

Epidemiology, Care and Prevention of Hemoglobinopathies

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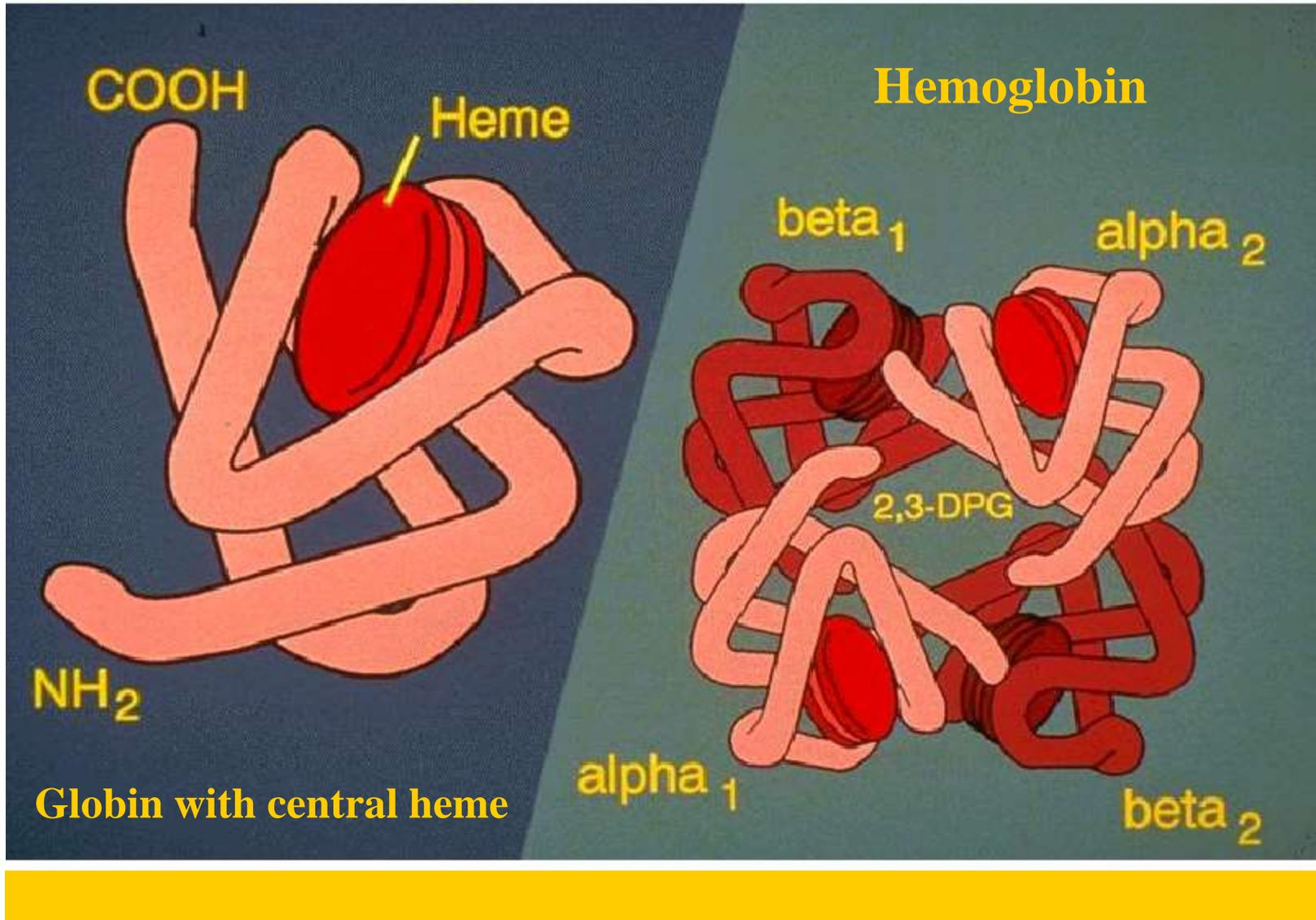


From Research to Practice
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Hemoglobinopathies

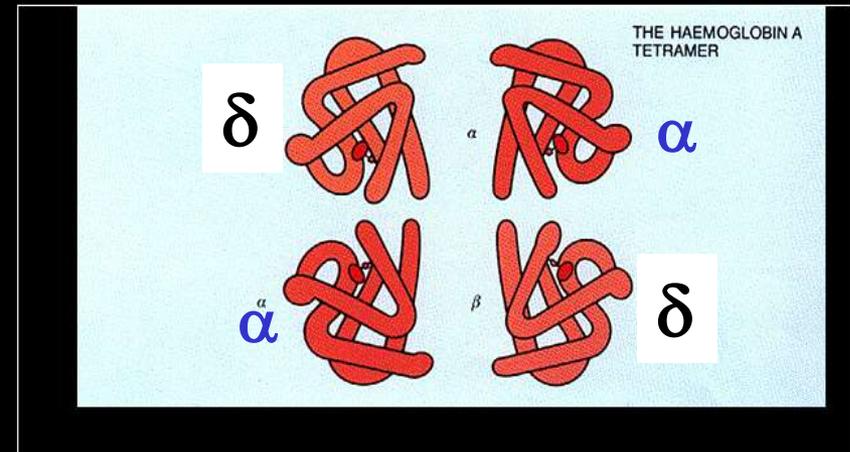
- Are inherited disorders affecting either the rate of synthesis and/or the structure of globin chains of Hemoglobin.



Hemoglobin is a complex protein. All Normal Hemoglobins consist of two pairs of globin chains, at the centre of each is one heme group.

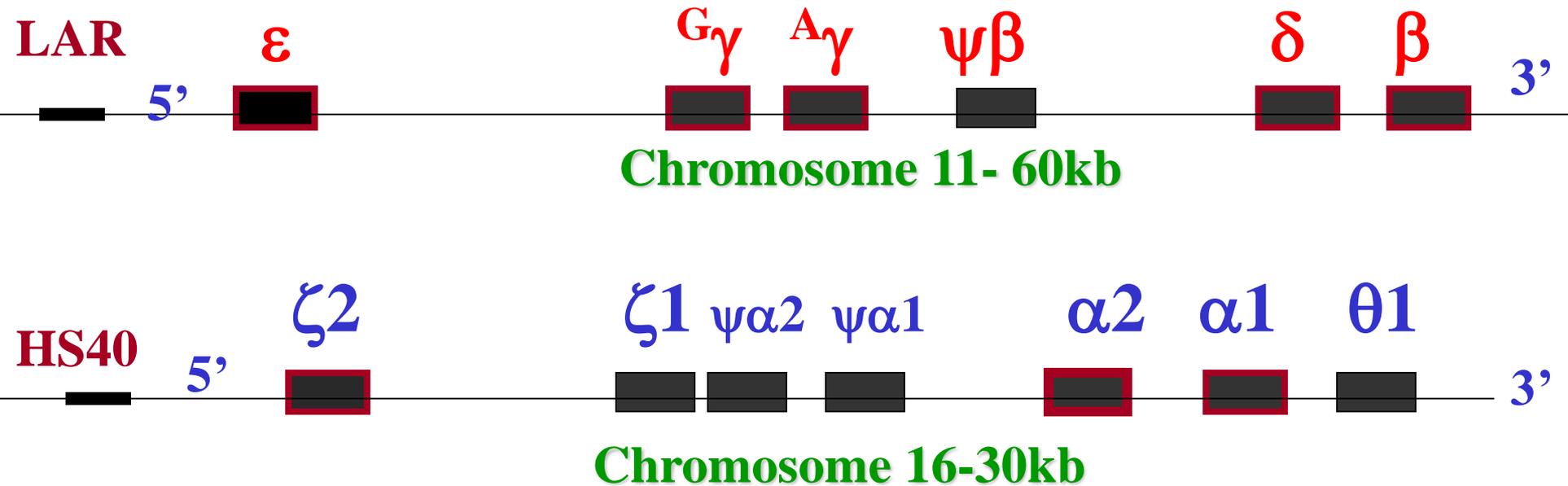
Types of normal Hemoglobins

- After the age of six months, the following are the normal hemoglobins with respective proportions :
- Hb A (Adult Hb) : $\alpha^2 \beta^2$ (~96%).
- Hb F (Fetal Hb) : $\alpha^2 \gamma^2$ (<1.0%).
- Hb A₂ (minor Adult Hb) : $\alpha^2 \delta^2$ (1.8-3.5%).
- These percentages are for those from age of 6 months through adult life.
- During most of fetal life and at birth the major Hb is Hb F, and it decreases to adult level of <1% by six months of age.



Normal Hb (Hb A₂ ; $\alpha^2 \delta^2$)

The globin chain production is directed by two set of genes (Gene Clusters) one on chromosome 16 (alpha gene cluster), the other on chromosome 11 (Beta gene cluster).



The Globin Gene Clusters

The alpha gene cluster includes one functional embryonic ($\zeta 2$) and two functional α genes ($\alpha 2$ & $\alpha 1$). The beta gene cluster includes one functional embryonic ϵ gene and two fetal $G\gamma$ & $A\gamma$ as well as one functional δ and β genes.

** *LAR* : Locus Activating region

Hemoglobinopathies

Either

- Quantitative Hbpathies like **thalassaemia**
- Qualitative like **Sickle cell**
Hb C, E, D and other less common variants



Pattern of Inheritance

- Hemoglobinopathies are inherited as Autosomal recessive disorders.
- Thus heterozygotes (carriers) are not usually symptomatic, while Homozygotes or compound heterozygotes are obviously symptomatic.



Some Basic Facts About the Global Epidemiology of Hemoglobinopathies

- Hemoglobin disorders constitute a significant health problem in more than 160 countries worldwide. These countries are responsible for 89% of worldwide births.
- Over 330 000 affected infants are born annually (83% sickle cell, 17% thalassemia).
- Hemoglobin disorders account for 3.4% of deaths in children < 5 years.
- Globally 5.2% of world population (and >7% of pregnant women) carry a significant Hb disorder, and over 1% of couples are at risk of getting affected infants.

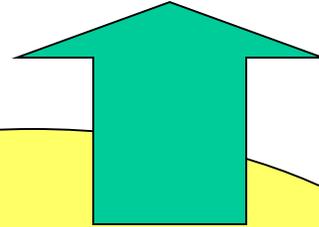
Thalassemias

Definition of thalassemia

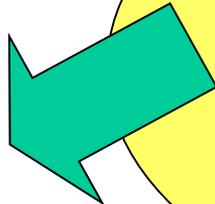
- A group of inherited disorders of Hemoglobin synthesis, characterized by reduced or absent synthesis of one or more of the globin chains of Hemoglobin.
- They are labeled α thalassaemias, if it is the alpha chain that is affected, or β thalassaemias, if it is the Beta.

β thalassaemias

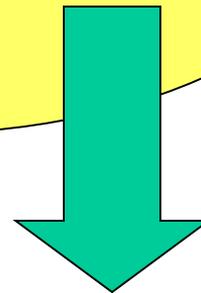
Thalassemia Major



**Clinical Phenotypic
classification of β -
thalassemia**



Thalassemia Intermedia



Thalassemia Minor

Clinically β thalassaemia could be classified into :

• β Thalassaemia Major :

Severe clinical manifestations presenting before the age of 2 years, usually transfusion dependent. Due usually to homozygosity to β thalassemic gene defect.

• β Thalassaemia minor :

Mild or no clinical manifestations, usually does not require specific management. Due usually to heterozygosity to β thalassaemia gene defect.

• β Thalassaemia Intermedia :

Moderate manifestations, intermediate between major and minor.

Management of β Thalassaemia major



Mainstay for management is Adequate Transfusion with Iron chelation.

-Maintain Hb >9.5 g/dl.

-Use of Iron chelation therapy whether subcutaneous Desferoxamine and/or oral Chelators.

- Bone marrow transplantation (the only cure, but expensive and risky)**

However there are various issues including provision of adequate and safe transfusion (usually every few weeks throughout life), as well as cost and compliance with various chelation therapies. Moreover transfusion transmitted infections particularly viral hepatitis constitutes a major issue particularly in less developed countries.



Prognosis in thal major

- If no Transfusions, death usually occurs in the first few years of life.
- If the patient is transfused regularly but iron overload is allowed to occur then death in 2nd or early third decade, most commonly due to progressive cardiac damage due to iron deposition, with heart failure or arrhythmias, often precipitated by infections.
- However if transfused regularly with measures to prevent Iron overload by Iron Chelation are instituted early on, Iron overload consequences maybe limited, although delayed puberty and stunted growth may still be encountered, but otherwise patients may develop normally.



Alpha thalassaemias

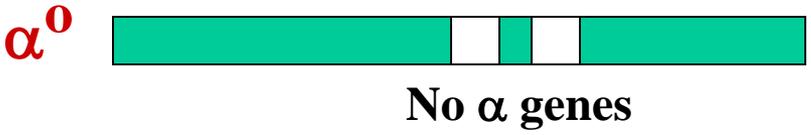
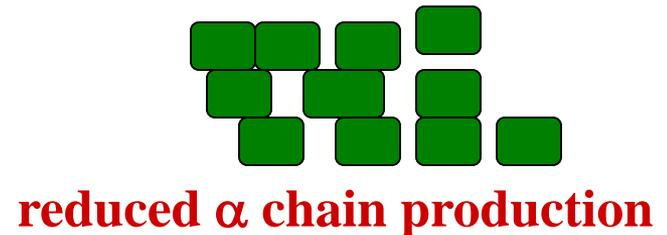
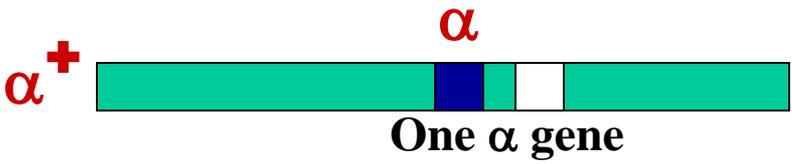
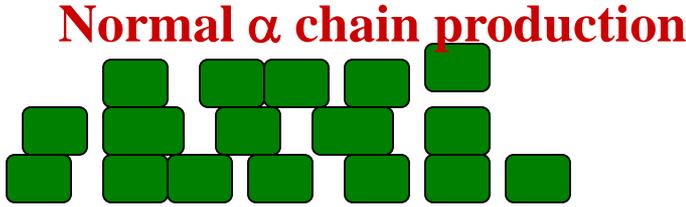
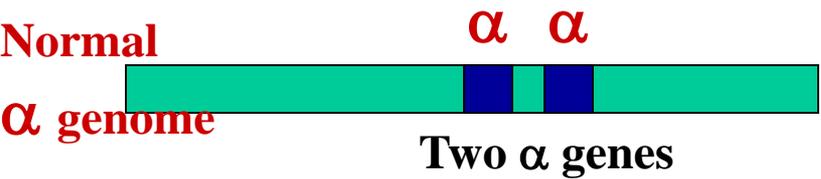
- **Due to reduced or absent synthesis of alpha (α) globin chains of hemoglobin (alpha (α) chains are constituents of all three normal Hbs A, A₂ and F)**



Genetics of Alpha thalassemias

- There are two α genes on each of chromosome 16, so there is a total of 4 α genes in the human genome.
- The defects leading to alpha thalassemias are usually *deletions* removing one or both alpha genes.

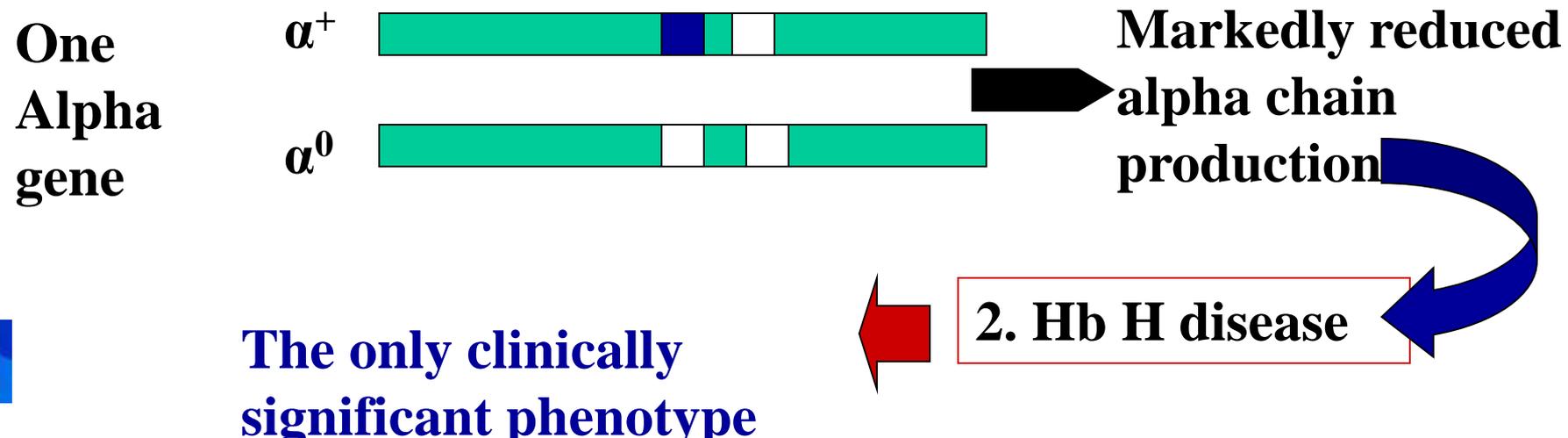
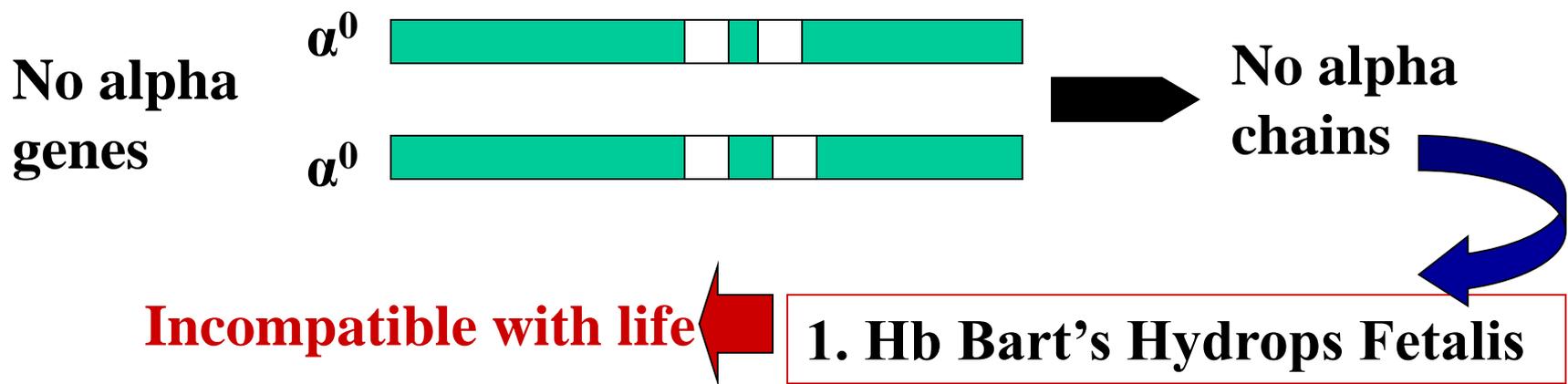
An over-Simplified diagrammatic representation of the thalassaemia genetic defects



No Alpha chain production

- Normal α gene
- Deleted α gene

Clinical Phenotypes of Alpha thalassaemia (relevant to number of alpha genes remaining):

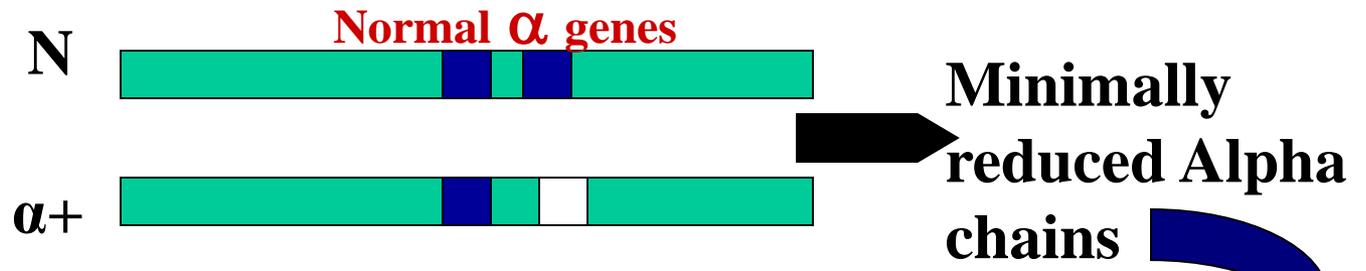


**Two
alpha
genes**



3. α Thalassemia minor

**Three
Alpha
genes**



4. Silent α thal. carrier state

Sickle cell Disorders



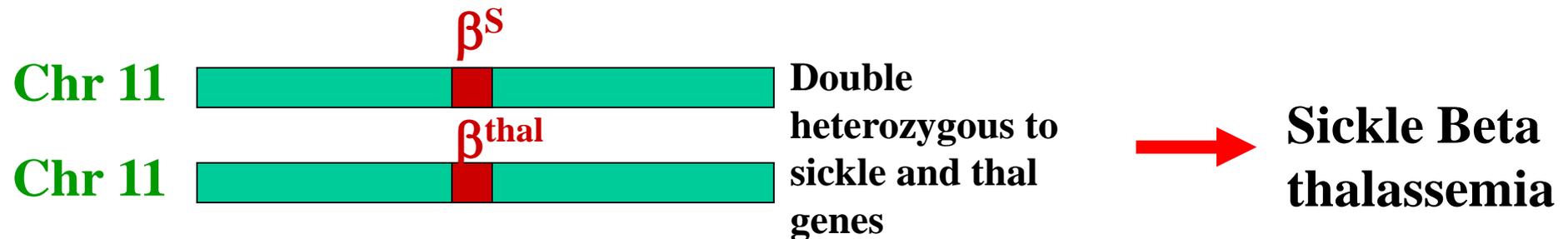
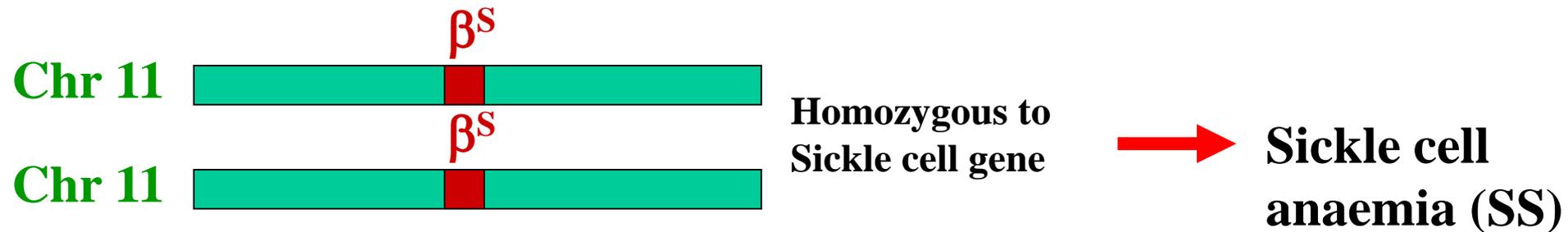
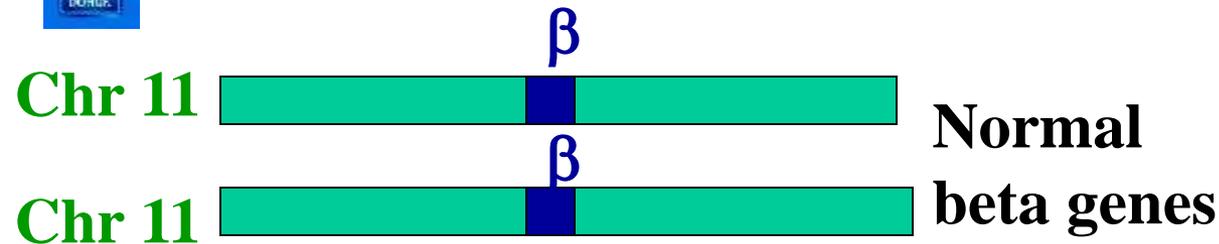
Definition of Sickling Disorders

- Disorders characterized by red cells which undergo sickling upon deoxygenation.
- Sickling is due presence in red cells of **Hemoglobin S** (Beta chain variant), due to a substitution of the amino acid (Glutamic acid) position 6 of the beta chain by Valine
- It is common in African populations and those of African origin, Indian subcontinent Arabian peninsula and southern Europe.



 **Defective β gene**

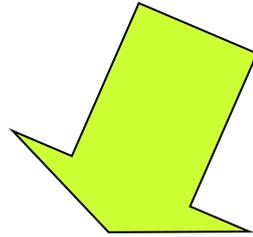
 **Normal β gene**





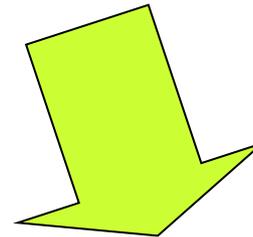
CLINICALLY sickle cell disorders could be classified into :

Sickle Cell Disorders



Symptomatic :

**Sickle cell
Disease**



Asymptomatic :

Sickle cell trait

Sickle cell Disease (SCD):

- A group of inherited disorders, characterized by sickling upon deoxygenation, with prominent clinical manifestations.
- It includes :
 1. Sickle cell anaemia (SCA): Homozygosity to Sickle cell gene (SS)
 2. Sickle cell/ β -thalassemia
 3. Sickle cell/ α thalassaemia
 4. Sickle/Hb C disease.
 5. Others.
- Sickle cell disease does not include Sickle cell trait (SA), since it is asymptomatic.

Pathophysiology of Sickle cell disease:

- 1. Vaso-occlusion:

The relative rigidity of sickle cells and their aggregation particularly in microvasculature, leads to vascular stasis, blockage of small vessels and tissue infarction.

- 2. Chronic Hemolytic anemia.

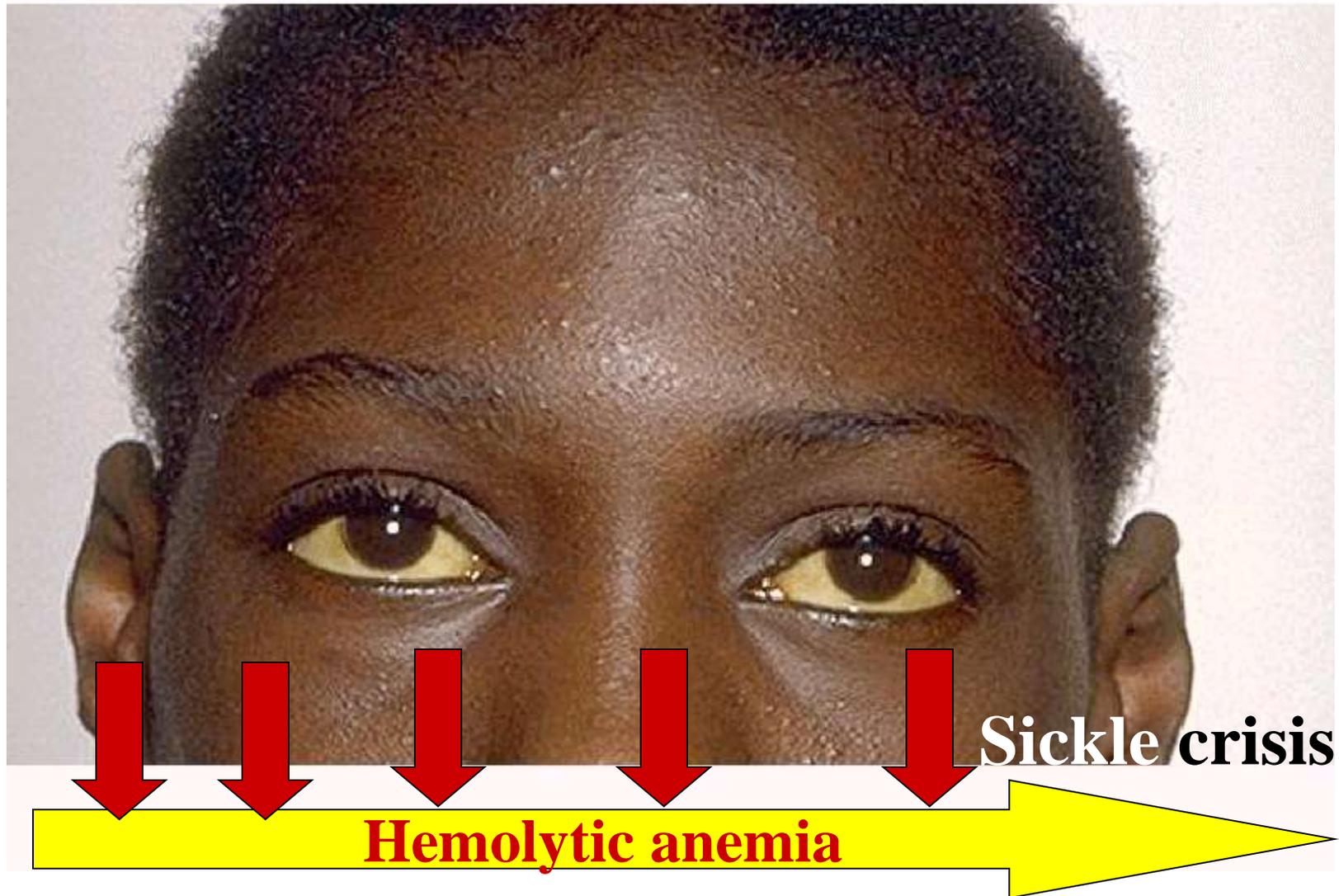
Sickled cells have increased mechanical fragility and hence shortened survival.

- 3. Propensity to infections.

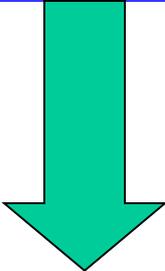
Clinical features of SCD :

- Extremely variable, features will not be apparent until the age of 4-6 months, and is characterized by variable degree of hemolytic anaemia (anemia and jaundice), extenuated by episodes of sickle cell crisis.
- Sickle cell crisis is any new syndrome developing rapidly in a patient with sickle cell disease due to the inherited abnormality. And they include :

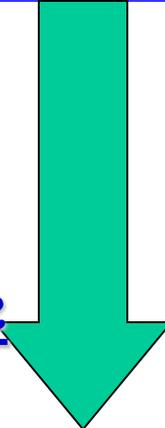
Sickle cell anemia with mild jaundice



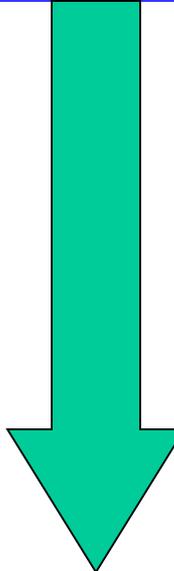
SICKLE CELL CRISES



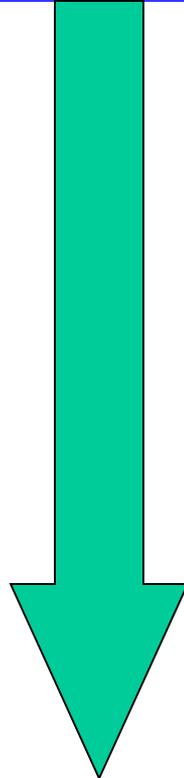
Vaso-Occlusive



Sequestration



APLASTIC



Hemolytic



1. VASO-OCCLUSIVE CRISES

Painful Bone Crisis

Most common of VOC;
1st manifest. Is usually
Hand-foot syndrome;
shift from the peripheral
to central skeleton with
age.

Acute Chest syndrome

Pul. Infarction; pneumonitis,
common cause of death.

Acute Abdomen

Mesenteric sickling

Acute Brain syndrome

Stroke, maybe fatal

Priapism

Painful persistent
penile erection

Management of Sickle cell disease:

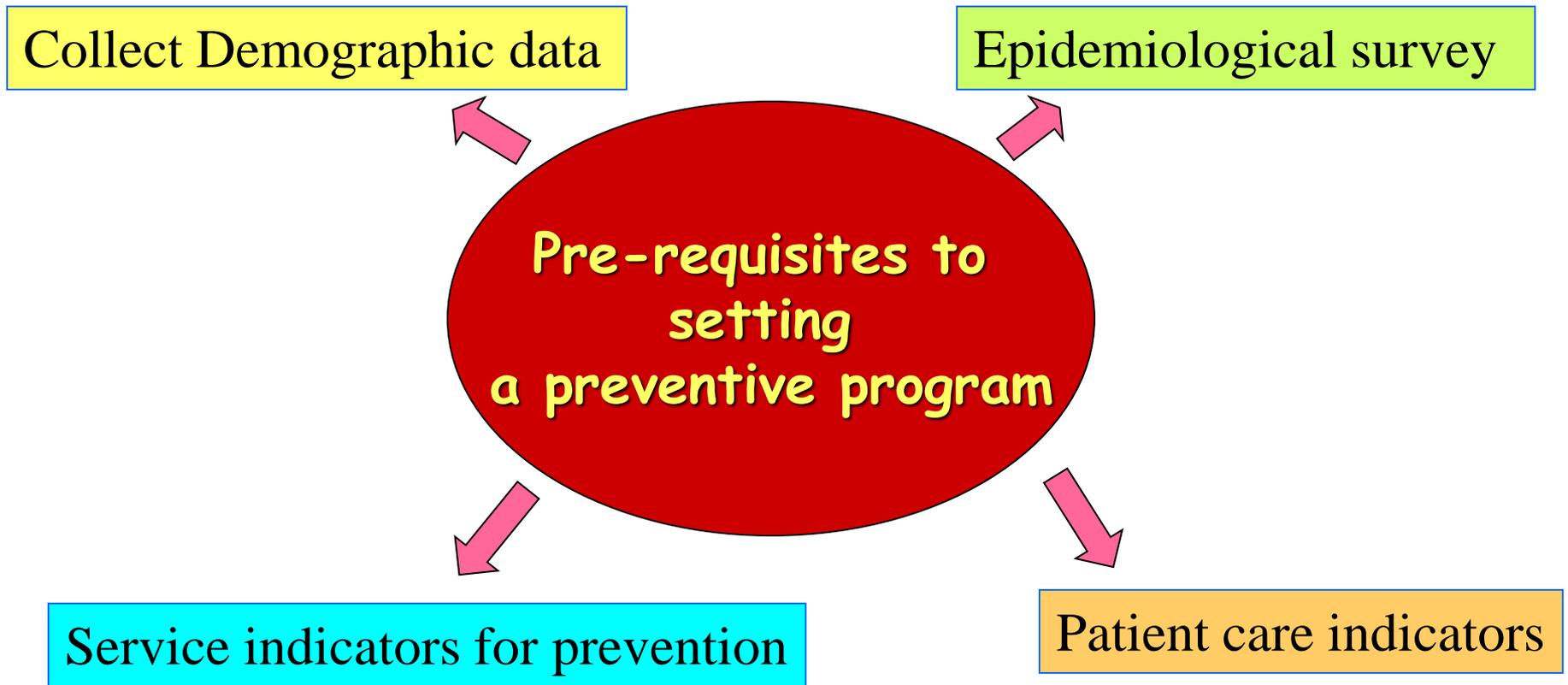
- Early Diagnosis (neonatal screen practiced in some countries)
- Prompt management of vaso-occlusive crises.
- Early diagnosis and prompt treatment of other crises and infections.
- Transfusion therapy.
- Bone marrow transplantation.

Course and Prognosis of SCD:

- High mortality in first few years, (especially in under-developed countries), is due to pneumonia and meningitis, splenic sequestration, Later on , infection is still most frequent cause of death, brain syndrome follows.
- Most patients however survive well into adult life.

PREVENTION PROGRAM OF Hemoglobinopathies

- 1. Pre-requisites for the program**
- 2. The Setting of the Program**
- 3. Health Education**



1. DEMOGRAPHIC DATA

- Population size **Based on a recent census**
- Population characteristics
- Crude Birth rate
- Infant mortality rate
- Consanguinity rate.

2. Epidemiological survey

To establish the spectrum and frequency of hemoglobinopathies in a given population, and for beta thalassaemia determining the molecular basis.

- **Selection of samples :**
 - **Size.**
 - **Representation.**
 - **Suggested sample groups:**
 - **Premarital screen**
 - **School children**
 - **Army recruits**
 - **Blood donors**



3. Service indicators for Prevention

- Indicators for carrier screening per year.
- Indicator for carrier information and offer of partner testing.
- Indicator for expert risk assessment and genetic counseling.
- Indicator for number of prenatal diagnosis.

4. Service indicators for patient's care

- Total number of major Hbpathies per country/region
- Annual homozygous birth rates
- Geographical and ethnic distribution
- Patient age distribution



Practical Settings Hbpathy Screening Program

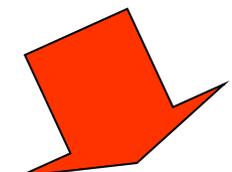
- To show how a Hbpathy screening program is organized we will describe how such a program could be set in a population with polymorphic frequencies of beta thalassemia and Sickle cell disorders, and we will focus as an example on the widely adopted **Premarital based screening program.**

The Prevention Scheme

- **First step - Premarital Screen** : to identify couples at risk of bearing affected children



Second Step - Genetic Counseling : to allow the couples at risk to take an informed decision.



Third step - Prenatal Diagnosis : to detect any affected fetus in early gestation in couples at risk and allow the partners the choice of termination.



Principles of Screening

- The diagnosis of beta thal minor (carrier state) could be based on reduced red cell indices (MCV and /or MCH) and increased Hb A₂.
- The diagnosis of Sickle cell trait could be achieved based on normal red cell indices with a positive sickling test and Hb AS pattern on Hb electrophoresis.
- Both partners should be carriers for them to **be at risk of bearing affected children** with a major Hemoglobinopathy. If only one of them is a carrier and the other is a non-carrier, they are not at risk.



Primary screening

- Couples attending a premarital screening center, should be first screened for **reduced MCV (mean Cell volume) and MCH (mean cell Hemoglobin)** using an daily calibrated electronic Hematology analyzer. Manual methods are not suitable for this purpose.
- The cut off points are set as **< 27 pg for the MCH and < 80 fL for MCV**. It is believed that these cut off points would virtually pick all beta thal carriers*.



Primary screening (cont)

- Simultaneously perform solubility test or sickling test on the couple to screen for sickle cell carrier state.
- If MCV and MCH are both above the cut off points and sickling/solubility test is negative in one or both of the partners, then the couple are considered **NOT** to be at risk of bearing children with a major Hemoglobinopathy and no further testing is done.
- Please remember that for the couple to be at risk of giving birth to an affected child, **both should be carriers**. Therefore it may be sufficient first to test only one of them (preferably the male), and if he has reduced red cell indices and/or sickling is positive, then we would proceed to test the female partner. This approach would make the screening more cost-effective and will reduce the number of further unnecessary testing.



Secondary Screening

- If both partners have either reduced red cell indices or positive sickling test, then **further testing** is done.
- If it is the red cell indices that are reduced then further tests include Hb A2 estimation and Serum Iron/TIBC (or S. Ferritin).
- If it is the sickling test that is positive then we have to perform Hb electrophoresis (alkaline) to confirm the diagnosis.



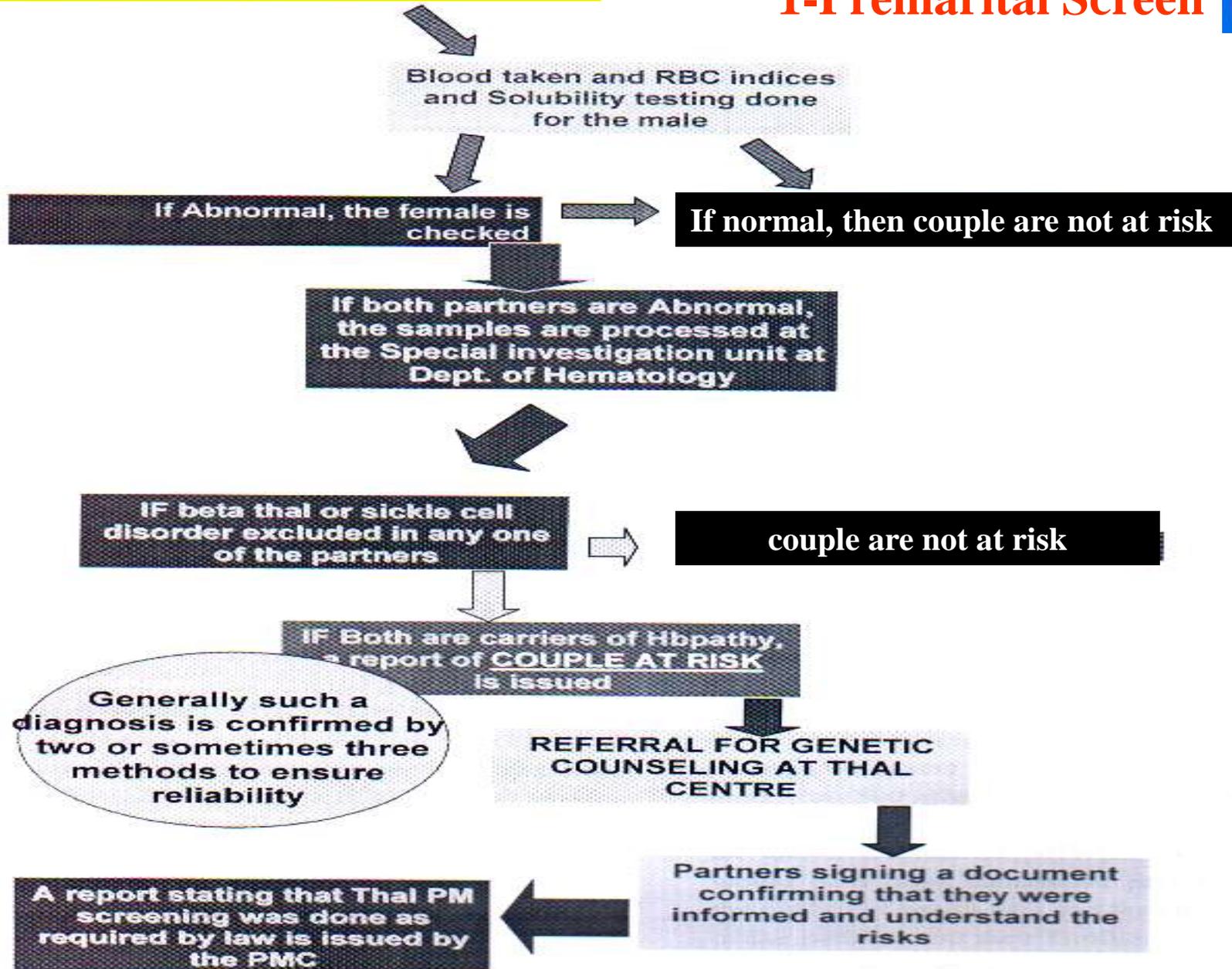
Secondary screening

- **If red cell indices are reduced and Hb A2 is increased $>3.5\%$ (NR 1.8-3.5%) then a diagnosis of β thal minor is given.**
- **If red cell indices are reduced and Hb A2 is $< 3.5\%$ and S. transferrin saturation $<15\%$ then a diagnosis of Iron deficiency is given.**
- **If red cell indices are reduced and S. transferrin saturation $>15\%$, then further testing for Hb F concentration is performed to exclude $\delta\gamma$ thal, and if Hb F is also normal, then the possibility of α thal minor is likely, although the remote possibility of β thal with normal Hb A2 may be considered. Further molecular studies are warranted in this situation.**



Methods for testing Hb A2

- Several Approved methods for Hb A2 estimation could be used in the context of the screening program including:
- **High pressure liquid chromatography (HPLC)** : Expensive but quite rapid and accurate, quite useful if large numbers of screened samples are to be handled.
- **Elution from cellulose acetate strips and Microcolumn chromatography**: accurate, much cheaper but labour intensive, so not suitable for high work loads.



A Flowchart showing the Steps of the premarital Hbpathy program

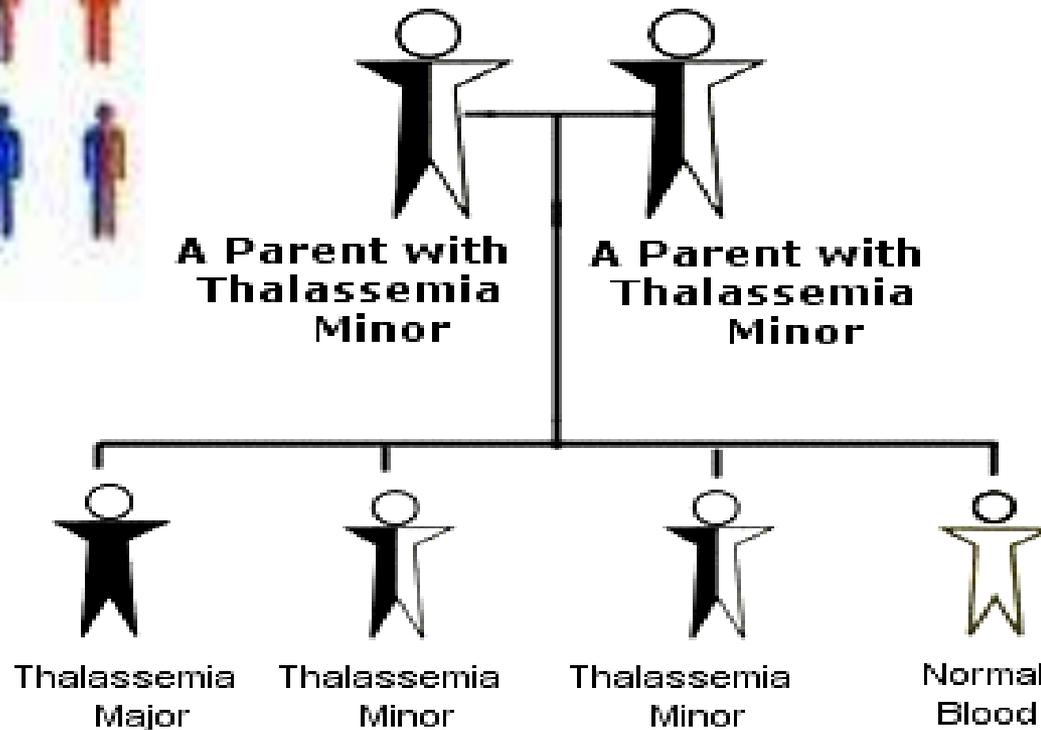
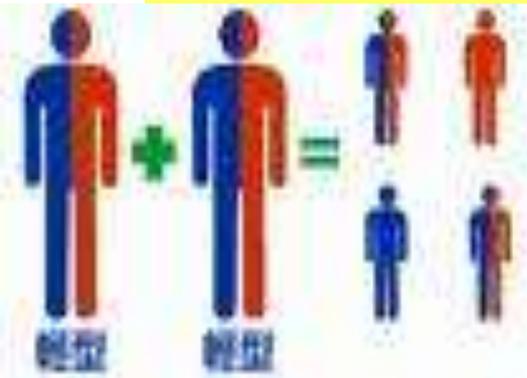
Other Screening programs

- Other than premarital screening, **antenatal screening** is also adopted in several countries, and follows the same general principles.
- An alternative to above screening programs, has been found to be affective, particularly where consanguinity is high, or the prevalence of thalassemia is low, and this **extended family screening (or inductive screening)**, in which on finding a patient or a carrier in a family, screening of other members of the family would help identify carriers and see whether any other couples are at risk*. This has been successfully applied in Pakistan and Sardinia.

The second Step : Genetic Counseling

- If the couple are at risk of a major Hemoglobinopathy as determined by screening (i.e. both are carriers), then they should be counseled accordingly.
- They would have a 1 in 4 risk of getting a child with a major hemoglobinopathy as with other autosomal recessive disorders.

IF BOTH PARTNERS ARE CARRIERS (THAL MINOR)



- **(1/4) are Homozygous (Thal Major)**
- **(1/4) are normal**
- **(1/2) are carriers (thal Minor)**

The genetic counselor

- The counselor should be extensively knowledgeable in hemoglobinopathies, and their care and complications, and it is best if he/she is medically qualified.
- She/he should ascertain that all relevant investigations are accurate and the couple are truly at risk.
- He/she should try to explain in plain words, the nature and prognosis of anticipated major hemoglobinopathy and its course and treatment options.
- She/he should explain the anticipated risk of getting an affected, unaffected or carrier child to the couple.
- She/he should Explain all the cons and pros of options available to the couple to allow them take the best informed decision possible.
- The decision on the option to be taken should solely rest with couple at risk. However the counselor should offer his support and assistance in whatever way possible.

So what are the couple's Options if they were found to at risk ?

** OPTION A :

- Either the couple will decide not to get married and find another partner. (???)
 - In Iran : 37 % did not proceed with the wedding, but the majority proceeded with the marriage*.
 - In Northern Iraq: 10% decided to separate.
 - In Cyprus : only 2% decided to separate, 98% proceeded with marriage.
- Dilemmas and complexities (Planned marriages!!!)

OPTION B

- Proceed to get married and then decide to have no children (maybe unrealistic), or limit the number of children to 2 only, and in this situation 56% of such marriages will not have an affected child.

OPTION C

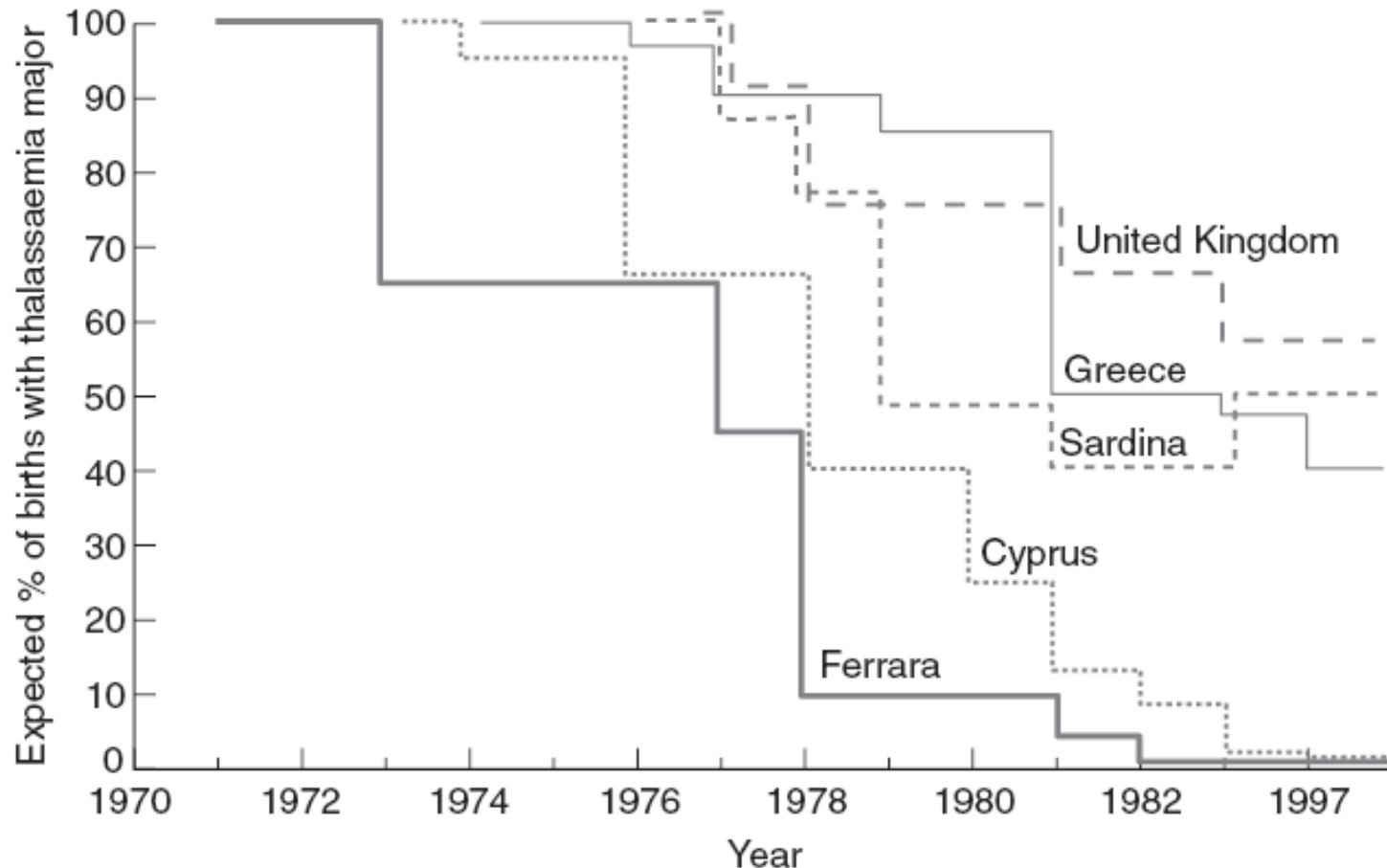
- Proceed to get married and then do prenatal DNA diagnosis early in each pregnancy, and if the child is affected then proceed to therapeutic abortion.



Opinion of Religion/law on abortion following confirmatory prenatal diagnosis

- From the religious point of view, several Islamic scholars allowed therapeutic abortion in early pregnancy, if there is a severe disease affecting the fetus.
- The same could be said about Christian authorities, and it is acceptable legally and ethically in Western countries and several Islamic countries to perform termination of pregnancy of affected children.

But does such a preventive approach really work??



The proportional decline in the birth of thalassaemia major in some countries applying the preventive program, including prenatal diagnosis, and shows clearly the value of this approach (Weatherall & Clegg 2001)

Another question, Is prevention cost effective??

- Studies from Cyprus revealed that the cost of eight weeks prevention program was equivalent to one week treatment of the thalassemic population.
- It was also demonstrated the cost incurred by terminating an affected pregnancy and subsequently having an unaffected child is 30% of the annual treatment cost and 2% of discounted lifetime cost of management of an affected child.

Angastiniotis et al, 1986;

Modell & Kuliev, 1991

A third question, Is prenatal diagnosis (PND) and subsequent termination justifiable in all Major Hemoglobinopathies?



- For β thalassemia major affected fetus, the prospect of life being only maintained by frequent transfusion and chelation or BMT, makes it justifiable to most to perform PND and termination.
- However the justification is not as clear cut for sickle cell disease, where the disease is not uniformly fatal in early life and with good management most will go well into adult life. Moreover the clinical course is unpredictable with a lot of modulating factors (some to be determined). However some countries practice prenatal diagnosis for Sickle cell disease.

A third question, Is prenatal diagnosis (PND) and subsequent termination justifiable in all Major Hemoglobinopathies?



- For a thalassemia, the situation is rather different. α^+ thalassemia although quite common, is relatively harmless, and only α^0 is important, because in homozygous state it will lead to Hb Barts hydrops Fetalis, and perinatal death, often with life threatening obstetric complications to mother. In the latter situation termination after PND maybe justifiable. However this is important only in the few areas of the world where α^0 defects are common.
- Where α^+ and α^0 are both prevalent, the chance of Hb H diseased fetus is a possibility but the relative moderate to mild clinical course of this disorder makes PND and termination less likely to be acceptable.

The Role of Health Education,

left to the last, but it is by no way the least!!

- This is probably one of the most important determinants for the success of the preventive program.
- It should include professional education, starting from undergraduates to doctors and nurses in practice.
- Public education, starting from school, to general media and certain target groups particularly the young generations.
- Seeking the support of policy makers, religious leaders and influential community figures through discussions and meetings tackling issues like the cost-effectiveness of prevention, the burden on the community and families, the religious precedents and ethical issues.



This Presentation was based on

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Thank you

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