Hemoglobinopathies: molecular genetics and prenatal diagnosis

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Introduction: The Hemoglobin Protein

Red blood cells:
Produced in the bone marrow
Non nucleated
70% hemoglobin

The Hemoglobin Protein:
Tetramer: 2 $\alpha$ and 2 $\beta$ chains
+ heme molecule,
In concert with iron binds $O_2$
Introduction: The Hemoglobin gene

The possible tetrameric products of the $\alpha$- and $\beta$-globin genes

- **Embryonic**
  - $\zeta_2\varepsilon_2$

- **Chromosome 11 Fetal**
  - $\zeta_2\gamma_2 / \zeta_2\alpha_2$
  - $\alpha_2 \varepsilon_2$
  - $\alpha_2 \gamma_2$
  - $\alpha_2 \alpha_2$
  - Hb F
  - Hb A2

- **Adult**
  - $\delta$
  - $\beta$
  - Hb A

Chromosome 16
Introduction: The Hemoglobin gene

Developmental pattern of expression of human hemoglobin

97% = Hb A
1% = Hb F
2% = Hb A₂
Introduction: $\beta$ - gene

Exon 1  Exon 2  Exon 3

LCR  IVS –1  IVS - 2

5'  3'
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.
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

SCD Incidence: 1/200-1/500
Molecular pathogenesis of Sickle cell anemia

Valine to glutamine replacement (position 6)

Deoxygenetted 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

no oxygen = damage and pain

- Anemia
- Pain episodes
- Stroke or brain damages
- Heart or kidney failure
- Increased infections
Molecular pathogenesis of Sickle cell anemia

Developmental pattern of expression of human hemoglobin

97 % = Hb A
1 % = Hb F
2 % = Hb A₂
Treatment of SCD

Classical treatment:
1. Pain treatment
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 $\beta$-globin, leading to $\beta$-thalassemia
- Either $\alpha$-globin, leading to $\alpha$-thalassemia

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

The distribution coincides with the frequency of malaria.
Molecular pathogenesis of Thalassemia

Normal RBCs

α-thal

β-thal

Precipitation of α₄
Very insoluble
Destruction of RBCs in marrow, spleen

Inclusion bodies of β₄ (Hb H)
\(\alpha\) - Thalassemia

Each chromosome 16 carries 2 functioning \(\alpha\)-globin genes:

\(\alpha\alpha / \alpha\alpha\)

\(\alpha\)-Thalassemias involve inactivation from 1 to all 4 genes

Wide range of severity:

\(\alpha-/\alpha\alpha\) (Africa \(\alpha\)-thal 2)
\(\alpha\alpha/-\) : Southeast asian \(\alpha\)-thal 1;
\(\alpha-/\) : Hb H disease

Moderate to marked anemia
Mean cell volume low

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

Carrier: reduced RBC volume
mild increase in Hb A₂ and F

The possible phenotypes depend on the level of transcription
β – Thalassemia: phenotypes

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

Nigerians: 78 %
(15% Hb AS)

Jamaica: 90 %
(Hb As mothers)

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

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


Cuba: 44% acceptability after recounselling

(Dorticos-Balea et al. *prenat Diagn.* 1997; 17:737-42)

India: 91.2% couples with an affected child


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 counselling prior to pregnancy
Genetic counselling

Theoretical acceptability of TAP vs PND

Cameroonian MD: 35% (vs 80%)

Nigerians: 45% (vs 78%)
(15% Hb AS)


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


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

# Genetic counselling

## Practical Attitudes to medical abortion

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<th>Country</th>
<th>n</th>
<th>PND</th>
<th>% TAP</th>
<th>ref.</th>
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<td>USA/Canada</td>
<td>1065</td>
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<td>39</td>
<td>Rowley PT. Ann NY Acad Sci. 1989; 565:48-52</td>
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<td>Geneva</td>
<td>30</td>
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<td>57</td>
<td>Wonkam et al. 2004</td>
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<td>USA</td>
<td>500</td>
<td></td>
<td>51</td>
<td>Wang et al. Prenat diagn. 1994; 14(9):851-7</td>
</tr>
</tbody>
</table>

**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 equipment
- Sampling equipment (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

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

The molecular diagnosis
Molecular diagnosis requirements

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


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 diag; 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 equipment
- 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

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!