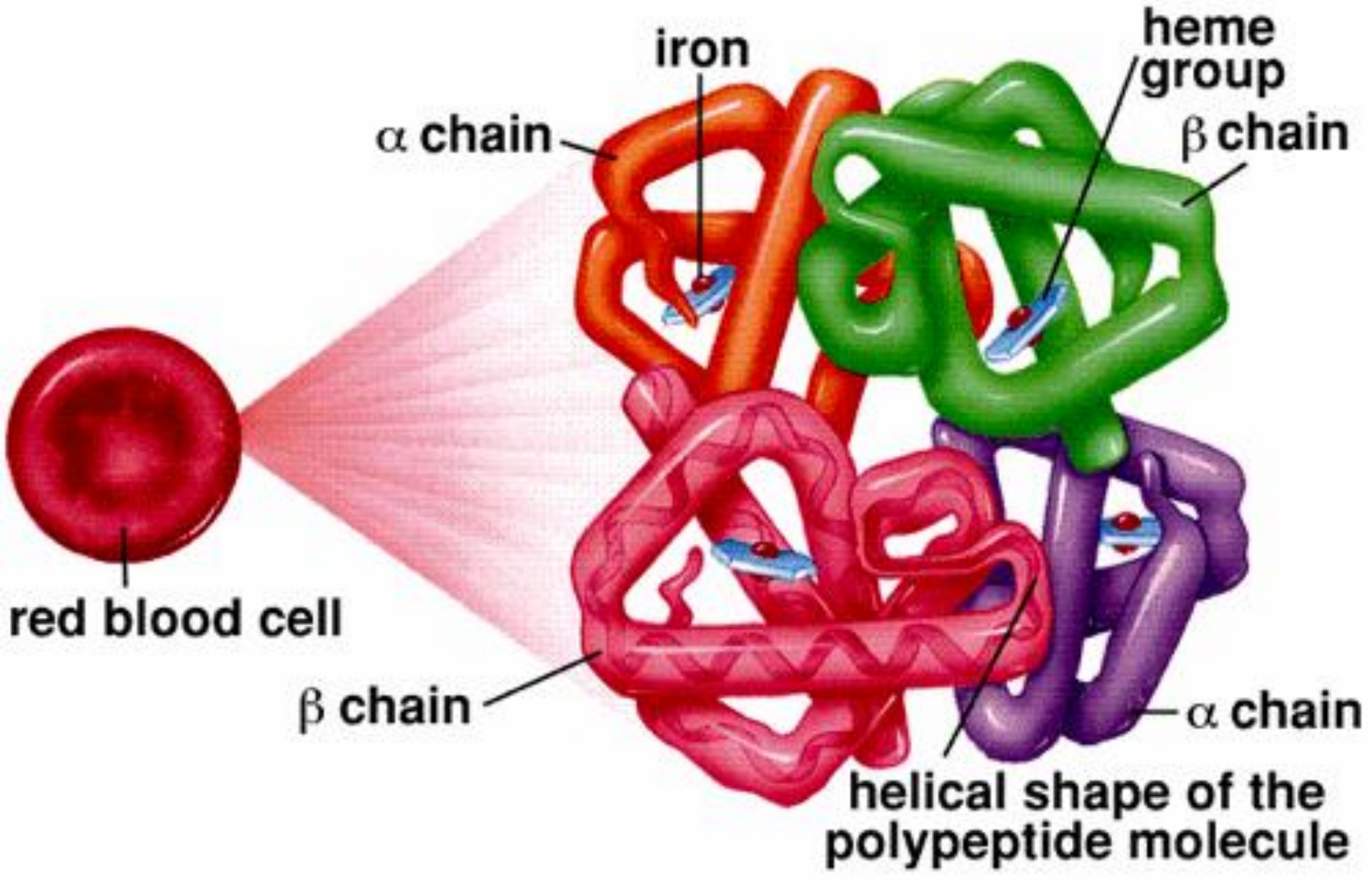
- On the surface:
  Sickle Hb
- Near the Heme Pocket:
  Hemolytic anemia (Heinz bodies)
  Methemoglobinemia (cyanosis)
- Interchain contacts:
  $\alpha 1\beta 1$ contact: unstable Hbs
  $\alpha 1\beta 2$ contact: High O_2 affinity: erythrocytosis
  Low O_2 affinity: anemia

Clinically Significant Hb Variants

- Altered physical/chemical properties:
  - Hb S (deoxyhemoglobin S polymerization): sickle syndromes
  - Hb C (crystallization): hemolytic anemia; microcytosis
- Unstable Hb Variants:
  - Congenital Heinz body hemolytic anemia (N=141)
- Variants with altered Oxygen affinity
  - High affinity variants: erythrocytosis (N=93)
  - Low affinity variants: anemia, cyanosis (N=65)
- M-Hemoglobins
  - Methemoglobinemia, cyanosis (N=9)
- Variants causing a thalassemic phenotype (N=51)
  - β-thalassemia
    - Hb Lepore (δβ) fusion
    - Aberrant RNA processing (Hb E, Hb Knossos, Hb Malay)
    - Hyperunstable globins (Hb Geneva, Hb Westdale, etc.)
  - α-thalassemia
    - Chain termination mutants (Hb Constant Spring)
    - Hyperunstable variants (Hb Quong Sze)

Modified and updated from Bunn & Forget: Hemoglobin: Molecular, Genetic, and Clinical Aspects. WB Saunders, 1986.

Hemoglobin Molecule



Hb Variants with Altered Functional Properties

Variants with Increased O2 Affinity	93
Variants with Decreased O2 Affinity	65
Unstable hemoglobins	141
Methemoglobins	9
TOTAL	**308**

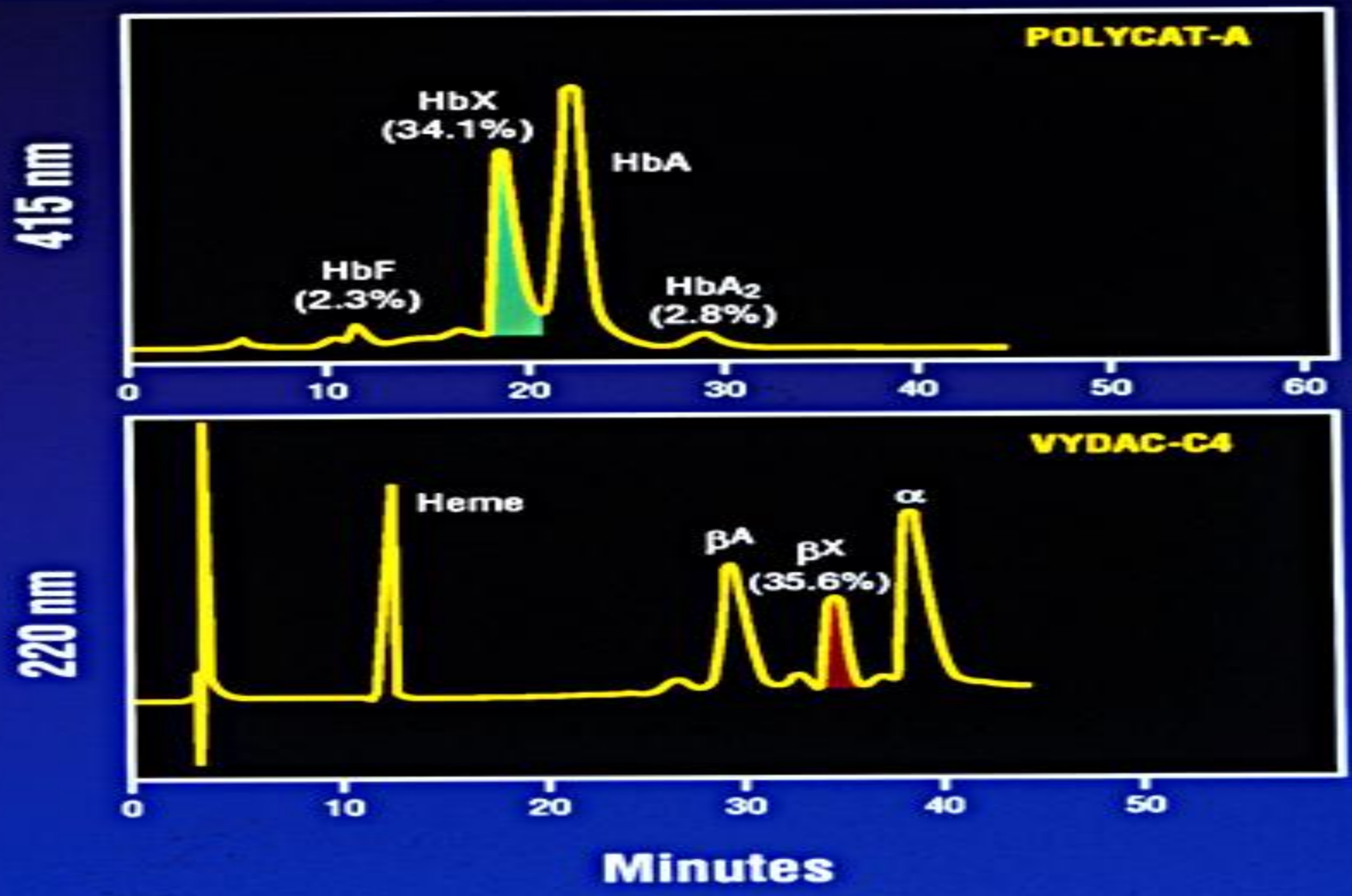
Case History: K.N.

- CLINICAL HISTORY:
  20 yo WF (Irish ancestry) evaluated during a routine prenatal visit. Mild erythrocytosis (Hb 15.2, Hct 52.4%, MCV 85.8)

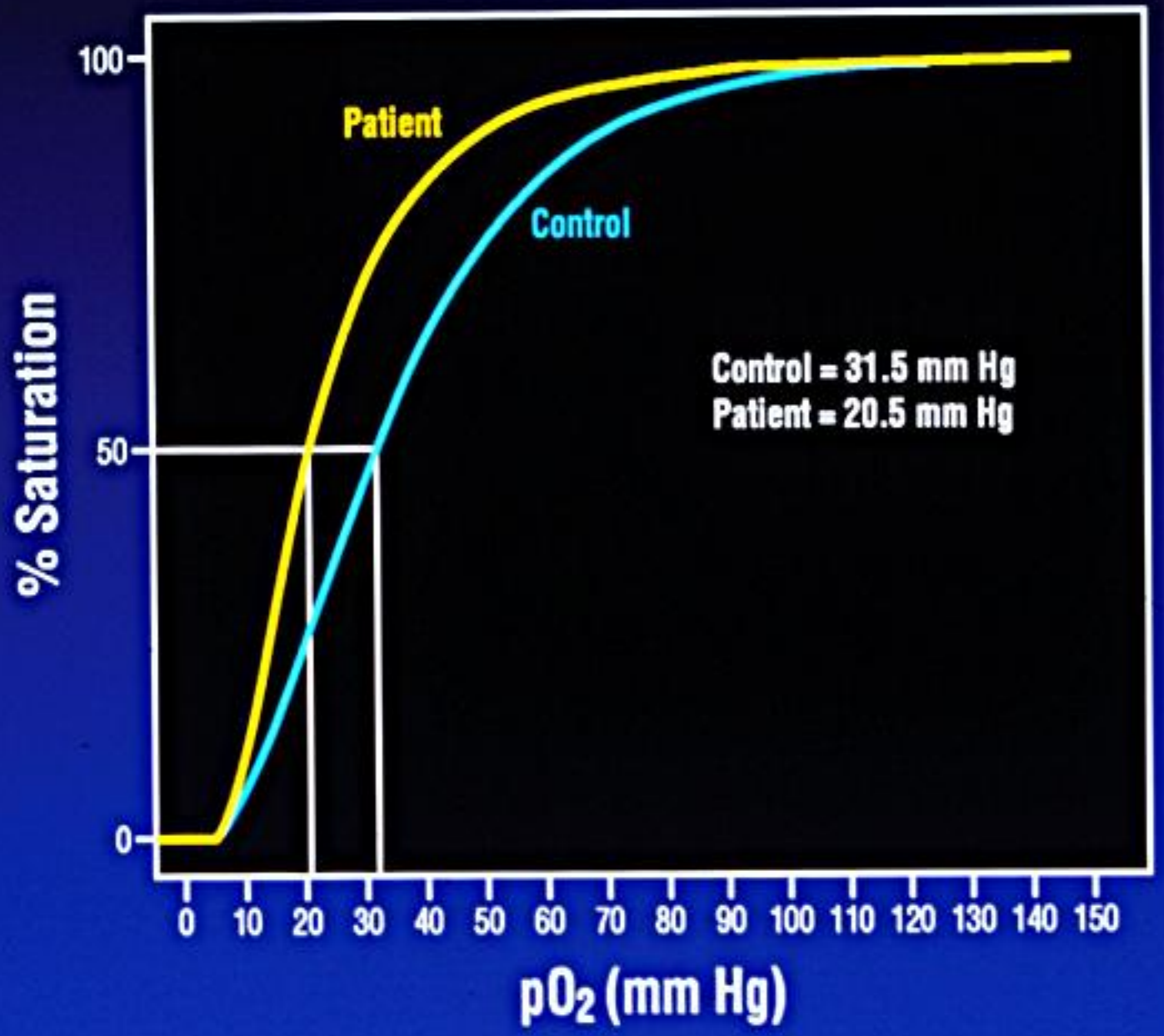
- HEMOGLOBIN ANALYSES:
  IEF: Hb X slightly anodic to Hb A
  HPLC: Hb X 35.6% eluting before Hb A
  Rp-HPLC: β^{x}
  O_2 affinity: Increased

HPLC Separation of "Hb Ty Gard"

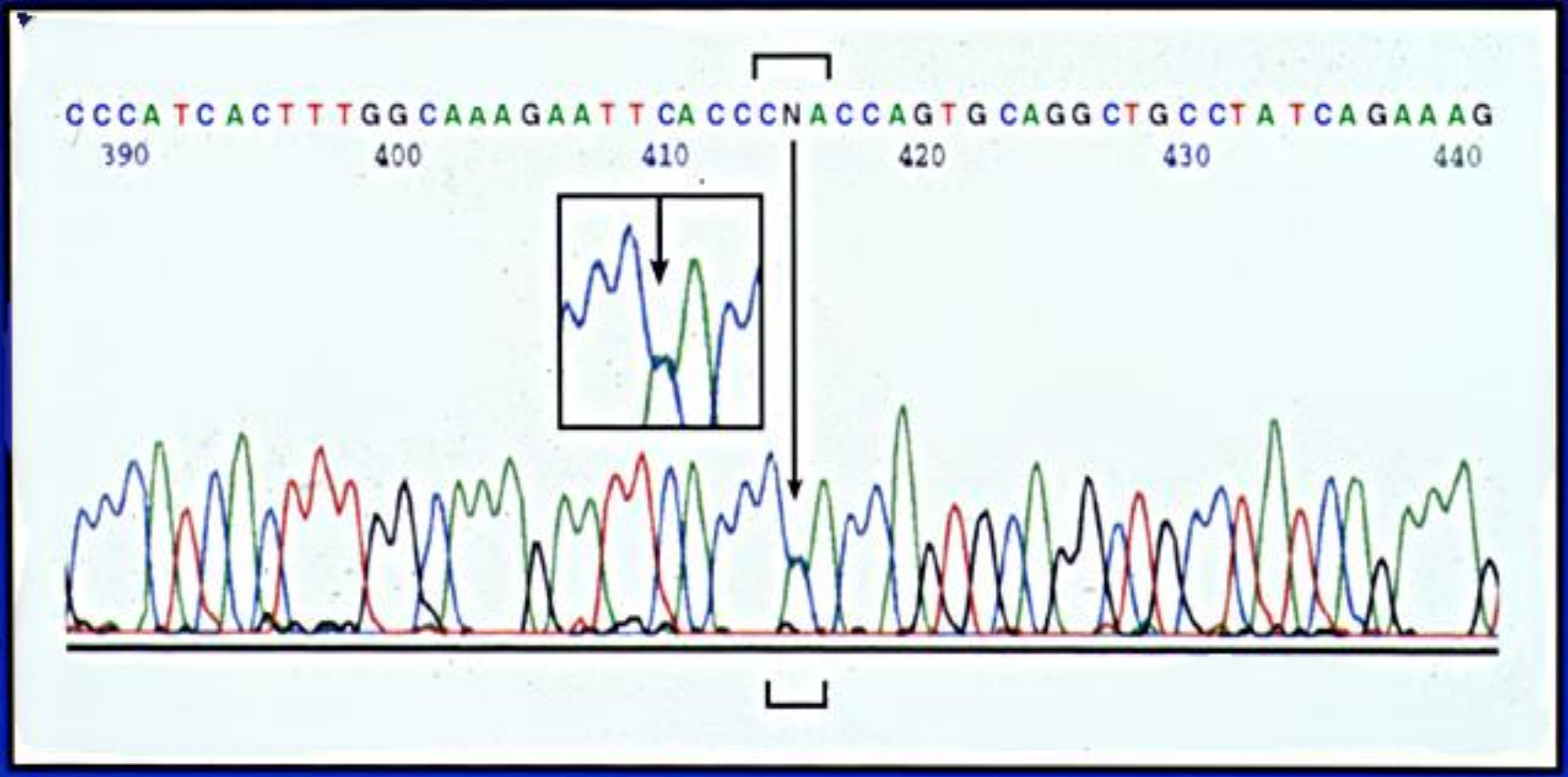
Top: Cation exchange HPLC separation of hemoglobins
Bottom: Reversed phase HPLC separation of globin chains



p50 of the patient with high oxygen affinity variant



Detection of the Heterozygote Hemoglobin "Ty Gard" (Pro 124 Gln; C→A) Mutation by Sequencing of RT-PCR Amplified Beta Globin cDNA



Family with Hb Hammersmith (β42 Phe→Ser)

- 5 month old AA infant girl seen in pediatric genetics clinic for developmental delay and found to have significant congenital anomalies including weakness of left lower extremity, tethered cord with sacral agenosis single right kidney and left-sided ptosis.
- Laboratory findings significant for anemia (Hb 9.8) and peripheral smear with poikilocytosis, polychromasia, schistocytes, basophilic stippling
- Pulse oximetry showed O2 sat of 80%
- Underwent successful release of tethered cord with O2 supplementation

Family with Hb Hammersmith (β42 Phe→Ser)

- Patient re-evaluated at 6 yo along with her monozygotic twin
- Both found to have a hemolytic anemia and significant congenital anomalies
- Laboratory testing significant for positive Heinz body prep, strongly positive isopropanol stability testing, IEF and cation exchange HPLC normal, rp-HPLC revealed earlier eluting βx at 14.1% and 12.7% in both twins
- PCR amplification and sequencing of β-globin gene revealed TTT→TCT (Phe→Ser) at codon 42 of β-globin gene (Hb Hammersmith)
- Both parents clinically and hematologically normal

Family with Hb Hammersmith (β42 Phe→Ser)

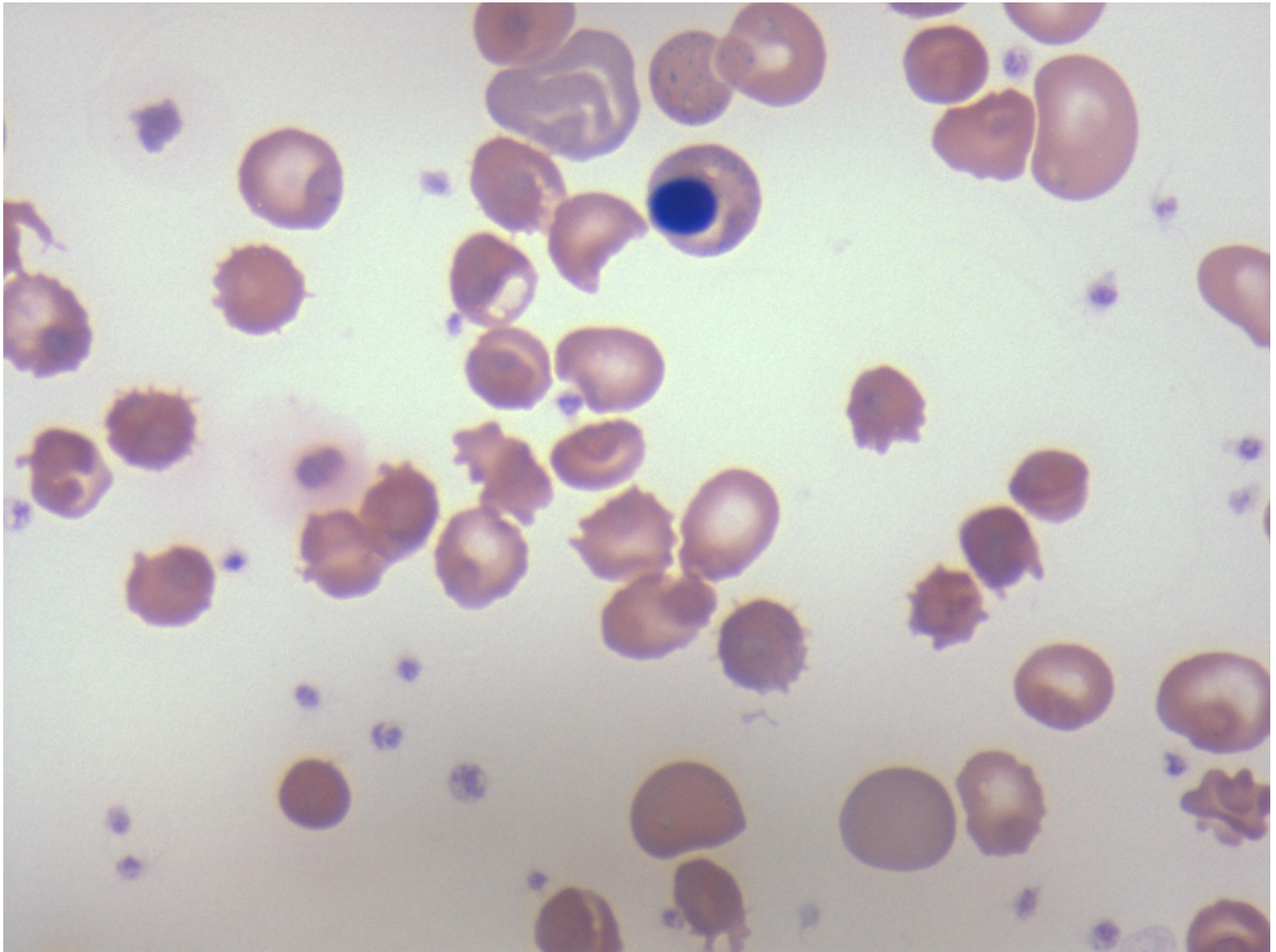
- At age 7 both twins had significant splenomegaly
- Splenectomy and cholecystectomy at age 9, both
- 1 twin died at age 20 due to infectious complications
- AD (the surviving twin) followed in the clinic, now at age 22

Family with Hb Hammersmith (β42 Phe→Ser)

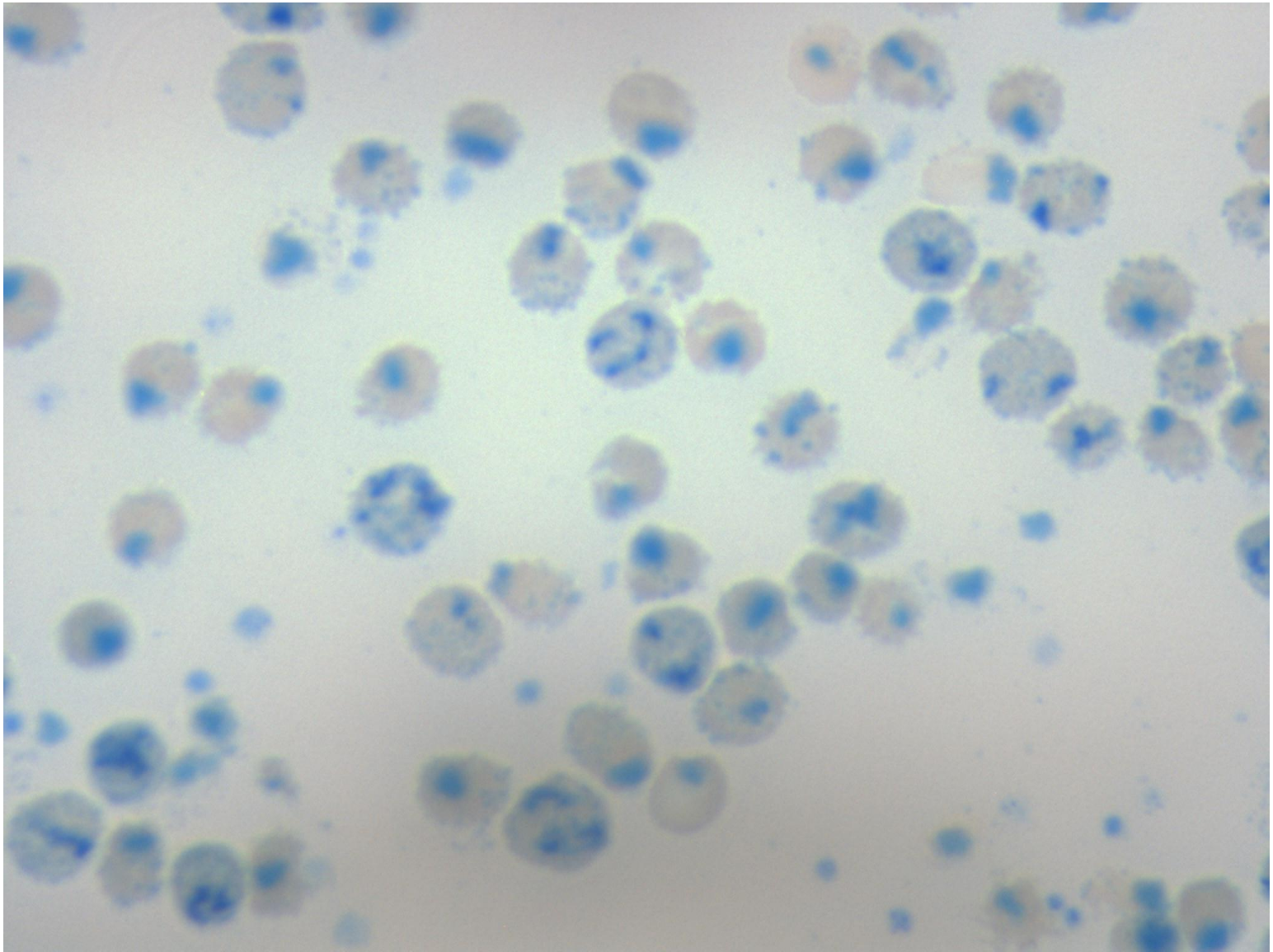
- Hematologic findings of AD

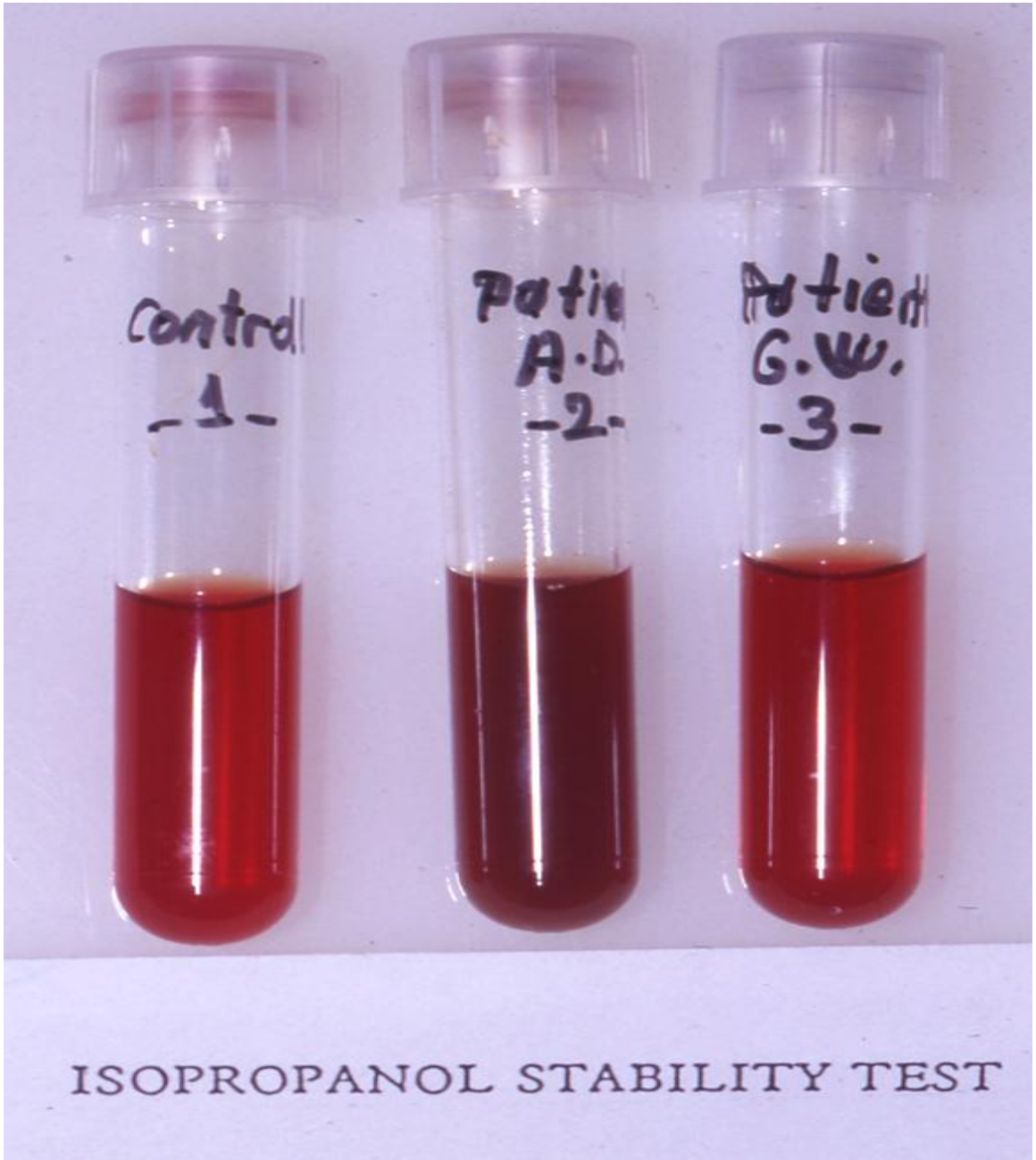
Hb	7.1
HCT	25.7
MCV	97.7
MCH	27
MCHC	27.6
RDW	18.5
Retics	11.3%

AD: Peripheral Smear



AD: Heinz Body Prep



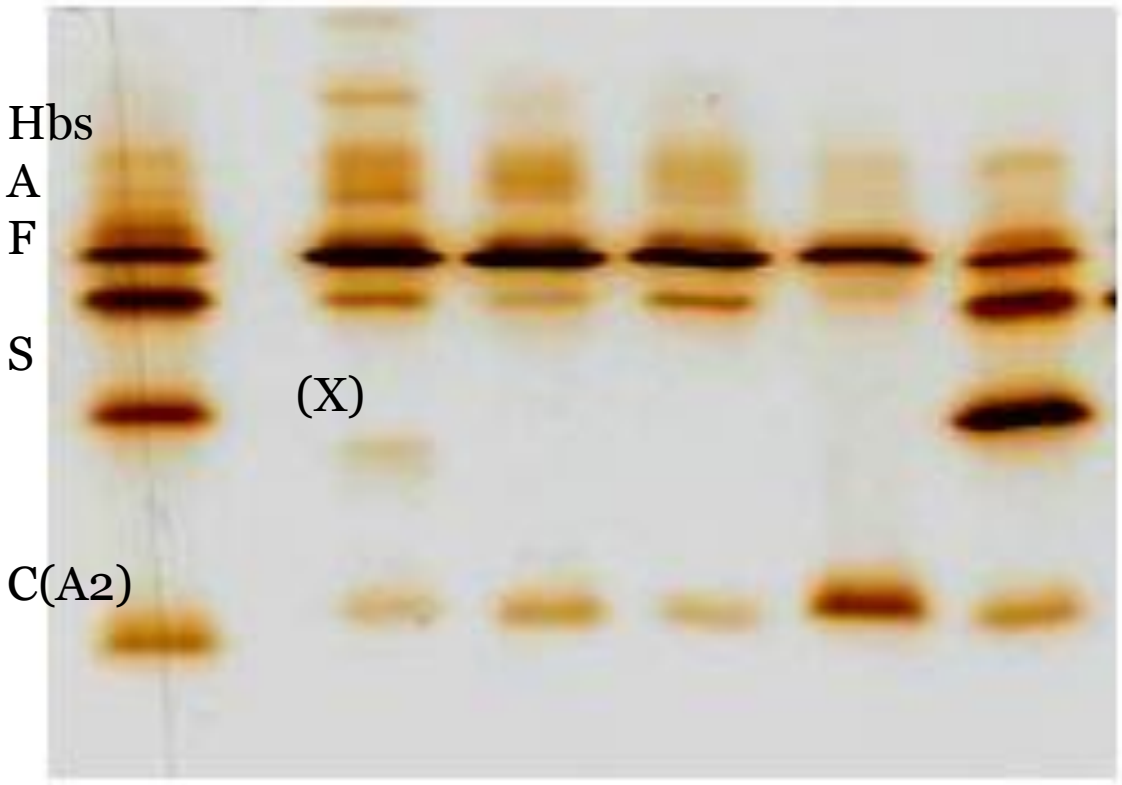


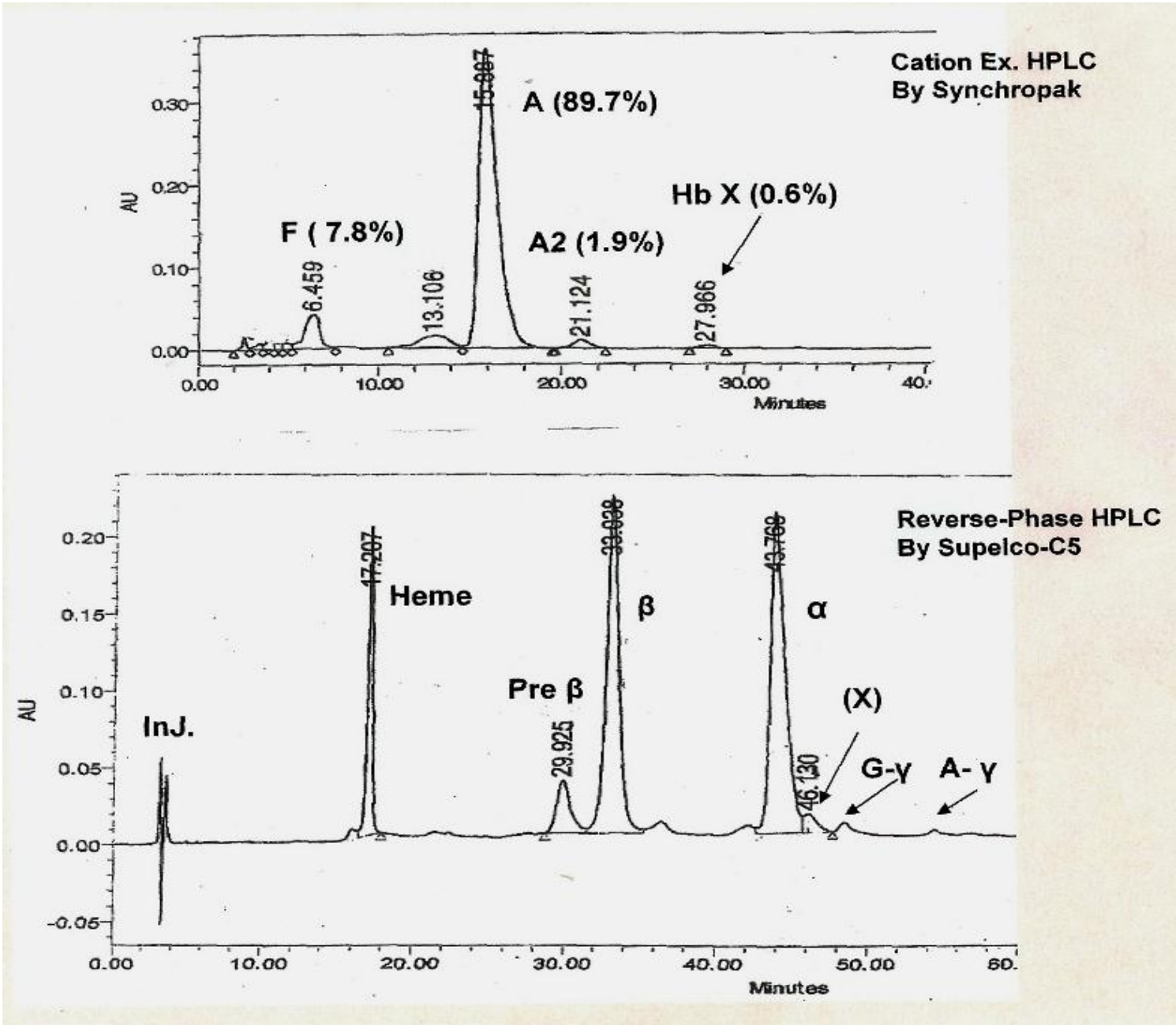
ISOPROPANOL STABILITY TEST

Hb Seal Rock

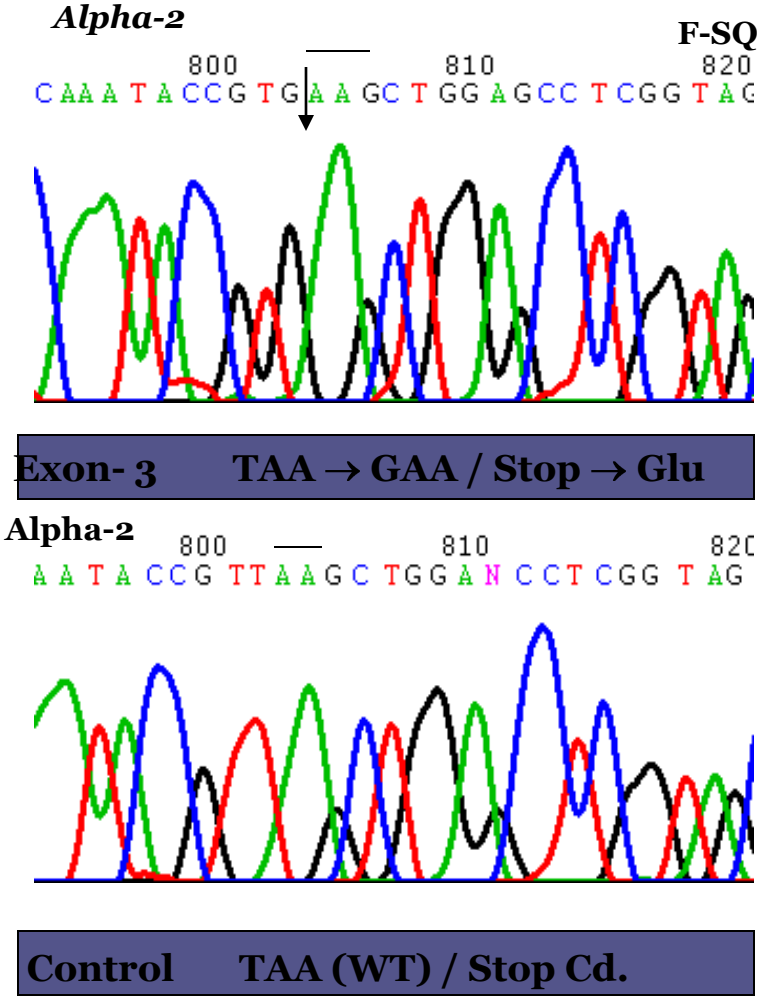
- 49 year old AAF with microcytic anemia, occasional transfusion requirements
- Hematology is comparable to mild Hb H disease.
- CB**C**:
  Hb.9.4; RBC: 3.5, Hct; 29, MCV; 84, MCH; 27.3, MCHC; 32.4
- **Iron studies**:
  Iron: 149 ug/dl, TIBC: 253 ug/dl, % saturation: 54, ferritin: 1506 ng/ml.
- **Hb Analyses:**
  IEF: Hb X slightly cathodic to Hb S
  HPLC: Hb X (0.6%) elutes after Hb A2
- **Molecular Diagnostic Studies**
  Alpha (– 3.7 deletion) : $(\alpha\,\alpha\,/\,\alpha\,-\,^{-3.7})$ / Heterozygous
  Alpha-2 chain variant at stop codon: TAA→GAA (STOP→Glu)-Hb Seal Rock
  Unstable, thalassemic alpha variant with 31 additional AA

IEF (Isoelectricfocusing) on thin-layer agarose: pH 6.0-8.0



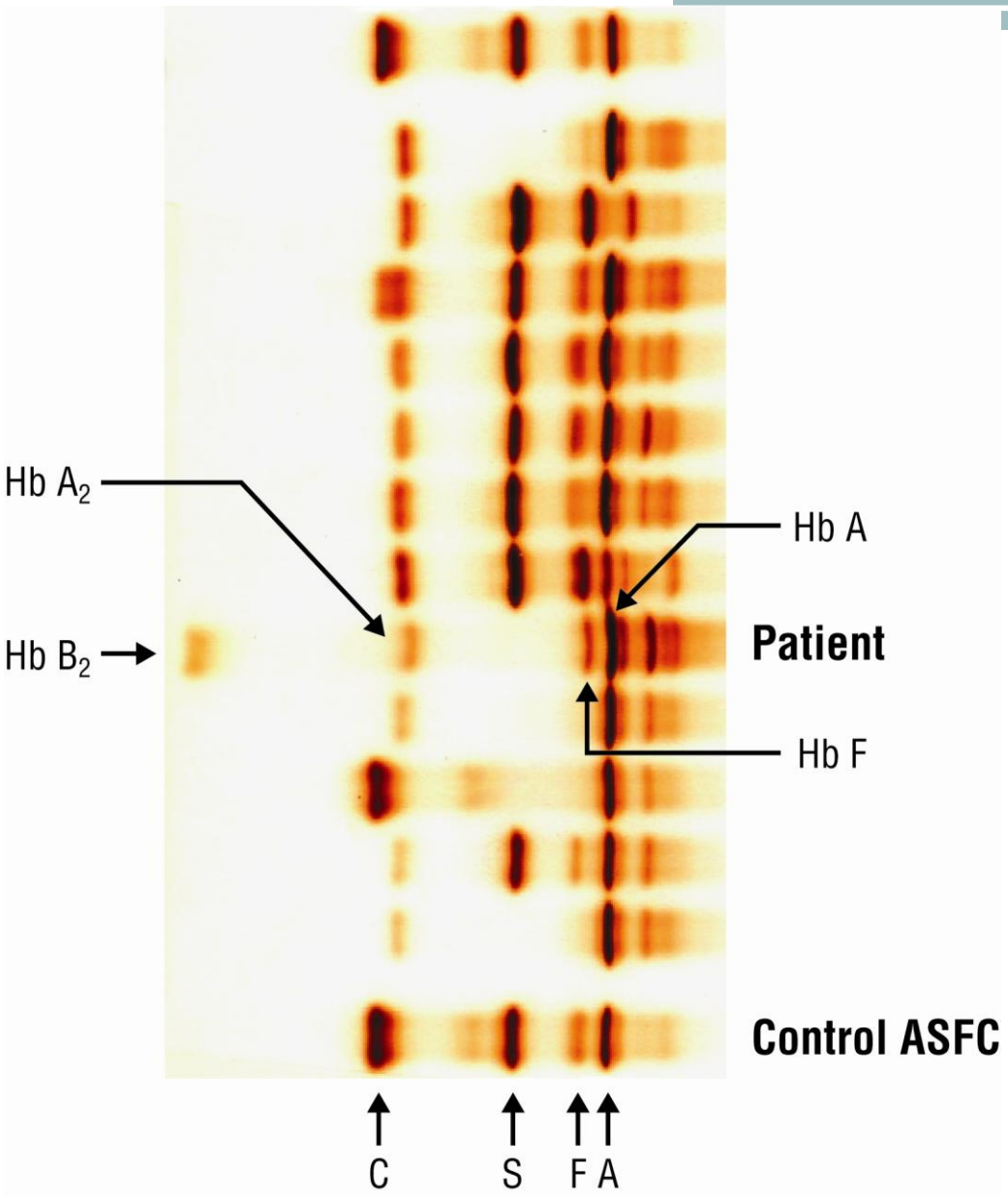

Cation Ex. HPLC
By Synchropak
A (89.7%)
F (7.8%)
A2 (1.9%)
Hb X (0.6%)
6.459
13.106
15.987
21.124
27.966
AU
Minutes
Reverse-Phase HPLC
By Supelco-C5
InJ.
Heme
Pre β
β
α
(X)
G-γ
A- γ
17.207
29.925
33.038
43.760
46.130
AU
Minutes

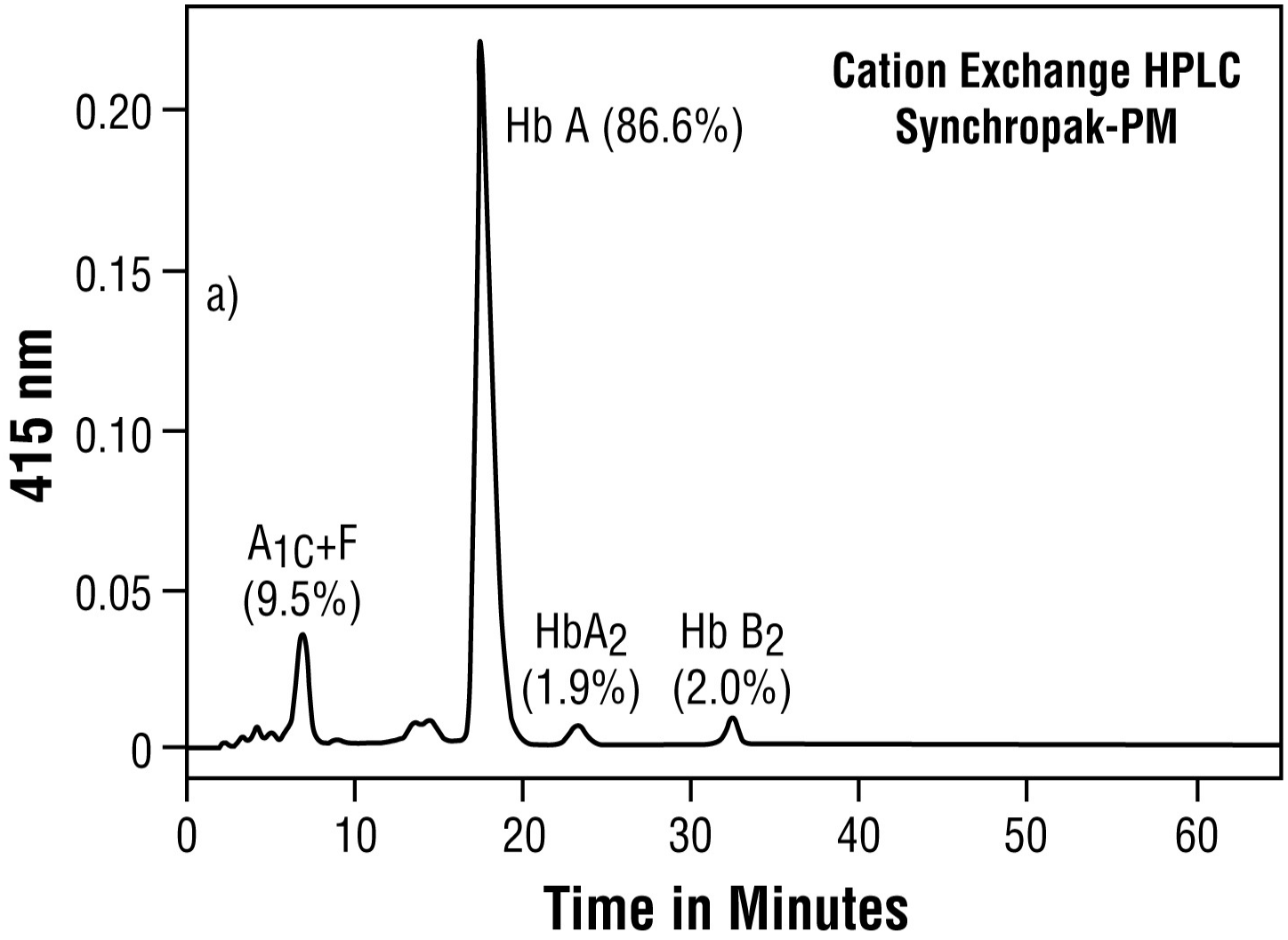
Unstable Hb Seal Rock:
Alpha 2 globin gene: Exon-3, STOP codon
TAA→GAA / Stop → Glu / homozygous



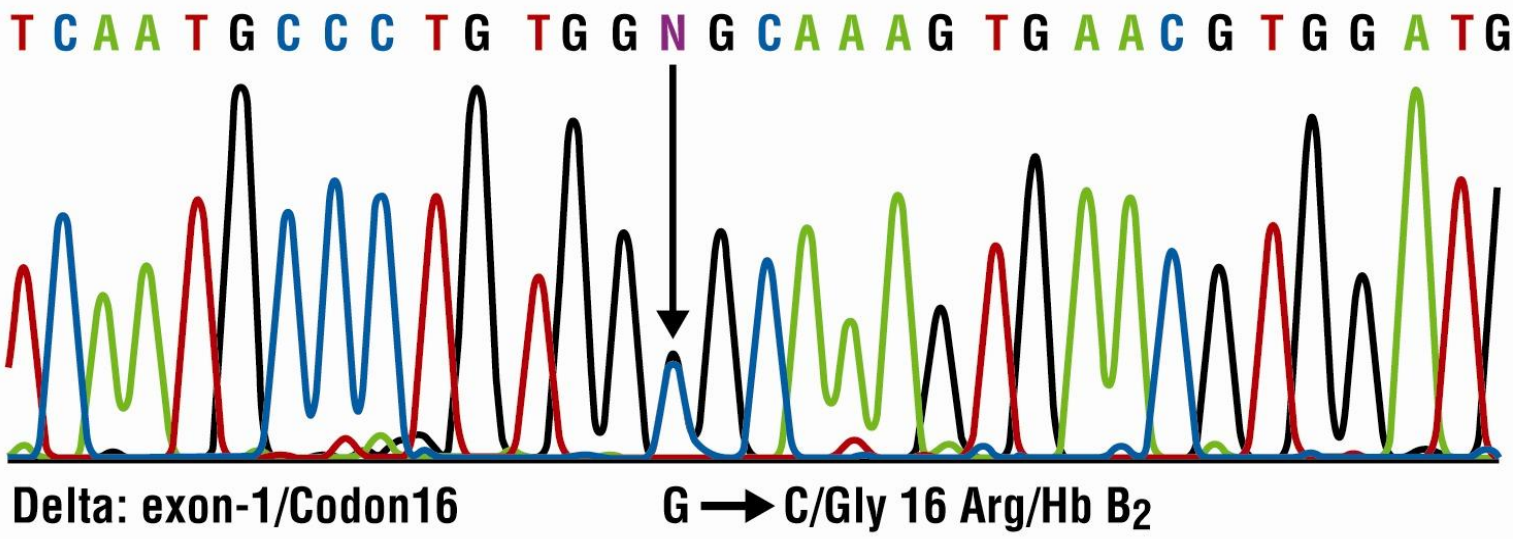
Hb Showa-Yakushiji (β110 Leu→Pro)

- 2 Year-old AAM presented with mild anemia, microcytosis (Winston-Salem, NC)
  Hb 9.9; Hct 31.3%; MCV 62.5 fl, MCH 19.8 pg; MCHC 31.7 g/dl; iron studies normal
- **Hb Analyses**:
  IEF: Hb A, F, A2 and a minor band cathodic to Hb A2
  HPLC: Hb A=91.0%, Hb F=5.0%, Hb A2= 2.0%, Hb A2'=2.0
- **Molecular Diagnostic Studies:**
  -$\alpha^{3.7}$ deletion (-α/αα)
  β-globin sequencing: β110 CTG→CCG (Leu→Pro)
  δ-globin sequencing: δ16 GGG→GCG (Gly→Arg) Hb A2' or B2

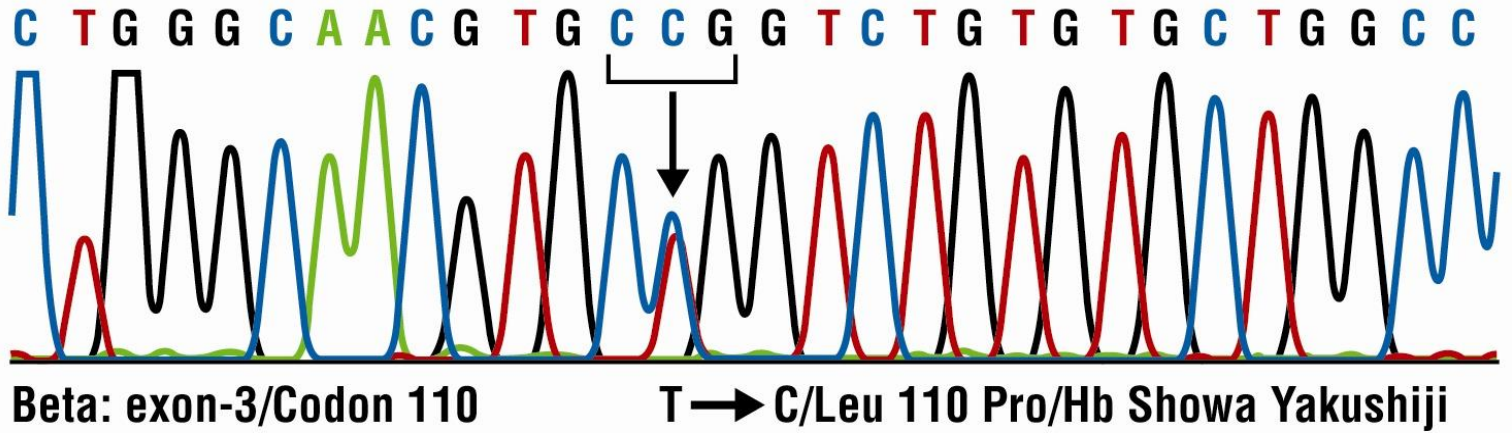

Hb A_2
Hb B_2
Hb A
Patient
Hb F
Control ASFC
C
S
F A


Cation Exchange HPLC
Synchropak-PM
a)
Hb A (86.6%)
A1C+F
(9.5%)
HbA2
(1.9%)
Hb B2
(2.0%)
415 nm
0.20
0.15
0.10
0.05
0
0
10
20
30
40
50
60
Time in Minutes

A) Hb B_2/Heterozygous



B) Hb Showa Yakushiji/Heterozygous



Hb Showa-Yakushiji (β110 Leu→Pro)

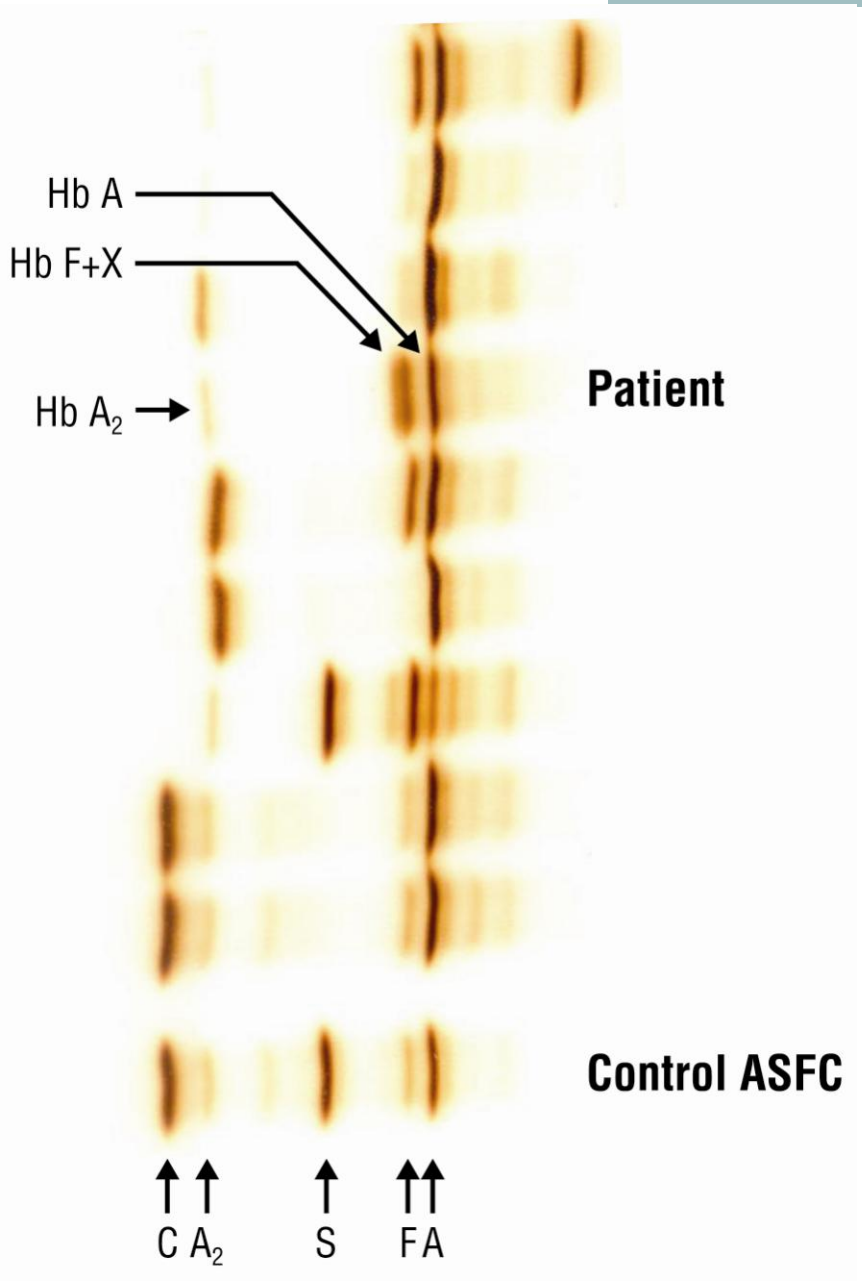
- Hyperunstable globin variant associated with a thalassemia phenotype
- First described in Japan; other families reported from India, Netherlands, Bengal
- First African American Case
- Co-existence of 3 globin mutants : Hb Showa-Yakushiji, Hb A2' and $-\alpha^{3.7}$ deletion
- Mechanisms of instability:

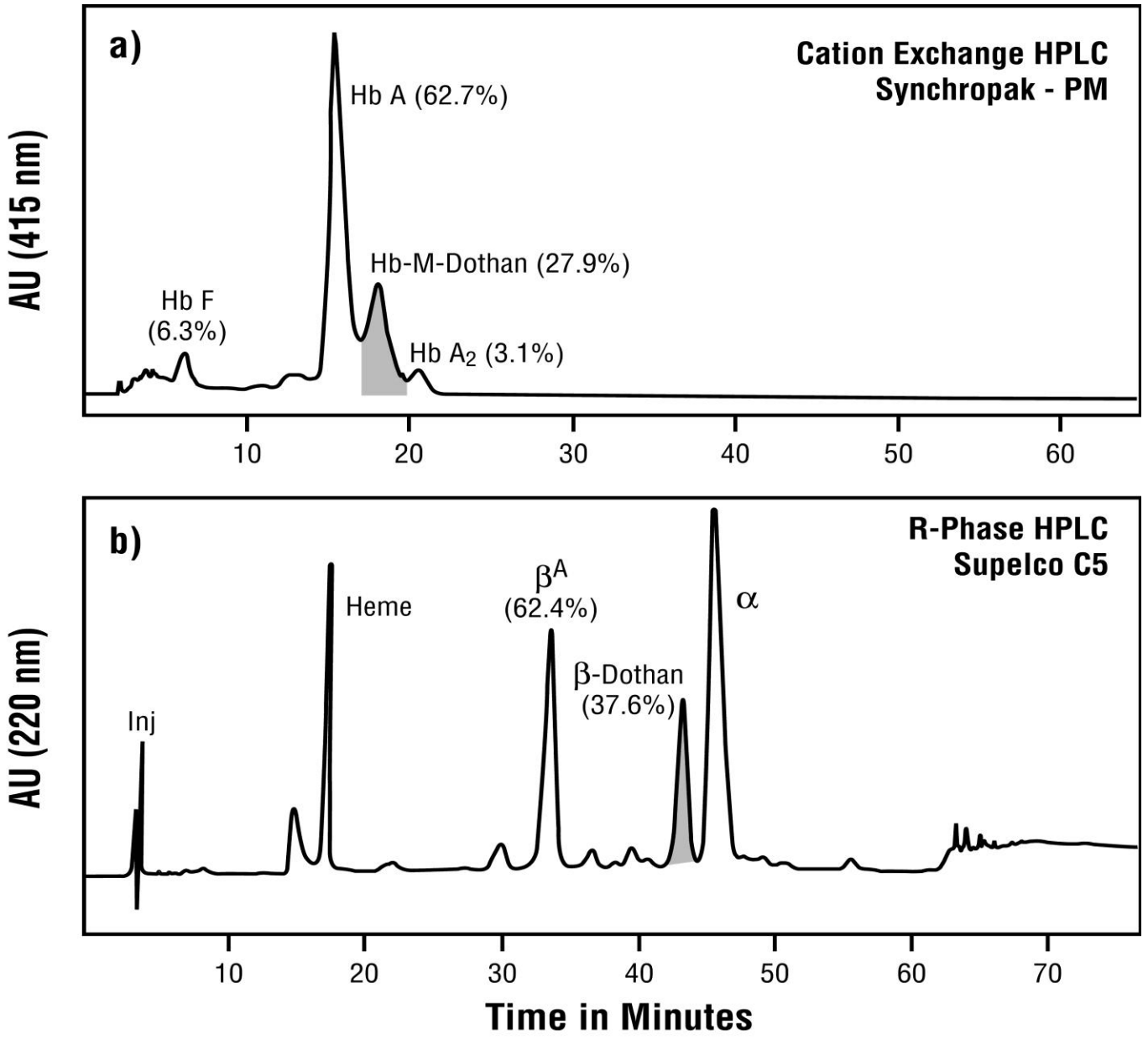
  Leu→Pro substitution disrupts the G-helix

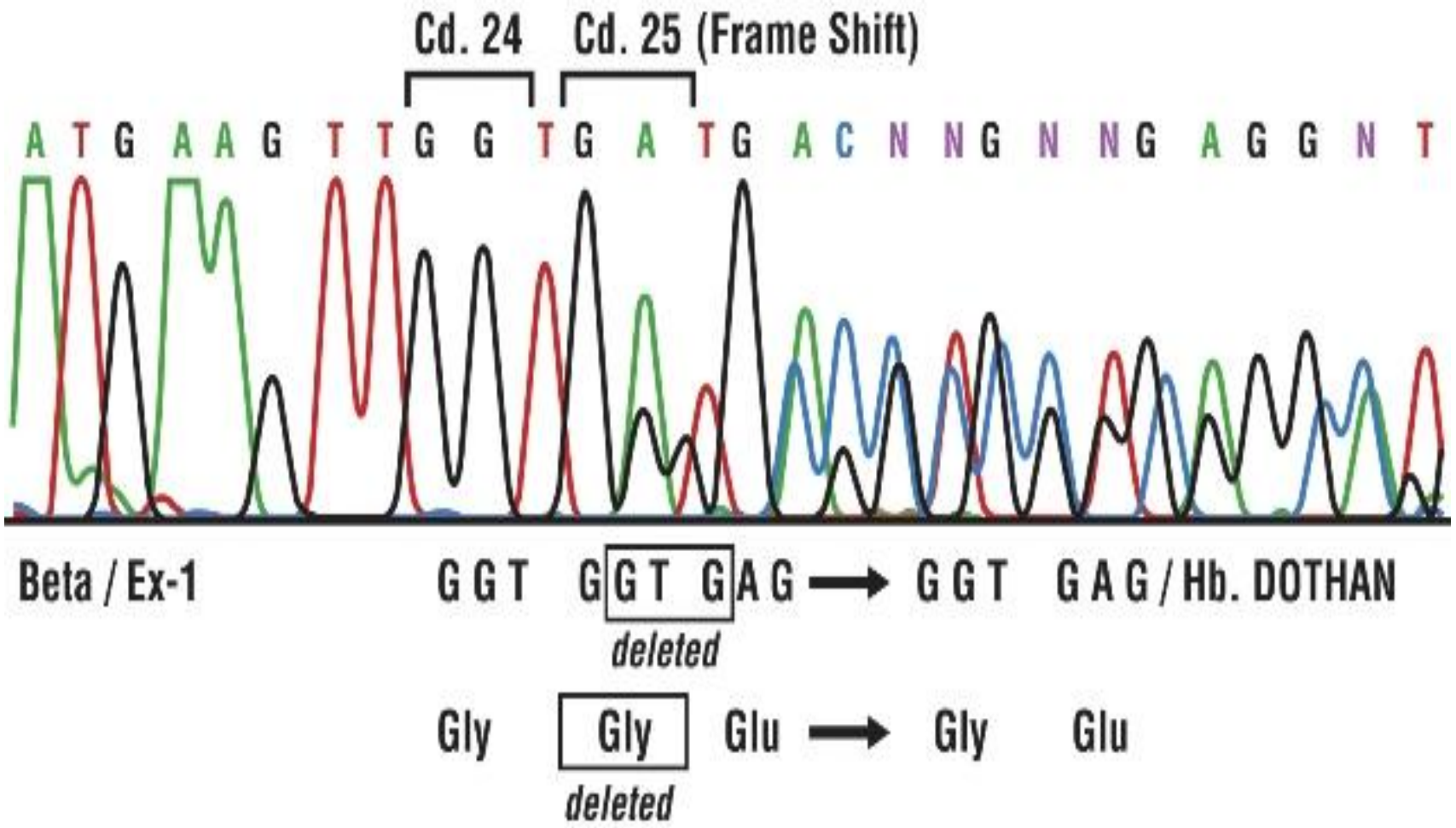
  Mutation at $\alpha_1\beta_1$ contact decreases $\alpha\beta$-dimer formation
- Phenotype likely ameliorated by concomitant α-thal (less severe anemia/hemolysis)

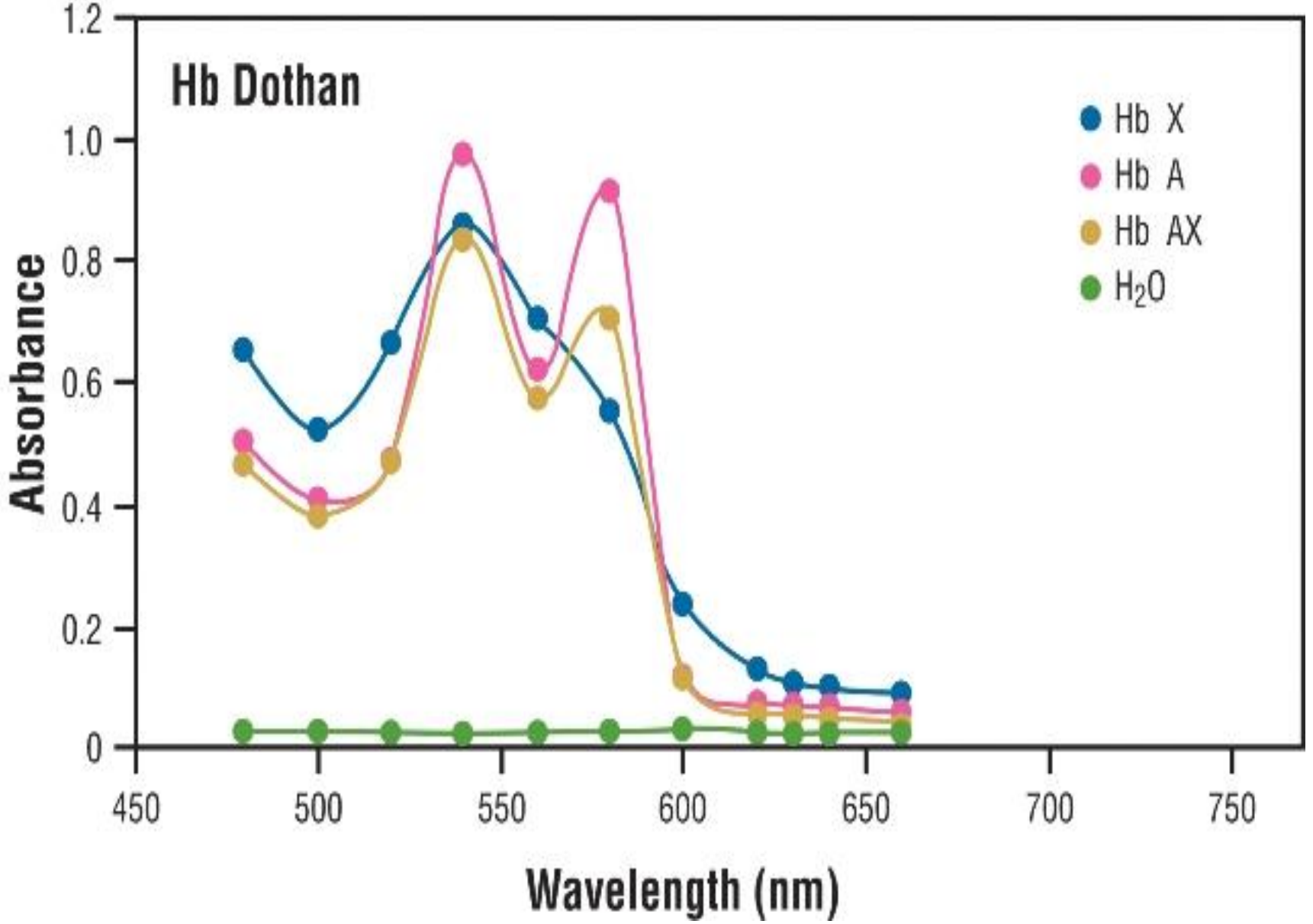
Hb M Dothan

- 9 mo Caucasian boy from Dothan, AL presented with cyanosis at 3 mo
- Found to have a low O2 saturation prior to ENT procedure
- Cardiac cath at UAB r/o congenital heart disease
- Co-oximetry showed normal PaO2 but confirmed low O2 saturation
- Found to have 20% metHb
- Cytochrome C5b reductase activity was normal
- Blood sample sent to GHSU Hemoglobin Lab


Hb A
Hb F+X
Hb A2
Patient
Control ASFC
C A2
S
F A


a)
Cation Exchange HPLC
Synchropak - PM
AU (415 nm)
Hb A (62.7%)
Hb-M-Dothan (27.9%)
Hb F
(6.3%)
Hb A2 (3.1%)
10
20
30
40
50
60
b)
R-Phase HPLC
Supelco C5
AU (220 nm)
Heme
βA
(62.4%)
α
β-Dothan
(37.6%)
Inj
10
20
30
40
50
60
70
Time in Minutes


Cd. 24
Cd. 25 (Frame Shift)
A T G A A G T T G G T G A T G A C N N G N N G A G G N T
Beta / Ex-1
G G T G G T G A G
deleted
G G T G A G / Hb. DOTHAN
Gly Gly Glu
deleted
Gly Glu


Hb Dothan
Hb X
Hb A
Hb AX
H_2O
Absorbance
1.2
1.0
0.8
0.6
0.4
0.2
0
450
500
550
600
650
700
750
Wavelength (nm)

Hb M Dothan

[β25/26 (B7/B8) / 9GGT/GAG→//Gly/Glu→Glu]

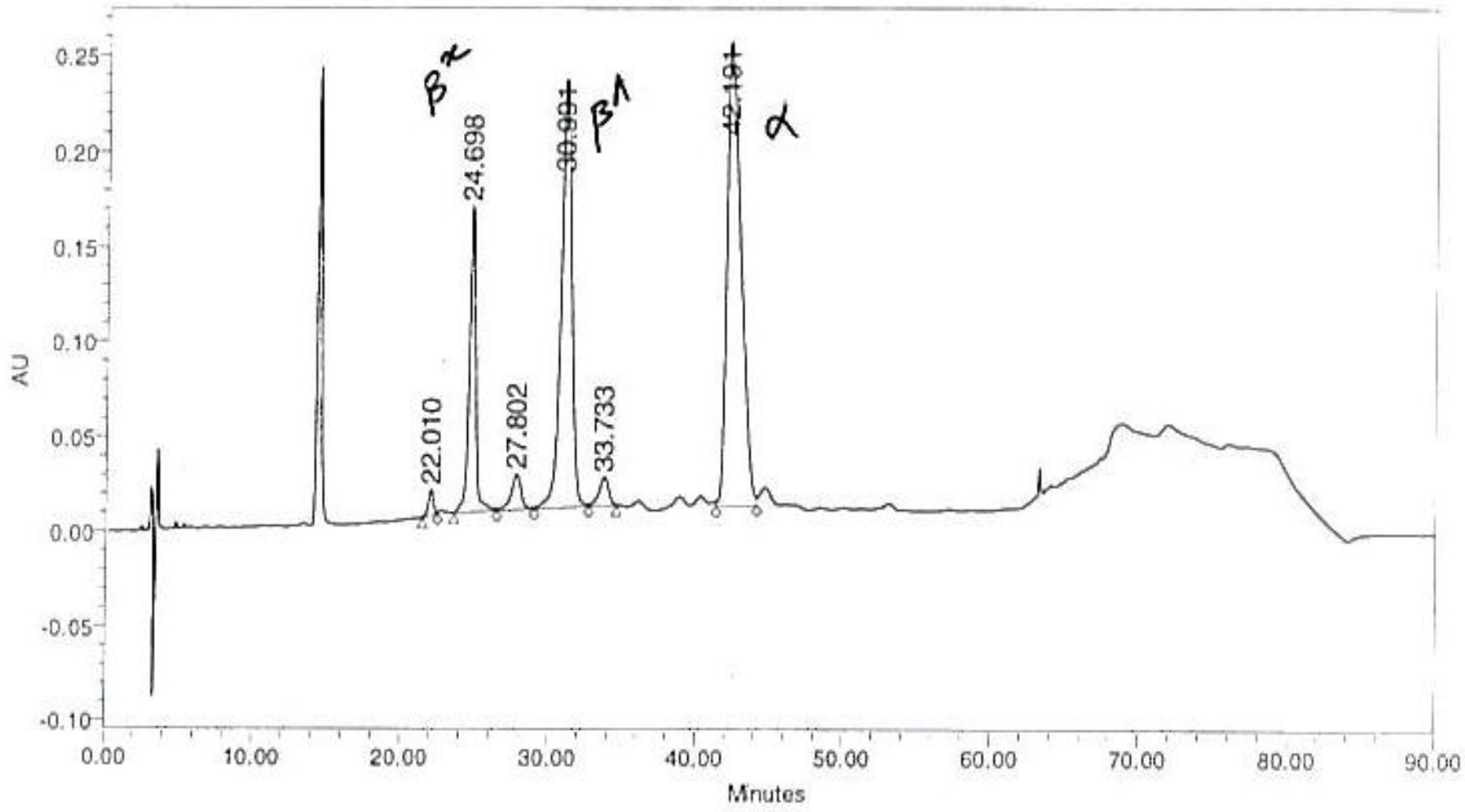
- Novel metHb variant resulting from the deletion of a Gly residue at β codon 25/26
- Deletion of a Gly residue at this location disrupts the close spatial contact between B and E helicis
- Likely affects the positioning of the distal histidine in E helix
- Similar variant (Hb Higashitochigi) reported from Japan

Kutlar F, et al. Blood Cells, Molecules, and Diseases 43 (2009), 235-238

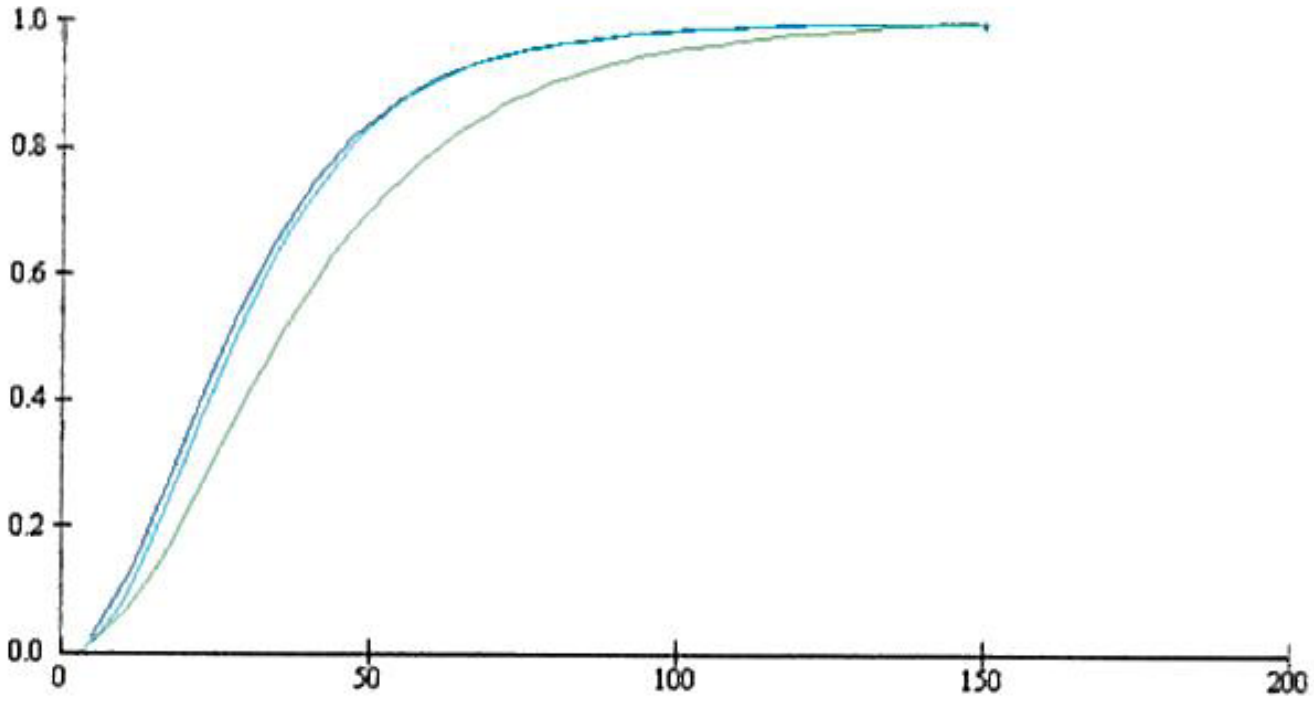
Hb Cheverly
β45 TTT→TCT (Phe→Ser)

- 18 mo Caucasian male seen in pediatric hematology for anemia
- Labs: Hb 10.2, HCT 30.8%, MCV 83.6, Retics 0.8%
- Hb analyses:
  IEF: Hb A + A2
  HPLC: Hb A 95.8%, Hb A2 3.2%, Hb F 1.0%
  rpHPLC: βX, βA, and α

Hb Cheverly


AU
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
22.010
24.698
27.802
30.991
33.733
42.191
0.00
10.00
20.00
30.00
40.00
50.00
60.00
70.00
80.00
90.00
Minutes

Hb Cheverly



Patient
P50=34.9 mmHg

Control
P50 = 6.6 mmHg

Diagnostic Approach to Hemoglobinopathies

- Hematologic Evaluation
  - CBC, retic count, peripheral smear, Heinz Bodies
- Isoelectric Focusing (IEF) on Agarose
- HPLC
  - Cation Exchange HPLC (Hb quantitation)
  - Reversed Phase HPLC (globin chain separation)
- Special Tests
  - Hb stability (isopropanol, heat)
  - O2 affinity (P50)
- Molecular Diagnostic Methods
  - PCR amplification and sequencing of globin genes
  - PCR based methods for detection of deletions
  - RT-PCR of globin mRNA

Conclusion

- Variant Hbs rarely cause a clinical and/or hematologic phenotype
- Most common phenotypes:
  - Heinz body hemolytic anemia (unstable Hbs)
  - Erythrocytosis (high oxygen affinity variants)
  - Anemia (low oxygen affinity variants)
  - Cyanosis (M hemoglobins)
  - Thalassemic hemoglobinopathies
- Family history may be helpful

Treatment

- Erythrocytosis due to high O2 affinity variant
  - Observation
  - Rarely phlebotomy required
- Heinz body hemolytic anemia (unstable Hbs)
  - Avoid oxidant stress
  - Splenectomy
  - Transfusions when required
  - Hydroxyurea ?
- Anemia (low oxygen affinity variants)
  - Observation, no treatment required
- Cyanosis (M hemoglobins)
  - Observation, no treatment required
- Thalassemic hemoglobinopathies
  - May require transfusions

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