Haemoglobinopathies

Due to resistance against malaria, carrier states of hereditary haemoglobinopathies are the most common monogeneic disorders in the world.

WHO has calculated, that about 7 per cent of the world's population carry a haemoglobinopathy gene.

Each year 300,000 - 400,000 children are born with a serious haemoglobin disorder.

Center for Haemoglobin Disorders at Herlev Hospital

Field of activity:
1. Diagnostic
2. Prenatal diagnosis
3. Genetic Counselling
4. Advising and education of health care persons
5. Treatment of patients with haemoglobinopathies
Hemoglobin
Hæmoglobinopathies

**Kvalitative mutations:**
Mutations leading to a change in an amino acid (missense):

Examples: Hb S, Hb C, Hb D, Hb Volga, Hb Philadelphia + approx. 600 others

**Kvantitative mutations:**
Mutations leading to reduced synthesis of globin chains (deletions, nonsense, frameshift, promoter mutations and mutations involving splicing.

Examples: α-thalassæmia, β-thalassæmia, δβ-thalassæmia, Hb Lepore, Hb E
Geographic Distribution of the Haemoglobinopathies

HB, 2007
Geographic Distribution of the Haemoglobinopathies

Migration

thalassaemia
sickle cell anaemia
Hb C
Hb D
Hb E

HB, 2007
Geographic origin of immigrants to the Nordic countries during the recent decade

In the period 1995-1999, the net annual immigration to

Norway: 18,000
Denmark: 16,000
Sweden: 53,000
Finland: 10,000
β-Thalassemia
Screening for Haemoglobinopathies
Screening for Haemoglobinopathies

Haemoglobinopathy

Svangreomsorg
Retningstlinier og redegørelse
Sundhedsvesens indsats i forbindelse med graviditer, fødsel og barselperioder

1998
Ny i 2008

HB, 2007
### Diagnosis

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β-thalassaemia:</strong></td>
<td>Homozygous β-thalassaemia, β-thalassaemia/ haemoglobin E disease</td>
</tr>
<tr>
<td><strong>α-thalassaemia:</strong></td>
<td>Homozygous α⁰-thalassaemia</td>
</tr>
<tr>
<td><strong>Sickle cell disease:</strong></td>
<td>Hb SS disease, Hb S/β-thalassaemia</td>
</tr>
</tbody>
</table>

**HB,2002**
Beta-Thalassemia major

- Tillväxtretardation, muskelatrofi, fettreduktion
- Hyperplastisk expanderande märg
  - Facies thalassemica.
  - Psudotumörer i rörben o kotpelare.
  - Generell osteoporos - patolog. frakturer
- Ökad plasmavolym pga
  extramedullär hematopoes och hepatosplenomegali
- Hypersplenism (hemolys, leukopeni, trombocytopeni)
- Gallsten
- Ökad järnresorption i tarmen
β-thalassaemia major
β-thalassaemia major

Modell & Berdoukas 1984
### α-Thalassemia

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>α+, silent carr.</th>
<th>α+, homozyg.</th>
<th>α₀, trait</th>
<th>HbH (β₄)</th>
<th>Hb Bart (χ₄)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Synonym</strong></td>
<td></td>
<td>α-thal. 2</td>
<td>α-thal. 1</td>
<td>α-thal. 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fung. Gener</strong></td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>α/β-ratio</strong></td>
<td>1.0</td>
<td>0.8</td>
<td>0.6</td>
<td>0.6</td>
<td>0.3</td>
<td>0</td>
</tr>
<tr>
<td><strong>Genotyp</strong></td>
<td>αα/αα</td>
<td>-α/αα</td>
<td>-α/-α</td>
<td>--/αα</td>
<td>--/-α</td>
<td>--/--</td>
</tr>
<tr>
<td><strong>MCV</strong></td>
<td>90 +/-5</td>
<td>81 +/-7</td>
<td>69 +/-4</td>
<td>69 +/-4</td>
<td>65 +/-7</td>
<td>110/120</td>
</tr>
<tr>
<td><strong>Hb</strong></td>
<td>u.a.</td>
<td>u.a.</td>
<td>mild anemi</td>
<td>mild anemi</td>
<td>mod/svår hemolytisk anemi, splenomegali, akut hemolytisk kris</td>
<td>död in utero/neonat.</td>
</tr>
</tbody>
</table>
Sickle cell anemia
Screening for haemoglobinopathies

Screening of pregnant immigrants:

All from the Mediterranean countries, Iran and Iraq with MCV < 78 fl.

All from Africa, including afroamericans, the Middle East, the Indian subcontinent, Southeast Asia and Polynesia.
Distribution of mean cellular volume in carriers for β-thalassaemia in Denmark (n=485) and α^0-thalassaemia (n=110)
Relationship between MCV and MCH in carriers of $\beta$-thalassaemia (+), $\alpha^+$-thalassaemia (°) or $\alpha^0$-thalassaemia (•)
Screening for Haemoglobinopathies

The frequency of serious beta-globin alleles among pregnant immigrants in Copenhagen County

1995: 2,8 % (14/205)
1996: 4,2 % (25/578)
1998: 4,2 % (16/381)

HB,2007
Mutations in β-thalassaemia

Promoter mutations ($\beta^+$)

- CACCC
- CCAAT
- TATA

5' UTR

5' Promoter

Mutations affecting transcription ($\beta^+$)

Mutations affecting initiation of translation ($\beta^0$)

Mutations affecting splicing of RNA from introns ($\beta^0$ and $\beta^+$)

- IVS I
- IVS II

Nonsense and frameshift mutations ($\beta^0$)

- 30
- 30(3)
- 104

Mutations affecting RNA cleavage ($\beta^+$)

- 105
- 146

3' UTR

3' Promoter

850 bp

130 bp
$\alpha$-thalassaemia deletions

[$\zeta$ $\psi\zeta\psi$ $\psi\alpha$ $\alpha_2$ $\alpha_1$ $\theta_1$]

**Geografi**

- $\alpha^{3.7}$ Iran, Irak, Indien, Sydøstasien, Indonesien, Afrika
- $\alpha^{4.2}$
- Middelhavet
- MED
- SEA
- Sydøstasien
- $-(\alpha)^{20.5}$ Middelhavet
- FIL
- THAI
- Sydøstasien
Haemoglobinopathy

Diagnostic Laboratory Methods:

**Screening:**
- High-pressure liquid chromatography (HPLC)
- Isoelectric focusing

**Unknown mutations in the β-globin gene:**
- PCR followed by sequencing
- Denaturing gradient gel electrophoresis (DGGE)

**Mutations in the α-globin gene:**
- Deletions: GAP-PCR (multiplex)
- Small mutations: PCR followed by sequencing
Haemoglobinopathies

HPLC

- Normal
- Heterozygot β-thalassæmi
- Heterozygot HbS
- Haemoglobin Lepore
Haemoglobinopathies

Isoelectric focusing
A new thalassaemic nonsense mutation in the b-globin gene (codon 37; tgg > tag) in an Afghanistani family

α₀-Thalassæmi PCR multiplex

Lane 1  - (α)²₀,₅ / - (α)²₀,₅
Lane 2  αα/αα
Lane 3  - (α)²₀,₅ / - MED
Lane 4  --SEA / --SEA
Lane 5  αα/ - (α)²₀,₅
Lane 6  αα/ --MED
Lane 7  αα/ --SEA
Lane 8  αα/ --FIL
Lane 9  αα/ --THAI
β-thalassaemia
Distribution of 245 mutations in the \(\beta\)-globin gene associated with \(\beta\)-thalassaemia in the Danish immigrant population.
a-thalassaemia deletions

Geografi

- $\alpha^{3.7}$ Iran, Irak, Indien, Sydøstasien, Indonesien, Afrika
- $\alpha^{4.2}$ Middelhavet
- MED Sydøstasien
- $\alpha^{20.5}$ Middelhavet
- FIL Sydøstasien
- THAI Sydøstasien
α-Thalassemia
### Prevalence of (--)SEA α-Thalassemia Deletion in Southeast Asia.

<table>
<thead>
<tr>
<th>Region</th>
<th>Proportions of Population who are Heterozygous Carriers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hong Kong</td>
<td>4.5</td>
</tr>
<tr>
<td>Northern Taiwan</td>
<td>3.5</td>
</tr>
<tr>
<td>Southern China</td>
<td>5.0-8.8</td>
</tr>
<tr>
<td>Northern Thailand</td>
<td>14.0</td>
</tr>
<tr>
<td>Central Thailand</td>
<td>3.7</td>
</tr>
</tbody>
</table>
Distribution of various α⁰-thalassaemia alleles in 109 patients with α⁰-thalassaemia and 32 patients with HbH disease in the Danish immigrant population.
Thalassemia in 165 immigrants with MCV < 80 fl

Total 165 samples:

β-thalassemia: 30 (18.2 %)
- β-thalassemia minor 29;
- β-thalassemia intermedia 1

α-thalassemia: 39 (23.6 %)
- αα/−α3.7 27;
- −α3.7/−α3.7 5;
- αα,−SEA 2;
- αα/−MED 2;
- αα/−(α)20.5 1;
- αα/αααanti 3.7 1;
- −α4.2/−(α)20.5 1

3 pts have α and β thalassemia simultaneously

Videncenter for Hæmoglobinsygdomme
Sickle cell anemia

percent of population that has the sickle-cell allele (Hemoglobin S)

- 14+  
- 12-14 
- 10-12  
- 8-10
The Procedure for Screening of Haemoglobinopathy in Pregnants of relevant ethnic origin in Copenhagen

Primary Health Care, General Practitioner

Blood sample

The Laboratory of General Practitioners in Copenhagen

The pregnant comes from a Mediterranean country, Iran or Iraq

MCV < 78

The sample is stopped

Haemoglobin analysis (HPLC)

If the sample shows heterozygosity for beta-thalassaemia, alpha0-thalassaemia, Hemoglobin S or hemoglobin E

The pregnant comes from Africa, the Middle East, the Indian subcontinent, or Southeast Asia

MCV > 78

All samples

A direct contact from the laboratory to the GP concerning a blood sample from the father
Chorion villus sampling
Prenatal diagnosis

Far. Cd5 -CT

Foster. Cd5 -CT

619 bp deletion

Lane 1 hetero controle
Lane 2 mother
Lane 3 fetus
Lane 4 fetus
Lane 5 mother
Lane 6 normal controle
α-Thalassemia
SEA deletion

Lane 1: Control heterozygous for SEA deletion
Lane 2: A newborn from Thailand with hydrops
Lane 3: The father of the child
Lane 4: The mother of the child
Number and results of CVS performed at Herlev Hospital in the period 2002-2007

**Beta-thalassaemia**
- Homozygous: 4
- Heterozygous: 2
- Normal: 1

**Sickle cell disease**
- Homozygous: 1
- Normal: 1

**Alpha-thalassaemia**
- Homozygous SEA: 1
- Heterozygous 3.7: 1
- Normal: 1