Thalassemias

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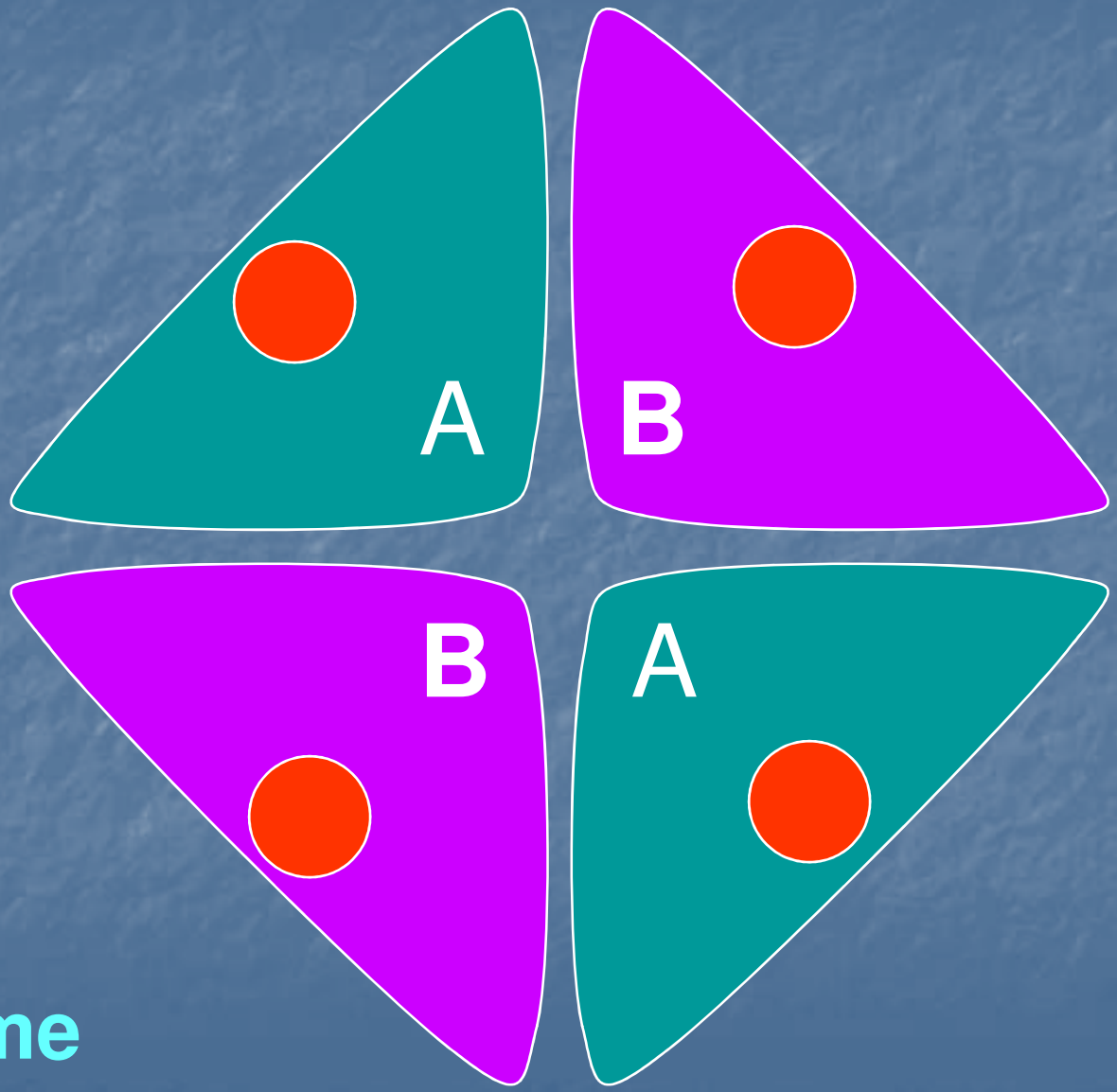
Review of Hb Structure

- Delivers oxygen to the cells
- Tetramer (4 subunits – 2 'A' and 2 'B') plus Heme groups

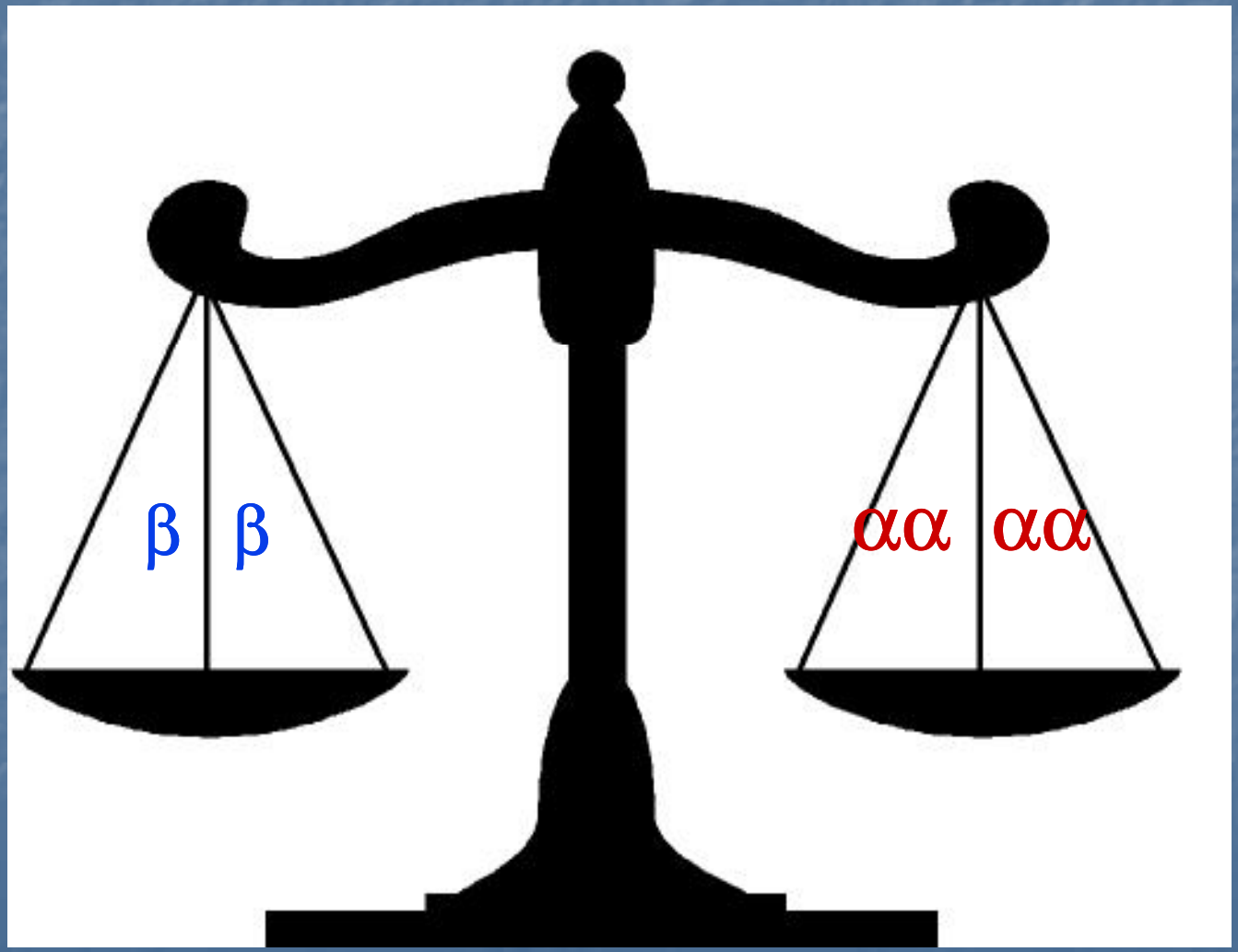
- A = Alpha like genes and pseudo-genes : 141 amino acid (Chromosome 16); ξ, α, θ

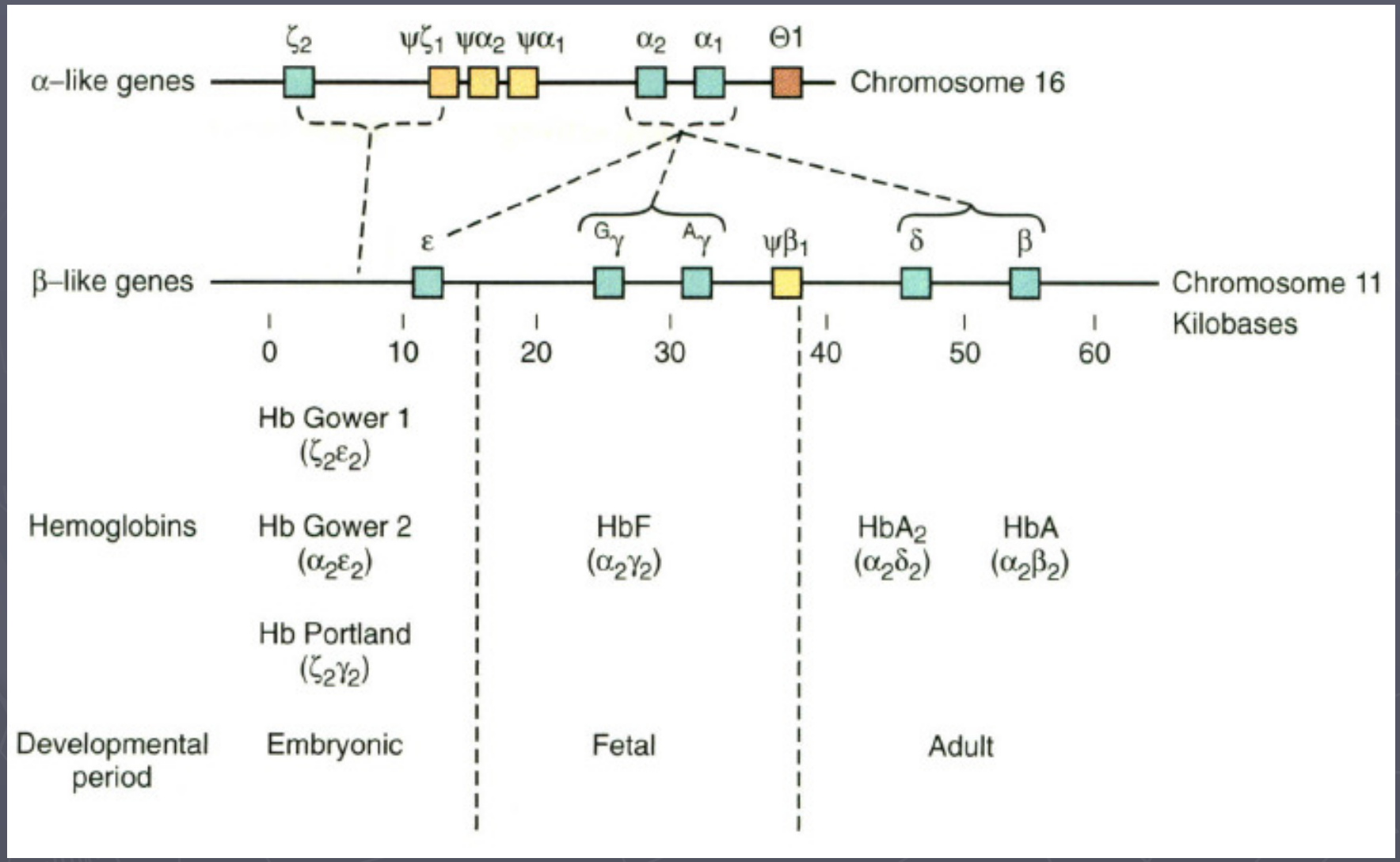
- B = Beta like genes and pseudo genes:146 amino acid (Chromosome 11); ε,γ,β,δ

Hemoglobin structure

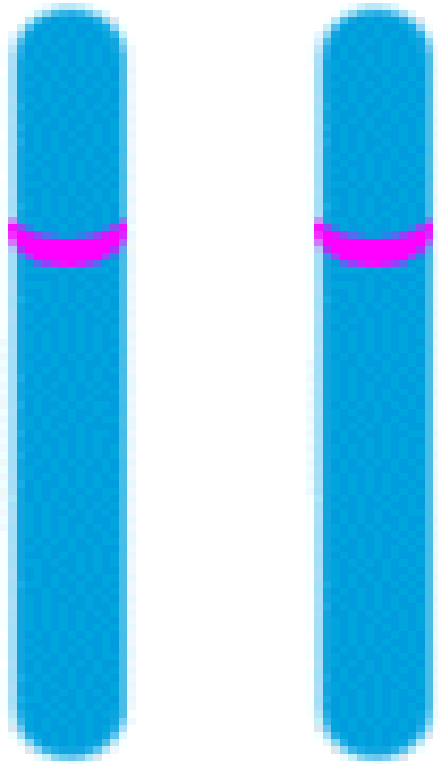


Normal hemoglobin production chain balance

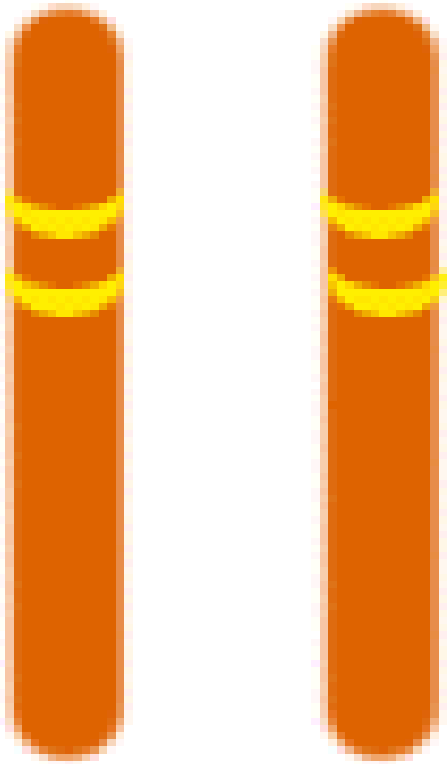



ζ2
ψζ1 ψα2 ψα1
α2 α1
Θ1
α–like genes
Chromosome 16
ε
Gγ
Aγ
ψβ1
δ
β
β–like genes
Chromosome 11
Kilobases
0
10
20
30
40
50
60
Hb Gower 1
(ζ2ε2)
Hemoglobins
Hb Gower 2
(α2ε2)
HbF
(α2γ2)
HbA2
(α2δ2)
HbA
(α2β2)
Hb Portland
(ζ2γ2)
Developmental period
Embryonic
Fetal
Adult

Chromosomes



Chromosome 11
β globin gene



Chromosome 16
α globin gene

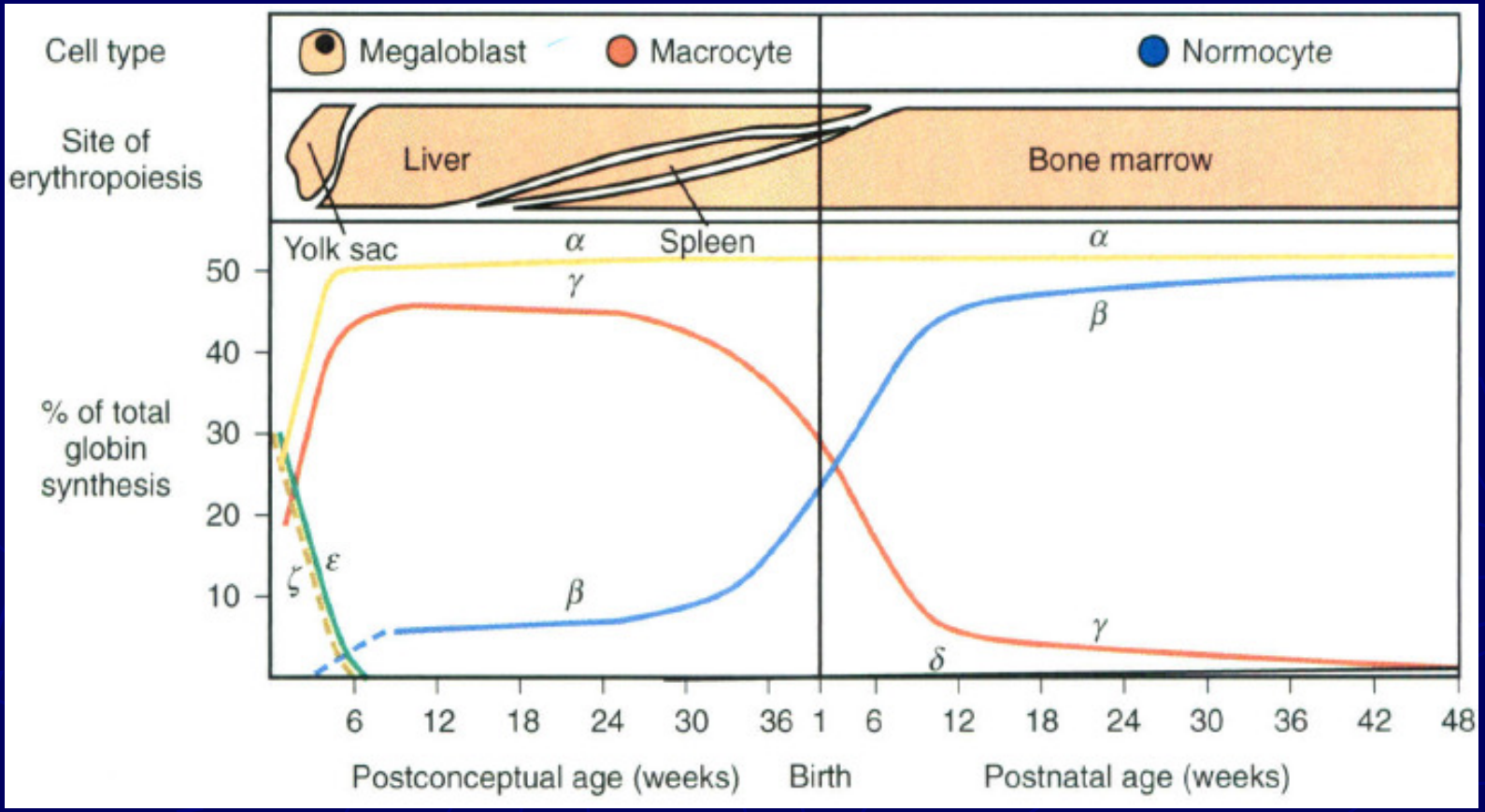


FIGURE 20-3. Sites of erythropoiesis and pattern of globin biosynthesis during development. Nucleated megaloblasts are produced predominantly in the yolk sac. They are replaced by macrocytic fetal red cells produced in the liver and subsequently in the spleen and bone marrow. The height of the *shaded area* approximates the proportion of circulating red cells produced by each organ. Globin biosynthetic measurements were made to obtain the data shown in the lower part of the figure through incubation of intact cells in the presence of radioactive amino acids followed by globin chain separation. *(Redrawn from Weatherall DG, Clegg JB. The Thalassemia Syndromes. Oxford, Blackwell Scientific, 1981, p 54.)*

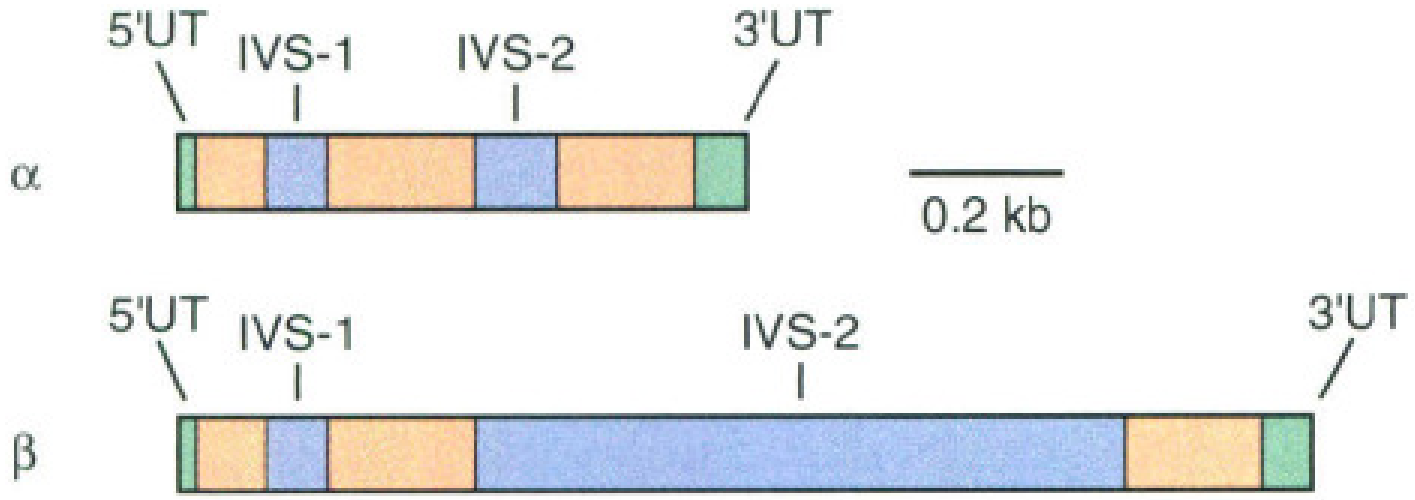
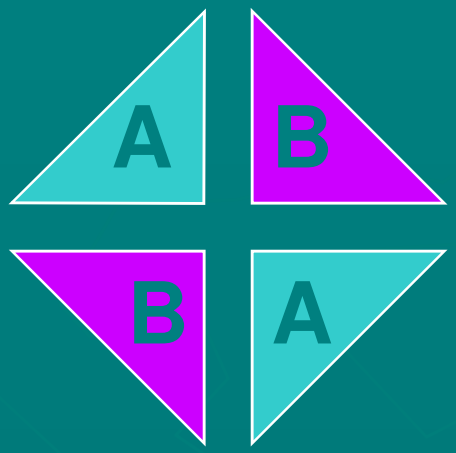
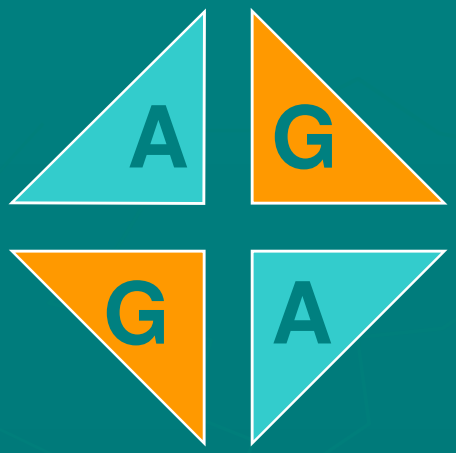


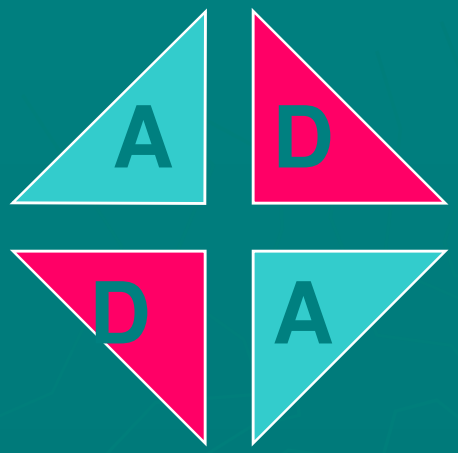
FIGURE 20-4. Structure of the human α- and β-globin genes. Untranslated (UT) regions, exon, and intervening sequences (IVS, introns) are depicted by green, salmon, and blue boxes, respectively.

Hemoglobin Type	Name	Components
Adult	A	$\alpha_2\beta_2$
	A2	$\alpha_2\delta_2$
Fetal	F	$\alpha_2\gamma_2$
Embryonic	Portland	$\zeta_2\gamma_2$
	Gower 1	$\zeta_2\varepsilon_2$
	Gower 2	$\alpha_2\varepsilon_2$
Abnormal	H	$\beta 4$
	Bart's	$\gamma 4$

Hemoglobins in normal adults







HbA	HbF	HbA_2
98%	~1%	<3.5%

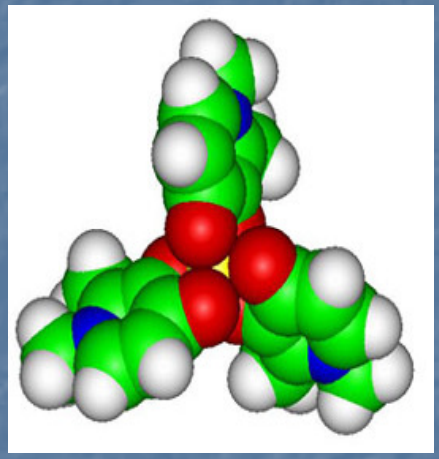
Disorders of Hemoglobin

1. Thalassemia: globin chains structurally normal(*quantitative*), but have imbalance in production of two different types of chains
2. Hemoglobinopathies: globin chain is abnormal (*qualititative*). Hb S, Hb C, Hb E

- **Hb S** (b6 Glu ⟶ Val) mutation, a A ⟶ T substitution at codon 6
- **Hb C** (b6 Glu ⟶ Lys) mutation, a G ⟶ A
- **Hb E** (b26 Glu ⟶ Lys) mutation, a G ⟶ A
- **Hb O Arab** (b121 Glu ⟶ Lys) mutation, a G ⟶ A
- **Hb D** (b121 Glu ⟶ Gln) mutation

What is Thalassemia ?

Thalassaemia is a group of inherited disorders of hemoglobin synthesis characterized by a reduced or absent one or more of the globin chains of adult hemoglobin .



The name is derived from the Greek words Thalassa = Sea" and "Hemia = Blood" in reference to anemia of the sea.

The first description of Cooley's anaemia. From the *Transactions of the American Pediatric Society, 37, 29–30, 1925.*

SECOND SESSION

A Series of Cases of Splenomegaly in Children, with Anemia and Peculiar Bone Changes. Presented by Dr. Thomas B. Cooley and Dr. Pearl Lee.

Five cases are reported, four from the Children's Hospital of Michigan and one from Dr. Abt's clinic.

All five presented the clinical syndrome ordinarily known as Von Jaksch's disease or pseudoleukemic anemia. There was anemia, splenomegaly, and some enlargement of the liver, discoloration of the skin, and in some of the sclerae, without bile in the urine. The blood showed normal or increased resistance to hypotonic solutions. There was moderate leukocytosis in all, not of the leukemic type, nucleated red cells, chiefly normoblasts, and in two, many reticulated cells. In all of these cases the symptoms were noted by the parents as early as the eighth month, when they were apparently well advanced. Rickets was not probable in any, and in only one was there definite ground for believing that syphilis might be a contributing factor.

In addition to the splenomegaly and the blood picture, in the four cases from the Children's Hospital attention was called to a peculiar mongoloid appearance, caused by enlargement of the cranial and facial bones, combined with the skin discoloration. In Dr. Abt's patient the cranial enlargement was also noted. Roentgen-ray examination of the skulls showed peculiar alterations of their structure, which the roentgenologist considered pathognomonic of this condition. The long bones also showed striking changes. These changes were identical in kind, varying only in degree in all four of the Detroit cases, while gross and microscopic examination in Dr. Abt's case showed a condition which would have given a similar picture.

Three of the patients died. One, who went through a course of antisyphilitic treatment because of a not thoroughly substantiated diagnosis of congenital syphilis, began to improve nearly a year after cessation of all treatment, and seems to be on the road to recovery. The fifth is living, after splenectomy, which is not believed to have improved his condition. He had, in addition to the ordinary symptoms, achlorhydria and some peculiarities in calcium and phosphorus metabolism, which could not be shown to be related to the anemia. He shows frequent hemoglobinuria and hemoglobin is constant in the blood serum. Since splenectomy he has had, for seven months, enormous numbers of nucleated red cells in his blood, reaching as high as 200,000. The only results in treatment have been with a mixture of spleen and red bone marrow, combined with administration of hydrochloric acid. One transfusion caused only slight, transient blood change, and urine examination showed that the transfused blood underwent rapid hemolysis. A more recent transfusion was followed by a better blood picture and less hemolysis.

Microscopic study of the tissues shows fibrous hyperplasia of the spleen, pigment deposit in the liver, and general leukoblastic hyperplasia of all of the bones, with erythroblastic aplasia. This general aplasia of the red cell-forming tissue seems probably to be the cause of the clinical manifestations, and from the early period at which they were noted, and apparently well advanced, it is suggested that the aplasia is congenital, and the disease to be considered a form of myelophthisic anemia. Case 3 may be considered to show that the body may compensate, through secondary hematopoietic areas, for the primary aplasia.

The desirability of roentgen-ray studies of the bones in other forms of anemia with splenomegaly is suggested.

Acknowledgments are made to Drs. P. F. Morse, E. R. Witwer and Lawrence Reynolds for pathologic and roentgenologic studies, and to Drs. A. Abt and O. T. Schlutz for the loan of their material, with Dr. Schlutz's complete analysis.

Thomas B. Cooley (1871–1945). Cooley was born at Ann Arbor, Michigan, graduated MD in 1895 and interned at the Boston City Hospital. After studying in Germany he returned to Boston to work in contagious disease. After further appointments in Ann Arbor and Detroit, and a period of service in France during the First World War, he settled in Detroit, where he spent the rest of his life in paediatric practice.

Thalassemia

- Results in overall decrease in amount of hemoglobin produced and may induce hemolysis.
- Results in microcytic, hypochromic anemias of varying severity.
- May be either homozygous defect or heterozygous defect.

- May contribute protection against malaria.

Demographics: Thalassemia

- **Found most frequently in the Mediterranean, Africa, Western and Southeast Asia, India and Burma**

Genetic Types of Thalassemia

two basic groups of thalassemia:

- Alpha (α)Thalassaemia
- Beta (β)Thalassaemia

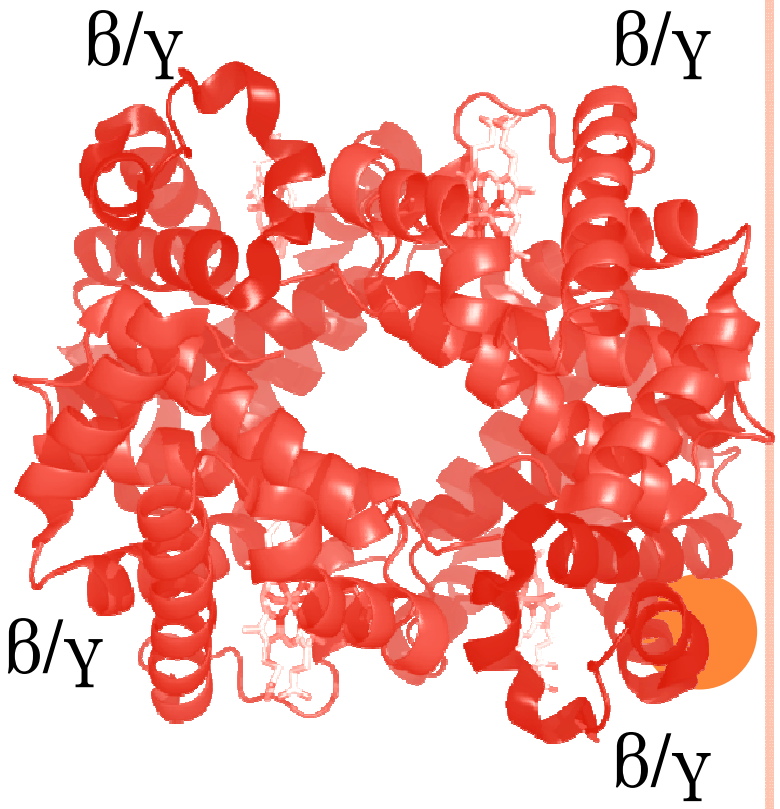
- Genetic: autosomal recessive
- Alpha thalassemia usually caused by gene deletion; Beta thalassemia usually caused by mutation.

Other Thalassemias Caused by Defects in the Beta-Cluster Genes

- Delta Beta Thalassemia
- Hb Lepore
- HPFH
- Beta Thalassemia with Hb S
- Beta Thalassemia with Hb C
- Beta Thalassemia with Hb E

Alpha Thalassemia

- Alpha Thalassemia: deficient/absent alpha subunits
  - Excess beta subunits
  - Excess gamma subunits newborns
- Tetramers formed:
  - Hemoglobin H adults
  - Hemoglobin Bart's newborns
- Five types:
  - Silent Carrier
  - Trait (Minor)
  - Hemoglobin H Disease
  - Major (Hemoglobin Bart's)
  - Hemoglobin Constant Spring



Genetic basis of Alpha Thalassemia

- Encoding genes on chromosome 16 (short arm)
- Each cell has 4 copies of the alpha globin gene
  - Each gene responsible for ¼ production of alpha globin
- **4 possible mutation states:**
  - Loss of ONE gene → silent carrier
  - Loss of TWO genes → thalassemia minor (trait)
  - Loss of THREE genes → Hemoglobin H
    - Accumulation of beta chains(β_4)
  - Loss of FOUR genes → Hemoglobin Barts(γ_4)
    - NO alpha chains produced ∴ only gamma chains present

- Deletions named according to their size
  - 3.7kb; 4.2kb; 5.2kb; 20.5kb
- Deletions named according to their origin
  - Med, Fil, Thai, SE Asian

Common Alpha Thalassaemia deletion mutations

Disorder	Deletion mutation
α^0 thalassaemia	$--^{SEA}$
	$--^{MED}$
	$-(\alpha)^{20.5}$
	$--^{FIL}$
	$--^{THAI}$
α^+ thalassaemia	$-\alpha^{3.7}$
	$-\alpha^{4.2}$

Non-Deletional Alpha Thal

- Usually represented as αα/α^{T}α

- May be more severe than deletional form – there is an abnormal product made; can cause more cellular damage
- Most common is Hb Constant Spring

Classification & Terminology

Alpha Thalassemia

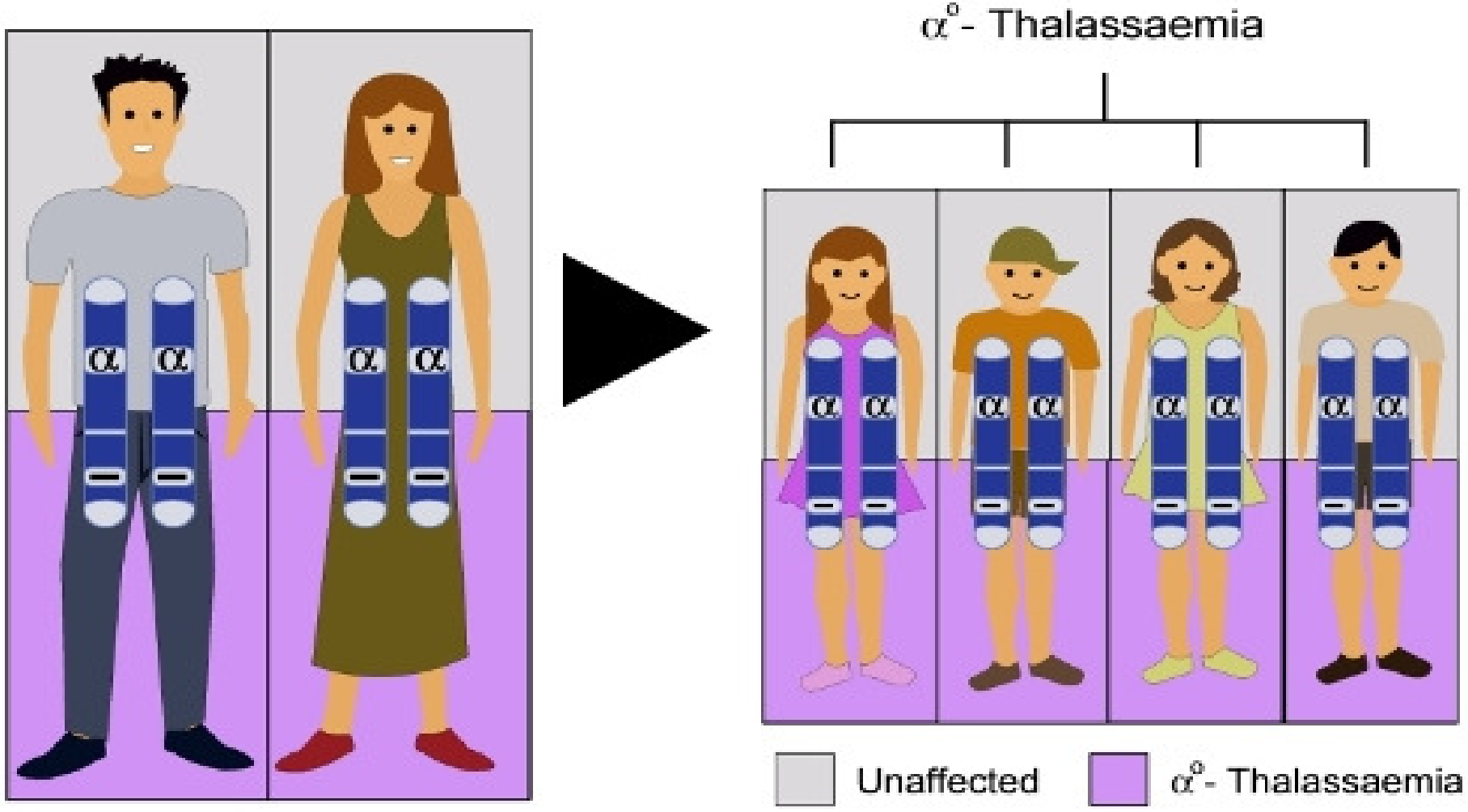
- Normal αα/αα
- Silent carrier - α/αα
- Minor(trait) -α/-α
  --/αα
- Hb H disease --/-α
- Barts hydrops fetalis --/--

TABLE 20-6 α-Thalassemia Syndromes

Syndrome	Clinical Features	Hemoglobin Pattern	α-Globin Genes Affected by the Thalassemia Mutation
Silent carrier (α-thal-2)	No anemia, normal red cells	1-2% Hb Bart's (γ_4) at birth; may have 1-2% Hb Constant Spring; remainder HbA	1
Thalassemia trait (α-thal-1)	Mild anemia, hypochromic and microcytic red cells	5-10% Hb Bart's (γ_4) at birth; may have 1-2% Hb Constant Spring; remainder HbA	2
Hemoglobin H (HbH) disease	Moderate anemia; fragmented, hypochromic, and microcytic red cells; inclusion bodies may be demonstrated	5-30% HbH (β_4); may have 1-2% Hb Constant Spring; remainder HbA	3
Hydrops fetalis	Death in utero caused by severe anemia	Mainly Hb Bart's; small amounts of HbH and Hb Portland also present	4

Both parents have α^{o}- Thalassaemia trait

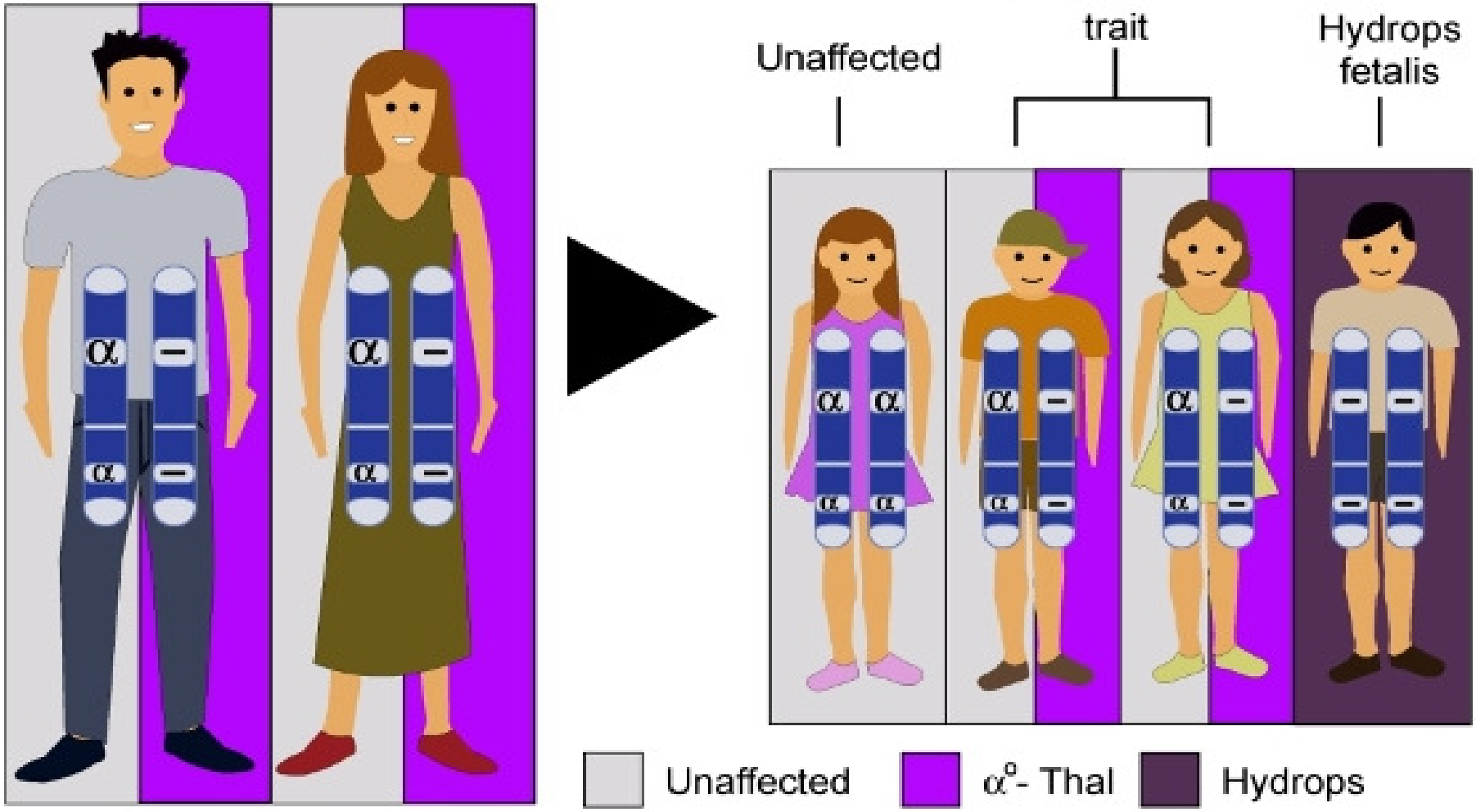
Thalassaemia genes on different chromosomes



If both parents are α^{o}- Thalassaemia carriers with a functional and a non-functional gene on each chromosome, then all their children will be carriers, exactly as their parents.

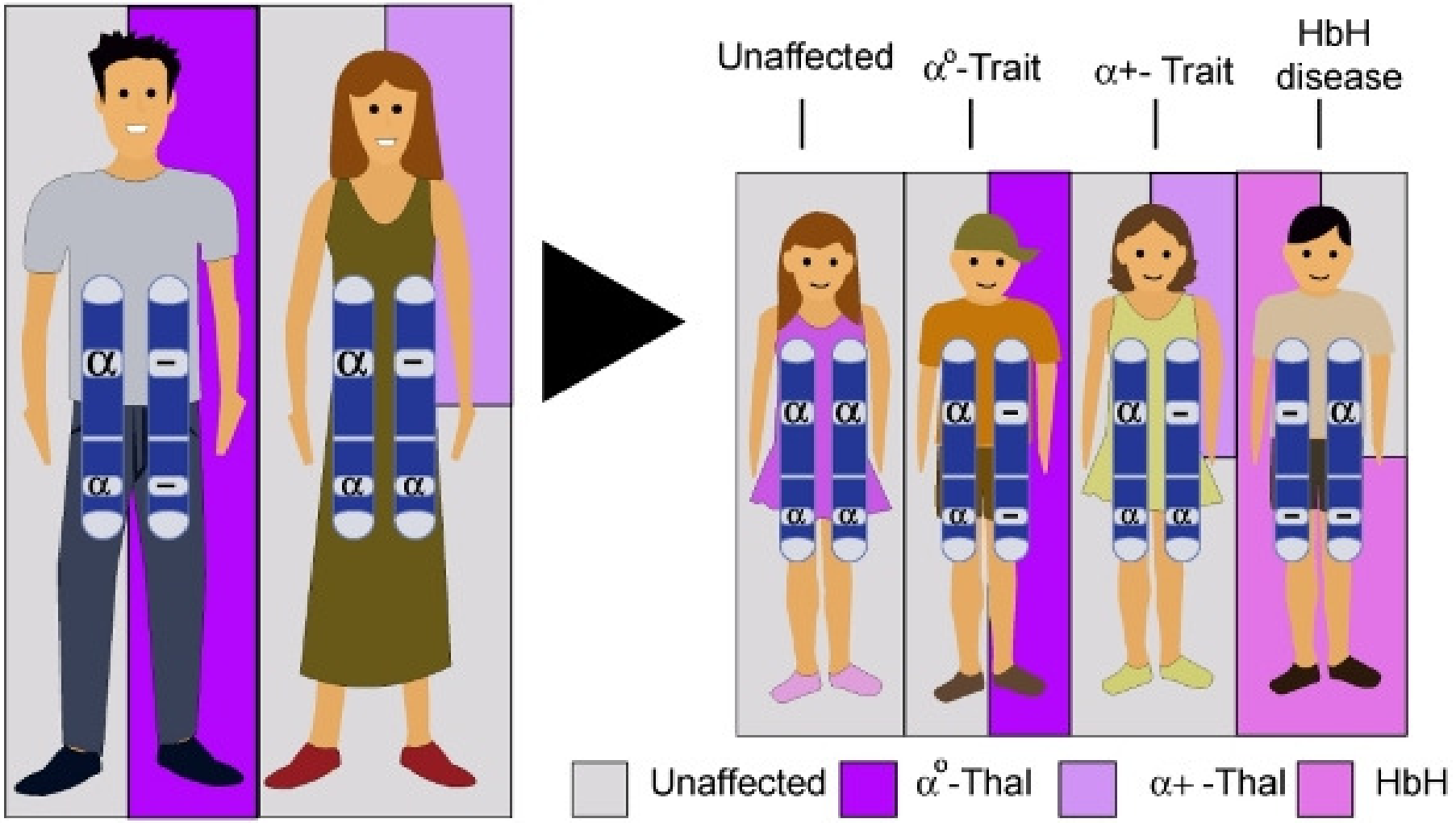
Both parents have α^{o}- Thalassaemia trait

Thalassaemia genes on same chromosome



If both parents have two non- functioning genes on the same chromosome and normal genes on the other then there is a 1:4 chance of a child inheriting the normal genes, a 1:2 chance of being a carrier like the parents, but also a 1:4 chance of inheriting only the non functioning chromosomes which means that this child will have hydrops fetalis.

Parents are different types of carriers



One parent (the father in this case) has two non functional genes on one chromosome while the other chromosome is "normal" (α^o- trait). The other parent (in this case the mother) has one non functional gene (α+ trait). Each Child has a 1:4 chance of being either totally unaffected, or have the α^otrait, or the α+ trait or being affected by HbH disease.

Clinical Outcomes of Alpha Thalassemia

- Silent carriers
  - asymptomatic , normal
- Alpha Thalassemia minor (trait)
  - no anemia
  - microcytosis
    -unusually small red blood cells due to fewer Hb in RBC
  - “normal”
- Alpha Thalassemia intermedia (Hb H)
  - microcytosis & hemolysis
  - clinically variable, results in severe anemia
  - Mild bone changes
  - splenomegaly
  - Hb H is susceptible to oxidation, therefore oxidant drugs and foods are avoided
  - Cells: "golf ball" appearance, especially when stained with brilliant cresyl blue

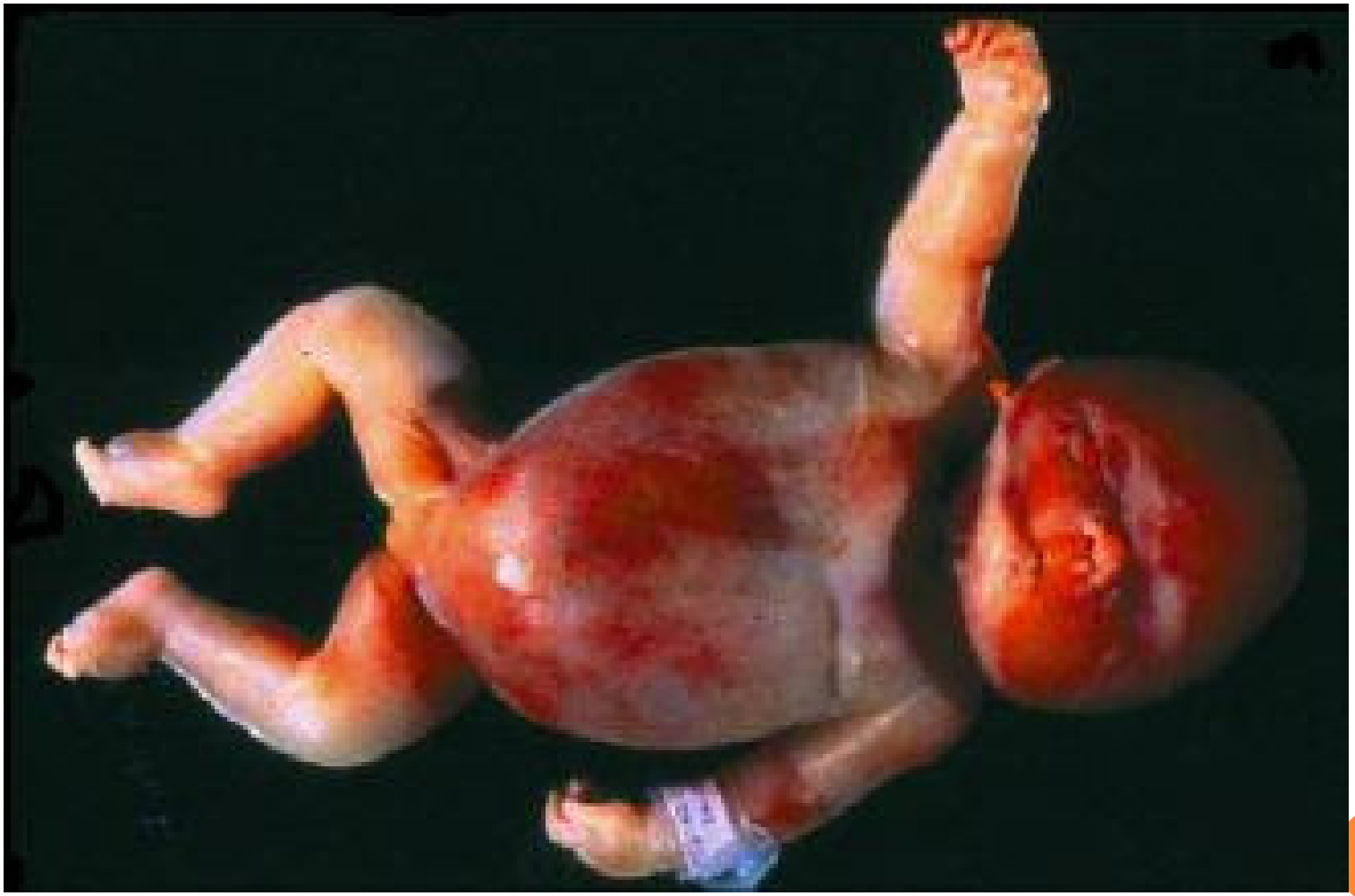
Clinical Outcomes of Alpha Thalassemia

- Alpha Thalassemia major

  - occurs in utero
  - Since alpha chains are synthesized in fetal life, symptoms of Alpha-Thalassemia Major begin in fetal life
  - Hb Bart's has high oxygen affinity so cannot carry oxygen to tissues

  - At birth, see severe hypochromic, microcytic anemia with numerous NRBCs
  - fatal hydrops fetalis
    - Edema , ascites caused by accumulation serous fluid in fetal tissues as result of severe anemia. Hepatosplenomegaly, cardiomegaly., leads to death

Hydrops Fetalis



Beta Thalassemia

- Commonly found in Mediterranean, Middle East, Asia, and Africa

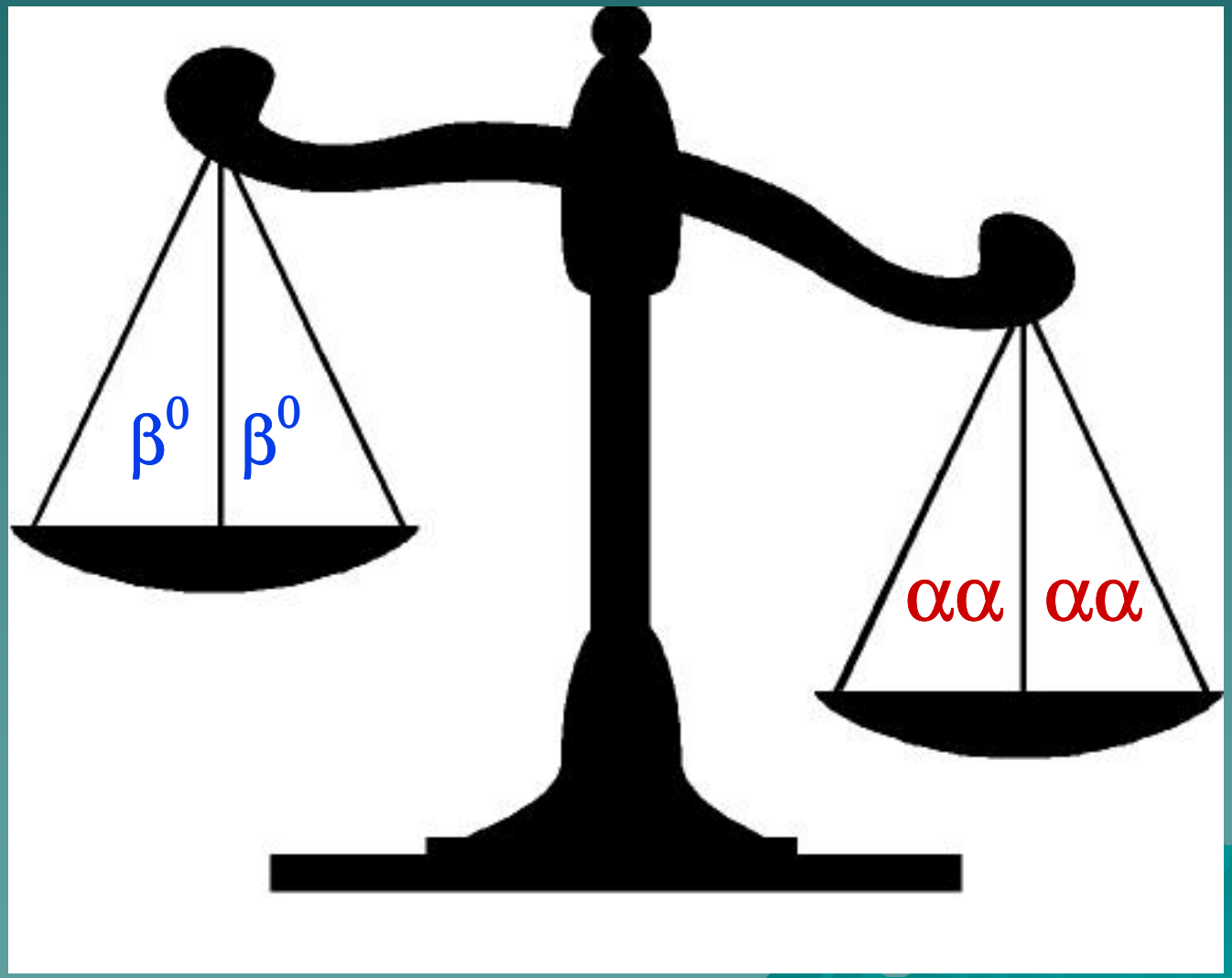
- **Three clinical types:**
  - Minor
  - Intermedia
  - Major (Cooley anemia)
    - May be asymptomatic at birth as HbF functions

Classification & Terminology
Beta Thalassemia

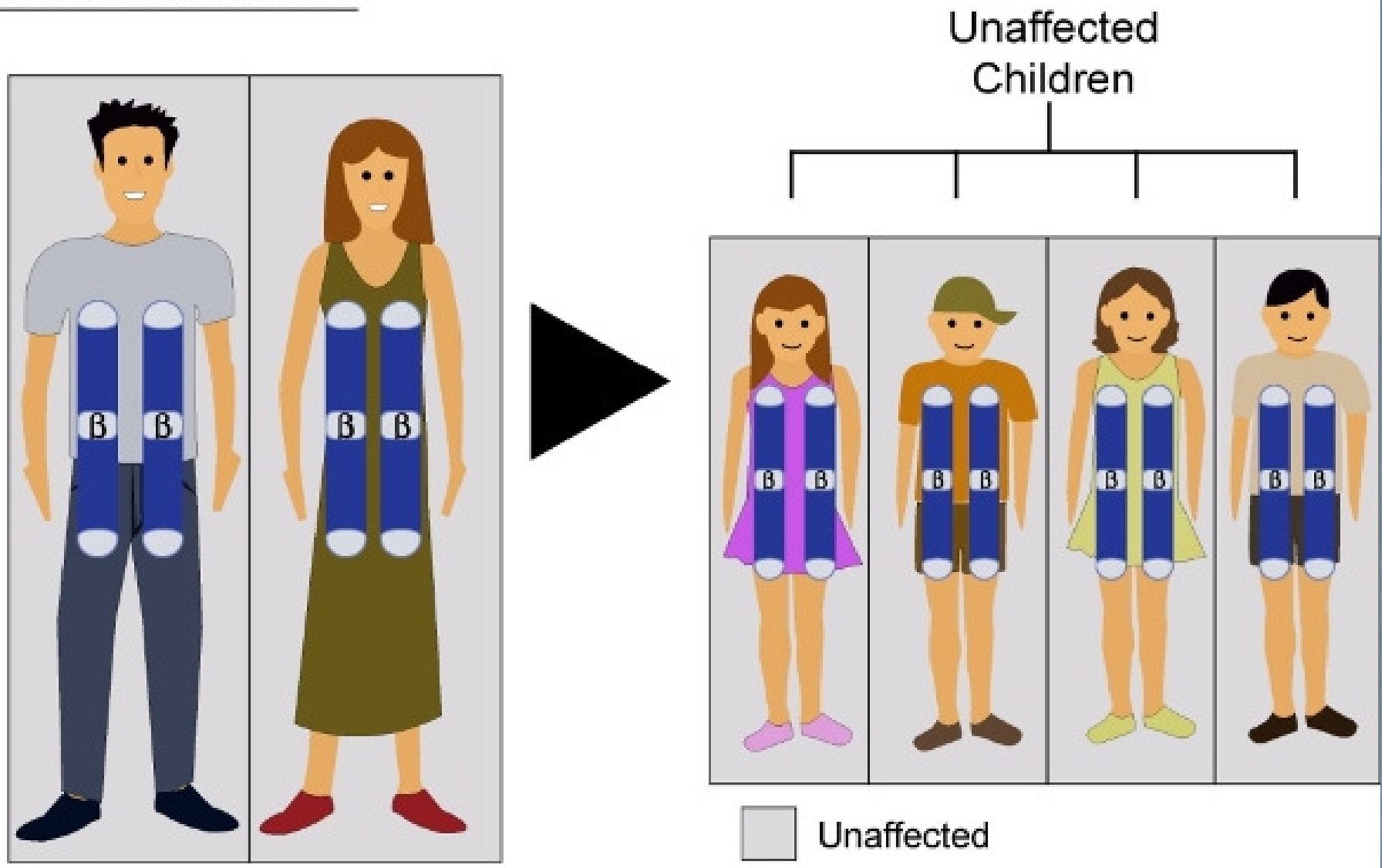
• Normal	β/β
• Minor	β/β^0
	β/β^+
• Intermedia	β^0/β^+
	β^+/β^+
• Major	β^0/β^0
	β^+/β^+
	β^0/β^+

Beta Thalassemia

Chain imbalance

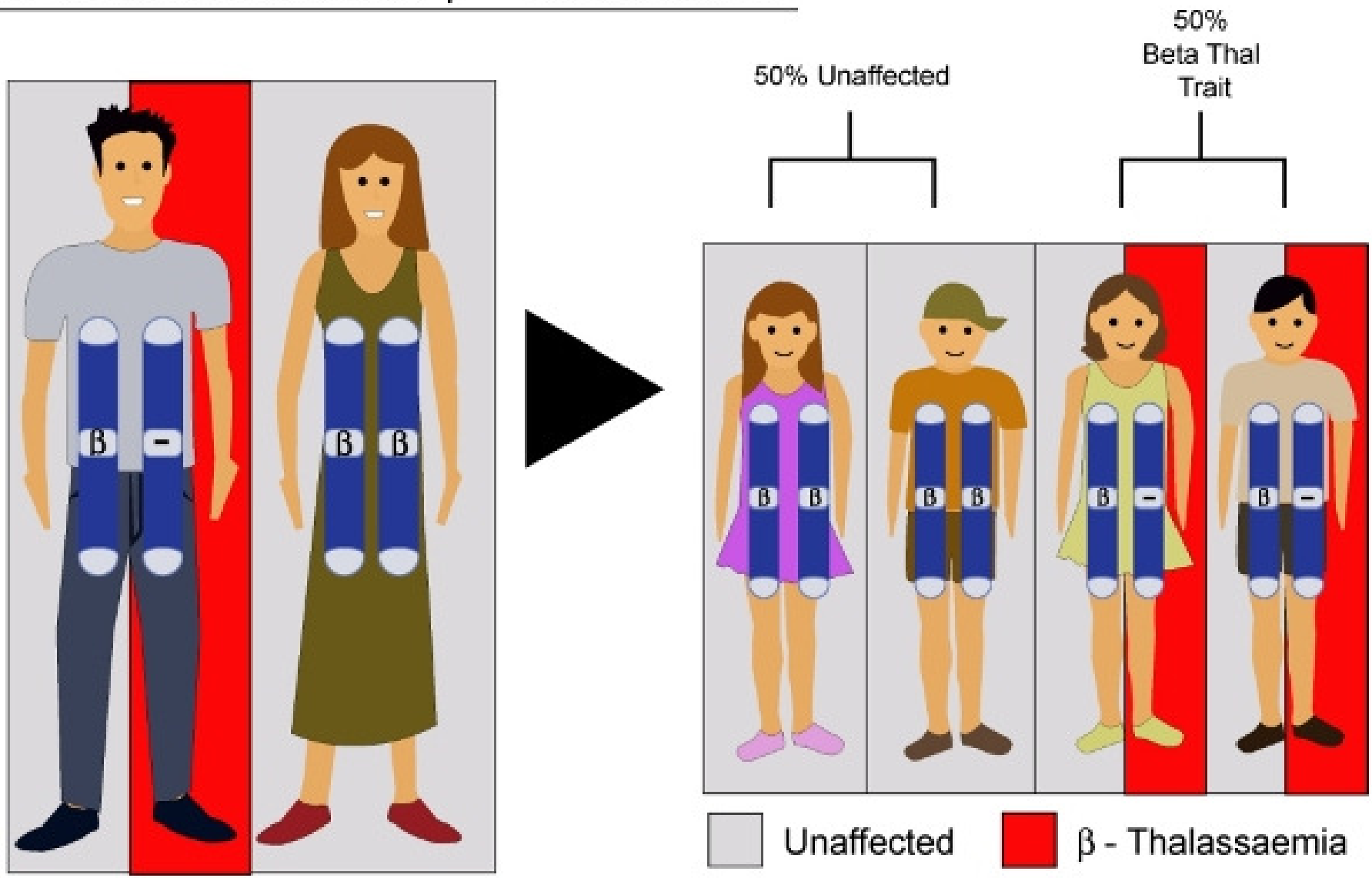


Normal Inheritance



When both globin genes of each parent are functioning normally, then all the children will carry functioning genes and none will have the Thalassaemia trait.

One Parent is a carrier of β – Thalassaemia

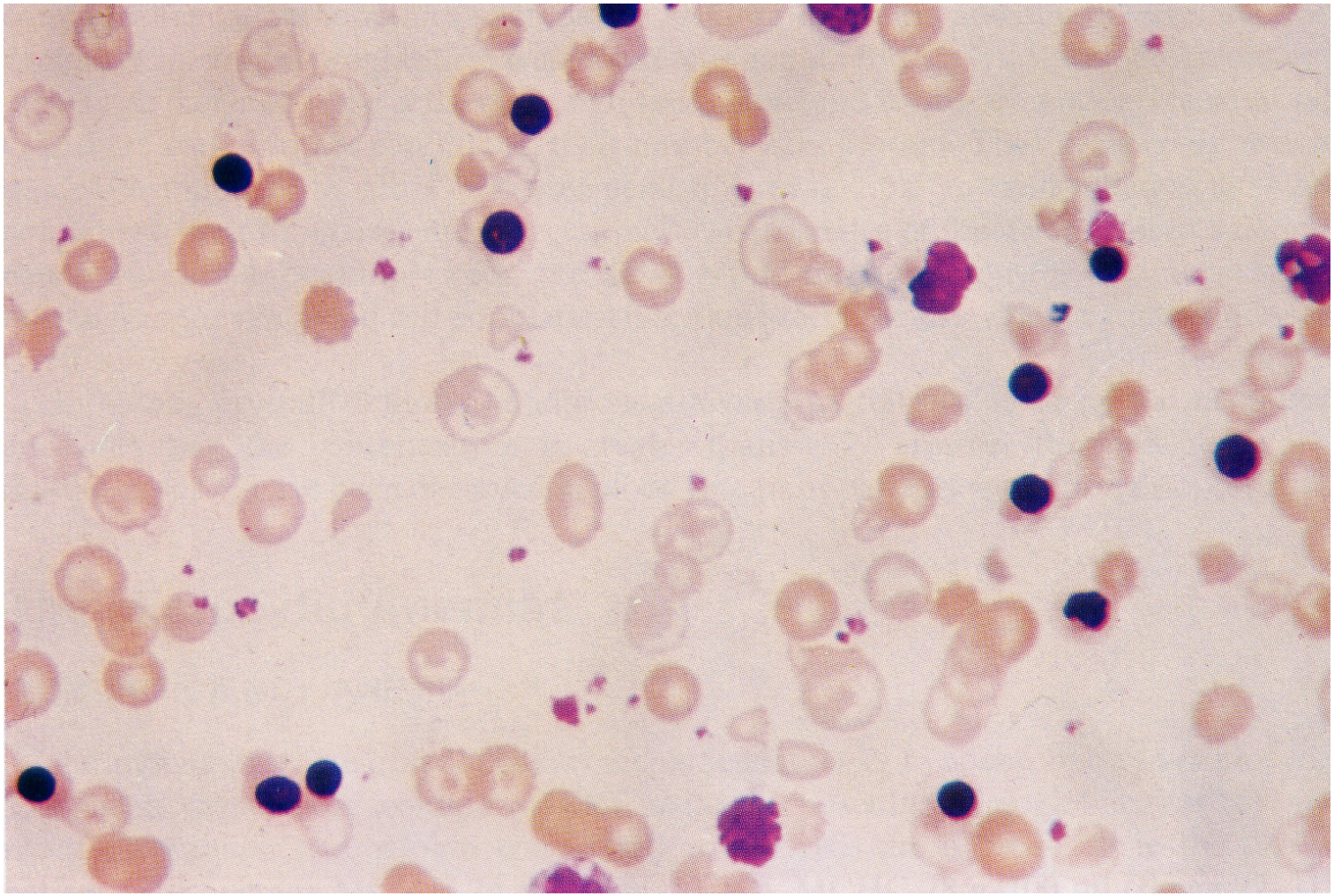


When one parent carries a β - Thalassaemia gene, then each child will have a 50:50 chance (1:2) of also being a carrier (or have the trait, or be heterozygote or have Thalassaemia minor)

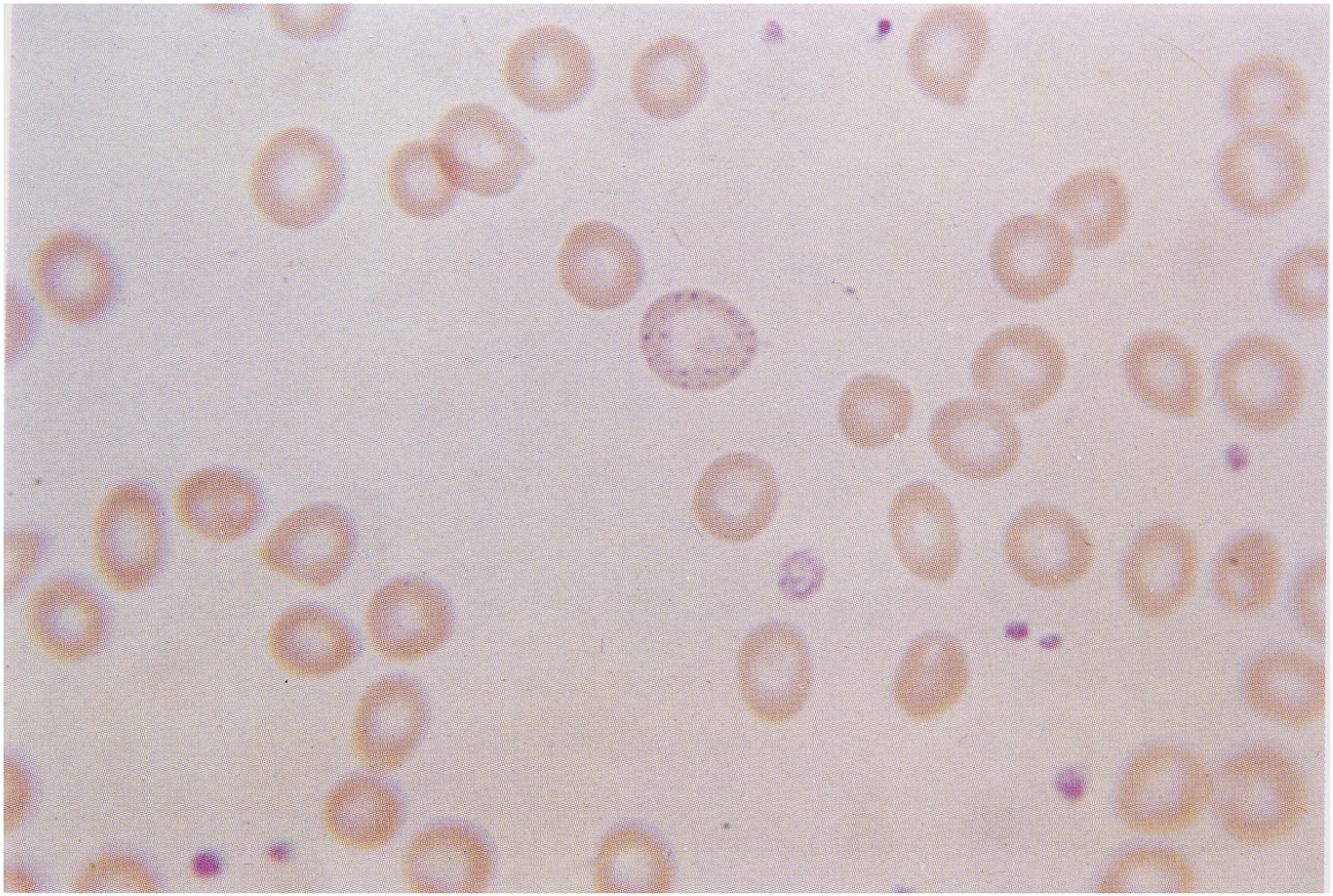
Clinical Outcomes of Beta Thalassemia

- Beta Thalassemia minor (trait)
  - asymptomatic
  - microcytosis
  - mild anemia
- Beta Thalassemia intermedia
  - symptoms similar to Cooley Anemia but less severe
- Beta Thalassemia major (Cooley Anemia)
  - most severe form
  - moderate to severe anemia
  - intramedullary hemolysis (RBC die before full development)
  - peripheral hemolysis & splenomegaly
  - skeletal abnormalities (overcompensation by bone marrow)
  - increased risk of thromboses
  - pulmonary hypertension & congestive heart failure

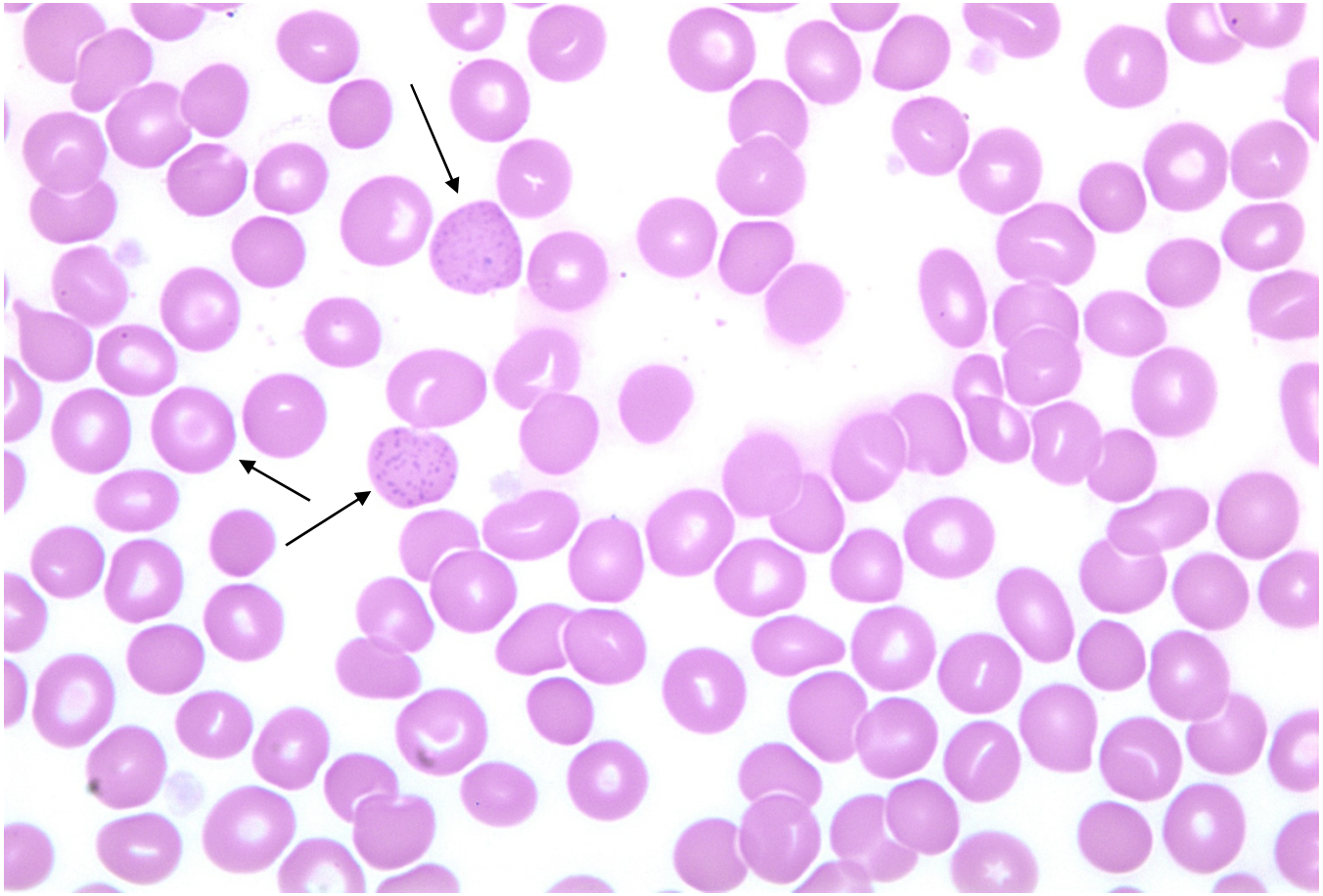
Thalassemia major



Thalassemia minor



Basophilic stippling, Target cell



Pathophysiology

- Disturbance of ratio between Alpha & non alpha globin chain synthesis then absent or decrease production of one or more globin chains
- Formation of abnormal Hb structures
- Ineffective erythropoiesis
- Excessive RBCs Destruction
- Iron Overload
- Extra-medullary hematopoiesis

Pathophysiology

- α-chain excess
  - unstable
  - Precipitates within the cell, causes damage
  - Macrophages destroy the damaged RBCs in the bone marrow, leads to ineffective erythropoiesis
  - Spleen also removes damaged RBCs, leads to chronic extravascular hemolysis

Pathophysiology

- **β-chain excess**
  - Unstable
  - Combines to form hgb molecules with 4 β-chains (hemoglobin H)
    - Infants: excess gamma chains combine with hgb molecules (hemoglobin Bart's)
  - High oxygen affinity, poor transporter of oxygen

Signs & Symptoms

- **Thalassemia Minor :**
  Usually no signs or symptoms except for a mild anemia.

Signs & Symptoms

- **Thalassemia Intermedia:**
- clinical presentation typically occurs at 2-4 years of age
- Hb levels 7 g/dL without Tx support
- When their Tx requirements reach 8 units per year, they are reclassified as –TM
- Anemia, hyperbilirubinemia, hepatosplenomegaly
- The majority of the patients will require Tx at some point in their lives or when hemolytic or aplastic crises associated with acute infections, folate deficiency,hypersplenism, or pregnancy occur.

- Indications for regular Tx in TI
- growth failure or cosmetic facial and bony abnormalities
- Massive splenomegaly , hypersplenism
- progressive anemia, fatigue, cardiopulmonary complications

Signs & Symptoms

- **Thalassemia Major :**

  1. Severe anemia, Jaundice or yellow colored skin.
  2. Growth retardation.
  3. Bony abnormalities specially of the facial bones.
  4. Pathological fractures
  5. Hepatosplenomegaly

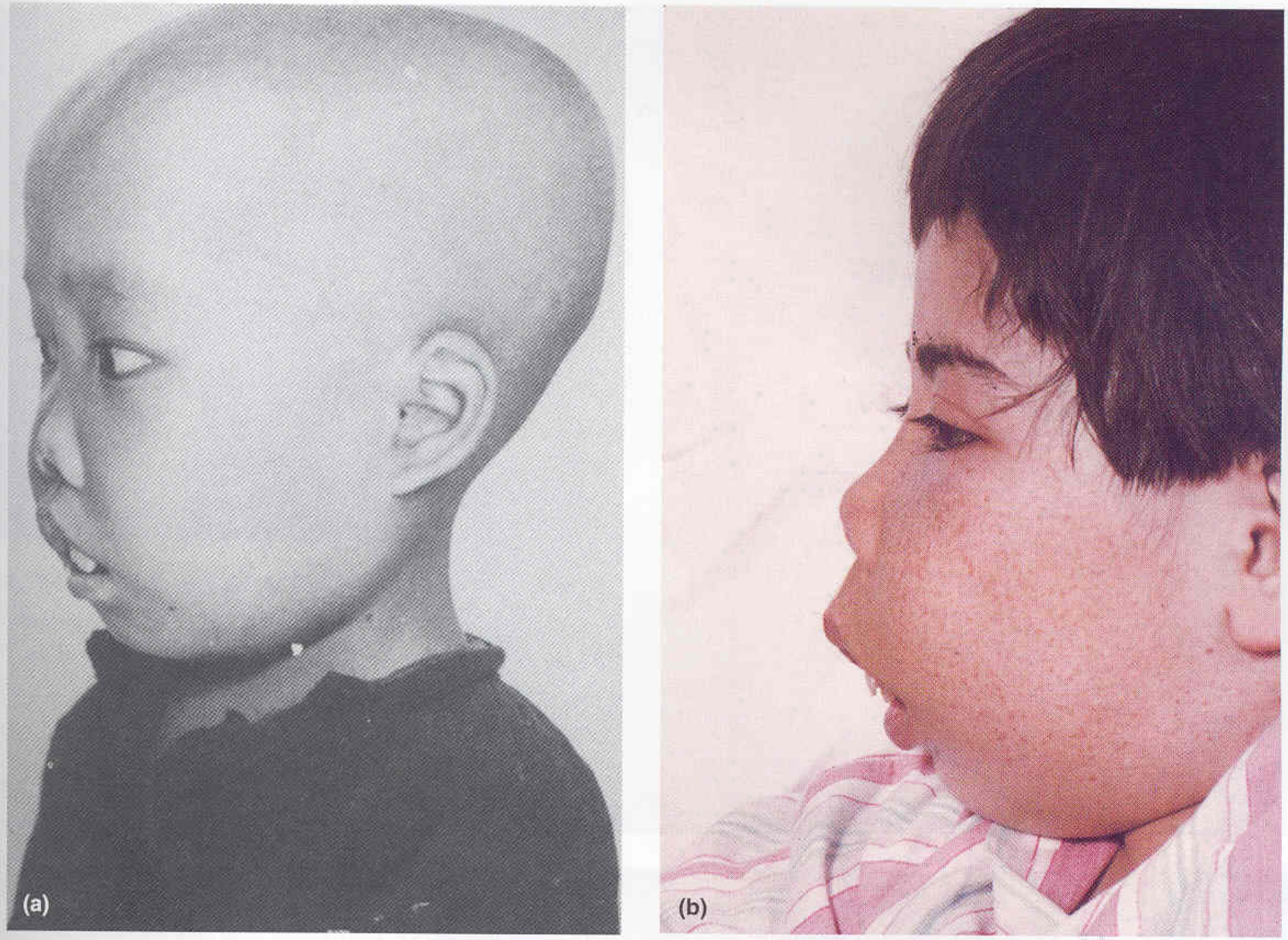


Fig. 7.2 Facial appearances in severe β thalassaemia. (a) Bossing of the skull. (b) Typical dental deformity.

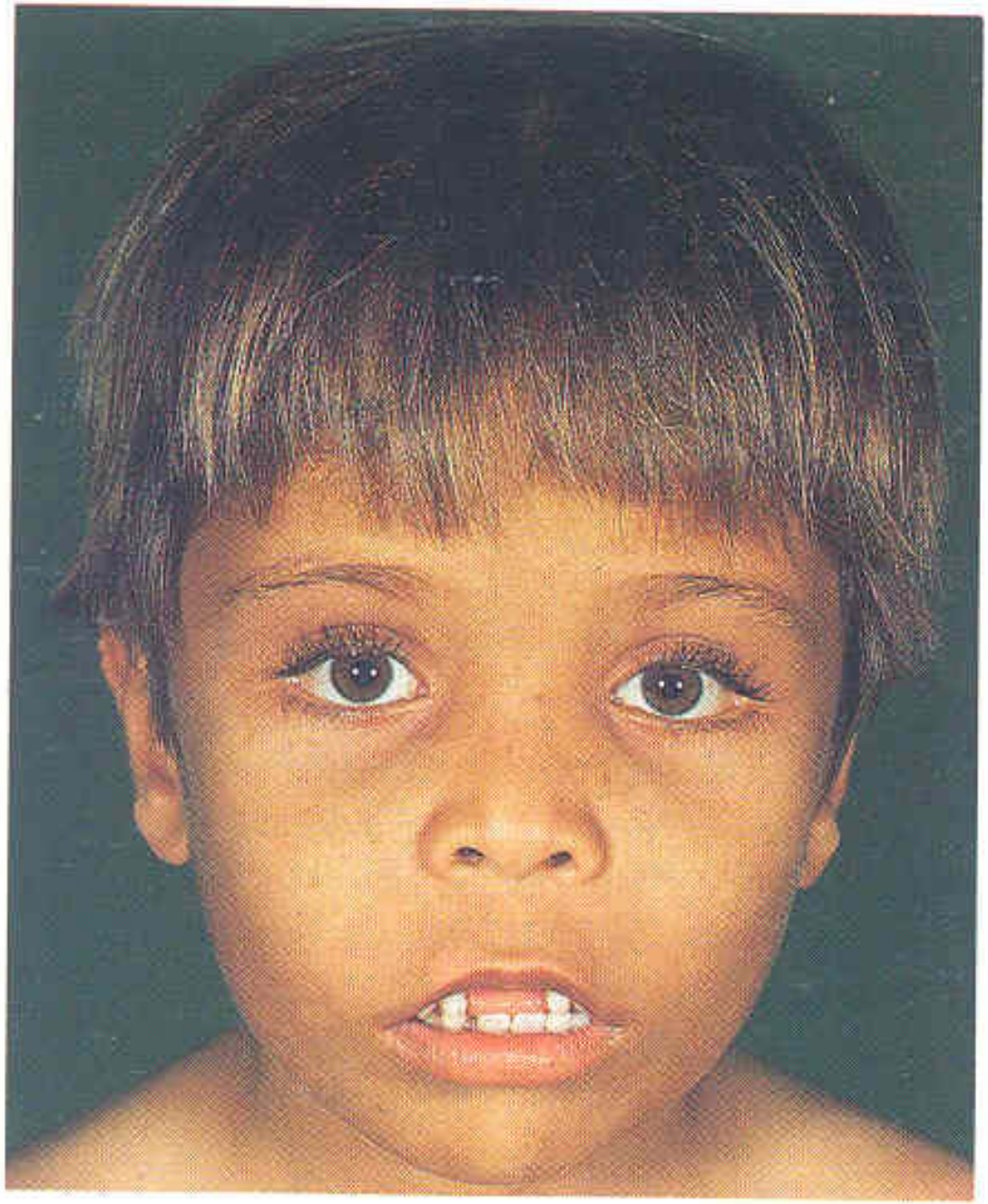
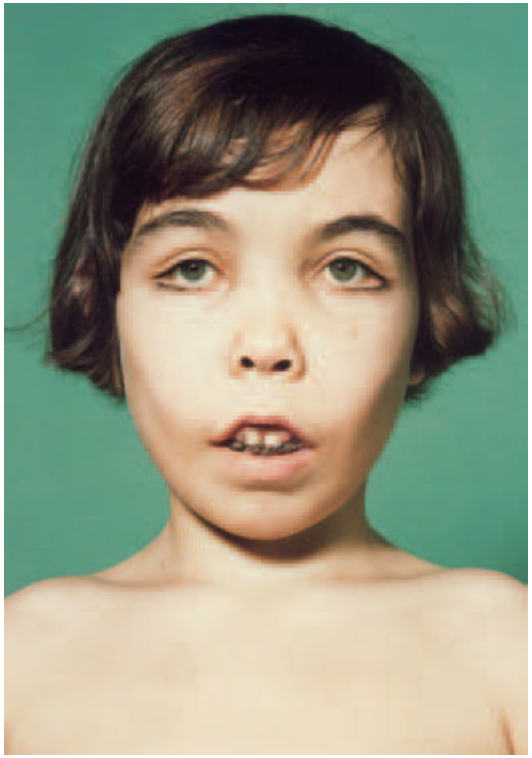
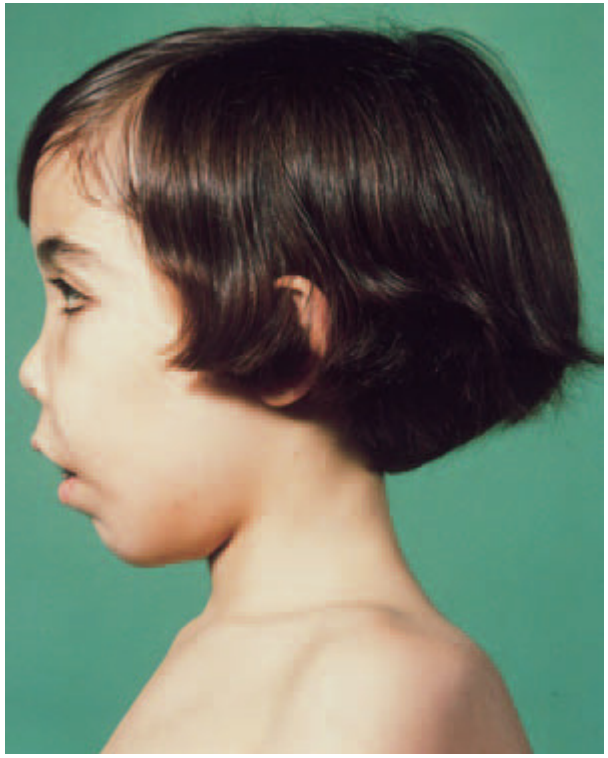
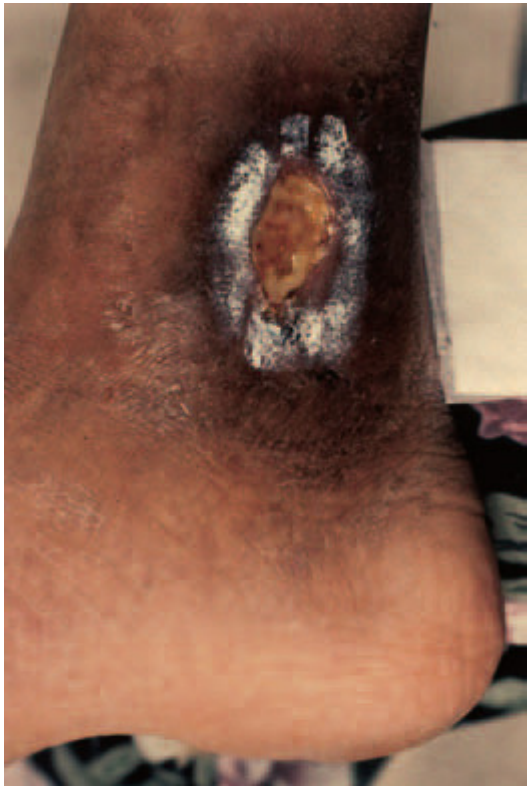


Fig. 5.6
β-Thalassaemia major: characteristic facies of a 7-year-old Middle Eastern boy includes prominent maxilla and widening of the bridge of the nose. There is also marked bossing of the frontal and parietal bones and zygomata giving a mongoloid appearance.

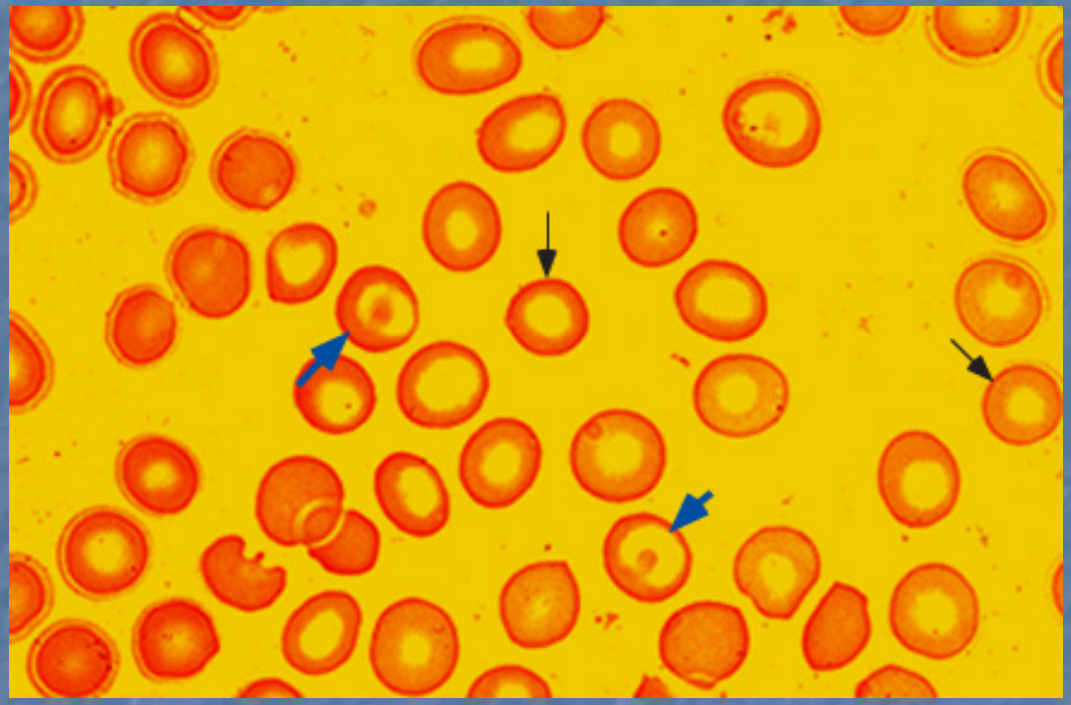
Clinical features of severe b thalassemia intermedia.
(a,b) Facial appearances; (c) chronic leg ulcer

Laboratory Diagnosis of Thalassemia

Laboratory Diagnosis

- Thalassemia minor:
- Hemoglobin : Hb level is usually normal or mildly reduced.
- PBS : Hypochromia and Microcytosis, basophilic stippling, target cell.
- Low MCV, Nl RDW, elevated RBC count .
- Reticulocyte Count increases
- Hb electrophoresis

Beta thalassemia trait



Peripheral smear from a patient with beta thalassemia trait.
The field shows numerous hypochromic and microcytic red cells (thin arrows), some of which are also target cells (blue arrows).

sebia

آزمایشگاه بیمارستان حضرت علی اصغر (ع)

زاهدان - خیابان آزادی - تلفن: ۳۲۲۹۶۸۸

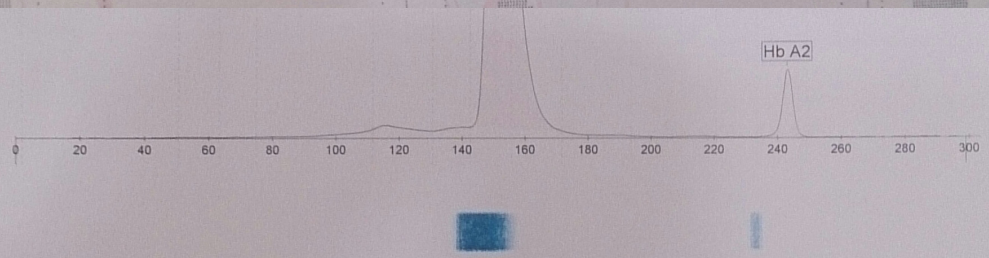
SYSMEX Hematology Analyzer

Patient Name: عبدالغفور هادزاده ID.NO: 38 Date: 167

Doctor: Sex/Age: Time: 10:

WBC	4.7	x10³/µl	[4-10]	RBC	6.62	x10⁶/µl	[3.9-5.8]	PLT 162 x10³/µ
Lymph	36.1	%		HGB	13.1	gr/dl	[12-17]	PDW 15.7 fl
Neut	51.0	%		HCT	44.9	%	[36-53]	MPV 10.6 fl
Mixed*	12.9	%		MCV	67.8	fl	[80-100]	P-LCR 31.8 %
*(Mono+Eos+Baso)				MCH	19.8	pg	[27-32]	
				MCHC	29.2	gr/dl	[31-36]	
				RDW-CV	15.9	%	[11.5-15]	
				RDW-SD	39.9	fl	[40-53]	

WBC RBC



Hemoglobin Capillary Zone Electrophoresis

Name	%		Normal Values %
Hb A	**94.5**	<	96.5 - 98.5
Hb A2	**5.5**	>	2.0 - 3.5

17 yr/ old male

Hb patterns in haemoglobin disorders

% Haemoglobin	A	F	A_2	S	Other
Normal	**97**	**<1**	**2–3**		
β thalassaemia trait	80–95	1–5	3–7		
β thalassaemia intermedia	30–50	50–70	0–5		
β thalassaemia major	0–20	80–100	0–13		
HPFH (Black heterozygote)	60–85	15–35	1–3		
HPFH (Black homozygote)		100			
α thalassaemia trait	85–95				Bart's 0–10% at birth
HbH disease	60–95				H 5–30% Bart's 20–30% at birth
HbBart's hydrops					Bart's 80–90%

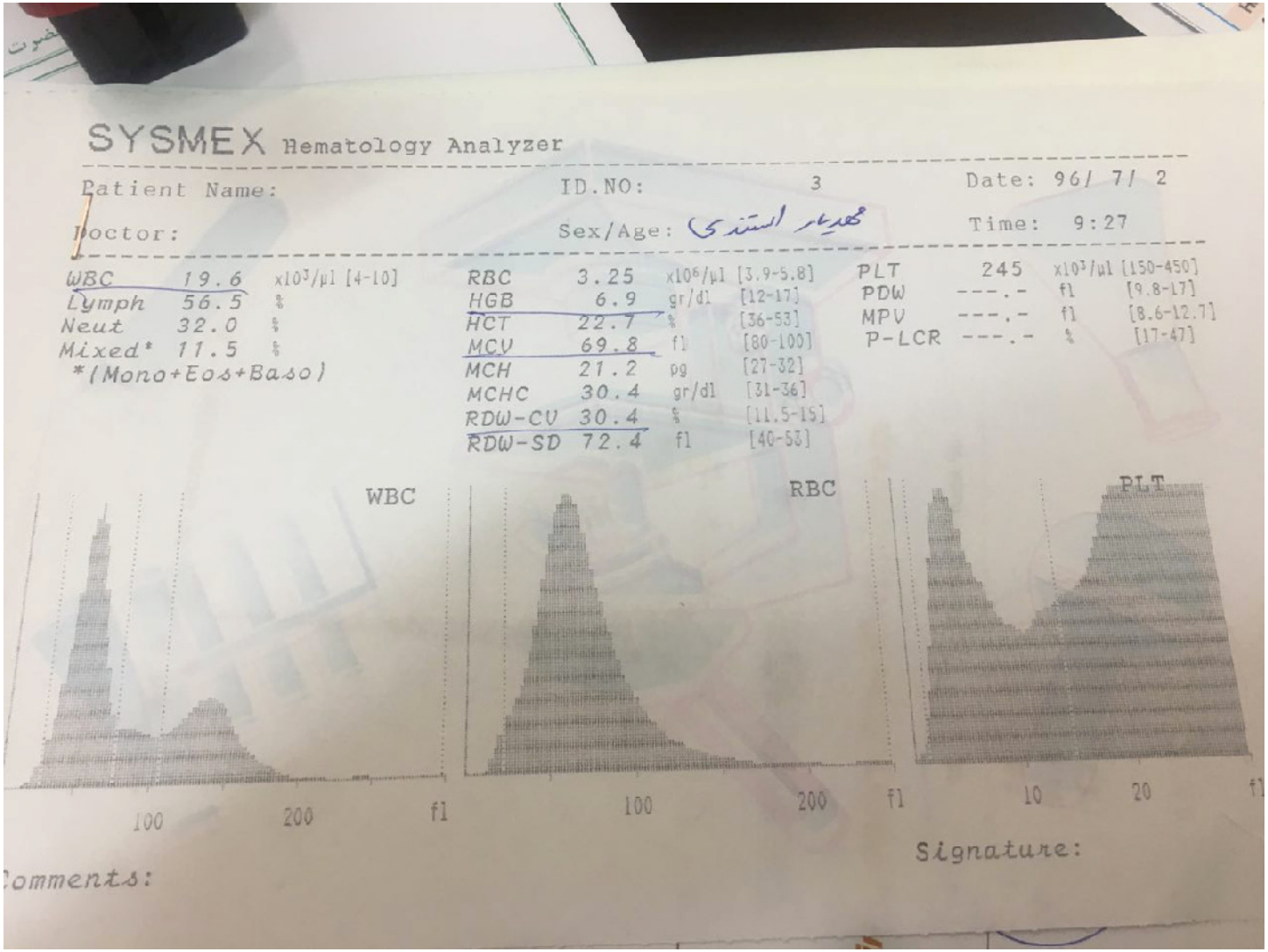
DDx of Microcytic, Hypochromic Anemia

- Iron Deficiency
- Alpha Thal
- Beta Thal
- Hb E Disease
- Anemia of Chronic Disease
- Sideroblastic Anemia
- Lead Poisoning

TABLE 20-8 Formulas for Differentiation of Thalassemia Trait from Iron Deficiency

	Thalassemia Trait	Iron Deficiency
Mentzer index (552)*		
MCV/RBC	<13	>13
Shine and Lal (562)		
(MCV) 2 × MCH	<1530	>1530
England and Fraser (563)		
MCV − RBC −(5 × Hb) − 8.4	Negative values	Positive values

*Numbers in parentheses are reference citations.
MCV, mean corpuscular volume; RBC, red blood cell.



SYSMEX Hematology Analyzer

Patient Name:
ID.NO: 3
Date: 96/ 7/ 2
Doctor:
Sex/Age: محمد استندی
Time: 9:27

WBC	19.6	x10³/µl	[4-10]	RBC	3.25	x10⁶/µl	[3.9-5.8]	PLT	245	x10³/µl	[150-450]
Lymph	56.5	%		HGB	6.9	gr/dl	[12-17]	PDW	---.-	fl	[9.8-17]
Neut	32.0	%		HCT	22.7	%	[36-53]	MPV	---.-	fl	[8.6-12.7]
Mixed*	11.5	%		MCV	69.8	fl	[80-100]	P-LCR	---.-	%	[17-47]
				MCH	21.2	pg	[27-32]				
				MCHC	30.4	gr/dl	[31-36]				
				RDW-CV	30.4	%	[11.5-15]				
				RDW-SD	72.4	fl	[40-53]				

*(Mono+Eos+Baso)

WBC

100 200 fl

RBC

100 200 fl

PLT

10 20 fl

Signature:

Comments:

7 months/old/ male