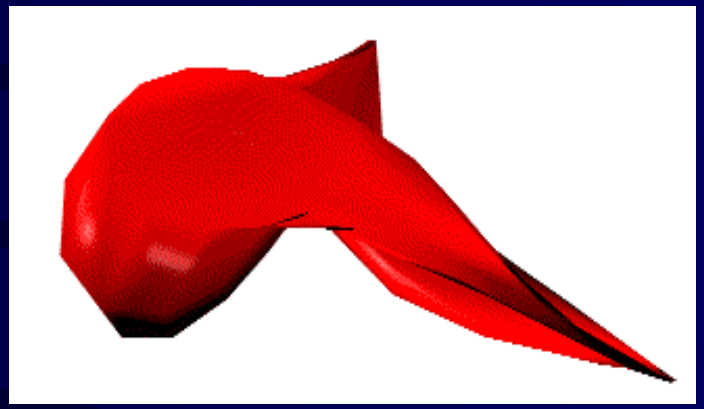
Hemoglobinopathies: molecular genetics and prenatal diagnosis



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Postgraduate Training in Reproductive Health Research

Faculty of Medicine, University of Yaoundé 2007

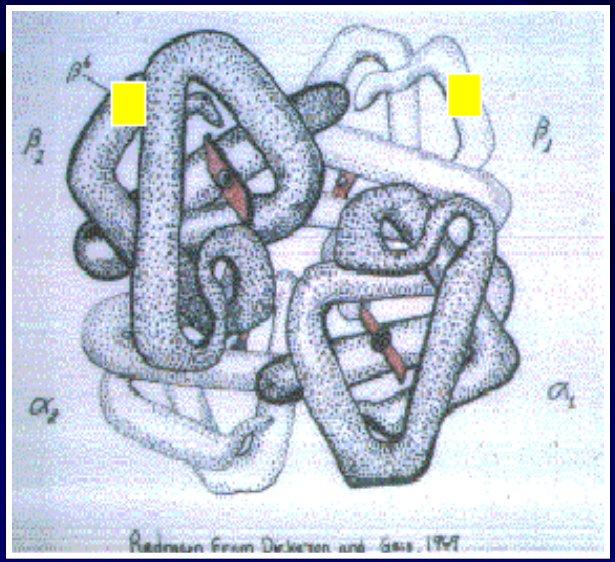
Introduction: The Hemoglobin Protein

Red blood cells:

Produced in the bone marrow

Non nucleated

70% hemoglobin



The Hemoglobin Protein:

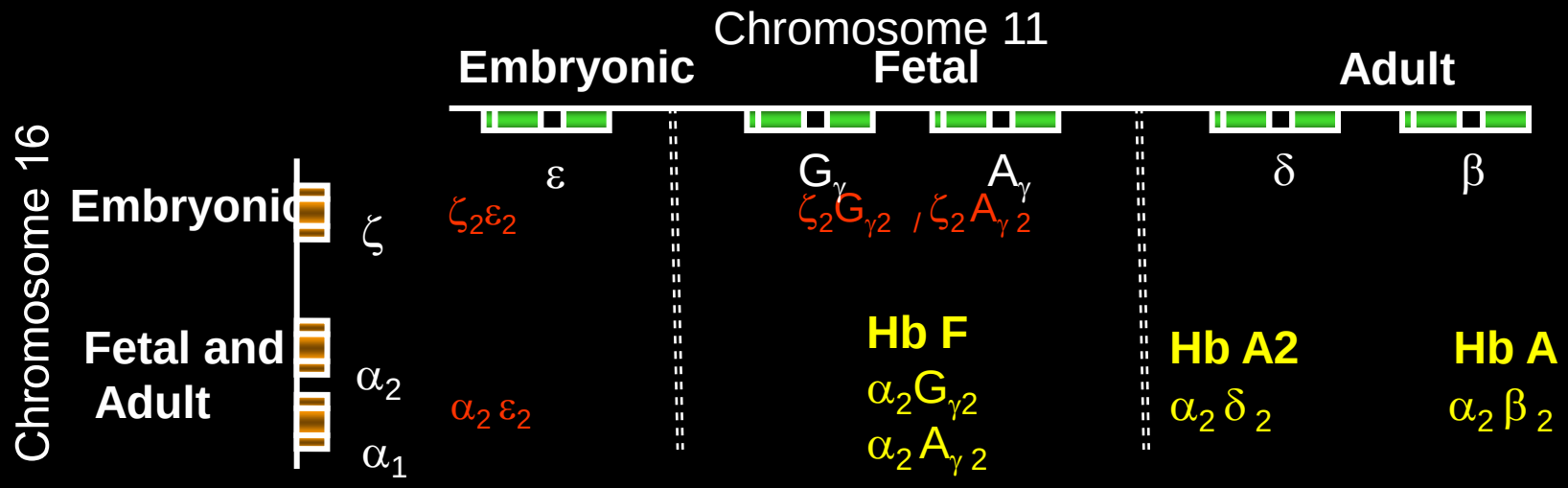
Tetramer: 2 α and 2 β chains

+ heme molecule,

In concert with iron binds O_2

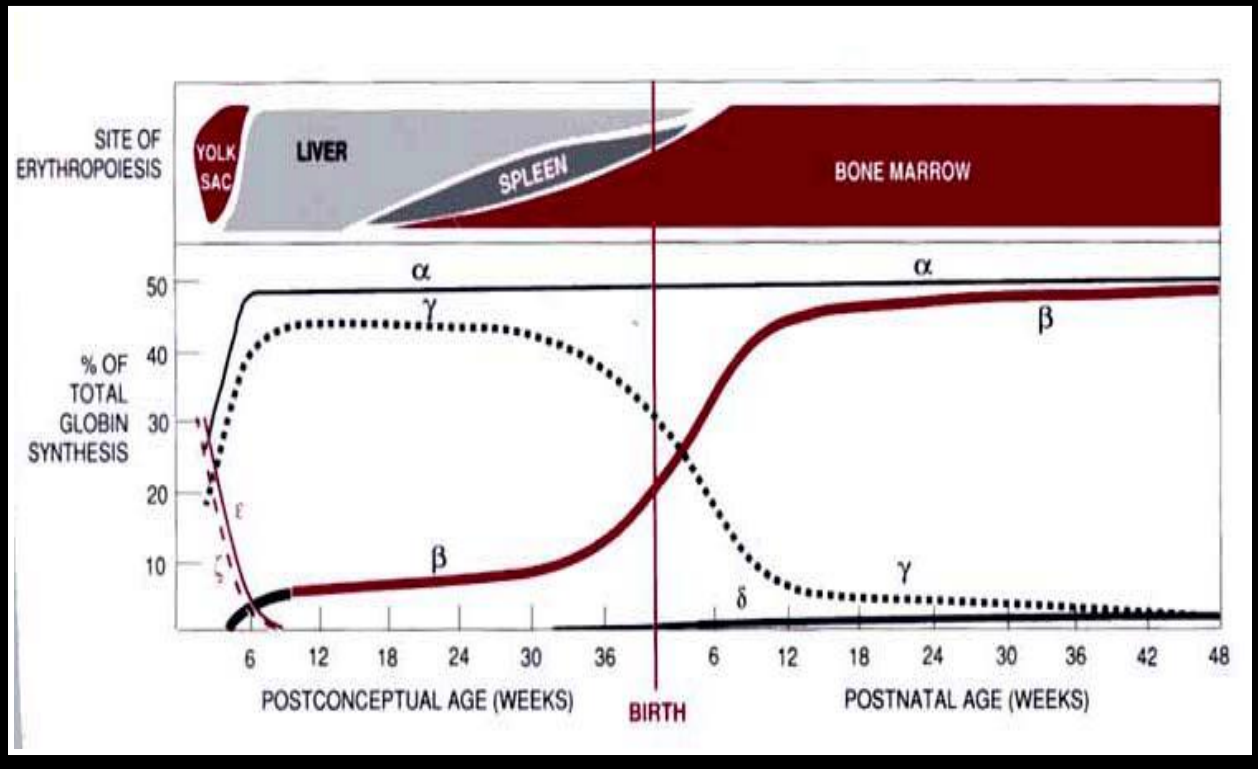
Introduction: The Hemoglobin gene

The possible tetrameric products of the α- and β-globin genes



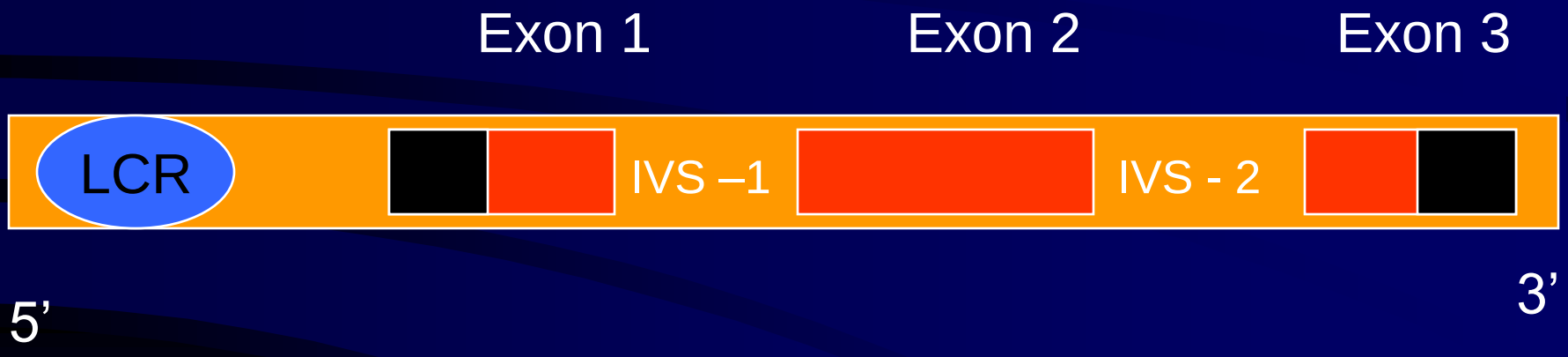
Introduction: The Hemoglobin gene

Developmental pattern of expression of human hemoglobin



97 % = Hb S
1 % = Hb F
2 % = Hb A_2

Introduction: β - gene



Introduction: Hemoglobinopathies

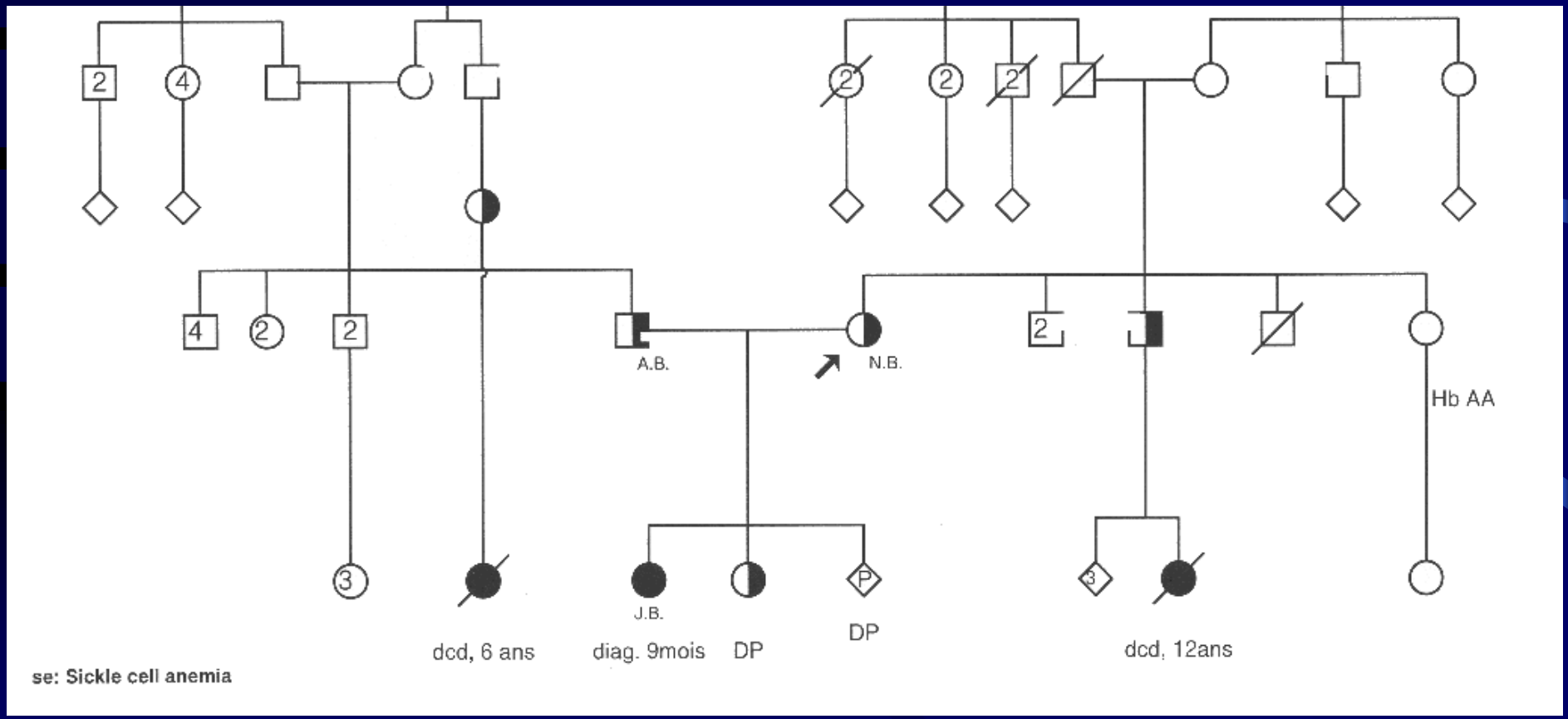
Two main types of Mutations :

Causing qualitative abnormalities: Sickle Cell Anemia

Causing quantitative abnormalities: Thalassemias

SCD:Definition

SCD is an autosomal recessive red blood cell disorder



se: Sickle cell anemia

SCD: epidemiology

- Incidence in the black population: 1/200-1/500
- Carrier frequency : from 8 to 25 %
- Homozygous SCD causes severe disease with shortening of life
- Heterozygotes are moderately protected from malaria parasite

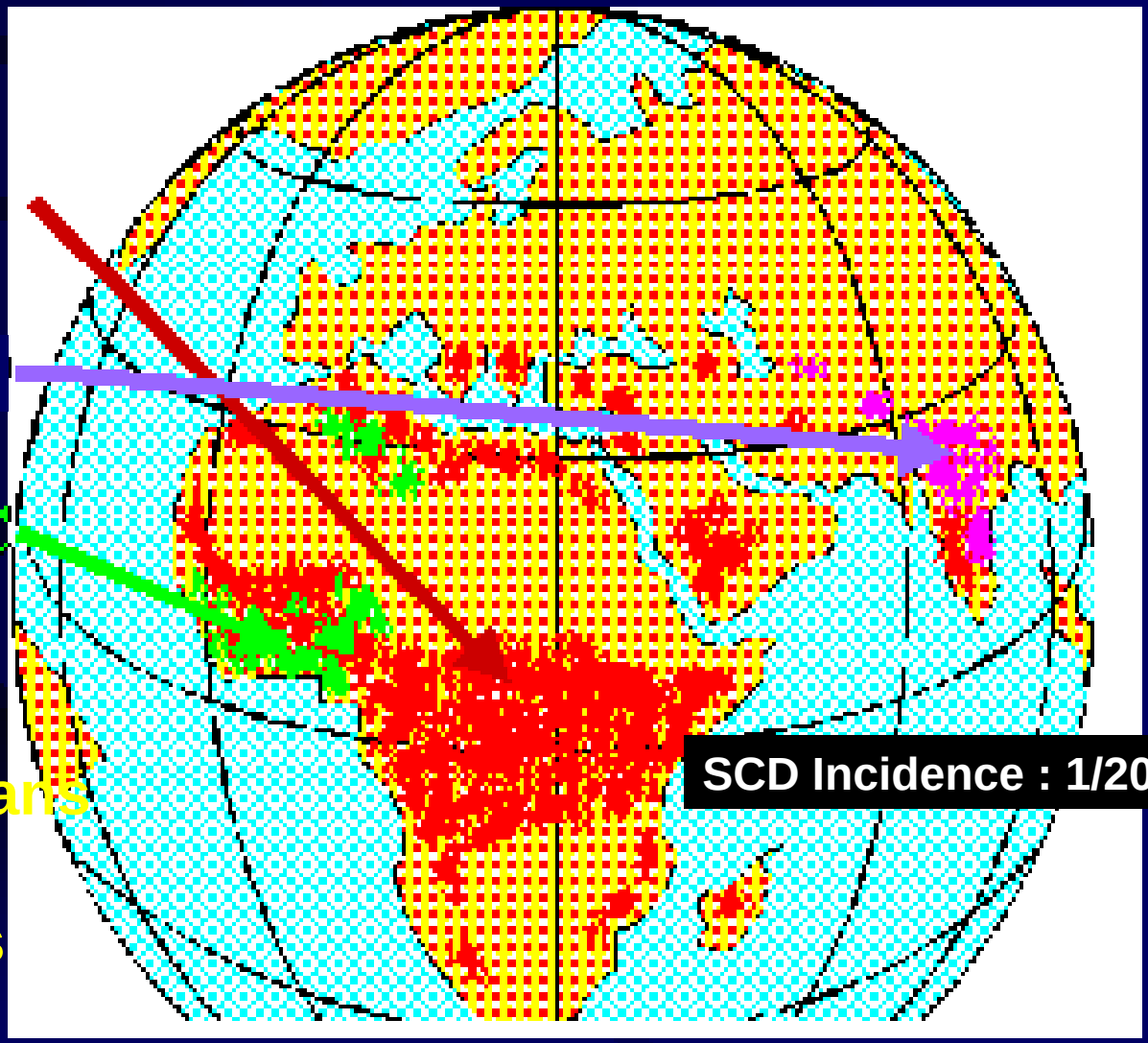
Moderate selection for heterozygotes has allowed the gene to reach its high frequency in area of the world were malaria has been endemic

Sickle cell disease: a worldwide distribution

Hemoglobin S

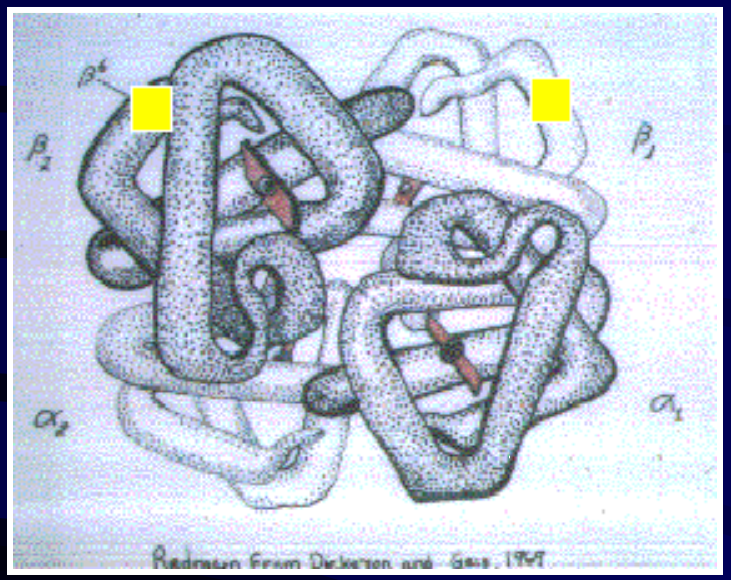
Hemoglobin C

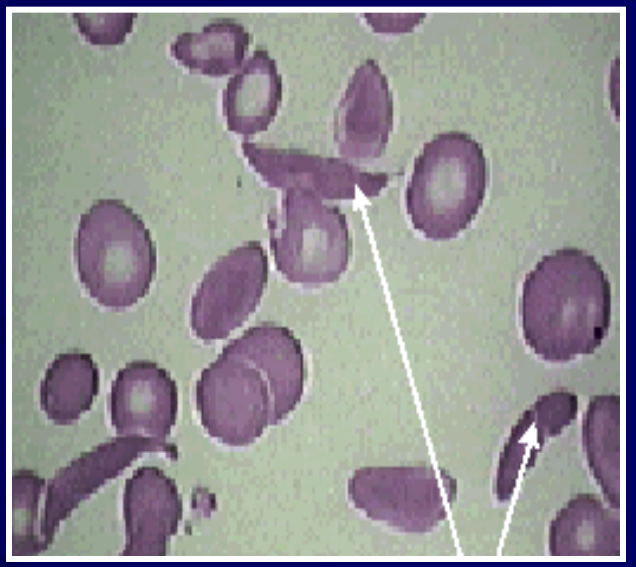
African
African Americans
Arabs
Greeks, Italians
India

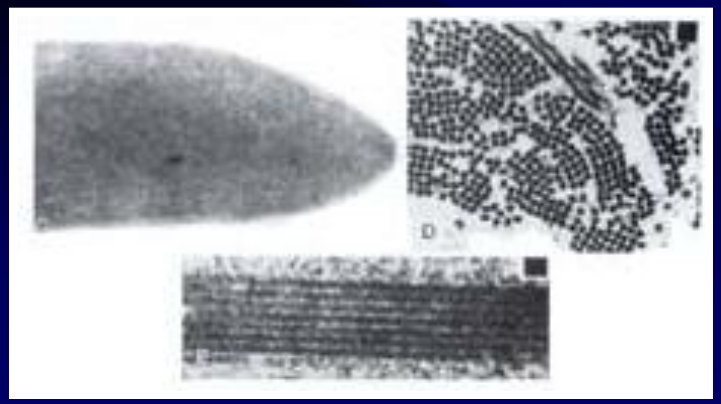


Molecular phathogenesis of Sickle cell anemia

Valine to glutamine replacement (position 6)



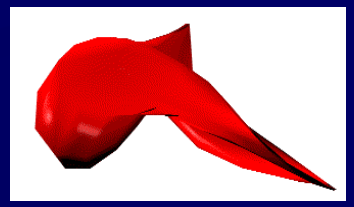




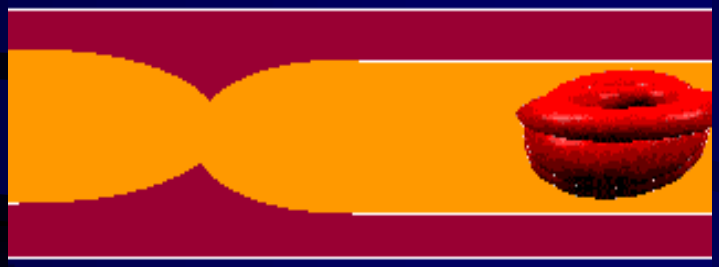
Deoxygeneted sickle hemoglobin crystallizes within the red cells

Leading to rigidity and inability to traverse small capillaries

Normal vs Sickle red cells



Disc-shape
Soft
Easily flow through
small blood vessels
Live for 120 days

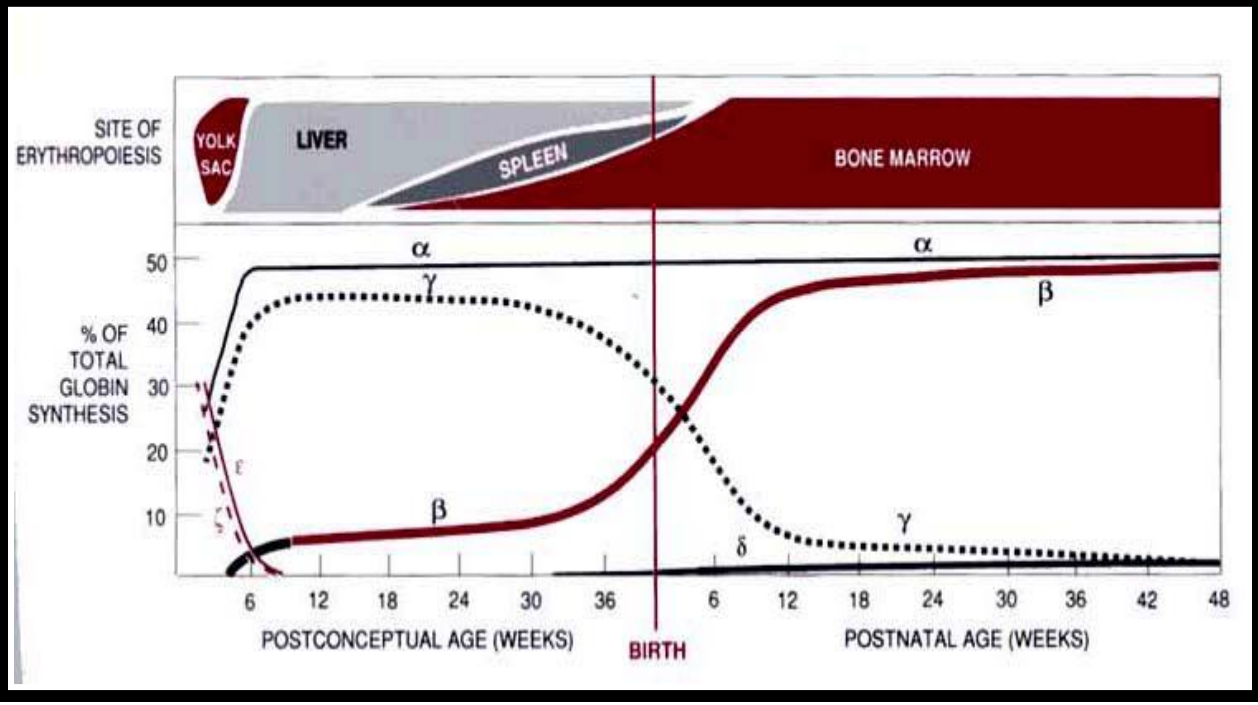


Sickle-shape
Hard
Often get stocked
in small vessels
Live for 20 days or less

- Anemia
- Pain episodes
- Stroke or brain damages
- Heart or kidney failure
- Increased infections

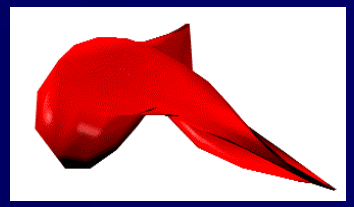
Molecular phathogenesis of Sickle cell anemia

Developmental pattern of expression of human hemoglobin



97 % = Hb S
1 % = Hb F
2 % = Hb A_2

Treatment of SCD



Classical treament:

1. Pain traitment
2. Antibiotherapy
3. Tranfusion
4. Rehydration

Emerging therapeutic agents:

1. Anti-adhesion
2. Hb F augmentation
3. Anti-oxydatitive therapy
4. Bone marrow transplantation
5. Gene therapy

Introduction: Hemoglobinopathies

Two main types of Mutations :

Causing qualitative abnormalities: Sickle Cell Anemia

Causing quantitative abnormalities: Thalassemias

Globin chain imbalance:
The thalassemias

Thalassemias are hereditary abnormalities of hemoglobin production in which the primary difficulty

Is a quantitative deficiency of :

Either β - globin, leading to β - thalassemia

Either α - globin, leading to α - thalassemia

The thalassemias are common not only in the mediterraneean area but also in parts of Africa and Southeast Asia

The distribution coincides with the frequency of malaria

Molecular phathogenesis of Thalassemia

Normal

Tetramers

αβ
βα

αβ
βα

αβ
βα

RBCs

α-thal

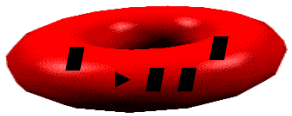
ββ
ββ

αβ
βα

β

ββ
ββ

ββ
ββ



Inclusion bodies of β_4 (Hb H)

β-thal

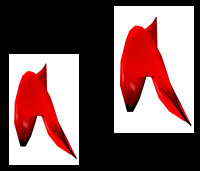
αβ
βα

αα
αα

α

αα
αα

αα
αα



Precipitation of α_4
Very insloluble
Destruction of RBCs in marrow, spleen

α - Thalassemia

Each chromosome 16 carries 2 functioning α-globin genes:
αα / αα

α-Thalassemias involve inactivation from 1 to all 4 genes

Wide range of severity :

α-/αα (Africa α-thal 2)
αα/- - : Southest asian α–thal 1;
α-/- - : Hb H disease
Moderate to marked anemia
Mean cell volume low

The most severe situation (--/--) (Southeast Asia)
Hydrops fetalis
Still birth or early neonatal death

β - Thalassemia

- In β-thal is the β-globin chains that are deficient
- Large number of mutations can result in decreased or absent function of β-globin gene
- Inherited as autosomal recesive
- Carrier: reduced RBC volume
  mild increase in Hb A_2 and F
- The possible phenotypes depend on the level of transcription

β – Thalassemia: phenotypes

β-thalassemia major : The Most severe; β^0 thal., No Hb A
Homozygous state of mutations preventing normal amount of β-globin protein

β-Thal minor : Heterozygous; are asymptomatic (1 normal globin gene)

β- Thalassemia intermedia: anemic and symptomatic but do not require transfusion

Usually not apparent at birth because the switch of fetal to adult hemoglobin is still incomplete and the deficiency of β-globin gene is not yet of consequence

β – Thalassemia: clinical manifestations

During the first year:

- Severe anemia
- Distortion of the bones of the face and skull
- Hepato-splenomegaly
- If not treated, death occurs in the first decade of life

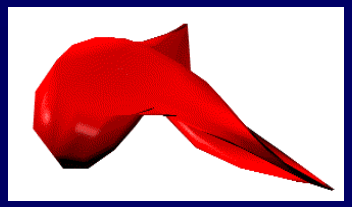
β – Thalassemia: treatment

Symptoms can be alleviated by blood transfusion

But, Total body level of iron rise continuously,
The iron deposits in heart, liver, pancreas and other organs leading to gradual failure of these organs

Bone marrow transplantation is potentially curative

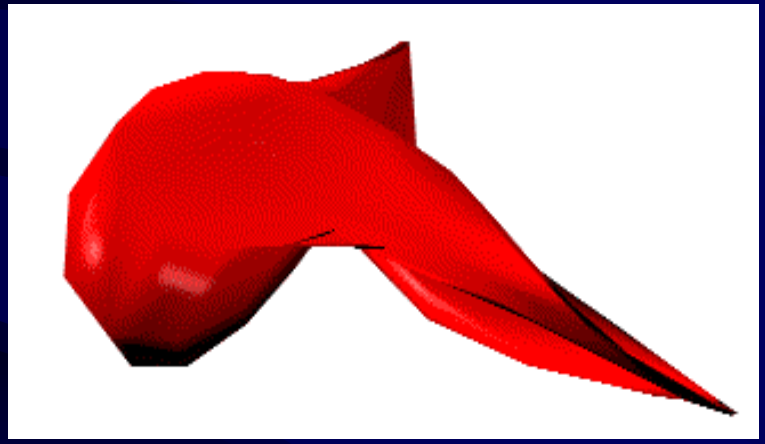
Hemoglobinopathies preventive approach



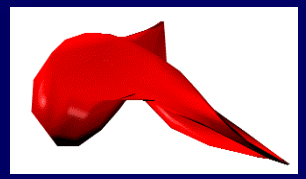
1 - Screening strategies

2 - Prenatal diagnosis

Sickle Cell Disease prenatal diagnosis



Prenatal diagnosis service



Acceptability

Cameroonians MD: 80 %

Wonkam et al. (2004)

Nigerians: 78 %
(15% Hb AS)

Durosinmi et al. *Afr j. med. Sc*. ,1997 26, 55-58

Jamaica: 90 %
(Hb As mothers)

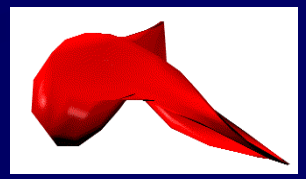
Jones et al. *W i med J*, 1988;37:12-15

Nigerians female SCD patients: 85 %
Mothers of SCD patients: 92 %
Fathers of SCD patients: 86 %

Durosinmi et al. *Soc. Sci. Med.* ,1995 41 (3) :433-436

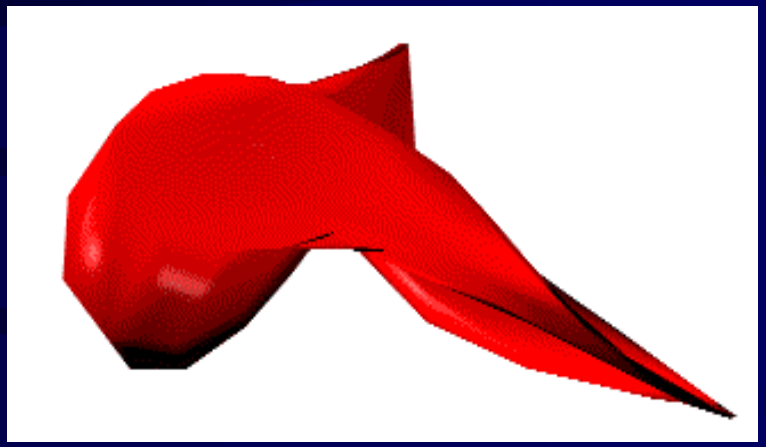
hemoglobinopathies are often the first conditions requiring set up of PND service (Alvan and Modell, *Nature genetics*; 2003)

Prenatal diagnosis service requirements



1. Genetic counselling service: risk assessment and free informed choice
2. Safe fetal sampling service
3. Molecular diagnosis labolatory

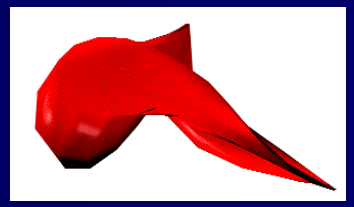
- Psycho-social support during and after medical abortion
- Hematologic and paediatric service: follow-up of babies
- Careful records



The Genetic counselling

Genetic councelling

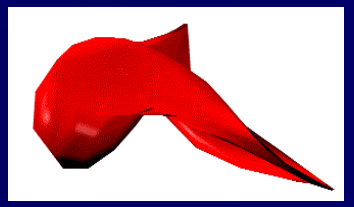
The core ethical principle



- The autonomy of the individual or couple: non directiveness
- Their right to full information
- The highest standard of confidentiality

Genetic counselling

Factors affecting the uptake of PND for SCD



20 years experience in UK : 2068 PND for Hb disorders:

National use of PND for SCD: 13 % (vs 50% for thal.)

Modell et al. *BMJ* 1997; 317:779-784

Couples at risk with pregnancy:

- 50 % requested prenatal diagnosis
- 82 % request in the first trimester
- 90% of couples already had an affected child

Petrou et al. *JMG* 1992; 29: 820-823

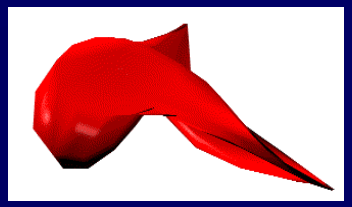
Cuba: 44% acceptability after recounselling (Dorticos-Balea et al.*prenat Diagn.* 1997; 17:737-42)

India: 91.2% couples with an affected child (Arora et al. *Natl Med J India*.2001; 14(6):340-2)

Geneva: 14/22 (63.6%) acceptability at the first opportunity (Wonkam et al., (2004))

Genetic counselling

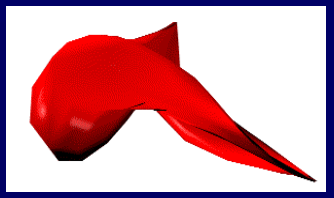
Factors affecting the uptake of PND for SCD



- Family history: a previously-affected child
- Time of referral: age of gestation
- Fear of abortion
- Socio - cultural
- Religious / Ethical

Importance of detecting and couselling prior to pregnancy

Genetic counselling



Theorical acceptability of TAP vs PND

Cameroonian MD :	35% (vs 80%)
Nigerians: (15% Hb AS)	45 % (vs 78 %)

Durosinmi et al. *Afr j. med. Sc.* ;1997, 26: 55-58

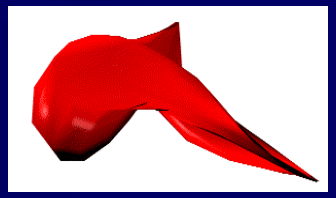
Jamaica:	46 % (vs 90 %)
(Hb As mothers)	

Jones et al. *W i med J*, 1988;37:12-15

Nigerians female SCD patients:	35%	(vs 85%)
Mothers of SCD patients:	63%	(vs 92 %)
Fathers of SCD patients:	51%	(vs 86 %)

Durosinmi et al. *Soc. Sci. Med*. ;1995, 41 (3) :433-436

Genetic counselling

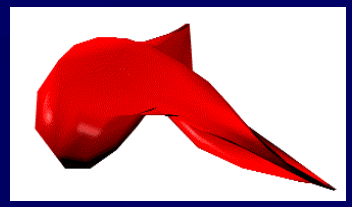


Practical Attitudes to medical abortion

Country	n	PND	% TAP	ref.
USA/Canada	1065		39	Rowley PT.*Ann NY Acad Sci*.1989;565:48-52
Nigeria	124		96	Akinyanju et al. *Prenat. Diagn*.1999; 19: 299-304
Geneva	30		57	Wonkam et al. 2004
USA	500		51	Wang et al. *Prenat diagn*. 1994;14(9):851-7

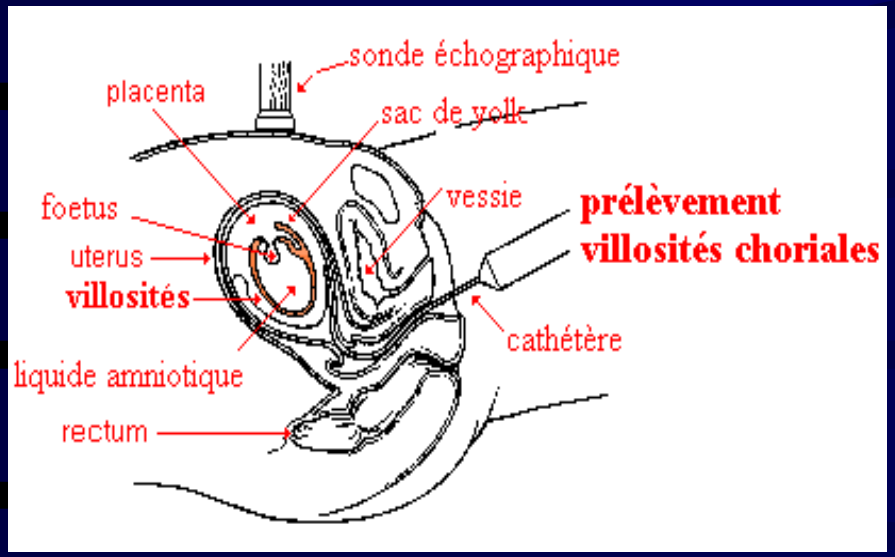
Critical factor affecting the mother decision:
Gestational age at the time of report (change-point: 20 WK)

Genetic counselling
The content

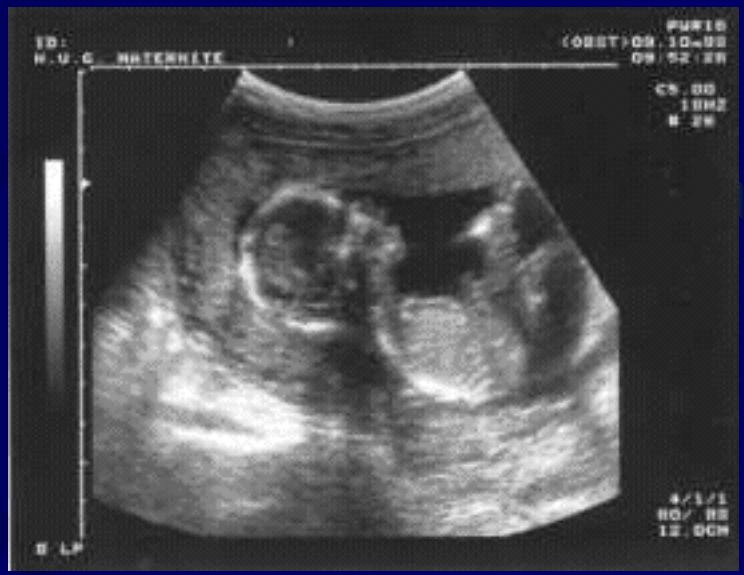


Medical geneticist
Genetic counsellors

- Accurate and comprehensive information: details of the SCD
- The risks of obstetric procedure
- The possibility of prenatal diagnosis (possibility of misdiagnosis)
- Attitudes to medical abortion
- Moral ethical and psychosocial problems are inevitable
- Written information for couple on risk and counseling choice

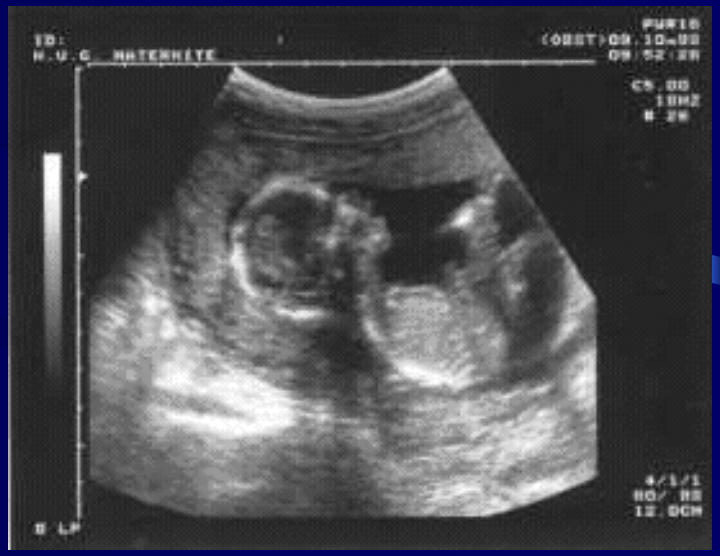


The fetal sampling



Fetal sampling requirement

- Obstetricians trained in fetal medicine (US and fetal sampling)
- US operator
- Nurses

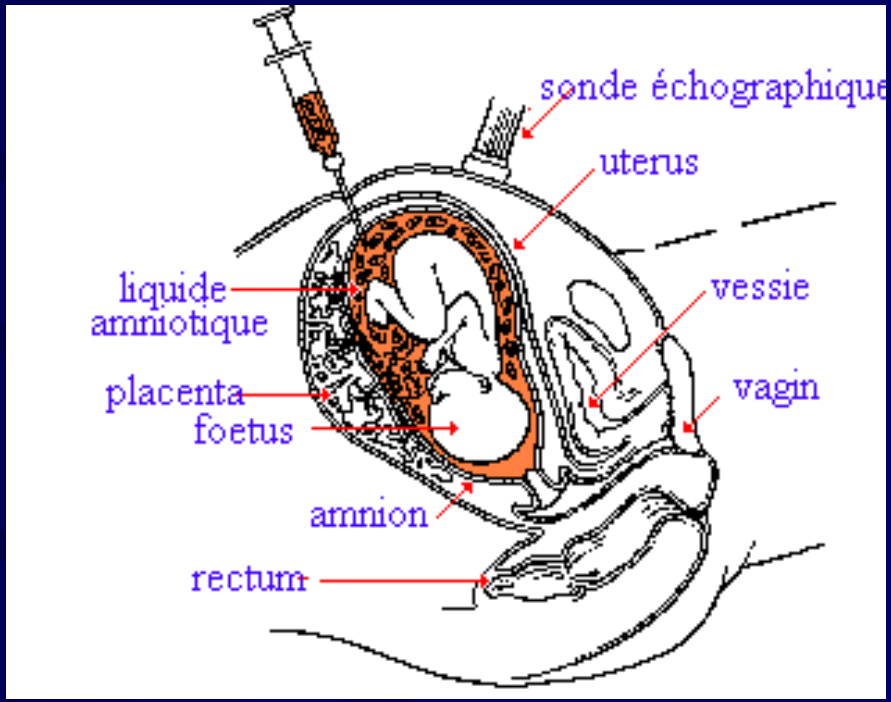


- High quality ultrasound equipement
- Sampling equipement (disposable and re-usable)
- Suitable sterile facilities for fetal sampling
- Suitable facilities for medical abortion in the 1st + 2nd trim.

Fetal sampling methods

Invasive:

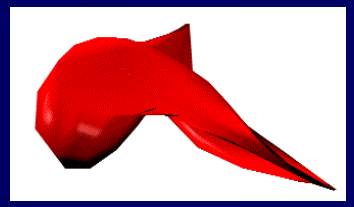
- CVS
- Amniocentesis
- Umbilical blood sampling



- Celocentesis (7-8 WK): ultrasound-guide aspiration of fluid from the extra-amniotic cavity

Makrydimas et al. *Ultrasound Obstet gynecol* 2004 ; 23 (5): 482-5

Fetal sampling methods

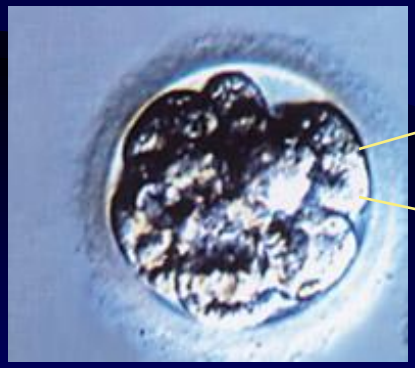


Non invasive:

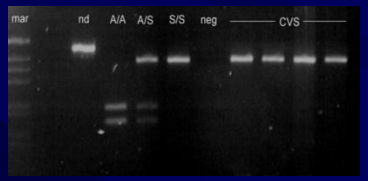
- Detection of fetal cell in maternal blood
- Fetal cell in transcervical sample

- Preimplantation genetic diagnosis

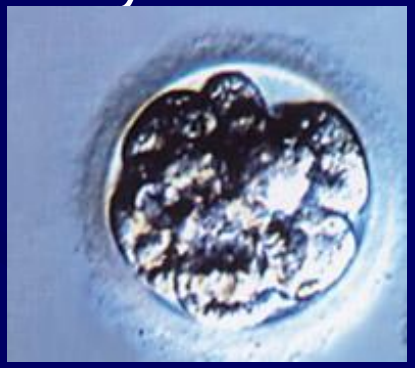
FIV/ ICSI



Single cell PCR



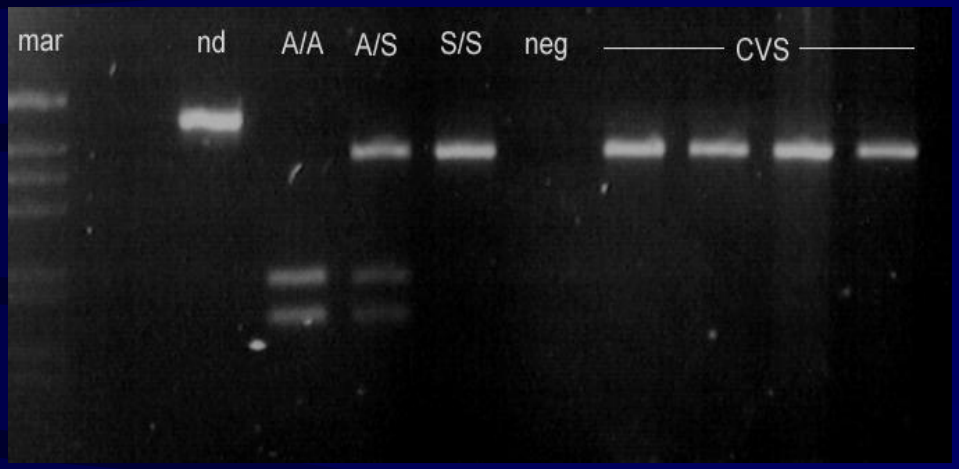
EmbryoTransfer



Chamayou et al. *Hum reprod* , 2002 may 17 (5):1158-65

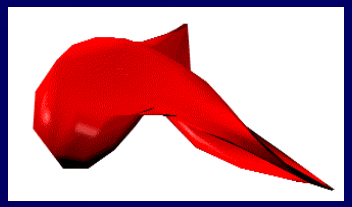
Merchand FA and Castleman KR. *Hum reprod update*. 2002 nov-dec 8 (6):509-21

Adinolfi M et Sherlock J., *J hum Genet*. 2001; 46 (3): 99-104



The molecular diagnosis

Molecular diagnosis requirements



1978: First DNA diag. By Kan and Dozy for SCD

Kan and Dozy. *Lancet ;*1978, ii:910-912

3254 PND in UK

808 homozygous (24.8%)

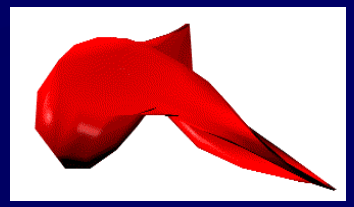
- Fetal blood analysis (error rate 1.55%)
- Southern blot (error rate 0.73%)
- PCR (error rate 0.1%)

Old et al. *Prenat diagn*; 2000; 20: 886-991

Source of errors:

1. Incorrect diagnosis of the parents
2. Contamination of fetal sample with maternal tissue
3. Mixing up samples, technical errors and misinterpretation
4. Non-paternity

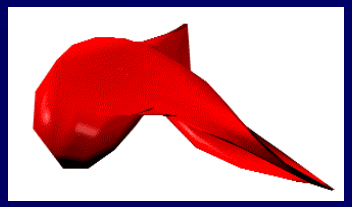
Molecular Diagnosis requirements (cont'd)



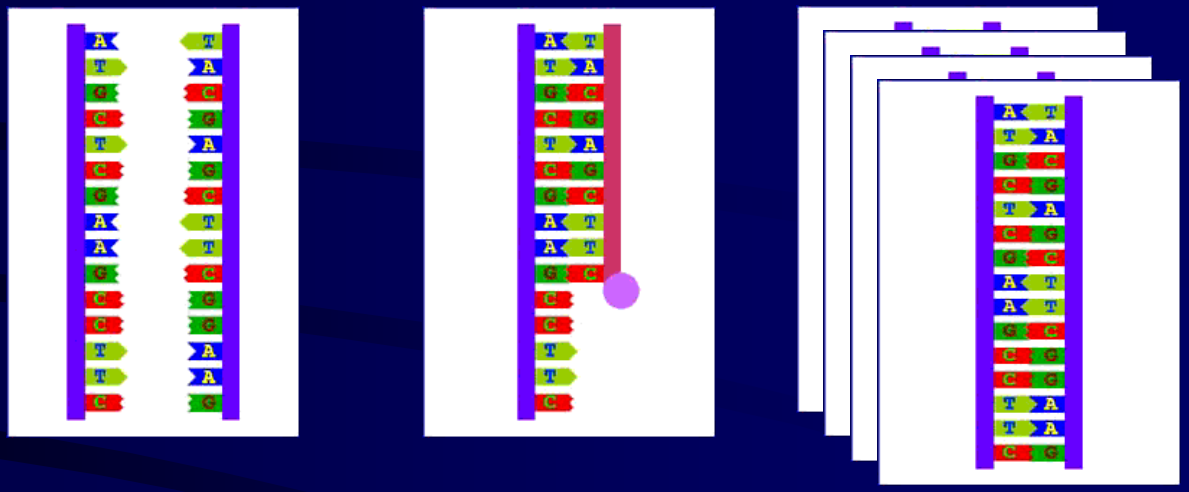
- Should be centralised in expert centres
- Minimum of 200 diagnoses / year
- A molecular geneticist
- Technicians
- Appropriate equiment
- Should be started using DNA and automated PCR

Molecular Diagnosis

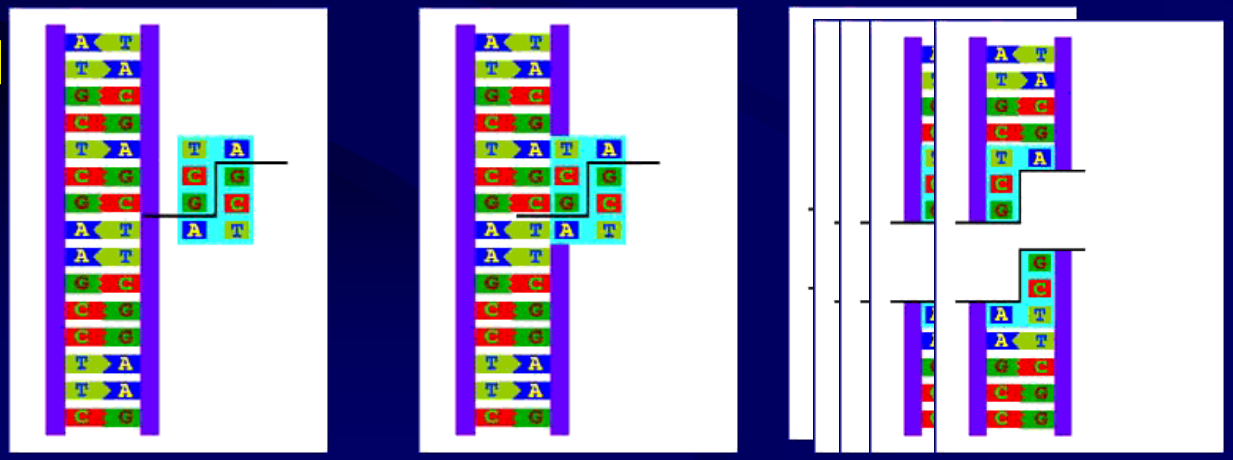
RFLP- PCR



PCR

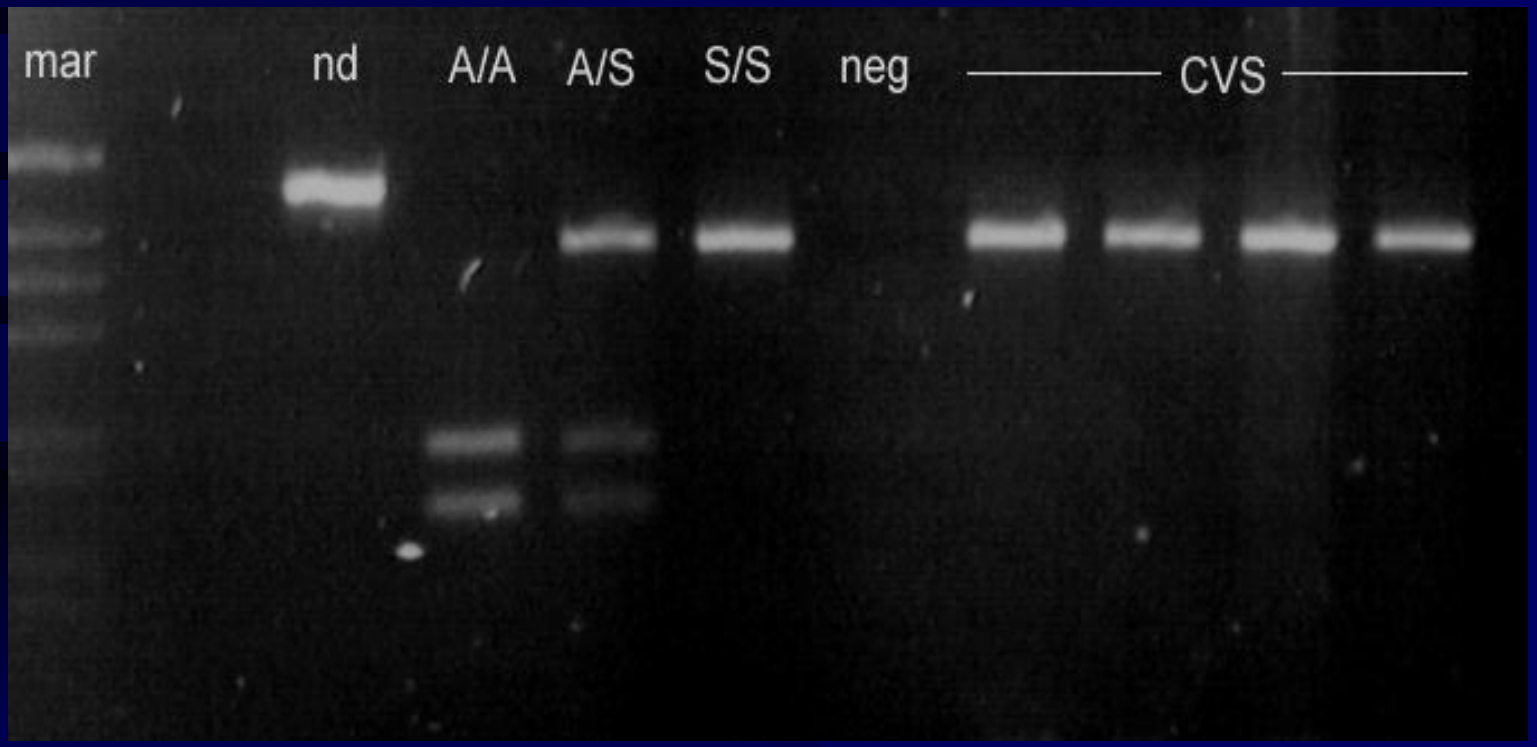


RESTRICTION ENZYMES

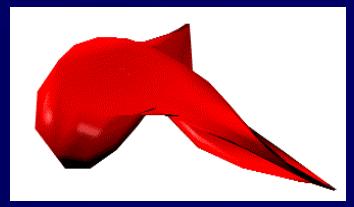


Molecular Diagnosis

RFLP- PCR



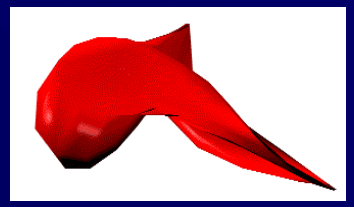
Controlling SCD



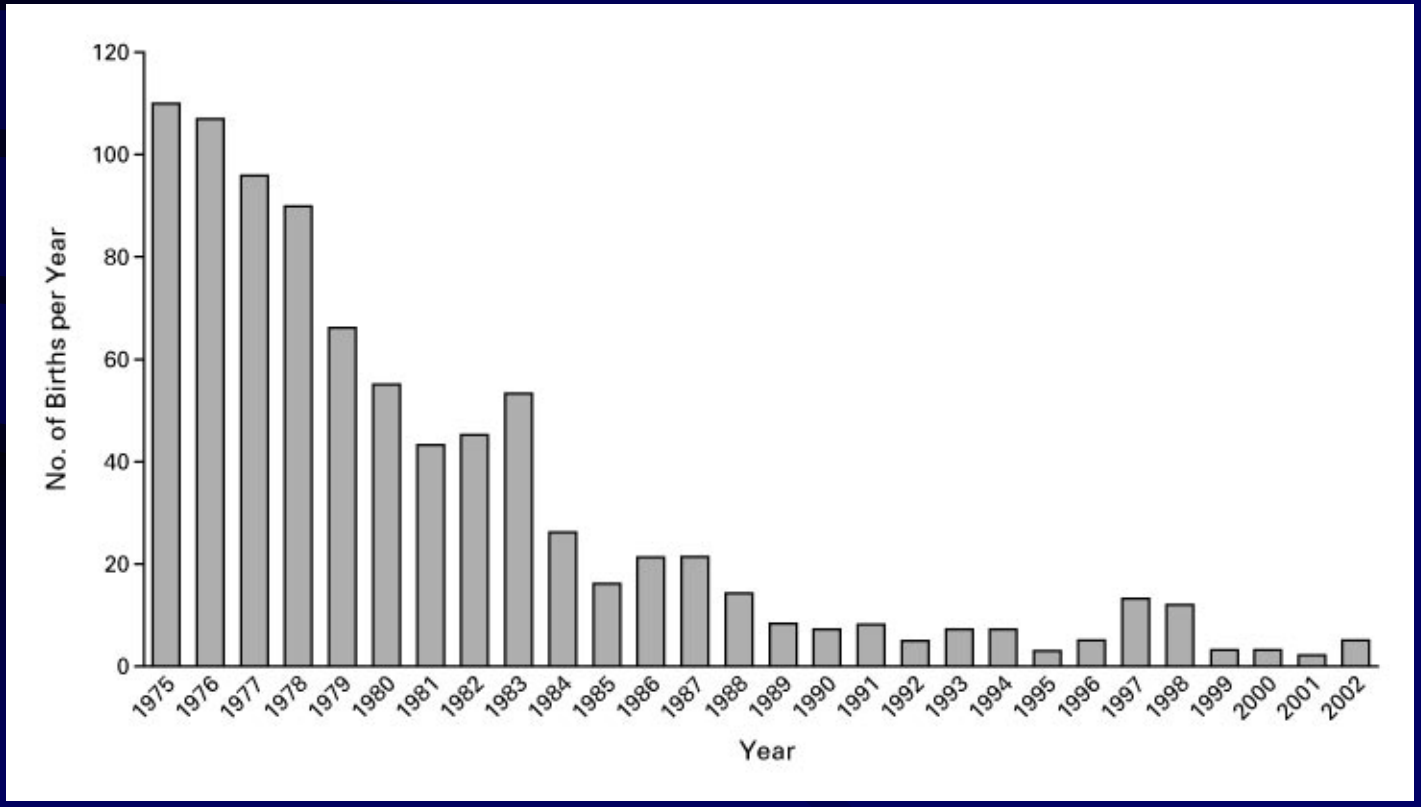
Screening

Controlling SCD

Screening and genetic diagnosis



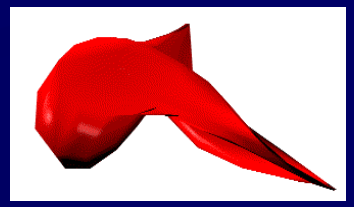
Declining Rate of Birth of Homozygous for Thal. in Sardinia



Cao A. and Galanello R., *N Eng J Med*, 2002 ; 47(15):1200-03

Controlling SCD

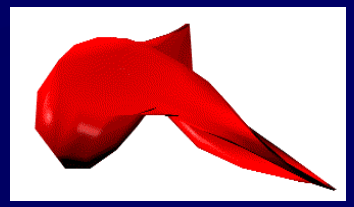
Screening Strategy



1- Family-centred approach: couples at risk
and « retrospective » genetic counselling

2- Population screening : « prospective » carrier diagnosis
- Antenatal
- Premarital
- Community
- High school
- Neonatal

Conclusions



- Consultants from « at-risk areas » should be proposed genetic counselling for hemoglobinopathies, ideally before pregnancy.
- PND is always a couple’s (and in the end the pregnant woman’s) free choice
- Information should be neutral, complete and updated about all available options
- The ethical aspects must be addressed thoroughly
- Each case is an individual one !