PATOGENESIS DAN DIAGNOSIS THALASSEMIA

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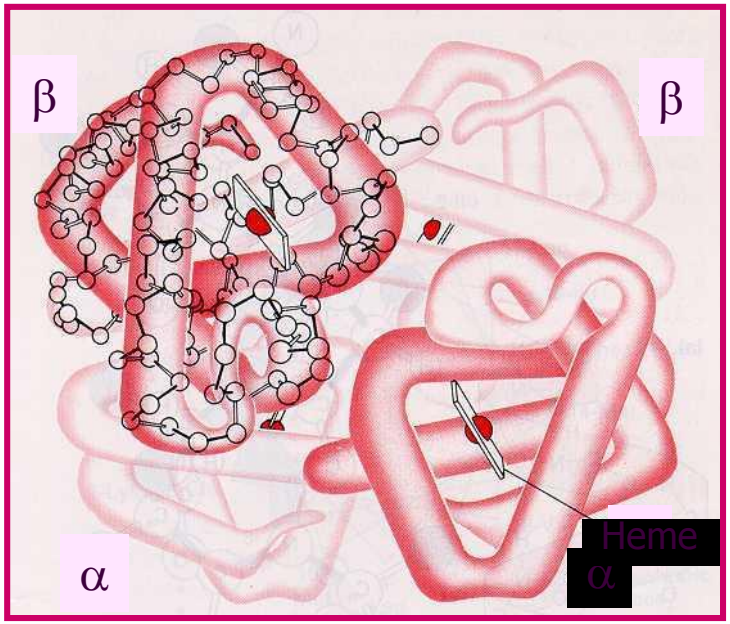
Definisi Thalassemia

- Kelainan hemoglobin bawaan yang ditandai dengan penurunan/tidak ada sintesis rantai globin beta (*thalassemia beta*) atau rantai globin alpha *(thalassemia alpha)*
- Kelainan hemoglobin lain:
  - Perubahan jenis asam amino yang menyusun rantai globin beta atau alpha tanpa ada penurunan sintesis rantai globin alpha atau beta – *Hemoglobin variant (Hemoglobinopati)*
  - Defisiensi besi

KOMPOSISI HEMOGLOBIN

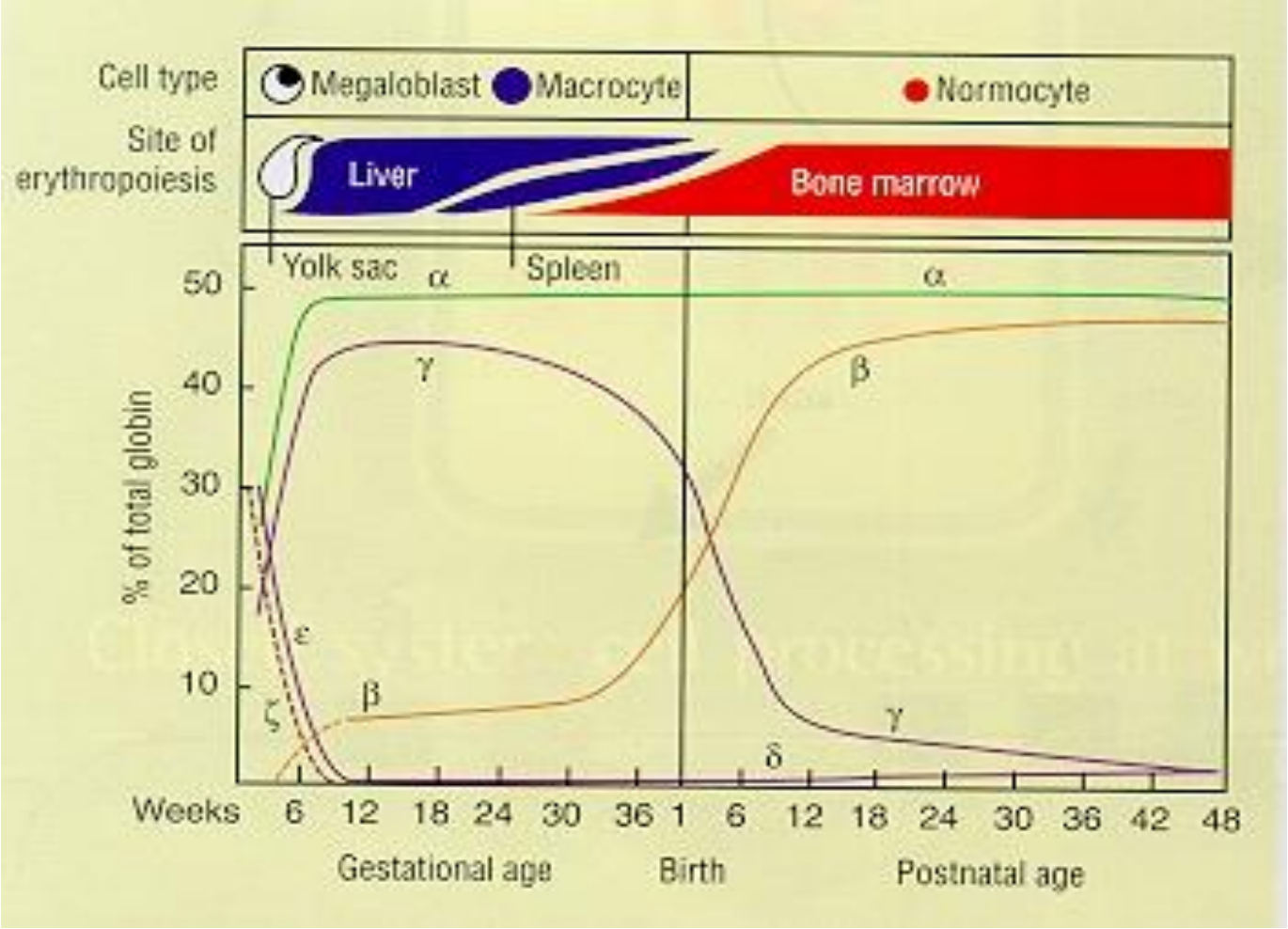
Molekul Hemoglobin



- 2 rantai globin-α
- 2 rantai globin-β
- 4 molekul heme

- Komposisi Hb dewasa:
  - HbA (>98%) – $\alpha_2\beta_2$
  - HbA_2 (2,5-3,5%) - $\alpha_2\delta_2$
  - HbF (<1%) - $\alpha_2\gamma_2$

Jenis hemoglobin selama perkembangan



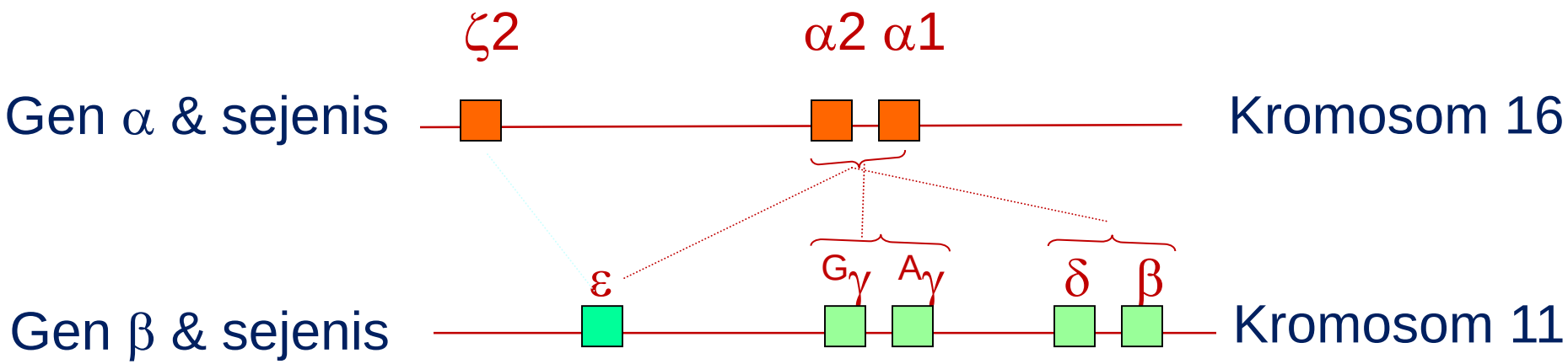
HbGower1
HbGower2
HbPortland

HbF

HbA & HbA_2
<<HbF

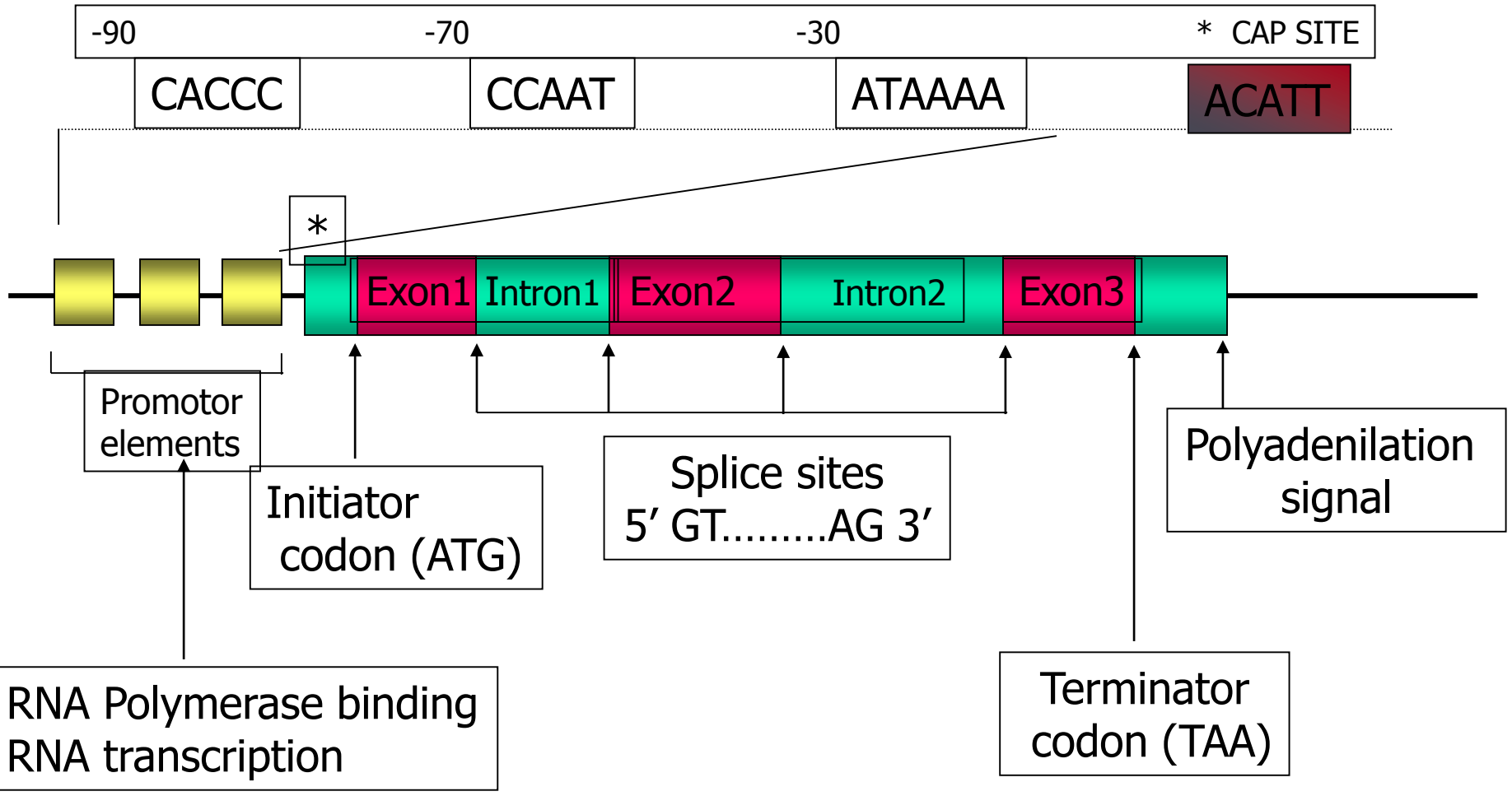
DASAR MOLEKUL THALASSEMIA

GEN PENYANDI SINTESIS RANTAI GLOBIN α DAN β

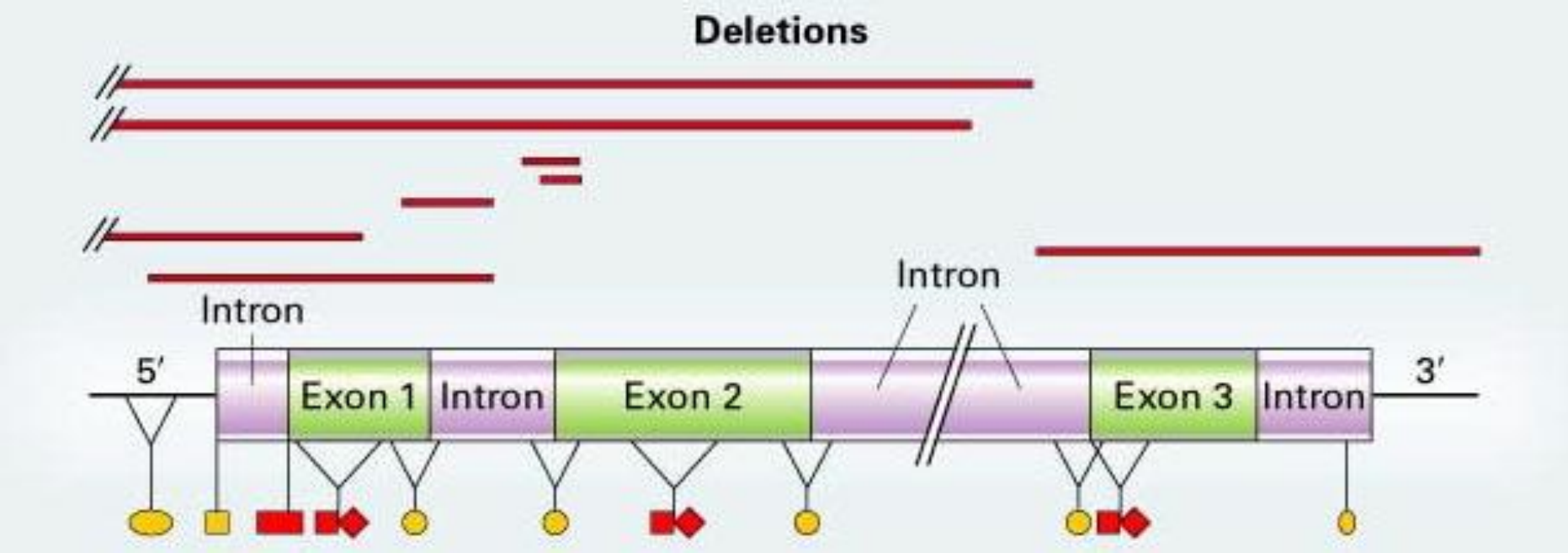


Hemoglobins	Hb Gower 1 ($\zeta_2\varepsilon_2$) Hb Gower 2 ($\alpha_2\varepsilon_2$) Hb Portland ($\zeta_2\gamma_2$)	HbF ($\alpha_2\gamma_2$)	HbA_2 ($\alpha_2\delta_2$)	HbA ($\alpha_2\beta_2$)
Masa Perkembangan	Embrio	Janin	Dewasa	

Struktur normal gen globin



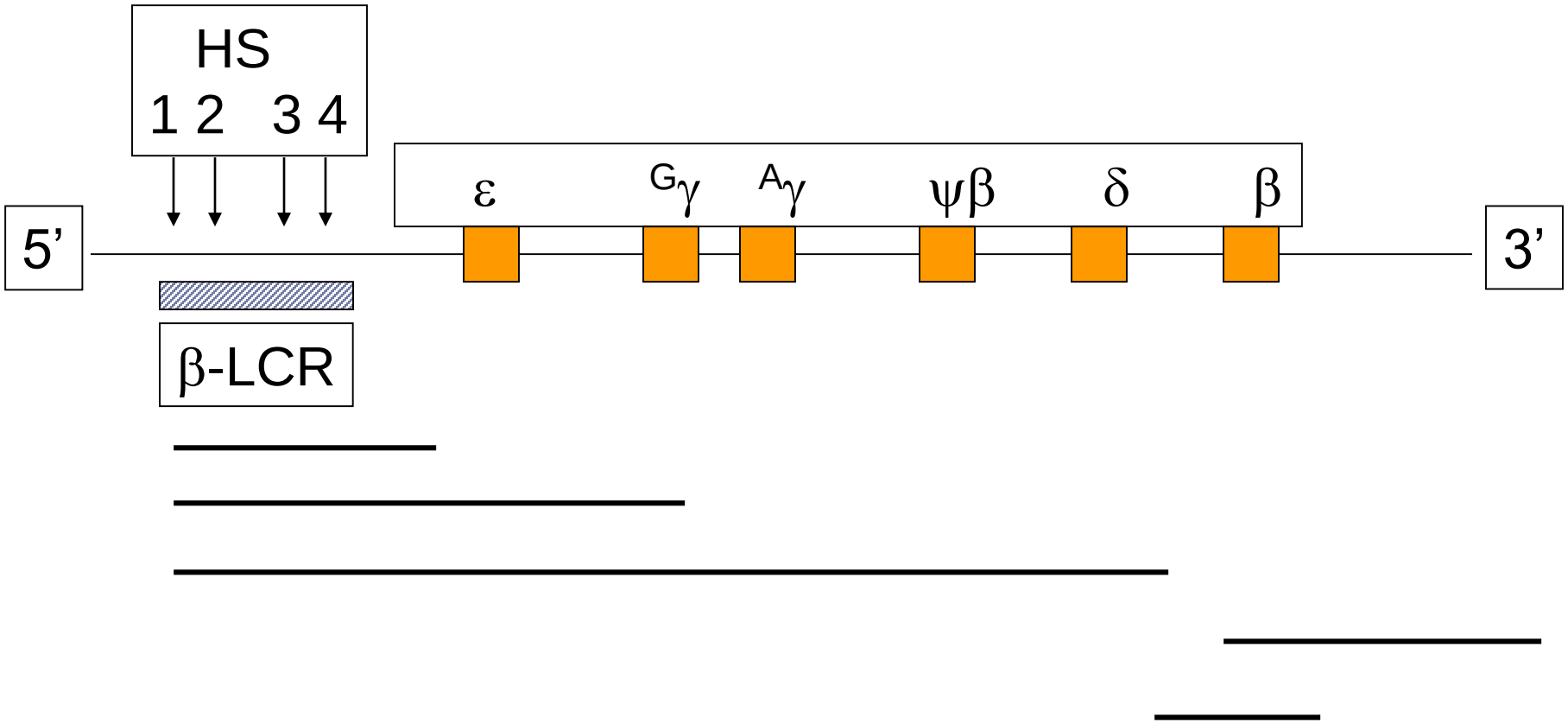
LOKASI DAN TIPE MUTASI PENYEBAB THALASSEMIA β



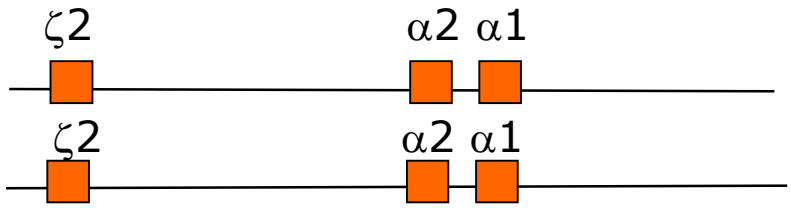
- Mutations affecting initiation of transcription located at the promoter regions (-28, -101, -) → β^{+} or β^{++}
- Mutation affecting of RNA from intron located at intron (IVS1-nt1, IVS1-nt5) or exon (HbE, HbMalay, Cd 30) → β^{o}, β^{+}, or β^{++}
- Polyadenilation signal mutation → β^{++}
- Mutation affecting initiation of translation (ATG to ACG, ATG to AGG) → β^{o} β^{+}
- Nonsense mutations → β^{o}
- Frameshift mutations → β^{o}

LOKASI DAN TIPE MUTASI PENYEBAB THALASSEMIA β

Delesi besar

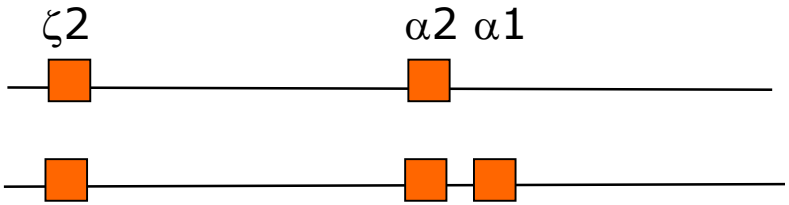


Mutasi penyebab thalassemia-α

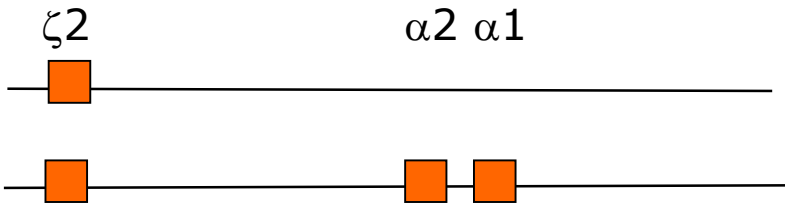


Gugus gen globin-α normal

Common mutations



delesi satu gen globin-α



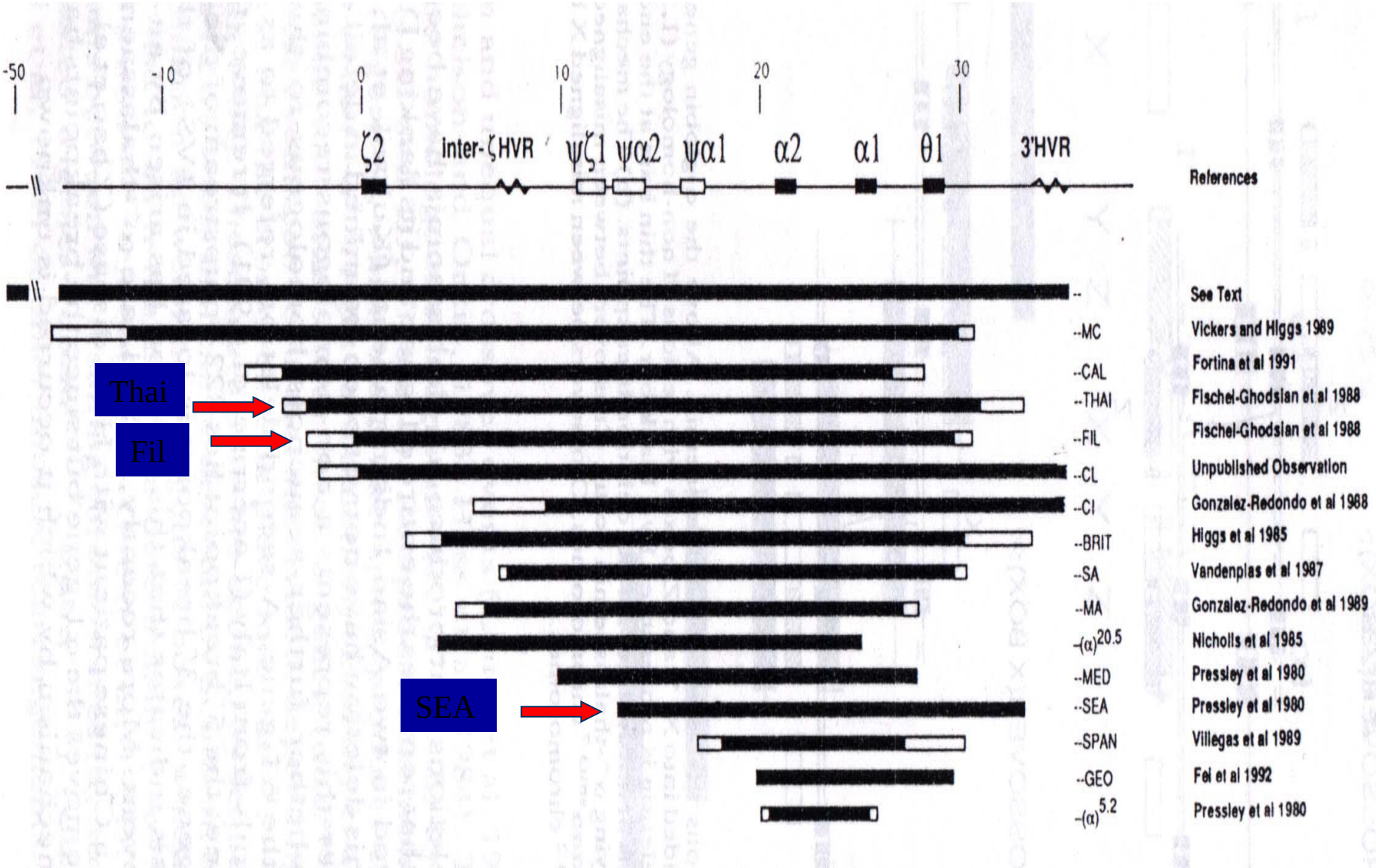
delesi dua gen globin-α

Less common mutations

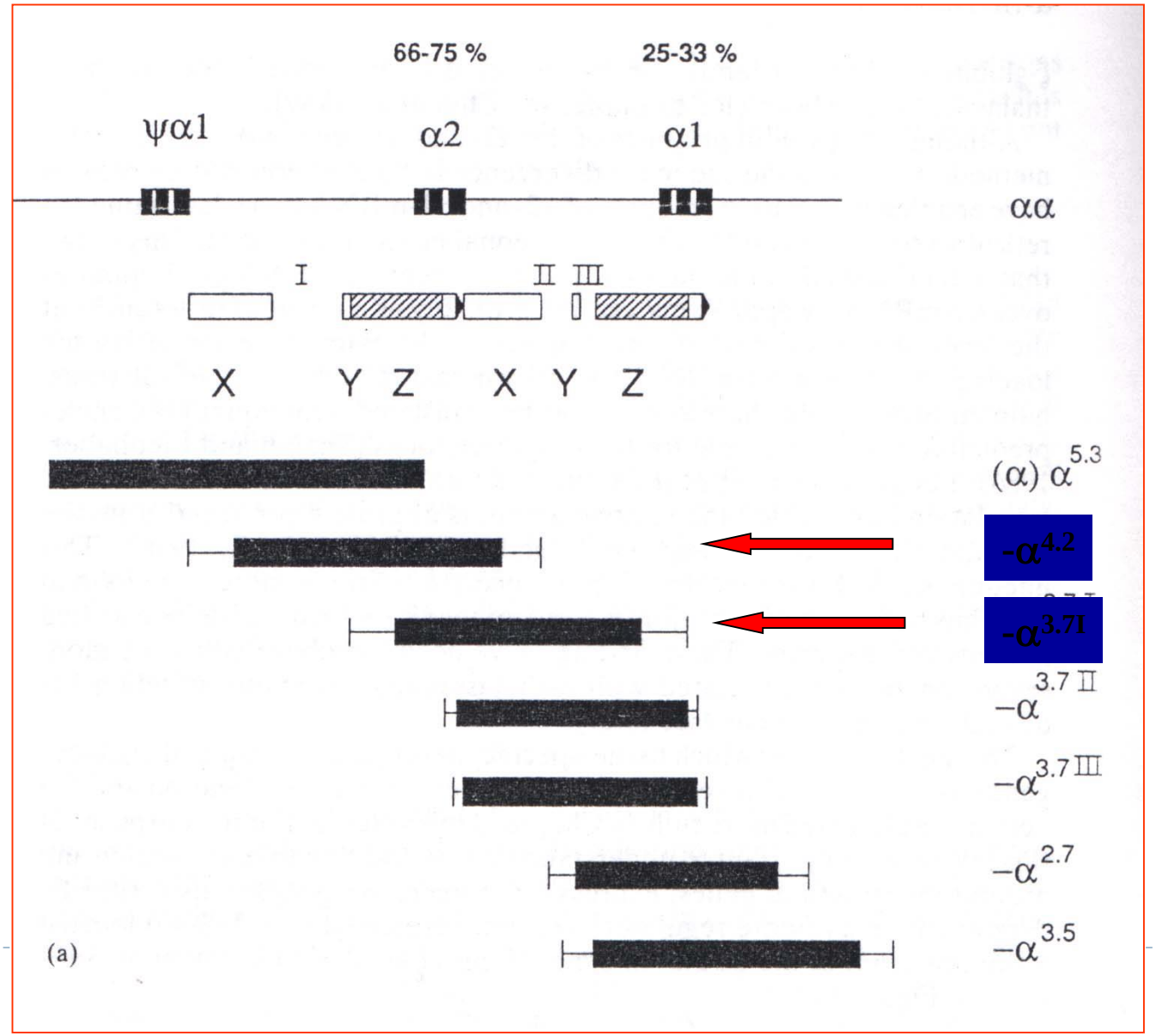
Umumnya mutasi *missense* atau mutasi pada codon terminasi yang menghasilkan:

- Hb tidak stabil
- rantai globin-α tidak stabil

MUTASI PENYEBAB THALASSEMIA-α^o (--/ $\alpha\alpha$)



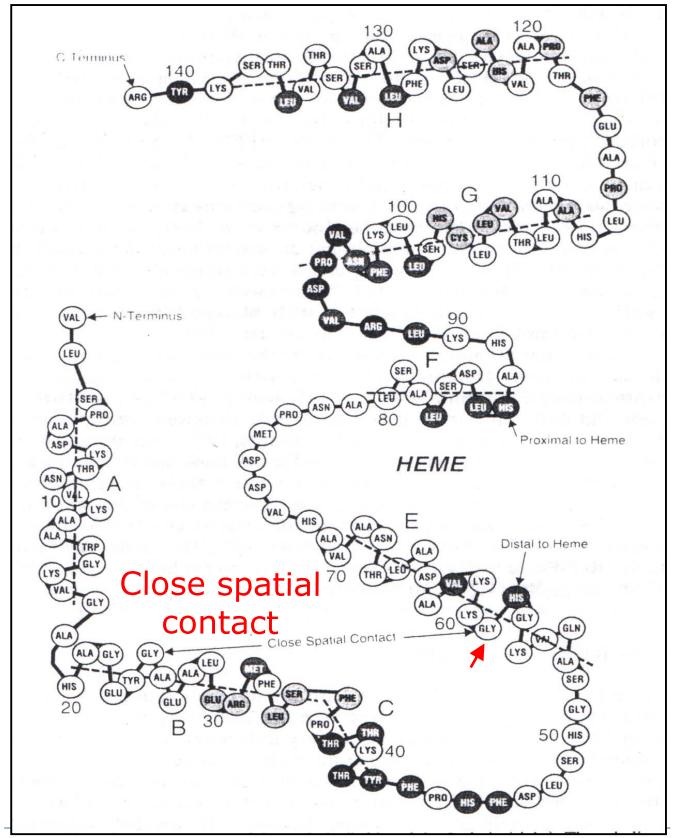
MUTASI PENYEBAB THALASSEMIA-α^+ (- $\alpha/\alpha\alpha$)



Mutasi thalassemia-α

Mutation causing unstable α-globin chain

- **Cd 59 (GGC^{Gly}→GAC^{Asp})**



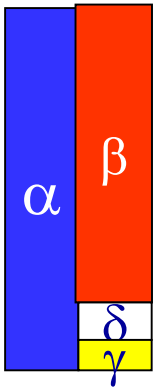
Mutation causing unstable hemoglobin

Cd 142 (TAA^{stop}→CAA^{Gln} + 30 asam amino)

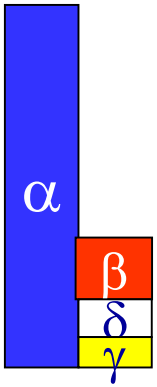
- Normal α-globin chain contains 141 amino acids
- This lengthened α-globin chain bind β-globin chain forms Hb variant known as Hb Constant Spring (HbCS)
- HbCS shows electrophoresis pattern different to that in HbA
- HbCS is an unstable Hb

PATOFISIOLOGI THALASSEMIA

Akibat mutasi gen globin-β



Normal Hb



Hb composition in β-thalasaemia

Mutasi di gen globin-β

↓

Produksi rantai globin-β (-) atau ↓

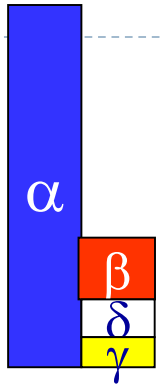
Kelebihan rantai globin-α

Sintesis Hb ↓

- Anemia
- mikrositik (MCV↓)
- hipokrom (MCH↓)

AKIBAT MUTASI GEN GLOBIN-β

Rantai globin-α berlebihan



⇩

α_4 (tidak stabil)

⇩ terurai

rantai globin-α bebas (tidak larut)

⇩

presipitasi

Sumsum tulang ⇩ ⇩ sirkulasi

Prekursor sel darah merah

- eritropoiesis tidak efektif
- destruksi di sumsum tulang

Sel darah merah matang

- trauma fisik
- perubahan metabolisme

Hemolisis di mikrosirkulasi (limpa)

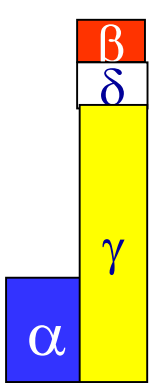
AKIBAT MUTASI GEN GLOBIN-α

Mutasi di gen globin-α

↓

Produksi rantai globin-α (-) atau ↓

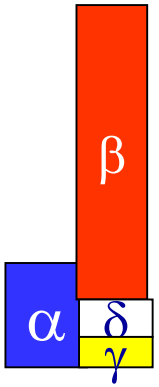
↓



←

Kelebihan:
Rantai globin-γ (janin)
Rantai globin-β (dewasa)

→



↓

γ_4 (Hb Bart)
β_4 (HbH)

↓

Presipitasi di sel darah merah matang

↓

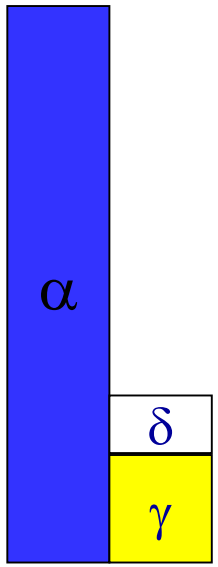
Hemolisis di limpa & mikrosirkulasi

DIAGNOSIS DAN MANIFESTASI KLINIS THALASSEMIA BETA

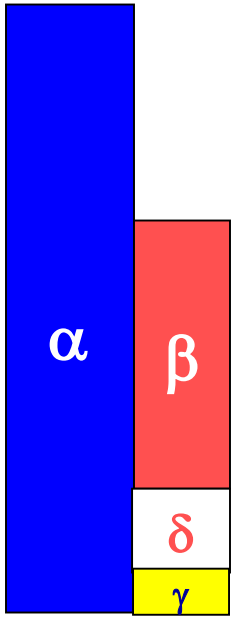
THALASSEMIA BETA MAYOR

Mutasi thalassemia-β^o atau -β^+ berat homosigot

- Sintesis rantai globin-β (-)
- Klinis anemia berat sejak umur 6 bulan (Thalassemia major)
- Morfologi SDM khas
- Hanya ada HbF (>90%) dan HbA_2 (normal atau sedikit meningkat), sedikit HbA (%) pada mutasi β^+ berat

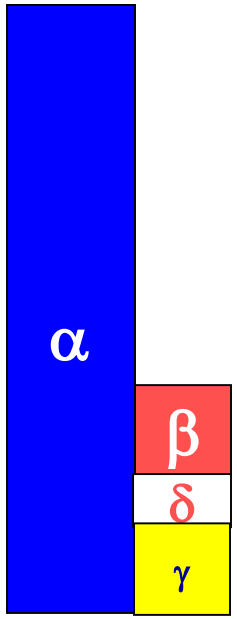


HETEROZYGOTE



Asymptomatic
Mild anemia to normal
Hb 8-15 g/dL(meanL 12)
MCV < 80 fl
HbA_2 > 3.5%
HbF > 1%

HOMOZYGOTE β+

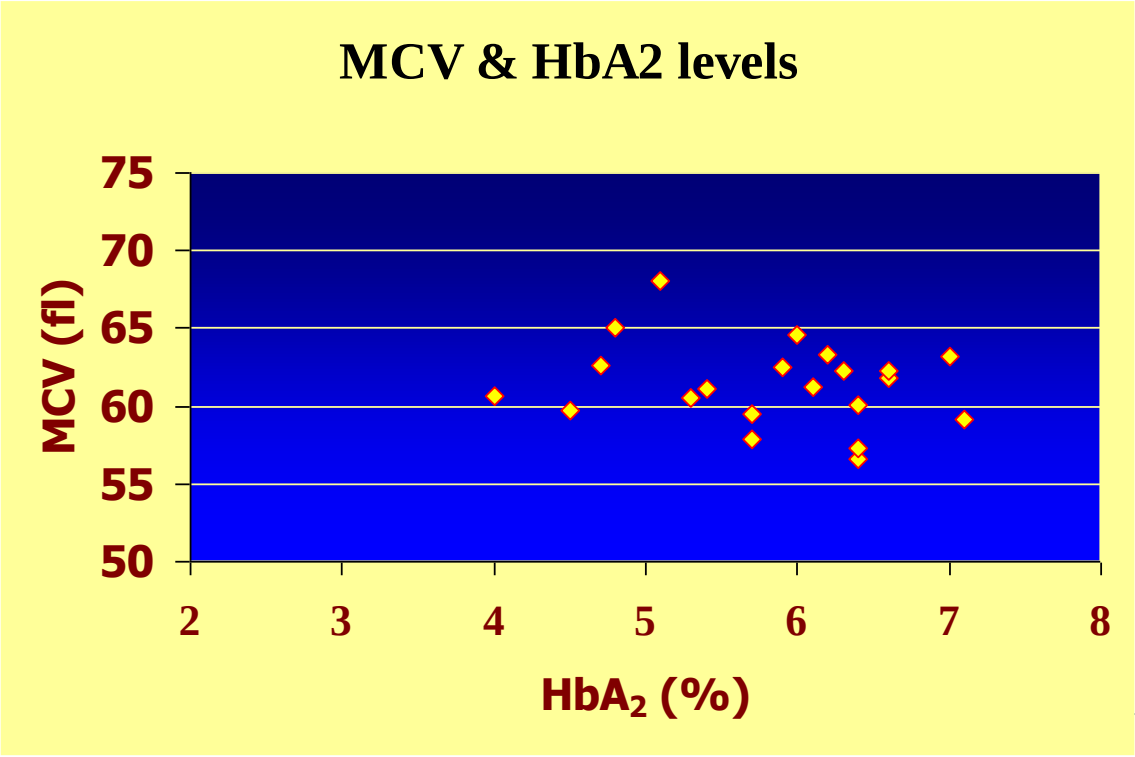


Mild to severe anemia
Liver & spleen may/not >>
Still has HbA
HbF range from 10 to > 90%

KARAKTERISTIK PARAMETER HEMATOLOGI ORANG TUA PENDERITA THALASEMIA MAJOR (MUTASI β^O ATAU β^+ BERAT)

Heterozygotes thalassemia-β^O atau β^+ berat

- klinis: thalassemia minor
- kadar Hb: 8.5 s/d 15 g/dl (X 12g/dl)



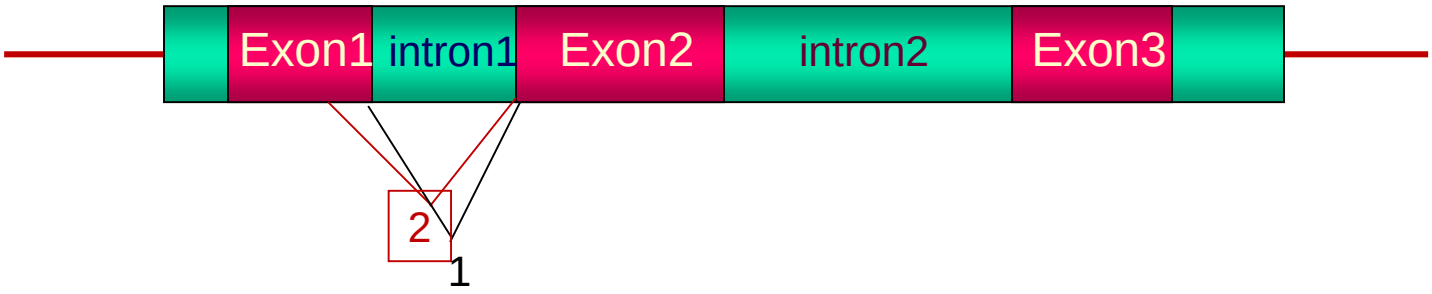
MCV<70fL
HbA_2>4%

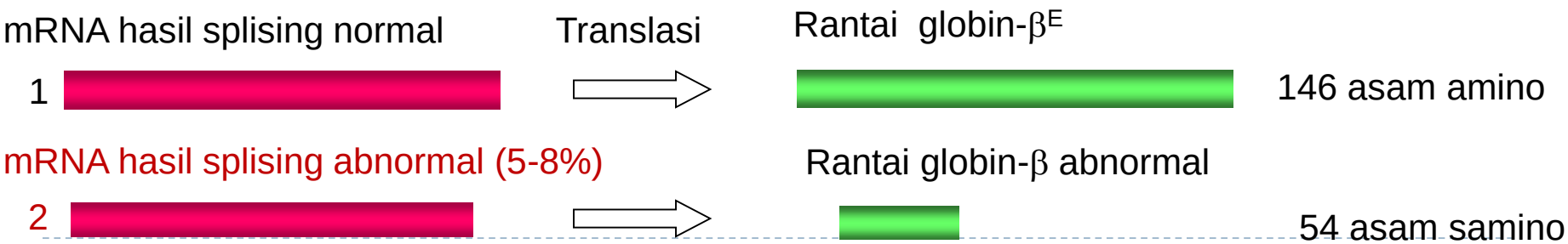
Hb variant

- Ada perubahan 1 asam amino pada rantai globin beta atau alpha
- HbE :
  - Hb variant (rantai globin beta) tersering di Indonesia
  - Juga termasuk kategori thalassemia karena mutasinya selain menyebabkan perubahan asam amino juga menyebabkan penurunan sintesis rantai globin beta (dalam hal ini β^E)

HbE

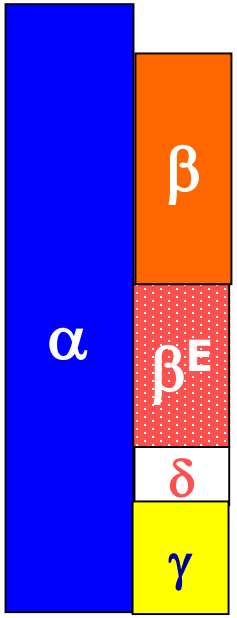
- perubahan asam amino (Codon 26, GAG^{Glu} → AAG^{Lys}) → Hb varian
- mengganggu proses splising → sintesis rantai globin beta (β^E) berkurang → Thalassemia





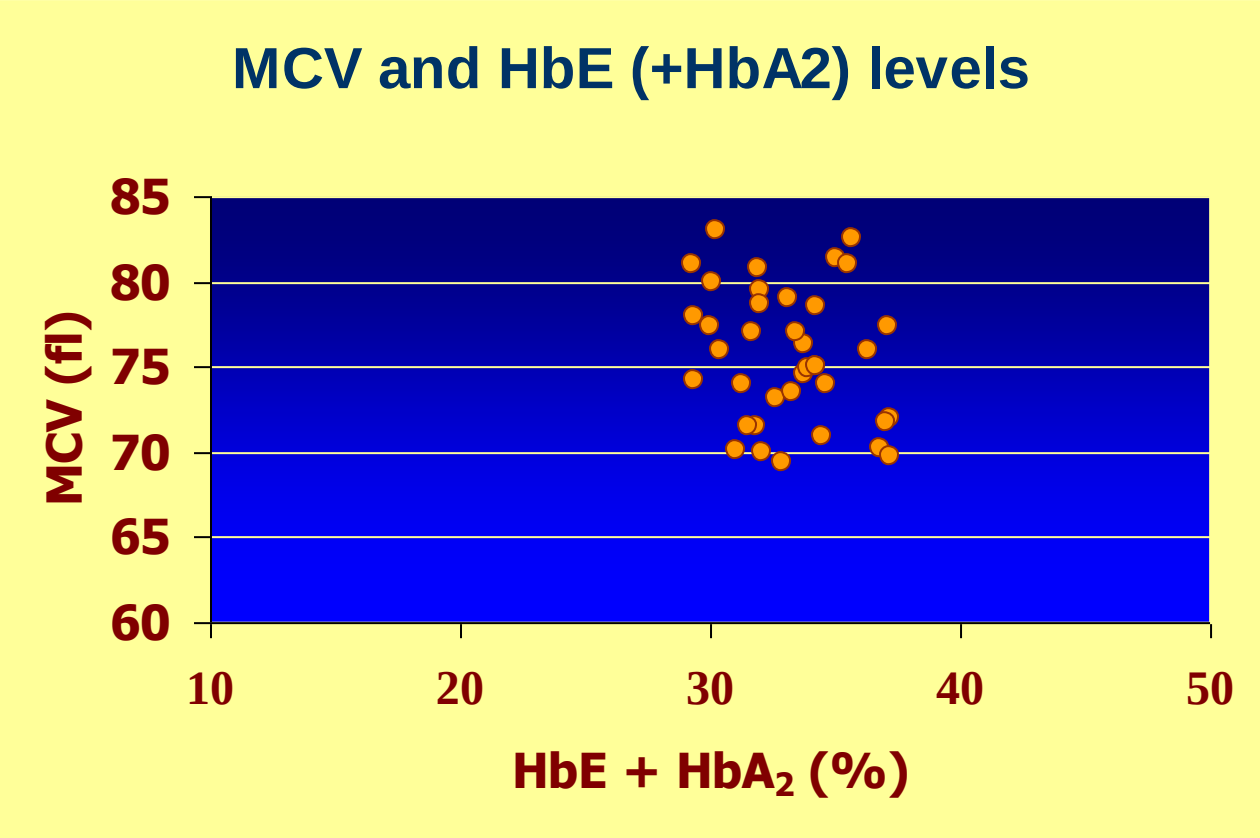
HbE heterozygote

HbE/N



- Usually normal Hb
- MCV > 70 fl
- HbE has same electrophoretic pattern as that in HbA_2, level of HbE= ~35%

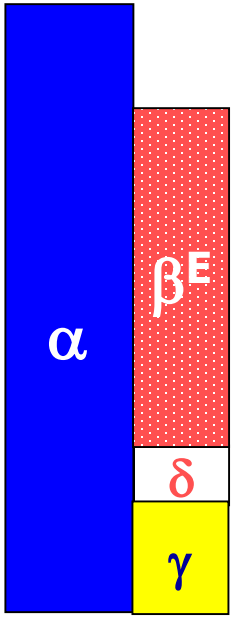
Clinical and hematological manifestations of Hb E (Codon 26, GAG^{Glu} to AAG^{Lys}) heterozygotes

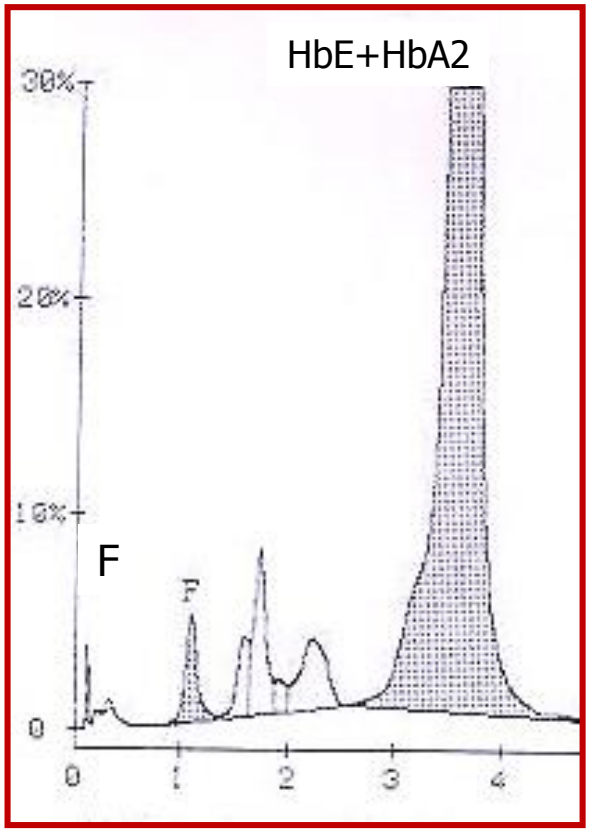


Important issue: MCV > 80 fl ??

HbE homozygote

HbE/HbE

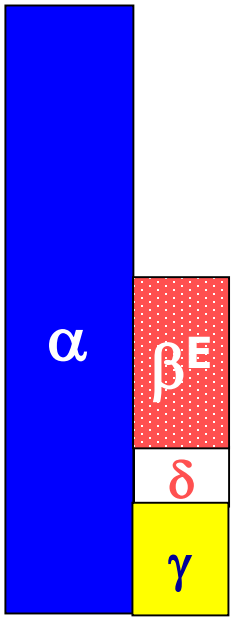




- mild anemia
- MCV < 70 fl
- only consists of HbE, HbA_2 and HbF

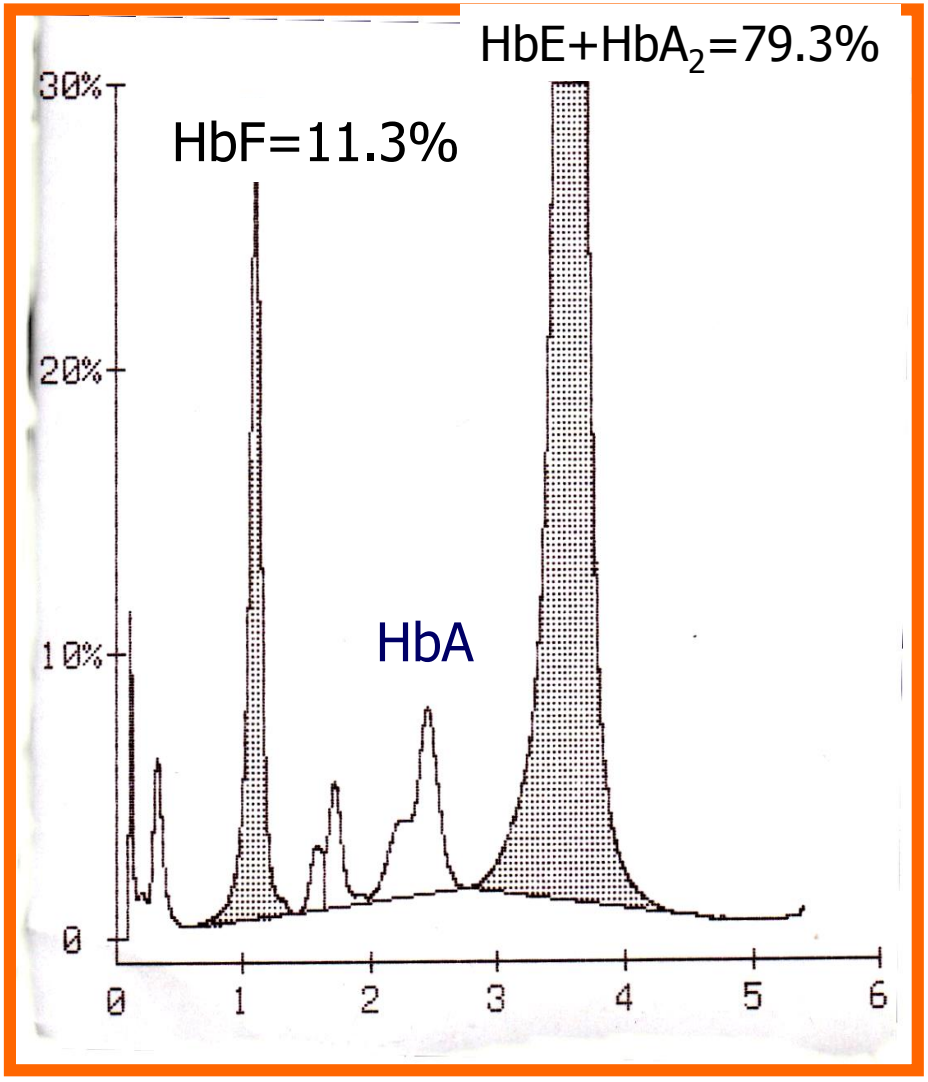
Compound heterozygote HbE with β-thalassemia mutations

HbE/ β^o or β^+



- Moderate to severe anemia
- consists of HbE, HbF, HbA2, HbA (**β+)**

HbE/IVS1-nt5 (β^+ berat)

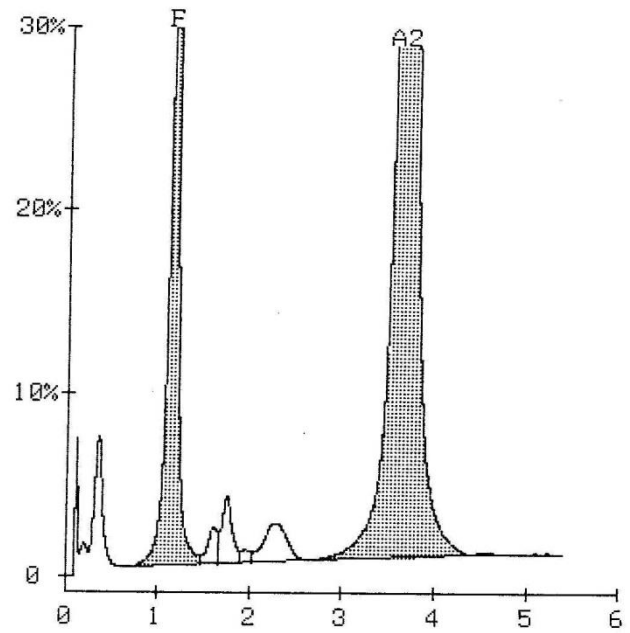


HbE/Cd41-42 (β^o)

NALYTE ID		%	TIME	AREA
F		20.5	1.15	263992
Unknown	1	0.9	1.60	12998
P3		1.9	1.74	27313
Unknown	2	0.3	1.96	4371
Ao		2.3	2.27	33390
A2		80.2	3.64	1013489

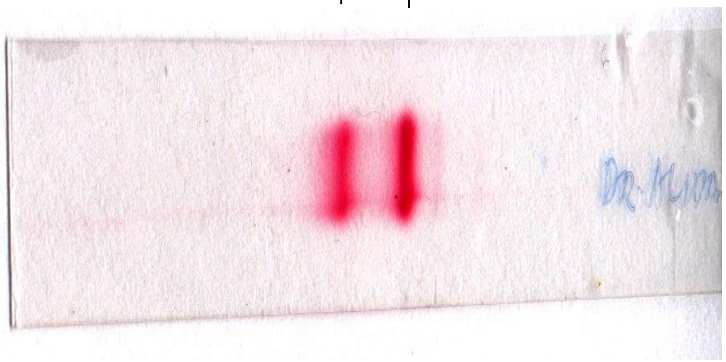
TOTAL AREA 1355553

F 20.5% A2 80.2%

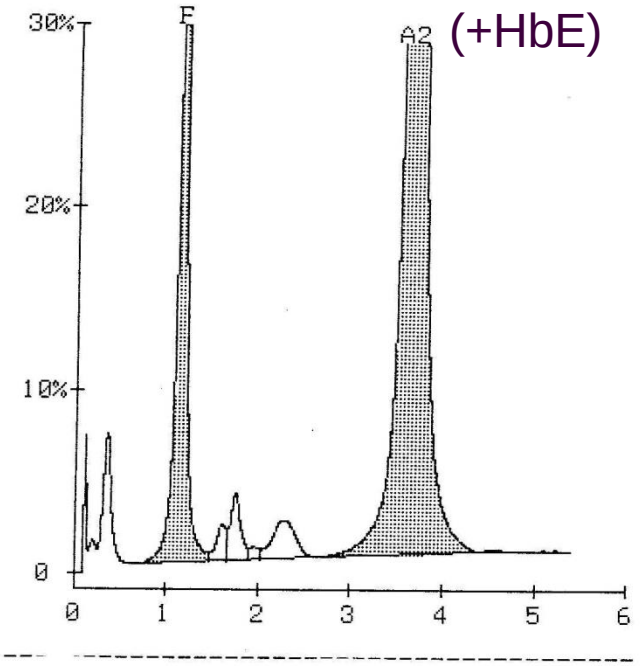


Electrophoresis

HbF HbA_2 (+HbE)

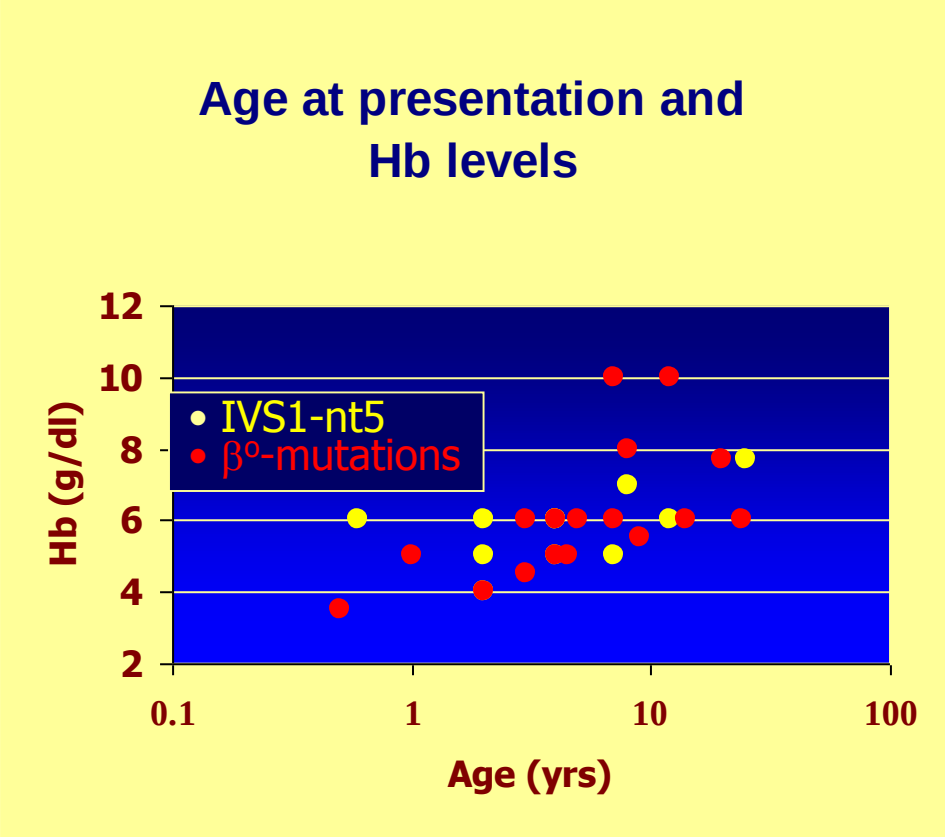


NALYTE ID	%	TIME	AREA
F	20.5	1.15	263992
Unknown 1	0.9	1.60	12998
P3	1.9	1.74	27313
Unknown 2	0.3	1.96	4371
Ao	2.3	2.27	33390
A2	80.2	3.64	1013489
	TOTAL AREA		1355553
F	20.5%	A2	80.2%



(+HbE)

Clinical and hematological manifestations of Hb E/β-thalassemia compound heterozygotes



Hb variant

- HbMalay :
  - Hb variant (rantai globin beta) tersering di populasi Melayu
  - Juga termasuk kategori thalassemia karena mutasinya selain menyebabkan perubahan asam amino juga menyebabkan penurunan sintesis rantai globin beta (dalam hal ini β^M)

Hb MALAY

- amino acid change (Cd 19, A<u>A</u>C^{Asn} → A<u>G</u>C ^{Ser})→ Hb variant
- affects mRNA process (splicing) → thalassemia
- similar pattern of electrophoresis as that in HbA

Normal splicing mRNA

1

Translation

Normal β^M-globin chain

146 amino acid

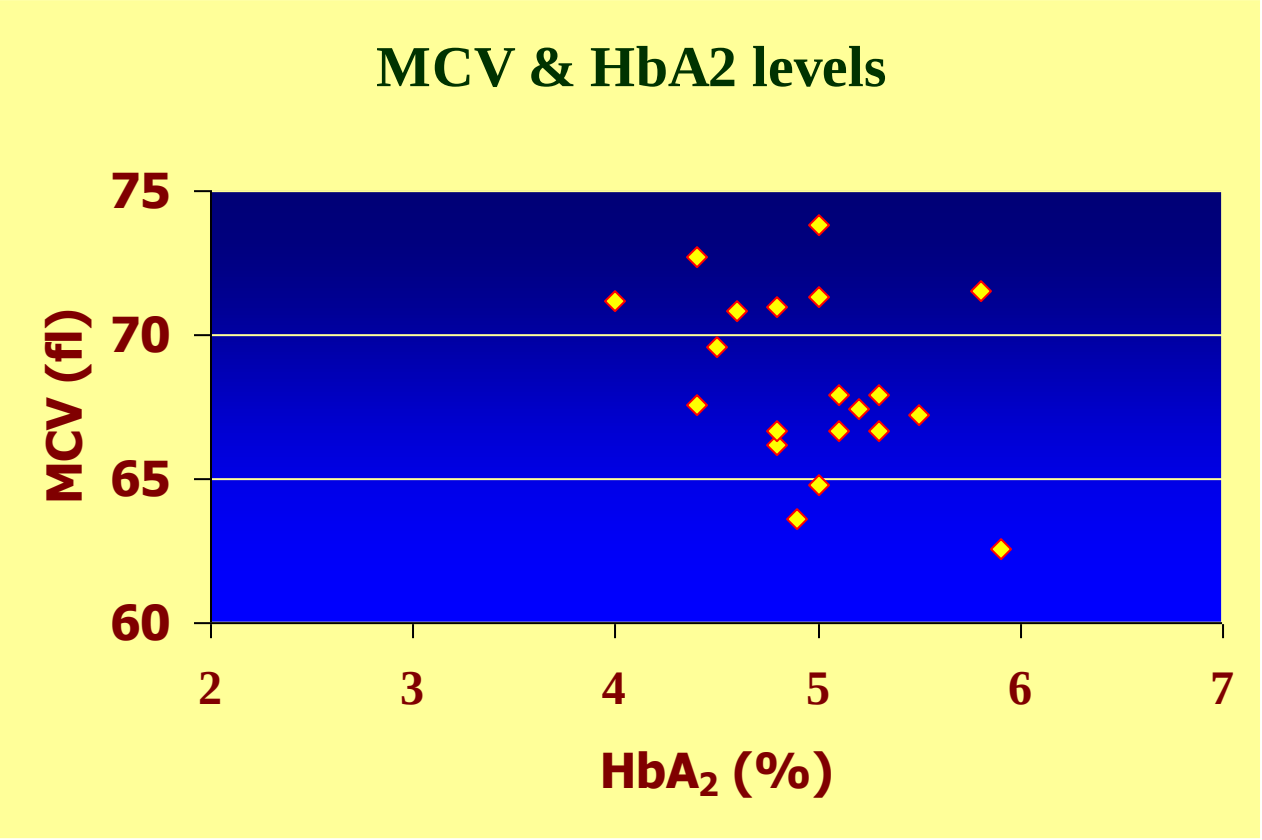
Abnormal splicing mRNA (25%)

2

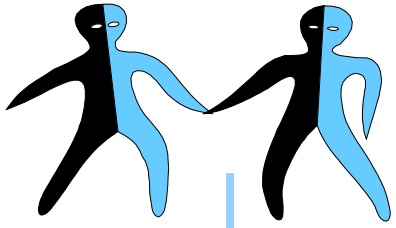
Abnormal β-globin chain

Stop at Cd 30 produces
29 amino acid

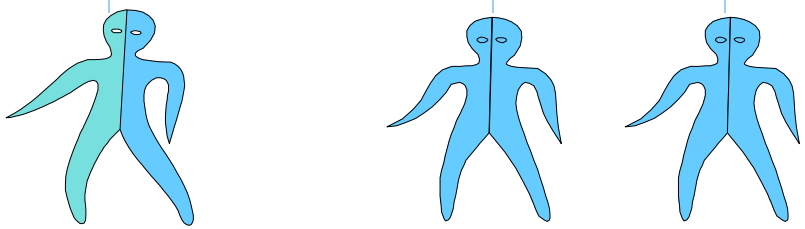
Hematological manifestations of Hb Malay heterozygotes



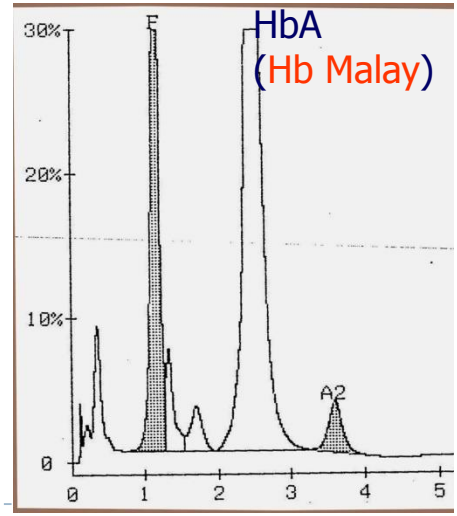
Clinical and hematological manifestations of Hb Malay



Hb (g/dl)	12.1	13.2
MCV (fl)	73.8	72.7
HbA2 (%)	5	4.4
HbF (%)	2.3	2.5
DNA analysis	Hb Malay het.	Hb Malay het.

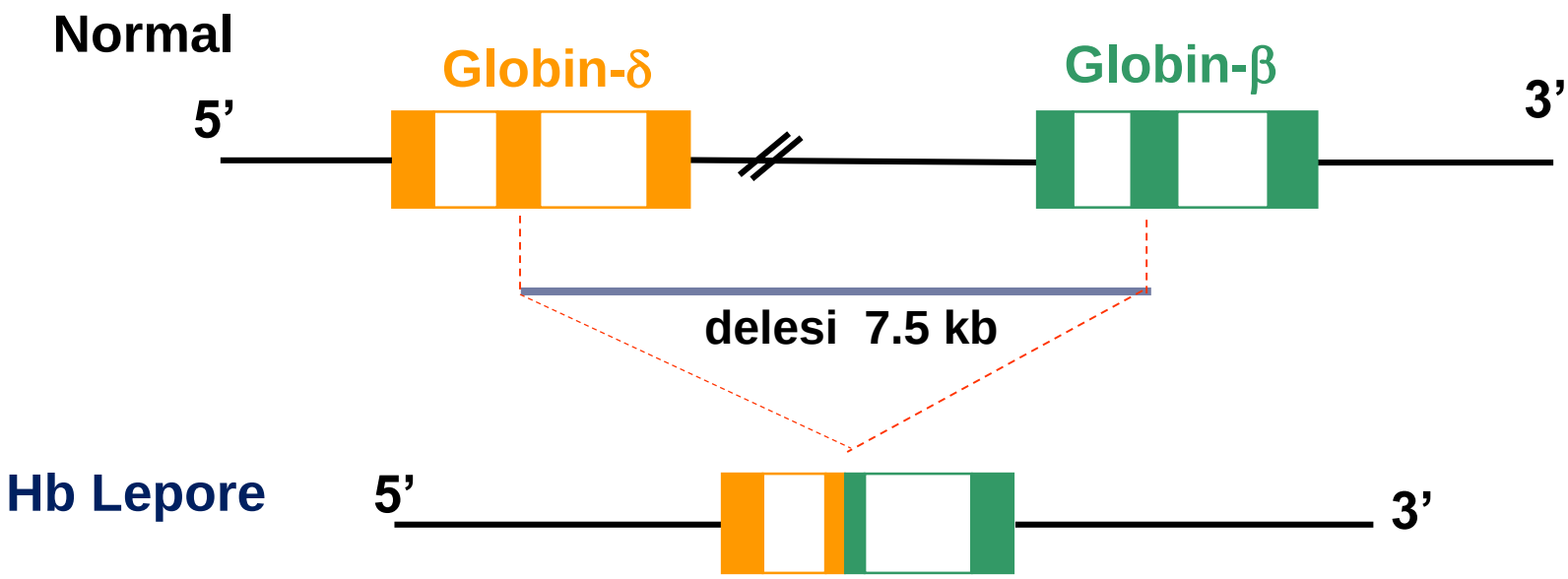


Hb (g/dl)	9.4	9.6	
MCV (fl)	74.8	70.7	
HbA2 (%)	3.9	4.5	
HbF (%)	50	29.3	
HbA (HbMalay)(%)	46	66	

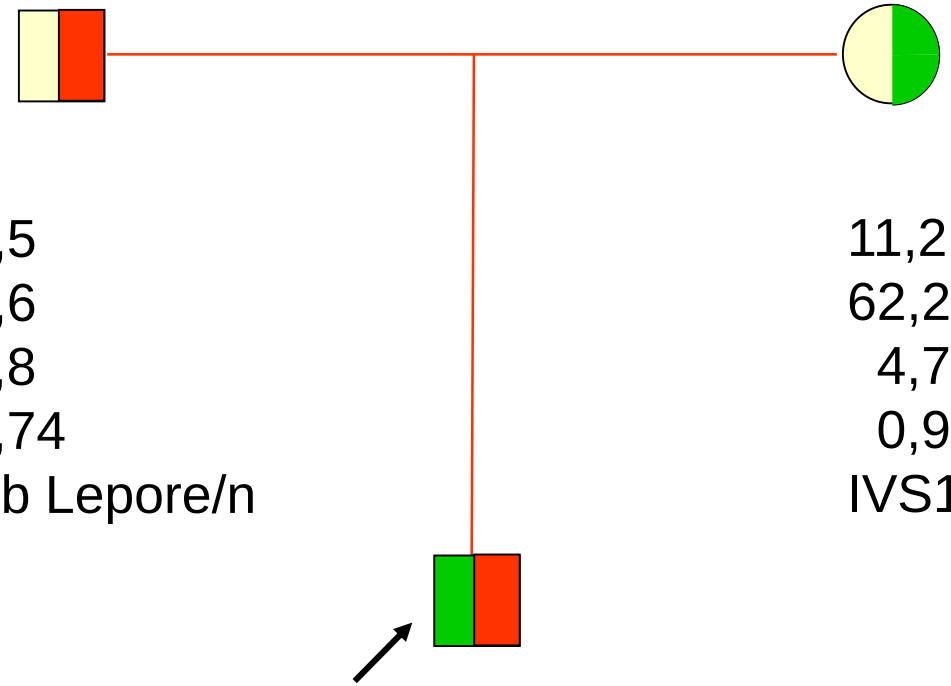


Hb Malay homozygotes are clinically thalassemia intermedia

Hb LEPORE



MANIFESTASI KLINIS & HEMATOLOGI Hb LEPORE



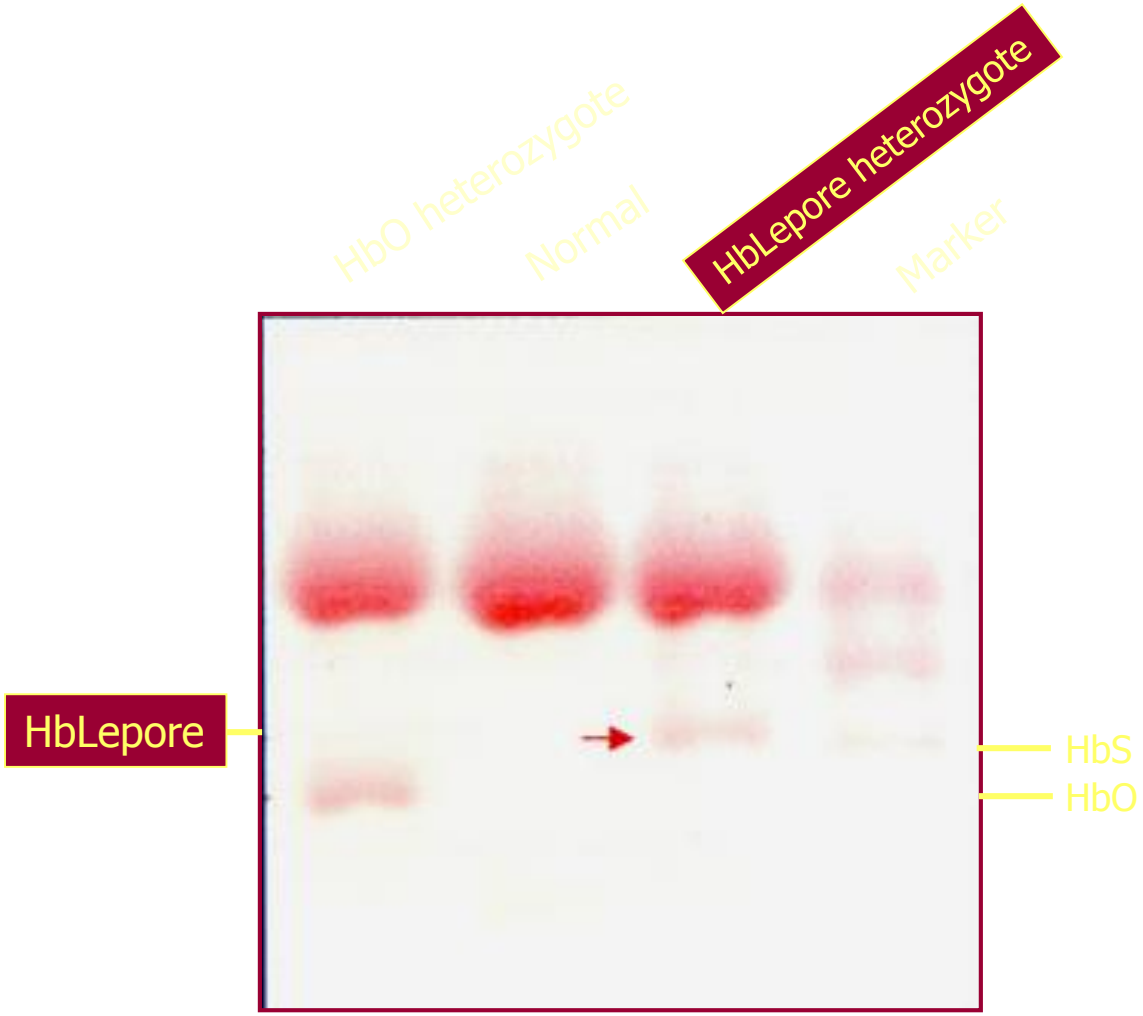
Hb (g/dl)	12,5	11,2
MCV (fl)	69,6	62,2
HbA_2 (%)	1,8	4,76
HbF (%)	1,74	0,97
Mutasi thal-β (PCR-RFLP)	Hb Lepore/n	IVS1-nt5/n

Klinis thalassemia mayor

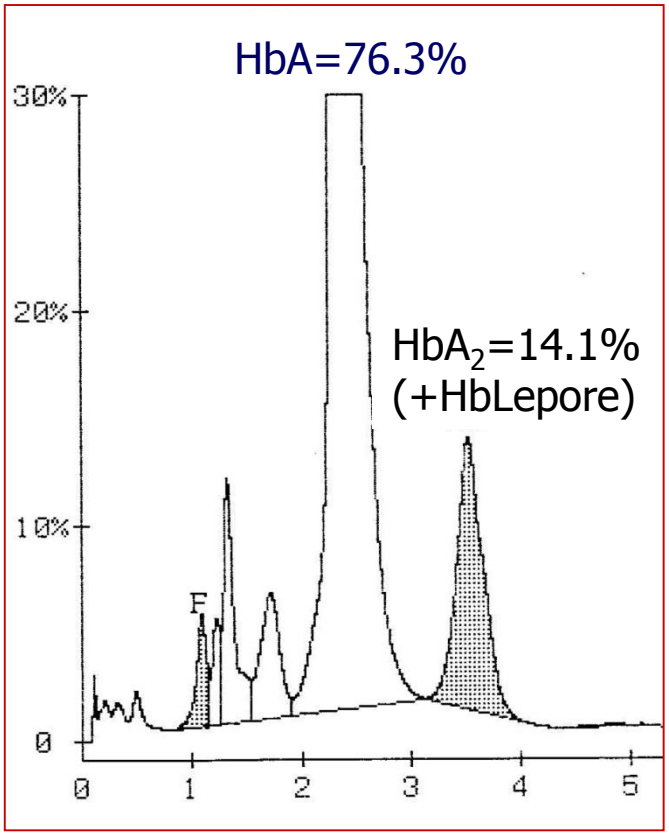
Transfusi setiap bulan

Hb Lepore/IVS1-nt5

Hb LEPORE HETEROZYGOTE



CELULOSE ACETATE ELECTROPHORESIS



β-THALASSEMIA SHORT VARIANT (HPLC)

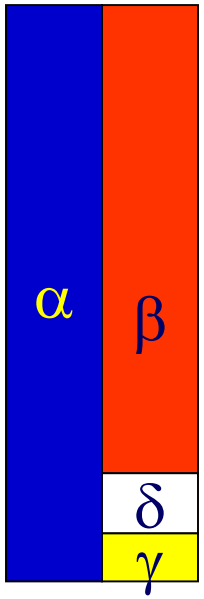
DIAGNOSIS DAN MANIFESTASI KLINIS THALASSEMIA ALPHA

Clinical manifestation of α-thalassemia

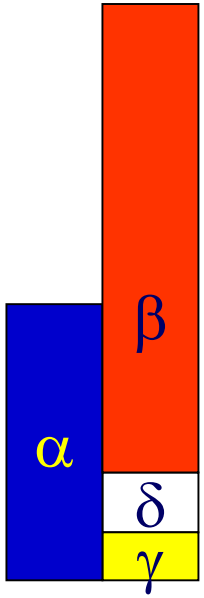
Normal

α-thalassemia

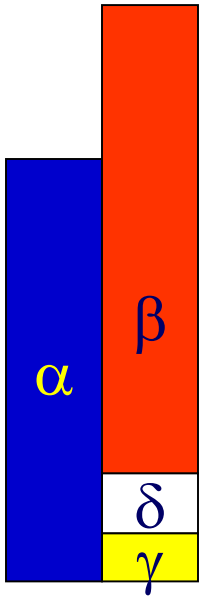
αα/αα



--/αα
-α/-α

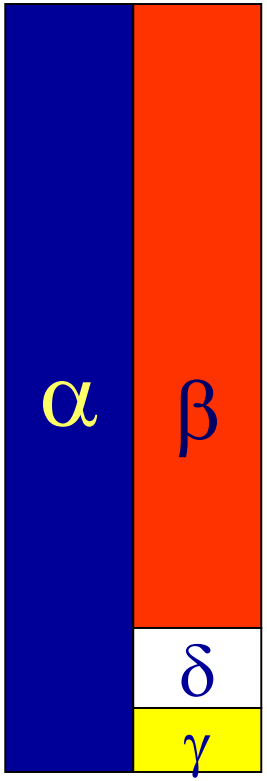


-α/αα



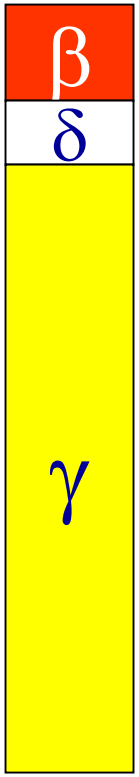
	normal	N/mild anemia	Normal
MCH :	26-32 pg	< 25 pg	25-27pg
HbA_2 :	2.5-3.5%	normal or low	normal or low
HbF :	<1%	low or absent	low or absent

Normal



α-thalassemia

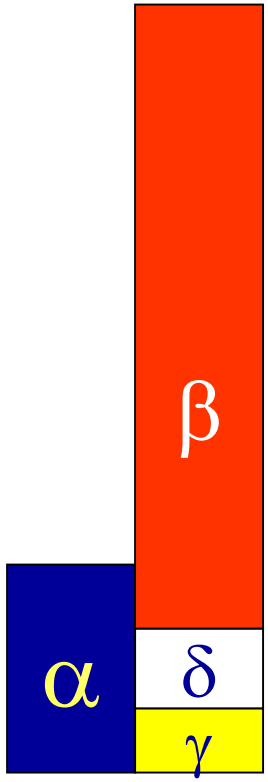
--/--



γ_4=HbBart

- death during fetal life
- Hydrops fetalis

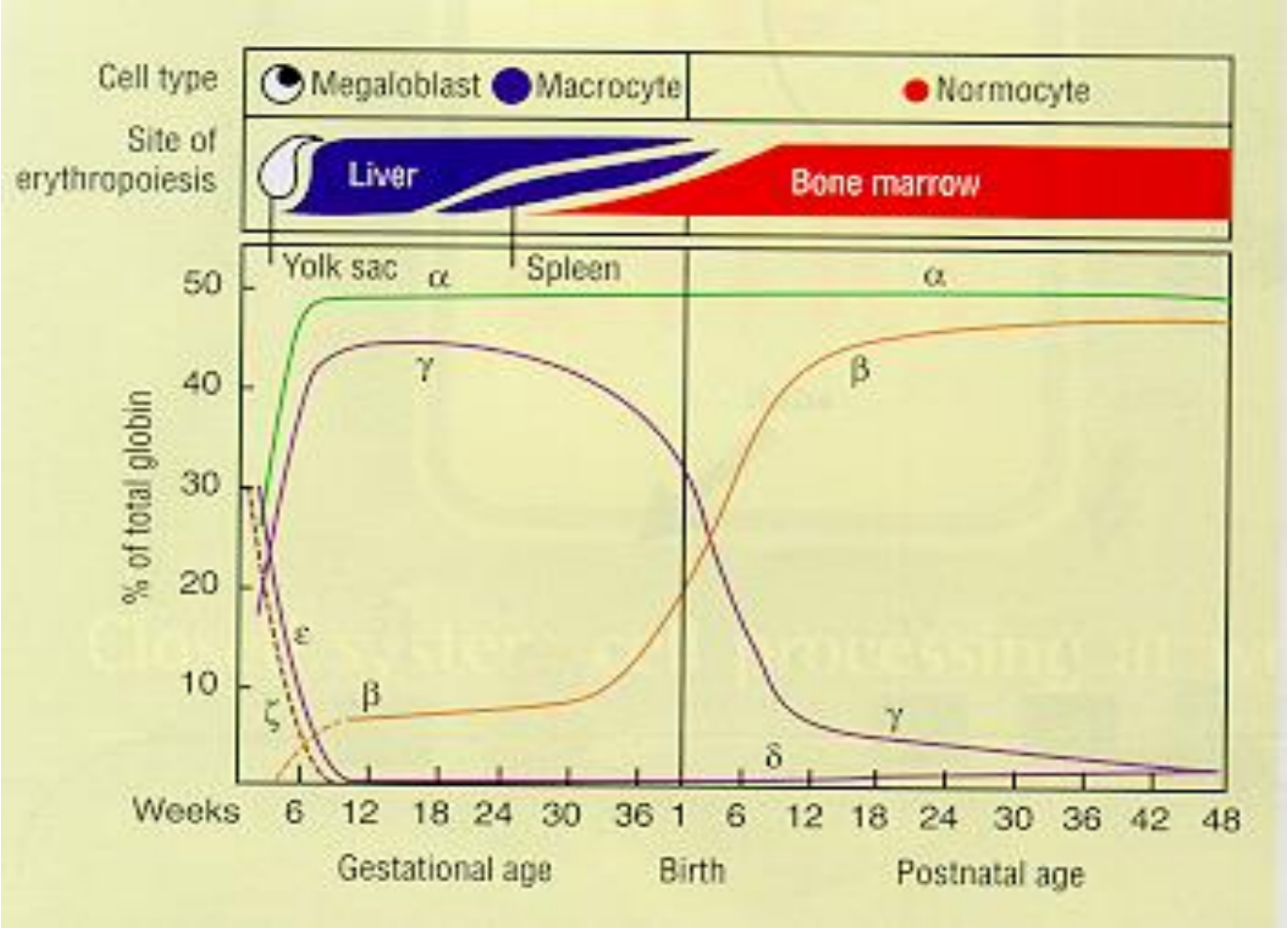
- -/-α



β_4=HbH

- HbH disease
- mild to severe anemia

Jenis hemoglobin selama perkembangan



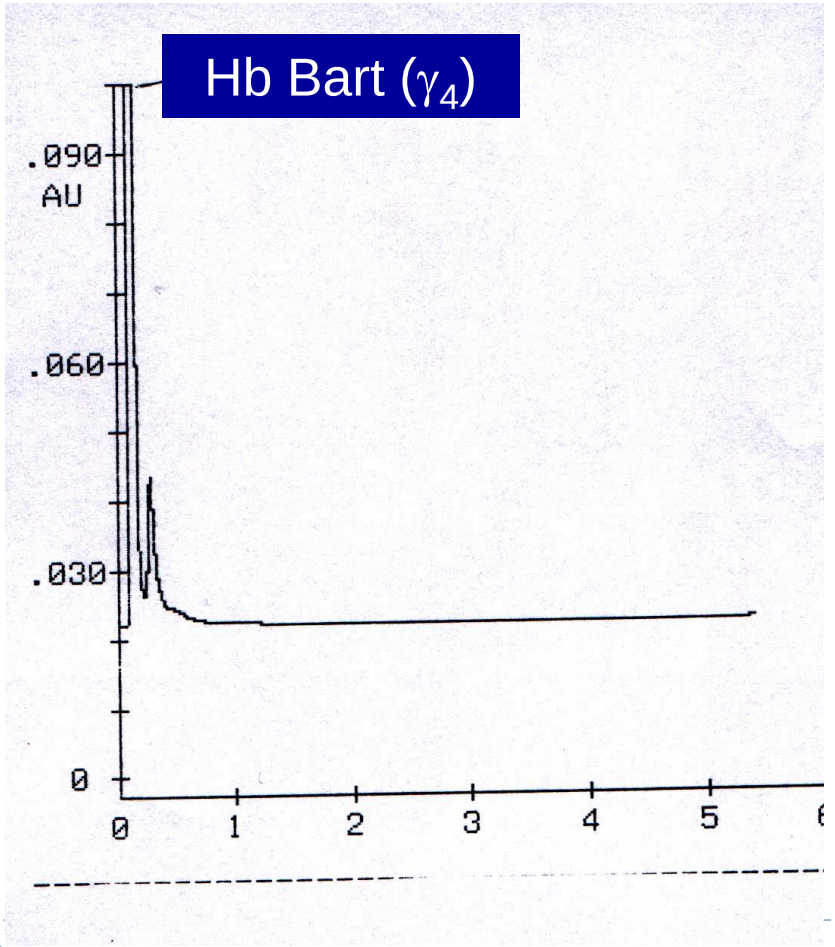
HbGower1
HbGower2
HbPortland

HbF

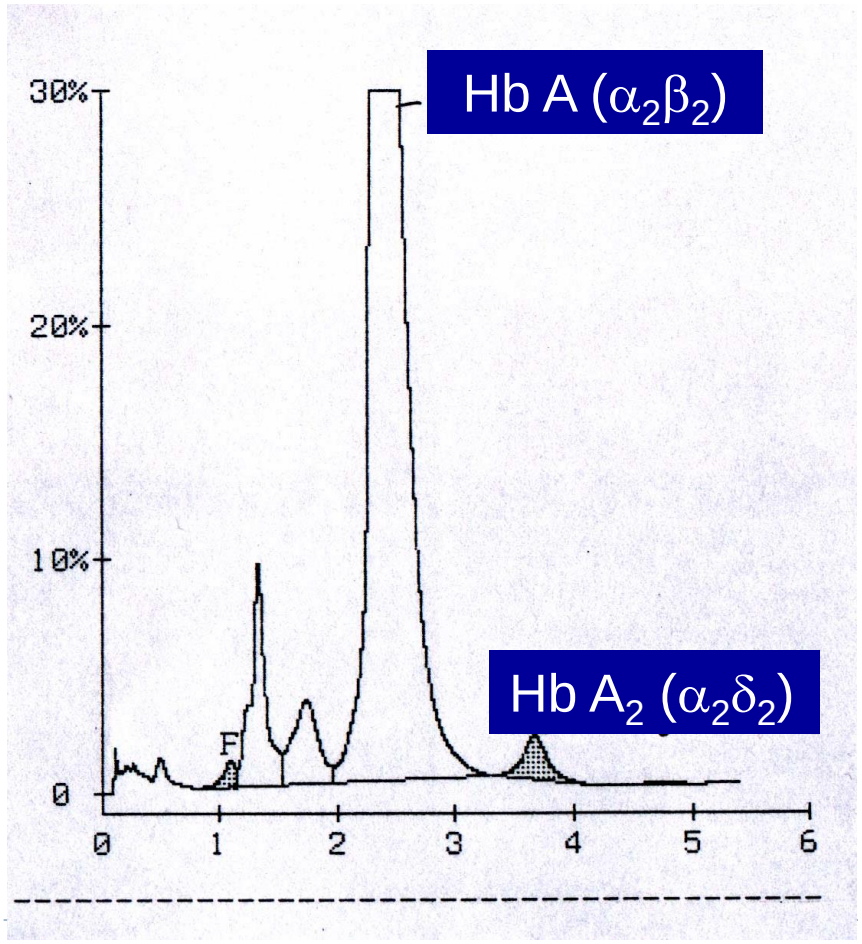
HbA & HbA_2
<<HbF

Beta-thalassemia short variant analyser (HPLC)

Fetus (--/--}

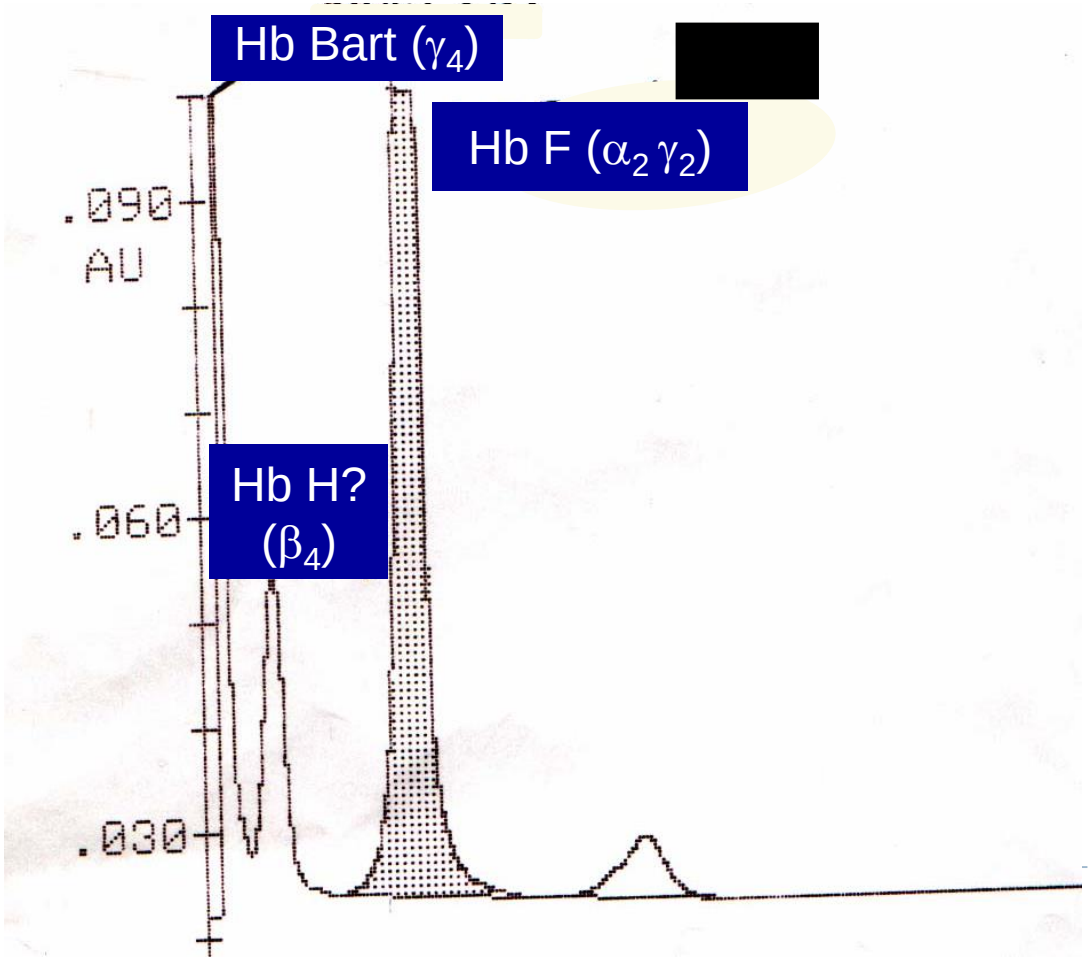


Parent (--/αα

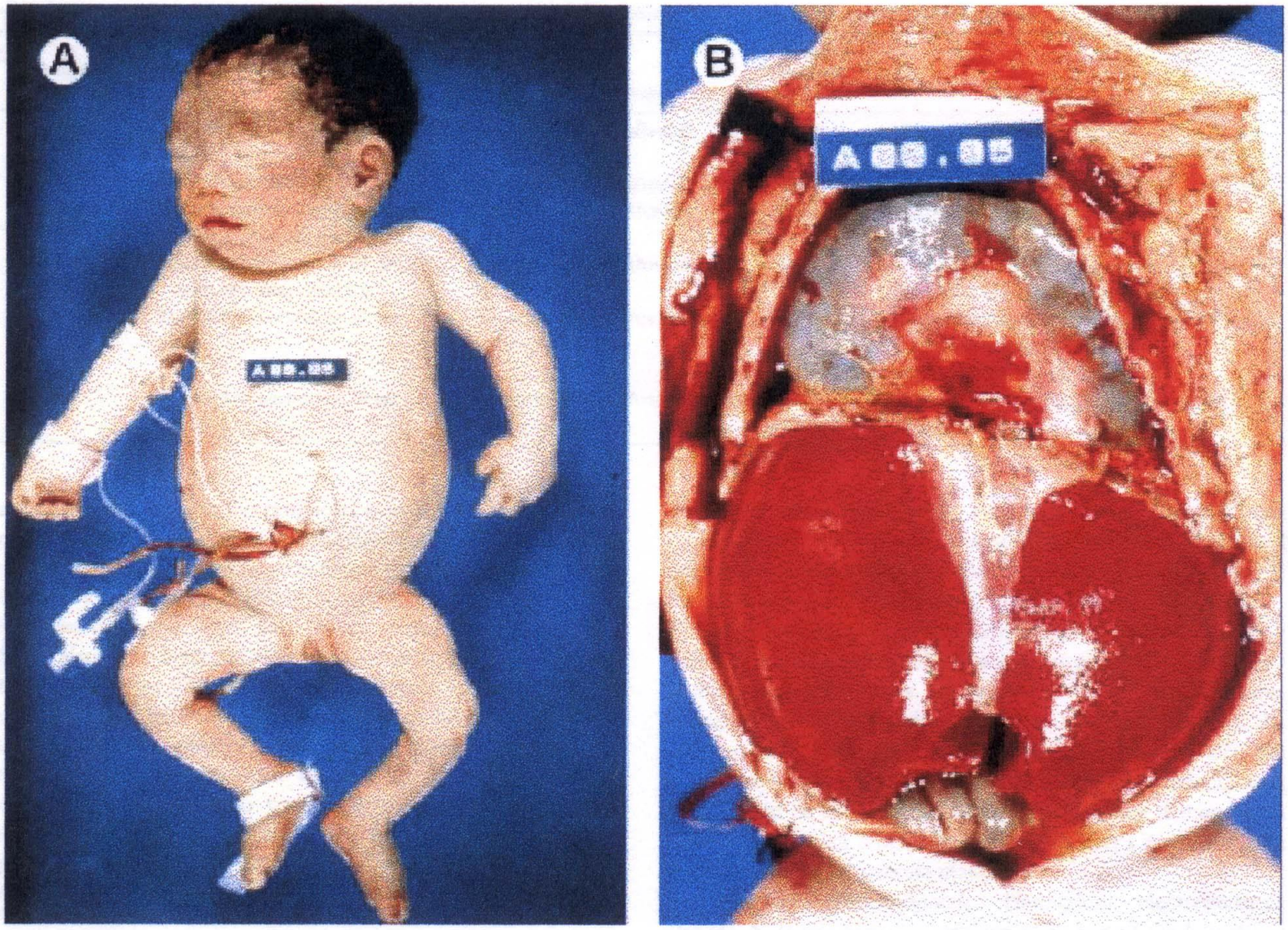


Beta-thalassemia short variant analyser (HPLC)

Fetus (--/-α)



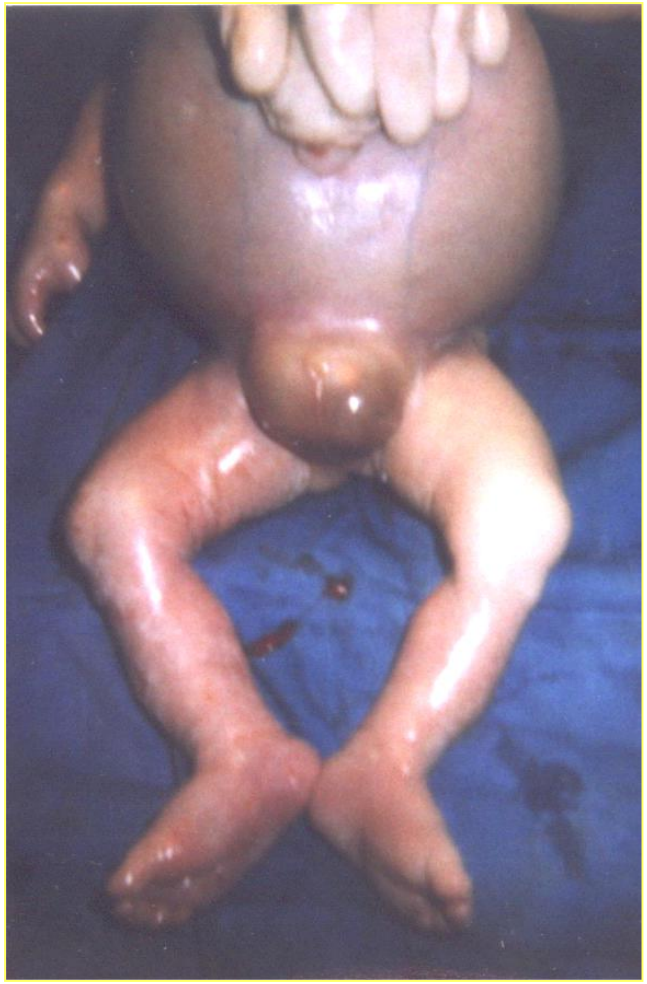
α^{o}-thalassemia deletions homozygote (--/--)

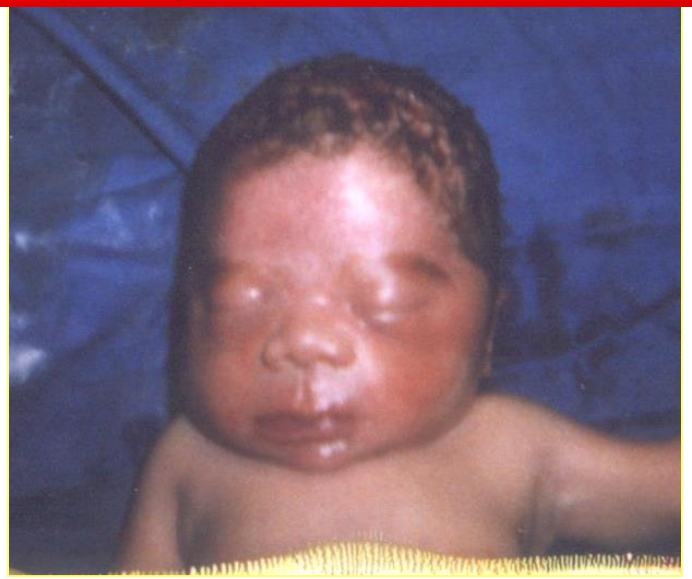


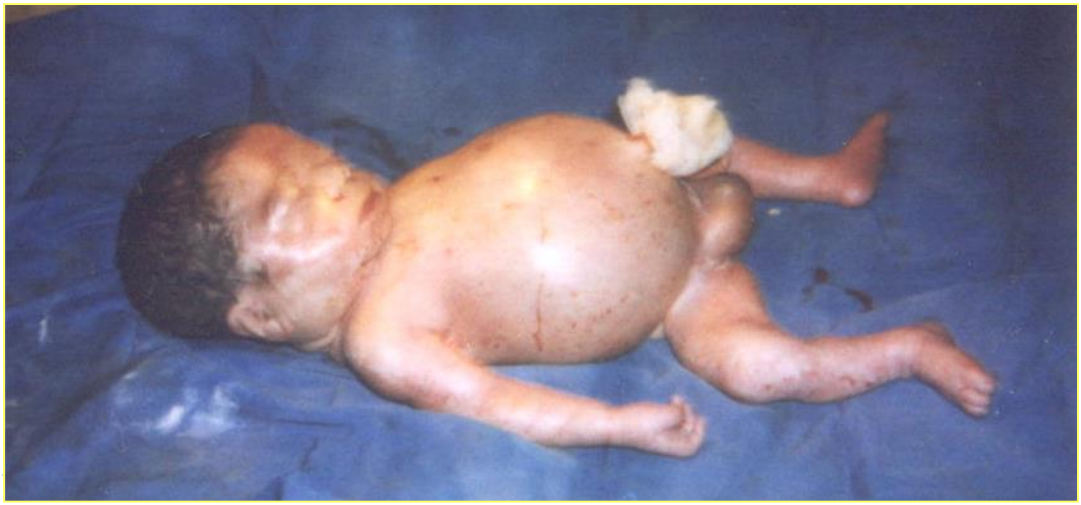
Case 1

- A pregnant woman, 32 weeks gestational age, $G_3P_2A_0$, high blood pressure, dyspnoe, edema
- Fetus with hydrops fetalis syndrome, anemia (Hb 8g/dl), young red cells in the cord blood >>
- Normal Hb (10.5 and 14.5g/dL), low MCV (66.8 and 63fL) and low HbA_2 (2.5%) in this couple
- Ethnic: Chinese

Hb Bart's Hydrops Fetalis ➡ SEA α-thalassemia homozygote (--/--)





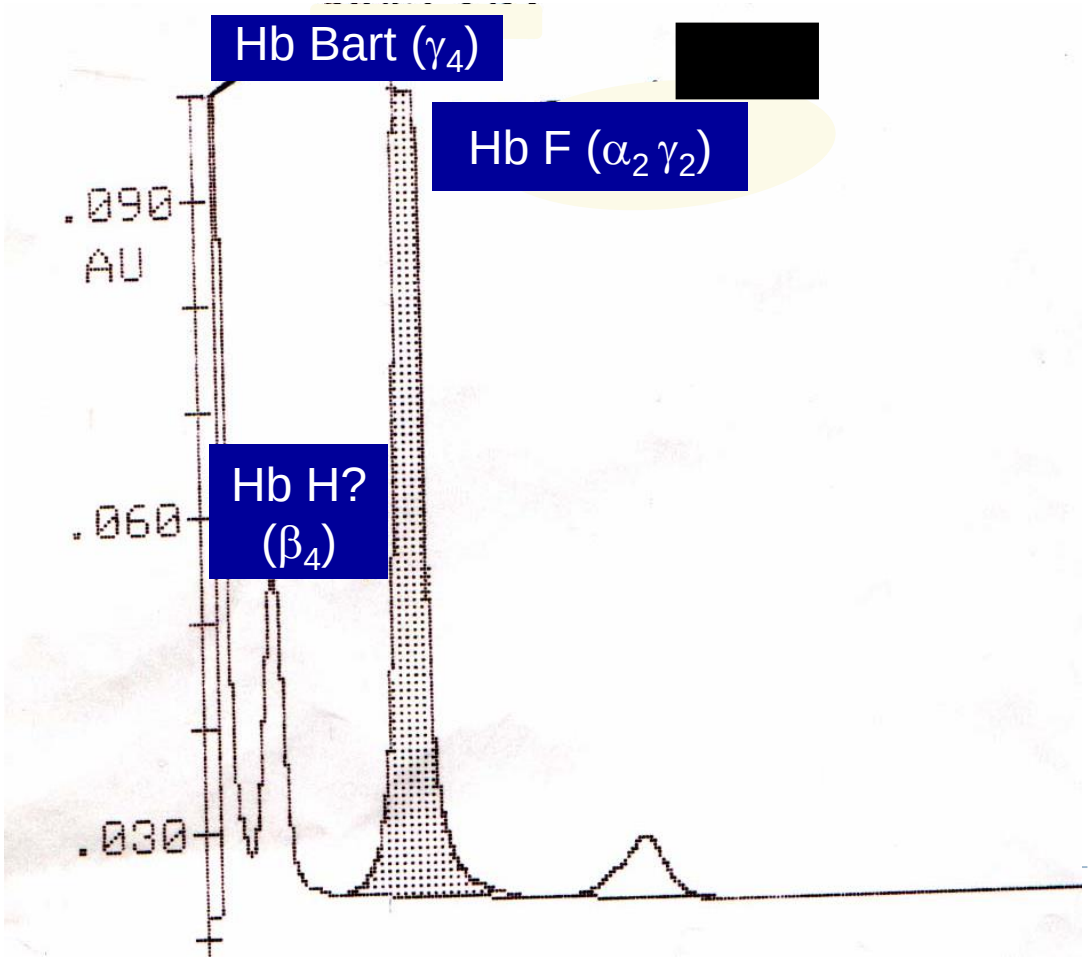


Case 2

- A pregnant woman, 22 weeks gestational age, $G_1P_0A_0$
- Fetus with hydrops fetalis syndrome, Hb 2g/dl and >> young red cells in the cord blood >>
- Normal Hb (10.7 and 15.2 g/dL), low MCV (63.5 and 75.3fL) and low HbA_2 (2.7% and 2.8%) in this couple
- Ethnic: Javanese

Beta-thalassemia short variant analyser (HPLC)

Fetus



DNA analysis

- SEA
- Thailand
- Filipino

α-thalassemia deletions were not detected

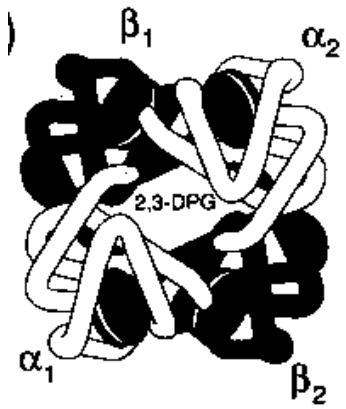
Sequencing analysis of α2-globin gene

- Mother: Cd 59 ($GGC^{Gly} \rightarrow GAC^{Asp}$) heterozygote
- Father: Cd 59 ($GGC^{Gly} \rightarrow GAC^{Asp}$) heterozygote
- Fetus: Cd 59 ($GGC^{Gly} \rightarrow GAC^{Asp}$) homozygote

$\alpha^{Gly59Asp}{}_2\beta_2$

- a highly unstable Hemoglobin
- reported in combination with either one- or two- α-globin gene deletions
- the reported cases also showed a high level of HbF

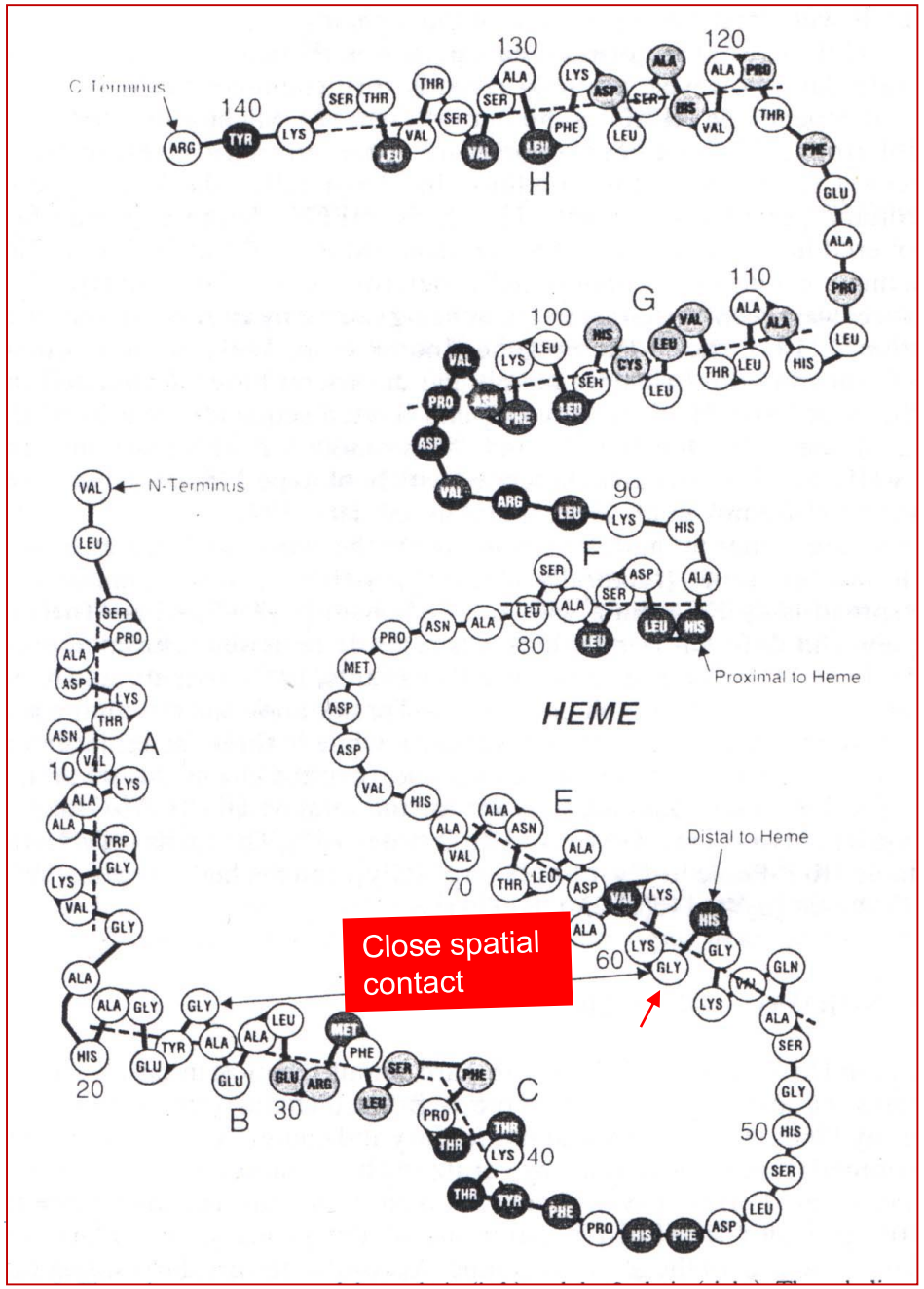
Mutation $\alpha^{Gly59Asp}{}_2\beta_2$ ➡ Why unstable ?



Glycine: non polar a.a.

Aspartic acid:

charged polar a.a.



PARAMETER HEMATOLOGY CODON 59 HETEROSIGOT

Hb (g/dL)	MCV (fL)	MCH (pg)	HbA_2 (%)	HbF (%)
10,7	63,5	21,3	2,7	1,5
15,2	75,3	25,2	2,8	0,5
13,5	78	26,5	2,5	0,5
15,6	75,8	25,2	2,6	0,3
9,4	73,2	24,5	3	1.9
14,4	74,5	24,5	3,1	0
11,5	78,5	26,1	3	0
13,7	75,3	24,1	3	0
13,6	71,4	23,4	2,4	0
13	74	25,1	2,8	0
15,1	75,6	25,4	2,9	0
14,1	76,3	25,8	**2,5**	0

Masalah diagnosis thalassemia alpha

- Parameter hematologi pada pembawa sifat (carrier/trait) thalassemia alpha yang disebabkan oleh mutasi titik tidak dapat memprediksi manifestasi klinis bentuk homosigot atau heterosigot ganda dengan mutasi lain
- Masalah yang serius terutama pada skrining antenatal
- Analisis DNA untuk memastikan diagnosis??

Kapan diperlukan analisis DNA untuk diagnosis thalassemia-hemoglobinopati

- Diagnosis Prenatal
- Hasil pemeriksaan indeks sel darah merah dan analisis Hb :
  - Tidak dapat menegakkan diagnosis
  - Tidak sesuai dengan manifestasi klinis

Diagnosis Thalassemia

- Thalassemia Beta
  - Umumnya tidak memerlukan analisis DNA
  - Pemeriksaan indeks sel darah merah (Hb, MCV, MCH, MCH, RDW), morfologi sel darah merah dan analisis Hb – dapat mendiagnosis sebagian besar kasus thalassemia beta heterosigot, homosigot atau heterosigot ganda

Diagnosis Thalassemia

- Thalassemia Alpha
  - Tidak semua jenis thalassemia alpha dapat didiagnosis dengan pemeriksaan hematologi (indeks sel darah merah, morfologi sel darah merah dan analisis Hb)
  - Yang selalu dapat didiagnosis dengan pemeriksaan hematologi:
    - HbBart hydrops fetalis yang disebabkan oleh delesi ke-4 gen globin alpha
    - Beberapa jenis penyakit HbH
    - Heterosigot delesi 2 gen globin alpha

Diagnosis Prenatal Thalassemia

- Diagnosis sedini mungkin – sampel vili khoriales (masa gestasi 10-12 minggu) – analisis DNA
- Sampel darah tali pusat dapat diperoleh pada masa gestasi > 18-20 minggu :
  - Morfologi sel darah merah dan analisis Hb – untuk diagnosis Hb Bart hydrops fetalis yang disebabkan oleh delesi 4 gen globin alpha
  - Analisis rantai globin (β/γ) } thalassemia beta
  - Analisis Hb (level HbA) }

SEBELUM MELAKUKAN ANALISIS DNA THALASSEMIA DIPERLUKAN:

1. Data klinis dan laboratorium hematologi (indeks sel darah merah dan analisis Hb)
   - Thalassemia alpha atau thalassemia beta
   - Pembawa sifat (heterosigot – 1 mutasi) atau penderita (homosigot atau heterosigot ganda – 2 mutasi)
   - Tidak dapat ditentukan jenis thalassemia dan klasifikasi klinis
2. Sampel sel berinti sebagai sumber DNA

Mengapa data klinis dan laboratorium hematologi penting?

Analisis DNA untuk mendeteksi mutasi thalassemia

- Spesifik gen ybs.:
  - Thalassemia beta – gen globin beta di kromosom 11
  - Thalassemia alpha – gen globin alpha di kromosom 16

- Spesifik jenis mutasi – menentukan metode deteksi mutasi:
  - Thalassemia beta: lebih sering mutasi titik (*single base substitution*)
  - Thalassemia alpha: lebih sering delesi besar (delesi 1 gen atau 2 gen globin alpha)

Mengapa data klinis dan laboratorium penting?

Ada anemia hemolitik lain yang manifestasi klinis seperti thalassemia

- Kelainan protein membran sel darah merah
- *Congenital Dyserythropoiesis Anemia*
- *AIHA*

RINGKASAN & KESIMPULAN

Thalasemia beta

- Diagnosis thalassemia beta dapat dengan mudah ditegakkan berdasarkan manifestasi klinis dan hematologi penderita + orang tua
- Diagnosis tingkat molekul diperlukan bila manifestasi klinis & hematologi tidak khas
- Pengetahuan dan hasil penelitian di tingkat molekul memberikan sumbangan bermakna untuk pengelolaan thalassemia yang lebih baik

RINGKASAN & KESIMPULAN

Thalassemia alpha

- Diagnosis thalassemia alpha lebih sulit karena sering seperti defisiensi besi – lebih sering diperlukan analisis DNA
- Mutasi thalassemia alpha di Indonesia lebih sering yang non-delesi – manifestasi kinis lebih kompleks
- Frekuensi thalassemia alpha di Indonesia mungkin tinggi tetapi tidak terdeteksi karena manifestasi klinis dapat sangat berat (kematian janin) dan anemia ringan (penyakit HbH).

Kesimpulan dan saran

- Deteksi mutasi thalassemia dan hemoglobinopati memerlukan diagnosis klinis atau data klinis dan laboratorium.
- Jenis mutasi thalassemia beta di berbagai etnik di Indonesia sangat bervariasi – data latar belakang etnik akan membantu pemilihan jenis mutasi yang dideteksi.
- Laboratorium diagnostik thalassemia dengan analisis DNA dapat dikembangkan di beberapa kota di Indonesia, terutama untuk keperluan diagnosis prenatal.