HEME

HEME

- Constituent of protein
- HB,MB,CYTCHROME,CATALASE,PER OXIDASE

Vital function

Oxygen storage and transport

Oxidation- reduction

Electron transport in respiratory chain

Destruction of harmful metabolite like H2O2

Heme is metalloporphyrin

- Iron is joined with porphyrin called protoporphyrin IX
- Porphyrin are tetrapyrrolle derivative
- Four pyrrole ring join by methenyl bridges
- Different porphyrin have different side chain attached to four pyrrole

- Heme is protoporphyrin IX
- Methyl,vinyl and propionyl group are attached to tetrapyrrole ring as side chain
- Type III isomer because asymmetric arrangement of side chain(methyl group) in ring IV

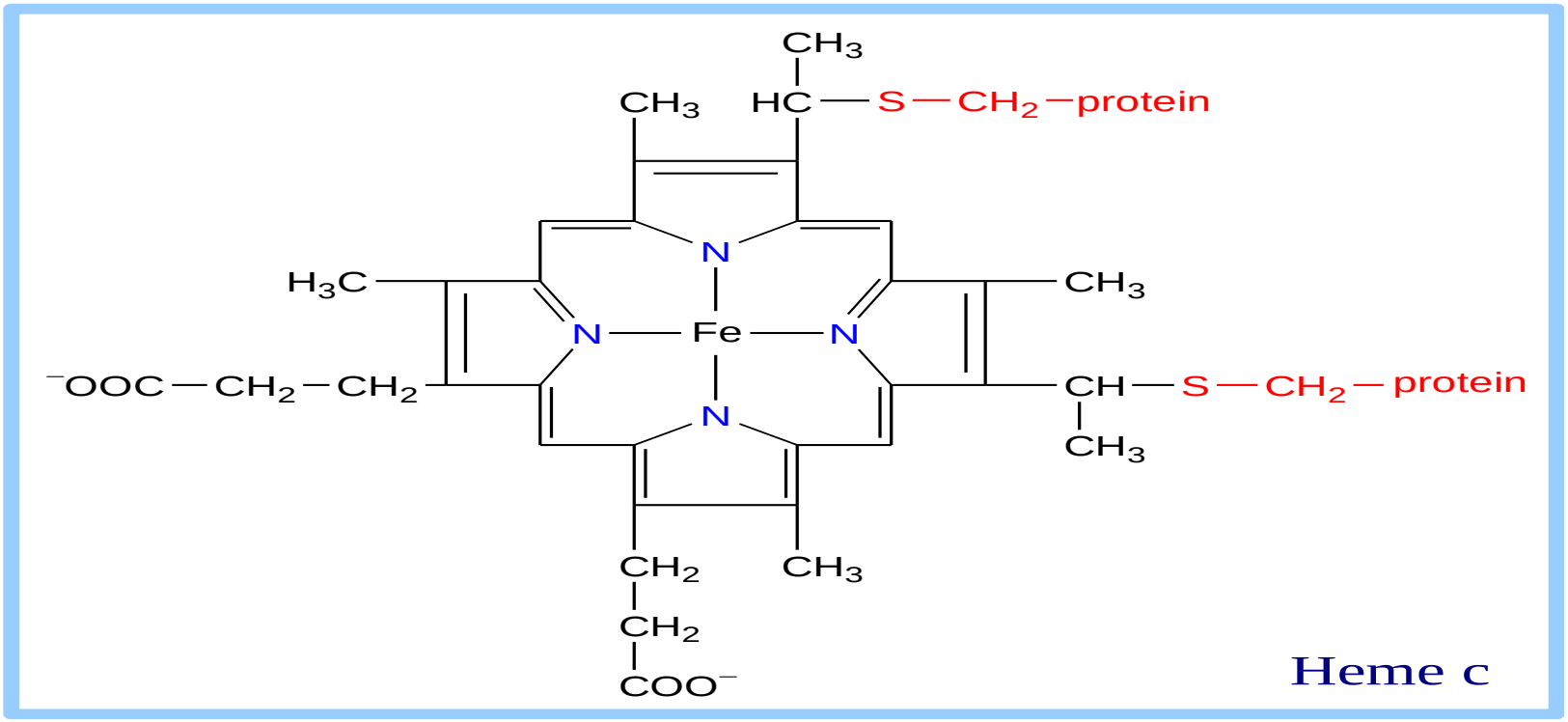
- Heme is metalloporphyrin in four N-atom of protoporphyrin III are joined to Fe atom
- Heme as prosthetic group attached to various protein to make heme-protein

- Pyrrole ring are named asI,II,III,IV and the bridges as alpha,beta,gammaand delta
- Possible group of substitution is 1 to 8
- Substituent group have symmetrical arrangement1,3,5,7 and 2,4,6,8----I series
- Asymmetrical distribution of substituent group 1,3,5,8 and 2,4,6,7-----III series

- Usual substitution are
- propionyl group
- Acetyl group
- Methyl group
- Vinyl group

- Type III is predominant in biological system.
- Called as series 9 -----FISCHER

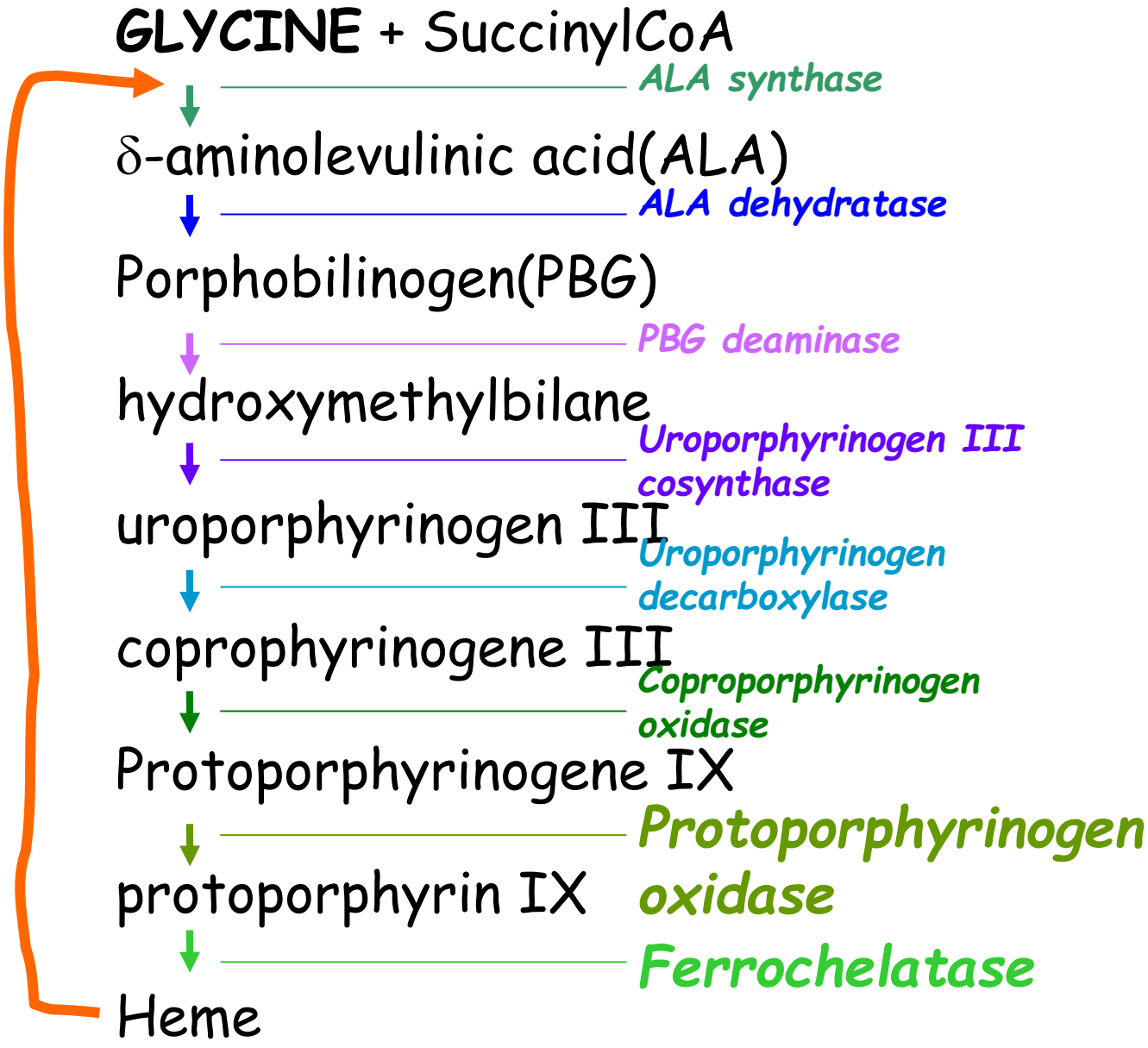
Structure of Heme

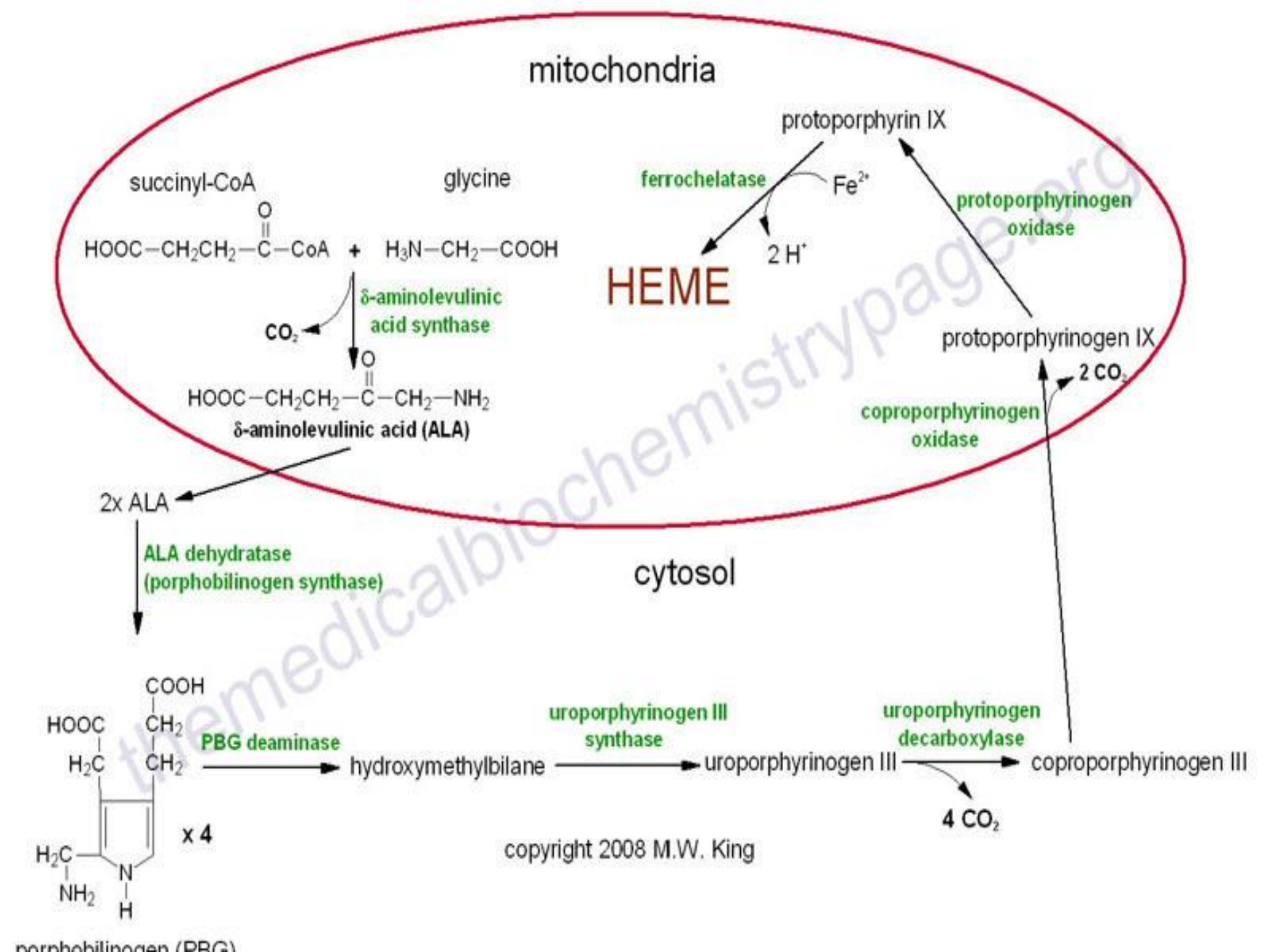


Heme is the prosthetic group of hemoglobin, myoglobin, & cytochromes

Synthesis of Heme

- Many tissue synthesis heme but liver and erythroid cells(bone marrow)


GLYCINE + SuccinylCoA
ALA synthase
δ-aminolevulinic acid(ALA)
ALA dehydratase
Porphobilinogen(PBG)
PBG deaminase
hydroxymethylbilane
Uroporphyrinogen III cosynthase
uroporphyrinogen III
Uroporphyrinogen decarboxylase
coprophyrinogene III
Coproporphyrinogen oxidase
Protoporphyrinogene IX
Protoporphyrinogen oxidase
protoporphyrin IX
Ferrochelatase
Heme



Synthesis of d-aminolevulinic acid

- Glycine+succinyl-coA-----ALA
- ALA synthase
- Pyridoxal phosphate---co-enzyme
- Rate-limiting and inducible
- ALA synthase has isoenzyme:
- ALA1(Liver)
- ALA 2(erythroid)

Formation of porphobilinogen

- Two molecule of ALA condense ---- porphobilinogen(pyrrole derivative)
- ALA dehydratase
- Cytosolic enzyme

Formation of UroporphyrinogenIII

- Four porphobilinogen condenses to form uroporphyrinogenIII
- uroporphyrinogenI synthase and cosynthase III
- UPGIII has pyrrole ring connected by methylene bridges
- Acetate and propionate side chain attach in asymmetric on ring IV giving type III isomer

Formation of coproporphyrin III

- UPG III decarboxylase converts UPGIII into coproporphyrinogen III by decarboxylating all four acetate group to methyl group

Formation of protoporphyrin IX

- CPG III diffuse into mitochondria where it undergoes
- Oxidative decarboxylation of propinate side chain of ringI and II to vinyl group.
- Protoporphyrinogen IX forms
- Protoporphyrinogen oxidase oxidises methylene bridges connecting four pyrrole rings to form protoporphyrin IX

Formation of HEME

- Fe +2 atom in corporated into proto porphyrin IX
- Ferrochelatase

REGULATION OF ALA SYNTHASE

- **Down regulated**

  Heme

- **UP regulated**

  Barbiturates, Steroids (e.g. testosterone)

➢ **These drugs are metabolized by the *microsomal***

***cytochrome P_{450} mono-oxygenase system*, a heme-**

containing protein. So increase utilisation of Cyt p450 depletes heme

- Barbiturates,griesofulvin,synthetic oestrogen and progesteron are inducer of ALA-1

ALA Synthase is the **committed step** of the heme synthesis pathway, & is usually rate-limiting for the overall pathway.

Regulation occurs through control of gene transcription.

Heme functions as a feedback inhibitor, **repressing transcription** of the ALA Synthase gene in most cells.

A variant of ALA Synthase expressed only in developing erythrocytes is regulated instead by availability of iron in the form of iron-sulfur clusters.

ferrochelatase

- Fe availability regulates heme synthesis through ferrocheletase
- Lead also inhibit ferrochelatase and decreases heme synthesis

Properties of porphyrins

- Porphyrin are coloured
- Porphyrinogen are colourless while porphyrin are brownish red in colour
- All porphyrinogen on oxidation are converted into corresponding porphyrins eg
- Uroporporphyrinogen ----->uroporphyrin
- Coproporphyrinogen------>coproporphyrin
- Protoporphyrinogen------>protoporphyrin

Soret band

- All porphyrins show sharp absorption peak at 400 nm referred to as soret band
- Double band of methylene bridges

Red fluorescence

- Exposed to ultra-violet light shows red fluorescence
- Double band of methylene bridges

Clinical significance

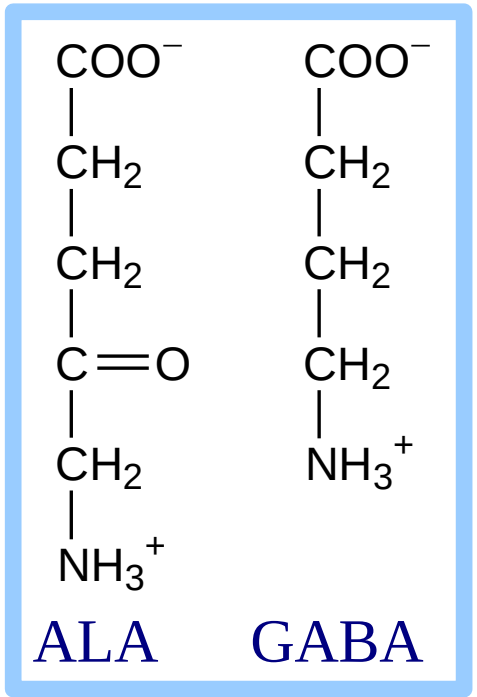
The **Zn^{++}** binding sites in the homo-octomeric mammalian Porphobilinogen Synthase, which include cysteine S ligands, can also bind **Pb^{++}** (lead).

Inhibition of Porphobilinogen Synthase by **Pb^{++}** results in **elevated** blood **ALA**, as impaired heme synthesis leads to de-repression of transcription of the ALA Synthase gene.

High **ALA** is thought to cause some of the neurological effects of **lead poisoning**, although Pb^{++} also may directly affect the nervous system.

ALA is **toxic to the brain**, perhaps due to:

- Similar ALA & neurotransmitter **GABA** (γ-aminobutyric acid) structures.
- ALA autoxidation generates **reactive oxygen species** (oxygen radicals).



LEAD TOXICITY

- **Inhibits multiple enzyme reactions including those involved in heme biosynthesis (PBG synthase & ferrochelatase)Binds to any compound with a sulfhydryl group**

- **One symptom of lead toxicity is increases in 5-ALA without concomitant increases in PBG**

Porphyrias

Porphyrias are **genetic diseases** in which activity of one of the enzymes involved in heme synthesis is decreased (e.g., PBG Synthase, Porphobilinogen Deaminase, etc…).

Symptoms vary depending on

- the enzyme
- the severity of the deficiency
- whether heme synthesis is affected primarily in liver or in developing erythrocytes.

Occasional **episodes** of severe **neurological symptoms** are associated with some **porphyrias**.

- Permanent **nerve damage** and even death can result, if not treated promptly.
- **Elevated δ-aminolevulinic acid** (ALA), arising from de-repression of ALA Synthase gene transcription, is considered responsible for the neurological symptoms.

Photosensitivity is another common symptom.

- **Skin damage** may result from exposure to light.
- This is attributable to elevated levels of **light-absorbing pathway intermediates** and their degradation products.

Types porphyria

- Depending on the tissue of involvement
- Hepatic
- Erythropoietic
- Mixed

Hepatic porphyria

- Acute intermittent porphyria
- Porphyria cutanea tarda
- Hereditary coproporphyria
- Porphyria varigata

Hepatic porphyria

Precipitated by various drugs alcohol, steroids, grisofulvin etc

Typical neurological symptoms

Pain in abdomen,nausea,vomiting

Pain in neck,chest,limbs

Abnormal sensation like numbness,tingling or loss of sensation

- Weakness in muscle presenting as fatigue or difficulty in walking
- Psychiatric symptoms like lack of sleep,nervouness,anxiety,depression,
- hallucination

Erythropoietic porphyria

- Congenital erythropoietic porphyria
- protoporphyria

Present as…..

- Anaemia
- Hemolysis
- spleenomegaly

Biological basis of symptoms and photosensitivity

- Due accumulation of product from enzyme deficiency
- Autonomic neuropathy
- ALA-----inhibit ATPASE interferes in nerve and muscle conduction
- Autonomic disturbance---alteration of GIT mobility producing abdomen pain,nausea,vomiting,diarrhoea

- Sensory and motor involvement leading in pain in limbs,chest,neck,weakness of muscle fatique,urinary retention
- Restlessness,anxiety,depresion,hallucination ,excessive sweating,trmors and hypertention due to sympathetic overactivity.

photosensitivity

- Excessive accumulation of porphyins
- Porphyrin react with UV light and generate free radicals with oxygen
- These radicals damage lysosomes and other cell organelle releasing many hydrolytic enzyme
- Skin damage and scarring

Acute intermittent porphyria(AIP)

- Hepatic type of porphyria
- Commonest variety 1:10,000 to 1:1,00,000
- UPG-I synthase deficiency
- Accumulation of ALA,PBG leading to neuropsychiatric symptoms
- Young adult
- Present as acute attack with acute pain in abdomen with or without other symptoms

- No photosensitivity
- Precipitated by drugs like steroid,alcohol,barbiturates,sulphonamide,
- carbamazepine,grisofulvin
- Diagnosis----high urine and blood ALA and PBG
- Enzyme deficiency in RBC

- Glucose inhibit induction of ALA synthase.
- and heme inhibit ALA synthase induction
- Hemealbumin or hemearginate is given

Characteristics of different porphyria

- X-linked sideroblastic anaemia
- ALA synthase defect
- Anaemia main symptoms
- Lab finding---decrease RBC and decrease Hb

Hepatic ALA dehydratase

- ALA dehydratase deficiency
- Abdominal pain
- Psychiatric symptoms
- Urinary ALA levels

Acute intermittent porphyria

- UROSYNTHETASEI deficiency
- Abdominal pain
- Psychiatric symptoms
- Blood ALA and PBG

Porphyria cutanea tarda

- Uroporphyrinogen decarboxylase
- Photosensitivity
- Uroporphyrinogen decarboxylase
- Photosensitivity
- Urophorphyrin +ve
- Uroporphyrinogen -ve

Hereditary coproporphyria

- Coproporphyrinogen oxidase
- Photosensitivity
- Pain abdomen
- Psychiatric symptoms
- Urophorphyrin +ve
- Uroporphyrinogen +ve(urine)

Varigata porphyria(hepatic)

- Protoporphyrinogen oxidase deficiency
- Photosensitivity
- Pain in abdomen
- Psychiatric symptoms
- Urophorphyrin +ve
- Uroporphyrinogen +ve(urine)

Proto-porphyria

- Ferrochelatase deficiency
- Photosensitivity
- Fecal protoporphyrin +ve also RBC +ve

Congenital erythropoietic porphyria

- Autosomal recessive
- Type 111 increase due loss offeedback inhibition
- Photosensitivity
- Dermatitis and scarring --→Monkey Face
- Erythrodontia
- Urine dark in color (port wine apperance)

- THANKS

HAEMOGLOBINOPATHIES

1. **Sickle cell anemia (Hb S)**
2. **Hemoglobin C disease (Hb C)**
3. **Hemoglobin SC disease (Hb S+ Hb C)**
4. **Thalasemia**

Sickle cell anemia

A 10-years-old African American male presented to ER with complain of pain "all over his body.“ His mother brought him into the ED at 4 pm .She reported that the pain began early that morning and had "gotten worse." She reported that it was not relieved by his usual doses of ibuprofen. He was medicated with strong IM pain killer. He reported minimal pain relief after receiving the medication. He reported that the slight relief was short-lived, and he continued to complain of unbearable pain through the night.

His past history is significant with many such hospital admissions and history of repeated chest infections and a non healing ulcer on his right ankle.

Family History: History of similar episodes of pain crisis and chest infection in two of the 5 siblings.

Sickle cell anemia

Examination: **Pale appearing child in agony oriented in time, space and person having a chronic ulcer on right ankle.**

Cardiovascular System: Moderate tachycardia, grade II/VI systolic murmur heard best over the upper left sternal border.

Gastrointestinal Tract: Abdomen: Moderate hepatosplenomegaly.

Complete Blood Count: Hb: 5gm/dl, TLC: 12,000/ul, Platelet count: 150,000/ul.

Reticulocyte Count: 12%.

Peripheral Film: Moderate poikilocytosis, anisocytosis, hypochromia, polychromasia target cells, many fragmented and sickle red cells.

Special investigations

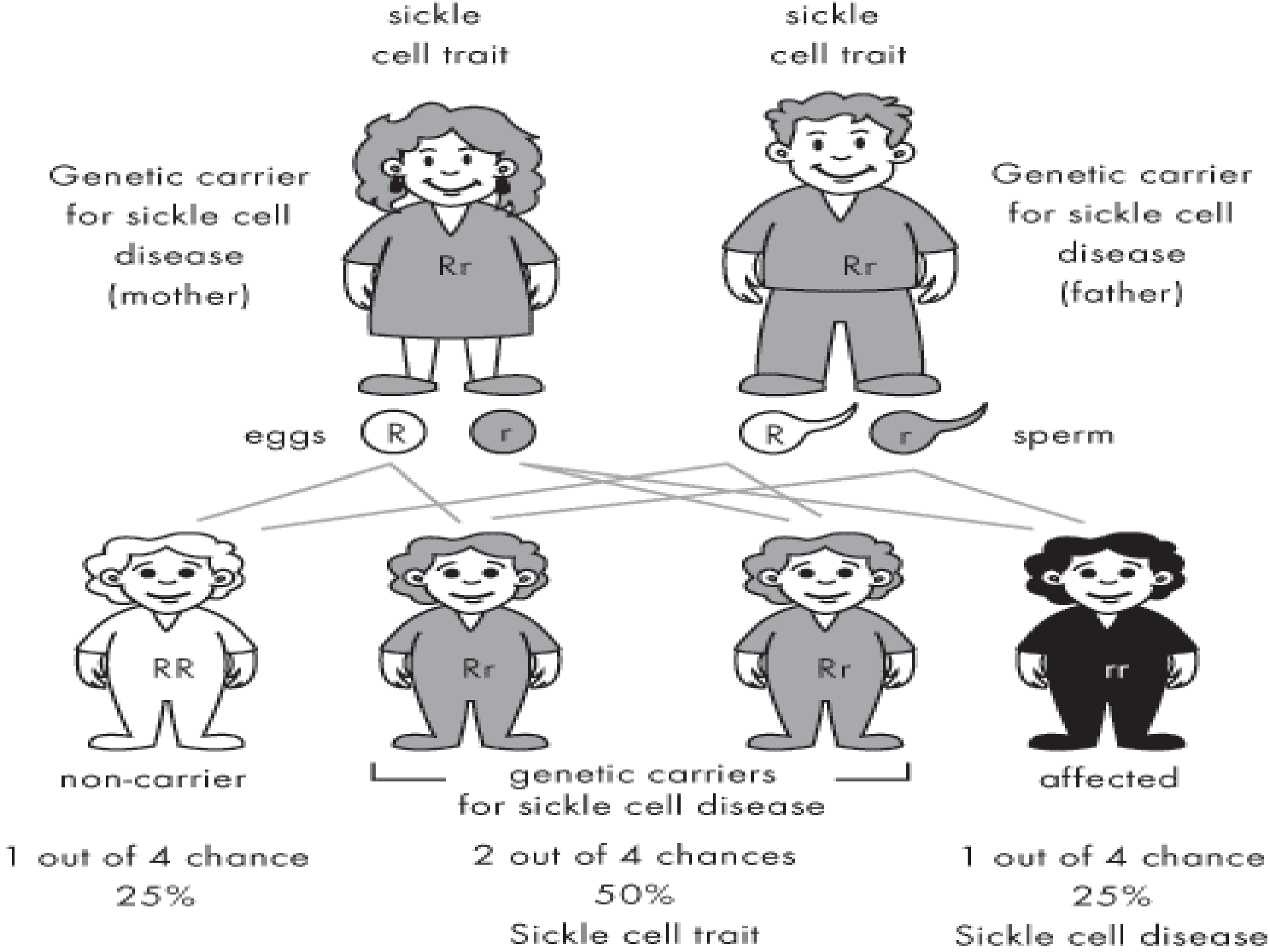
Sickle Screening Test: Positive
HbS: 70%, HbF: 13%, HbA: 17%

Sickle cell anemia (Hb S disease)

Homozygous recessive (2 mutant genes that codes for β–chains of globin)-----β^S ---------$\alpha_2\beta_2{}^S$ (Hb S)

Valine replaces glutamate in the 6th position of β–chains

- **Common in African blacks**
- **Confers resistance against malaria**
- **Hb crystallizes and take sickle shape under hypoxic conditions**
- **Increased RBC Sequestration**


sickle cell trait
sickle cell trait
Genetic carrier for sickle cell disease (mother)
Genetic carrier for sickle cell disease (father)
Rr
Rr
eggs
R
r
R
r
sperm
RR
Rr
Rr
rr
non-carrier
genetic carriers for sickle cell disease
affected
1 out of 4 chance
25%
2 out of 4 chances
50%
Sickle cell trait
1 out of 4 chance
25%
Sickle cell disease

SECTION OF GENE FOR HEMOGLOBIN

Normal DNA sequences:	GGA	CTC	CTC
Abnormal DNA sequences:	GGA	CAC	CTC

MESSENGER RNA CODON TABLE

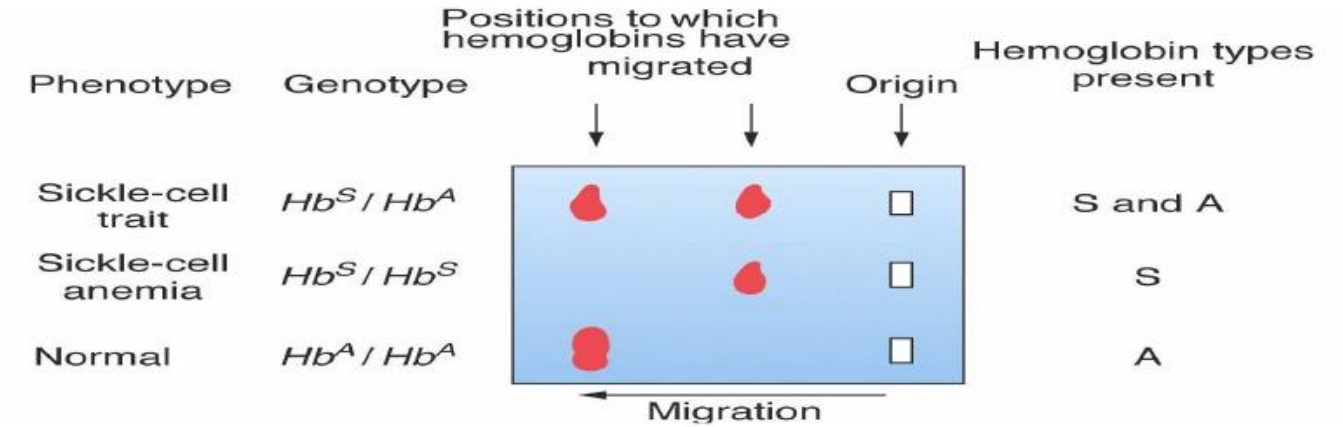
Codon	Amino Acid
GUG	Valine
CAC	Histidine
CUC	Leucine
ACU	Threonine
CCU	Proline
GAG	Glutamic acid

HBB Sequence in Normal Adult Hemoglobin (Hb A):

Nucleotide	CTG	ACT	CCT	GAG	GAG	AAG	TCT
Amino Acid	Leu	Thr	Pro	Glu	Glu	Lys	Ser
	3			6			9

HBB Sequence in Mutant Adult Hemoglobin (Hb S):

Nucleotide	CTG	ACT	CCT	GTG	GAG	AAG	TCT
Amino Acid	Leu	Thr	Pro	Val	Glu	Lys	Ser
	3			6			9


Phenotype
Genotype
Positions to which hemoglobins have migrated
Origin
Hemoglobin types present
Sickle-cell trait
Hb^S / Hb^A
S and A
Sickle-cell anemia
Hb^S / Hb^S
S
Normal
Hb^A / Hb^A
A
Migration

THALASSAEMIA

- **An 8 month old boy was brought by his parents with complaints of lethargy, marked pallor, inactivity and abdominal distension. Eight month old infant presented with the marked pallor and growth failure. There is also history of change in facial appearance. Initially symptoms were less marked. But now they have progressed further.**

- **Family History: History of death of sibling at the age of 15 months diagnosed as deficiency of blood**

Examination: **Pale appearing, inactive toddler**

- **Mild tachycardia as above, grade II/VI systolic ejection murmur heard best over the upper left sternal border.**
- **Moderate hepatosplenomegaly**

Complete Blood Count: **Hb: 5gm/dl, TLC: 18,000/ul, Platelet count: 150,000/ul.**

- **Reticulocyte Count: 10%.**
- **Peripheral Film: Marked poikilocytosis, anisocytosis, microcytosis, hypochromia, polychromasia target cells, many fragmented red cells.**
- **Radiology: X-ray skull show crew cut appearance and maxillary prominence**

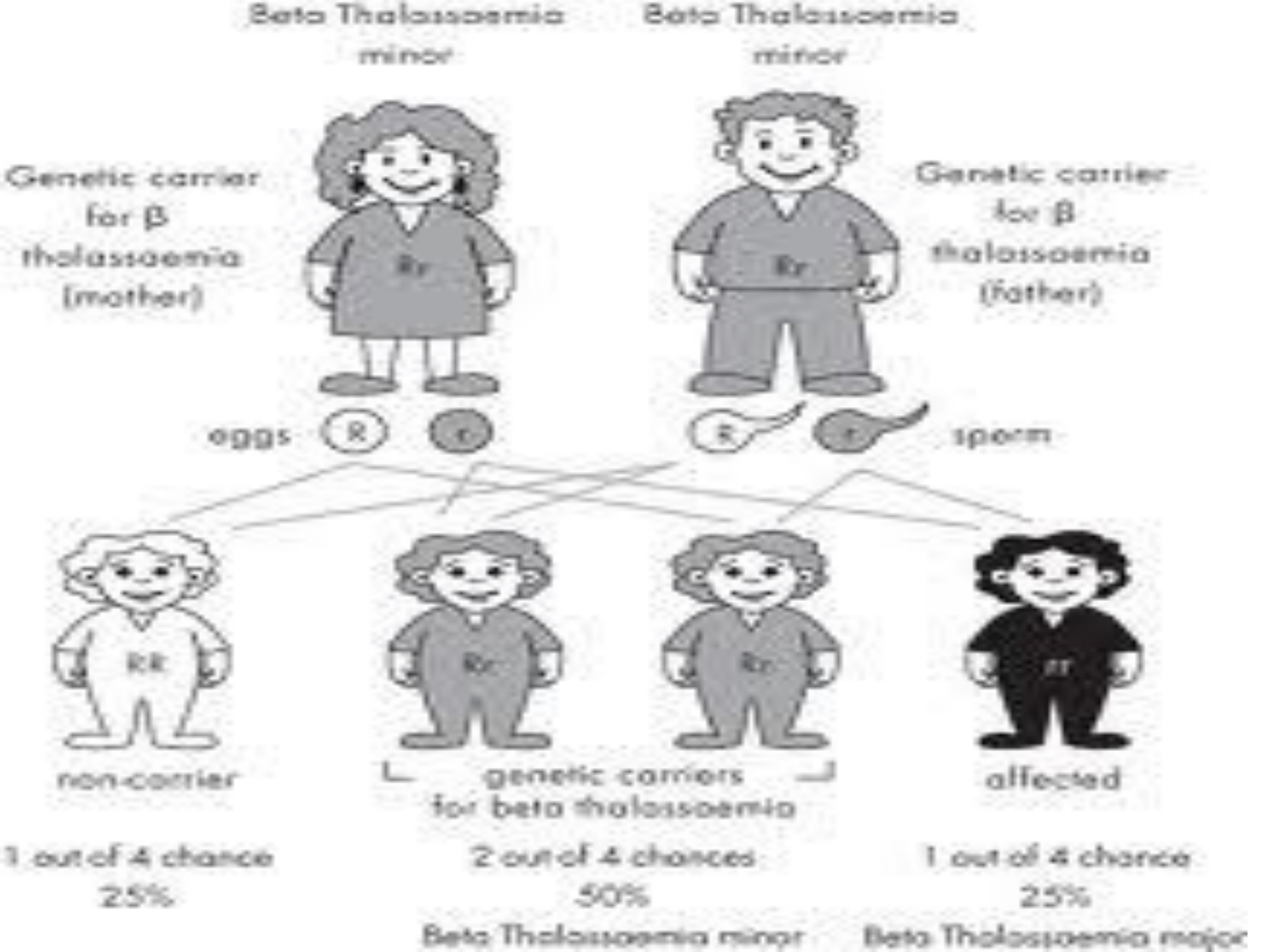
Special investigations

HbF: 90%, HbA: 08%, HbA2: 02%

-

THALASSAEMIA

- **Beta-thalassemias are a group of hereditary blood disorders characterized by anomalies in the synthesis of the beta chains of hemoglobin resulting in variable phenotypes ranging from severe anemia to clinically asymptomatic individuals.**
- **The total annual incidence of symptomatic individuals is estimated at 1 in 100,000 throughout the world and 1 in 10,000 people in the European Union. 1.5% of the global population (80 to 90 million people) are carriers of beta thalassemia, with about 60,000 symptomatic individuals born annually, the great majority in the developing world.**


Beta Thalassaemia minor
Beta Thalassaemia minor
Genetic carrier for β thalassaemia (mother)
Genetic carrier for β thalassaemia (father)
Rr
Rr
eggs
R
r
R
r
sperm
RR
Rr
Rr
rr
non-carrier
genetic carriers for beta thalassaemia
affected
1 out of 4 chance 25%
2 out of 4 chances 50% Beta Thalassaemia minor
1 out of 4 chance 25% Beta Thalassaemia major

THALASSAEMIA

- **Thalassemia Major,"Cooley's Anemia" and "Mediterranean Anemia"**
- **Thalassemia Intermedia and Thalassemia Minor also called"beta-thalassemia carrier",**
- **“Beta-thalassemia trait or"heterozygous beta-thalassemia".**

THALASSAEMIA

β-Thalassemia

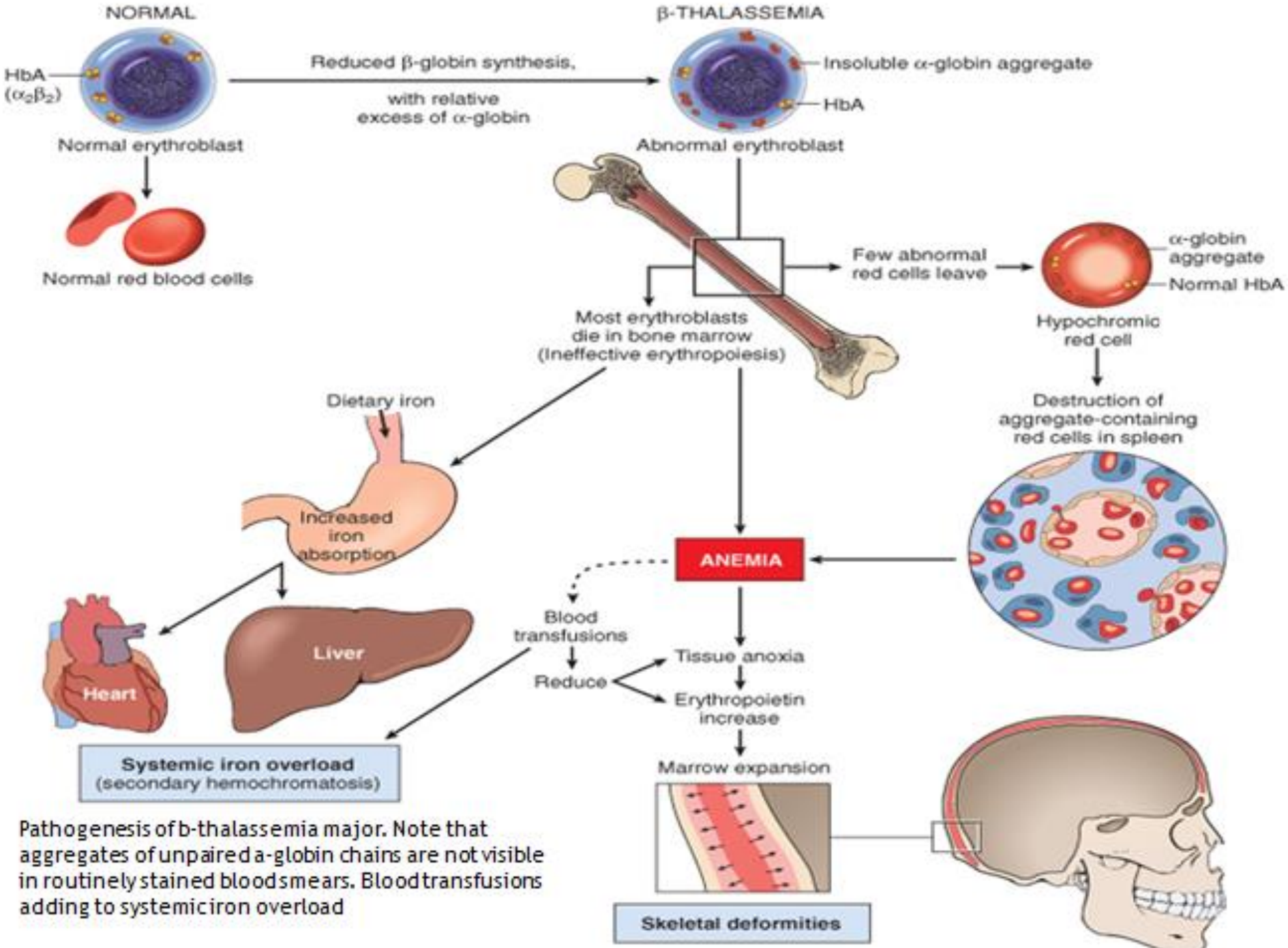
- **Reduced or absent synthesis of globin chains**
- **2 copies of β-globin gene on chromosome 11**
- **β^0 No globin chain synthesis**
- **β^+ Some globin chain synthesis**
- **β^+/β^+ Homozygote have anemia of variable severity**
- **β^+/β^0 Compound Heterozygote tend to be more severely affected**
- **β^0/β^0 Homozygote have severe disease**

THALASSAEMIA

- **Excess β-chains form a homotetramer, HbH(Useless for delivering oxygen because of high oxygen affinity)**
- **Inclusion bodies (HbH precipitates trapped and destroyed in the spleen)**
- **Ineffective erythropoiesis: Precipitated α-chains unable to form a stable tetramer**

THALASSAEMIA

- **β -Thalassemia Minor (Make some β-chains. No treatment required)**
- **β -Thalassemia Major (Seemingly healthy at birth , but severely anemic, usually first or second year of life due to ineffective erythropoiesis)**
- **Skeletal changes as a result of extramedullary hematopoiesis**
- **Iron chelation therapy and Bone marrow replacement**



Pathogenesis of b-thalassemia major. Note that aggregates of unpaired a-globin chains are not visible in routinely stained blood smears. Blood transfusions adding to systemic iron overload

THALASSAEMIA

α-Thalasemia

Deletion mutations

4 copies of the α-Globin gene (2 on each chromosome 16)

- **Silent carrier of α-Thalasemia: One of the four gene is defective no physical manifestation**
- **α -Thalasemia trait: 3 α-globin genes are defective (Hb β_4 disease)—Mild to severe hemolytic anemia**
- **(Hb Bart (γ_4 disease)—All 4 α-globin gene defective . Hydrops fetalis**

THANKS