Counselling and prenatal diagnosis

Antonis Kattamis, Greece
Epidemiology of Hemoglobinopathies

- 7% of world population carriers of hemoglobinopathies
- 500,000 newborns annually affected
  - 300,000: Thalassemias
  - 200,000: SCD, HbE and others
- 90% in Asia, India, Middle East

WHO estimates
Mediterranean Countries

2692 Thalassaemia expected births /year
Mediterranean countries
Thalassaemia: around 39000 patients
Who Should we Screen?

- Carrier screening for hemoglobinopathies should be offered to women/families from ethnic backgrounds with a reported increased carrier frequency
- MCV < 80 fl, or abnormal Hb electrophoresis
- Screening should be done in the pre-conception period or as early into the pregnancy as possible. (II-2A) (GRADE moderate/moderate)
<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose acetate electrophoresis alkaline pH</td>
<td>Low cost, extensive laboratory experience</td>
<td>Low resolution, not generally used in modern laboratories, may miss (\beta)-thalassemia in newborn period</td>
</tr>
<tr>
<td>Citrate agar electrophoresis acidic pH</td>
<td>Can distinguish uncommon variants from hemoglobin S or hemoglobin C, low cost</td>
<td>Low resolution, high-performance liquid chromatography can usually substitute</td>
</tr>
<tr>
<td>Supravital staining with brilliant cresyl blue</td>
<td>When positive, definitive diagnosis of (\alpha)-thalassemia, semiquantitative results allow presumptive genotyping</td>
<td>May be negative in milder forms of (\alpha)-thalassemia, labor intensive</td>
</tr>
<tr>
<td>Isoelectric focusing</td>
<td>Good resolution, widely used, relatively low cost</td>
<td>Cannot definitively identify some variants, not sensitive for (\alpha)-thalassemia, not quantitative, may miss (\beta)-thalassemia in newborn period</td>
</tr>
<tr>
<td>High-performance liquid chromatography</td>
<td>Quantitative, good resolution, rapid, widely adopted</td>
<td>Cannot definitively identify some variants, not sensitive for (\alpha)-thalassemia, may miss (\beta)-thalassemia in newborn period</td>
</tr>
<tr>
<td>Capillary zone electrophoresis</td>
<td>High resolution, can resolve hemoglobin A(_2) from hemoglobin E, quantitative</td>
<td>Cannot definitively identify some variants, not sensitive for (\alpha)-thalassemia, may miss (\beta)-thalassemia in newborn period</td>
</tr>
<tr>
<td>Mass spectrometry</td>
<td>Useful for rapidly characterizing hemoglobin variants</td>
<td>No role in diagnosis of thalassemia</td>
</tr>
<tr>
<td>DNA blot analysis</td>
<td>Can detect large deletions, specific breakpoints do not need characterization</td>
<td>Low resolution, requires specific probes, labor intensive, largely replaced by newer techniques, cannot detect point mutations (except for those affecting restriction sites)</td>
</tr>
<tr>
<td>Gap–polymerase chain reaction</td>
<td>Rapid, can be multiplexed, good for common deletions, especially in (\alpha)-thalassemia</td>
<td>Cannot detect point mutations, will miss deletions lacking specific primers</td>
</tr>
<tr>
<td>Multiplex ligase-dependent probe amplification</td>
<td>Can cover large chromosomal regions for deletion analysis, quantitative, exact breakpoints do not need characterization</td>
<td>Low resolution, cannot detect point mutations or small deletions</td>
</tr>
<tr>
<td>Comparative genomic hybridization</td>
<td>Can cover large chromosomal regions for deletion analysis, high resolution, exact breakpoints do not need characterization</td>
<td>Cannot reliably detect single (\alpha)-globin deletions due to cross-hybridization</td>
</tr>
<tr>
<td>Sanger sequencing</td>
<td>Best current method for characterizing globin variants and point mutations causing thalassemia</td>
<td>Not useful for detecting deletions</td>
</tr>
<tr>
<td>Allele-specific methodologies (allele-specific polymerase chain reaction, reverse dot-blot, arrays, etc)</td>
<td>Useful in genetically homogeneous populations, high throughput, economical</td>
<td>Less useful in ethnically diverse populations</td>
</tr>
<tr>
<td>Next-generation sequencing</td>
<td>Has potential to characterize mutations and deletions throughout all globin genes in parallel</td>
<td>Not yet developed for this application, may be subject to problems in repetitive sequences, particularly (\alpha)-globin genes</td>
</tr>
</tbody>
</table>

How Should we Screen?

- Carrier screening for thalassemia:
  - Complete blood count
  - Hemoglobin (Hb) electrophoresis (HE) or Hb high performance liquid chromatography (HHPLC)
  - Quantification of Hb alpha 2 and fetal Hb
  - Serum ferritin
  - H-bodies (blood smear stain using brilliant cresyl blue)
    (II-2A) (GRADE moderate/moderate)

- If the female thalassemia screening results are abnormal, screen the male partner.
  (III-A) (GRADE low/moderate)

- If both reproductive partners are carriers ➔ referred for formal genetic counselling (reproductive risks, recommended prenatal testing, and diagnostic management).
  (II-3A) (GRADE moderate/moderate)

Thalassemia: major health problem for many countries and needs urgent confrontation.

WHO Expert Committee recent publication on “The resolution on Thalassemia and other hemoglobinopathies”

Urges Member States:
- Implement and reinforce national programs on HB disorders
- Evaluate the impact of national programs
- Intensify the training of all health professionals
- Promote community education
- Promote international cooperation
- Develop and strengthen medical genetic services
- Support basic and applied research

Thalassaemia and other Haemoglobinopathies
EB118, May 2006 – Resolution EB118.R1

Sickle cell anaemia
WHA59, May 2006 – Resolution WHA59.20
Prevention strategies are being implemented

Cyprus and Sardinia: thalassemia awareness and control programmes
- Sardinia: TM declined from 1/250 to 1/4000 births

Greece
- Nationwide programme for carrier identification set up in the 1970’s; knowledge spread through mass media, schools

India
- Care included in 5-year Plan of the Government of India

Saudi Premarital Screening and Genetic Counselling Programme
- Prevalence decreased from 32.9 to 9.0/1000

Malaysia
- Prevention strategy implemented in Kuala Lumpur

Global prevention strategies

Current Status in EMR
National Prevention Strategies

1. Prenatal screening is mandatory or in place in most of EMR countries (both for thalassaemia & SCD)
   a. Iran - 1997
   b. Jordan – 2001
   c. KSA est 2002 (Royal Decree No. 3 issued on 7/11/1429
   d. Bahrain - 2004
   e. UAE - 2011
   f. Qatar - 2010

2. Genetic Studies/training – Genetics Centre Tunis

3. WHO Collaborating Centre for Genetic Riyadh

4. Arab Genomic Centre Dubai
Difficulties in setting up a National Thalassemia Prevention Program

- Population Size (Large/Medium/Small)
- Birth Rate (High/Medium/Low)
- Hb carrier rate (High/Low)
- Degree of awareness: public and professional
- Homogeneity/Heterogeneity of population:
  - Religion
  - Social/ Cultural background
  - Language
  - Ethnicity
- National plans and central coordination.
- Budgetary allocation.
Required Steps in the Prevention Programs

Accurate Epidemiological Data

• To assess the burden of a disease
  • Prevalence of carriers
  • Characterization of molecular defects
  • Accurate prevalence of patients and national cohort of patients (based on registries)
  • Incidence of annual birth rate of affected infants
  • Annual input of affected newborns

• To formulate and implement plans for prevention and treatment
• To follow efficacy and management

AC = amniotic cells; CVS = chorionic villus sampling; PCR = polymerase chain reaction.
Prevention strategies

1. Public education and awareness
2. Epidemiology Local
3. Population screening. Specialised labs
4. Identification couples at risk / Genetic counselling
5. Prenatal diagnosis
### Prenatal diagnosis

<table>
<thead>
<tr>
<th>Year</th>
<th>Methodology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1974</td>
<td>Fetal blood (end 2nd trimester) Chain synthesis</td>
</tr>
<tr>
<td>1979</td>
<td>DNA – amniotic cells (ACs, 2nd trimester) Southern blotting (HbS, α- and (δβ)-thal)</td>
</tr>
<tr>
<td>1982</td>
<td>DNA trophoblast (chorionic villi)(1st trimester) Southern blotting, oligonucleotide hybridization</td>
</tr>
<tr>
<td>1989</td>
<td>DNA (CVS, ACs, fetal blood) PCR-based techniques</td>
</tr>
<tr>
<td>1998</td>
<td>Preimplantation genetic diagnosis</td>
</tr>
<tr>
<td>2004</td>
<td>Celocentesis</td>
</tr>
<tr>
<td>????</td>
<td>Fetal DNA in maternal blood</td>
</tr>
</tbody>
</table>
Short history of PGD

1990
First successful clinical application
Hammersmith Hospital, London

1992
PGD for cystic fibrosis

1999
Preimplantation analysis of chromosome no.

1998
PGD for thalassaemia
PGD for cancer

2001
PGD for HLA compatibility

2002
PGD for late-onset disorders
Overall success depends on the positive outcome of all stages of PGD

- ART & fertilization
- Embryo biopsy
- Cell tubing & transport
- Genetic diagnosis
- Embryo transfer

• Fast: result within 24-36 hours
• Sensitive: single cell PCR
• Robust: Embryo genotype
• Accurate: 100% specificity

• Fiorentino et al., 2006; Van de Velde et al., 2009;
• Harper et al., 2010; Kahraman et al., 2011
Experience in Department of Medical Genetics, Athens (2010-2015)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of couples requesting</th>
<th>Number of cycles (number of couples)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PGD &amp; HLA-compatibility</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-thalassemia/sickle-cell syndromes</td>
<td>14</td>
<td>14 (8)</td>
</tr>
<tr>
<td>β- thalassemia AND sideroblastic anemia</td>
<td>1</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Diamond-Blackfan (DBA)</td>
<td>1</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Chronic granulomatous disease</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Hyper-IgM Syndrome</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>HLA-compatibility alone</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Childhood ALL</td>
<td>1</td>
<td>2 (1)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>20</td>
<td>19 (11)</td>
</tr>
</tbody>
</table>
PREVENTION PROGRAM
The example of Greece
Incidence of thalassemia trait in the Greek population

- β-thalassaemia trait 7.4%
- α-thalassaemia trait 8.5% (α+: 7.1%, α0: 1.4%)
- HbS trait ~ 1%
- Births of homozygotes during 1966–75: 2,850

HbS = sickle-cell haemoglobin.
HBB gene mutations population-specific

**SPAIN**
- CD 6 –A
- IVS I-1 G>A
- IVS I-110 G>A
- IVS II-705 T>G
- IVS II-745 C>G
- Unknown / Others

**PORTUGAL**
- IVS I-1 G>A
- IVS I-6 T>C
- IVS II-705 T>G
- IVS II-745 C>G
- Unknown / Others

**GREECE**
- IVS I-1 G>A
- IVS I-6 T>C
- IVS II-745 C>G
- Unknown / Others

**ITALY**
- TCR
- IVS I-1 G>A
- IVS I-5 G>C
- IVS II-1 G>A
- IVS II-745 C>G
- IVS II-848 C>A
- CD 106/107 +G
- Unknown / Others

**EGYPT**
- CD 5 –CT
- CD 6 –A
- CD 8 –AA
- IVS I-1 G>A
- IVS I-6 T>C
- IVS I-110 G>A
- IVS II-745 C>G
- IVS II-848 C>A
- CD 106/107 +G
- Unknown / Others

Mild mutations

HAEMATOLOGICAL RARE DISEASES: from genetic counselling - through bench - to bed
Main objectives of prevention program

- Program started in 1978
- Public education (high school)
- Massive screening of childbearing population to detect all couples at risk (voluntary)
  - Cyprus: obligatory premarital screening
- Prenatal diagnosis for couples at risk
  - The right to healthy children
  - The right to information
  - The right not to know
  - The right to choose
- Termination of pregnancy in case of affected fetus
Time trends in observed versus expected new β-TM cases
Longitudinal trends in the reduction of new affected births

**β-thalassemia**

<table>
<thead>
<tr>
<th>Period</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1980-1984</td>
<td>58.30%</td>
</tr>
<tr>
<td>1985-1989</td>
<td>78.70%</td>
</tr>
<tr>
<td>1990-1994</td>
<td>82.40%</td>
</tr>
<tr>
<td>1995-1999</td>
<td>87.80%</td>
</tr>
<tr>
<td>2000-2004</td>
<td>91.20%</td>
</tr>
<tr>
<td>2005-2009</td>
<td>94.70%</td>
</tr>
<tr>
<td>Overall</td>
<td>81.10%</td>
</tr>
</tbody>
</table>

**Sickle Cell Disease**

<table>
<thead>
<tr>
<th>Period</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1980-1984</td>
<td>76.20%</td>
</tr>
<tr>
<td>1985-1989</td>
<td>81.10%</td>
</tr>
<tr>
<td>1990-1994</td>
<td>86.70%</td>
</tr>
<tr>
<td>1995-1999</td>
<td>85.00%</td>
</tr>
<tr>
<td>2000-2004</td>
<td>89.60%</td>
</tr>
<tr>
<td>2005-2009</td>
<td>91.60%</td>
</tr>
<tr>
<td>Overall</td>
<td>84.60%</td>
</tr>
</tbody>
</table>
RECENT DEMOGRAPHIC CHANGES:
Distribution of new cases according to origin

1a. β-TM

1b. SCD
## Reasons of Prevention Program Failure for patients of Greek origin

<table>
<thead>
<tr>
<th></th>
<th>Unawareness</th>
<th>Program failure*</th>
<th>Parental choice</th>
<th>Miscellaneous</th>
<th>All causes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β-TM (%)</strong></td>
<td>189 (48,8)</td>
<td>129 (33,3)</td>
<td>44 (11,4)</td>
<td>25 (6,5)</td>
<td>387 (100)</td>
</tr>
<tr>
<td><strong>SCD (%)</strong></td>
<td>100 (57,8)</td>
<td>32 (18,5)</td>
<td>34 (19,7)</td>
<td>7 (4,0)</td>
<td>173 (100)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>289 (51,6)</td>
<td>161 (28,8)</td>
<td>78 (13,9)</td>
<td>32 (5,7)</td>
<td>560 (100)</td>
</tr>
</tbody>
</table>

*Program failure: Non-identification of carrier state
Incorrect prenatal diagnosis
Incorrect genetic advice - IVF
The Age Distribution (in 5 yr intervals) of thalassemia cohort on prevention and treatment programs born between 1980-2009 versus an expected cohort on conventional treatment alone.

On prevention:
- Gradual decrease of patients with age.
- Cohort ages 0-9: 121 (12%) versus 845 (63%) of 20-29 yrs.

On treatment:
- Homogeneous age distribution from 0-4 to 25-29 years age cohorts.
## Age distribution of Greek Patients with β-thal in 2010

<table>
<thead>
<tr>
<th>Age in yrs</th>
<th>Number</th>
<th>%</th>
<th>Ages group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pediatric: 0-14 yrs</td>
</tr>
<tr>
<td>&lt;5</td>
<td>48</td>
<td>1.5</td>
<td>Cohort: 220 (6.8%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Prevalence: 13.9</td>
</tr>
<tr>
<td>5-9</td>
<td>73</td>
<td>2.3</td>
<td>Young adults: 15-20yrs</td>
</tr>
<tr>
<td>10-14</td>
<td>99</td>
<td>3.0</td>
<td>Cohort: 790 (24.5%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Prevalence: 36.9</td>
</tr>
<tr>
<td>15-19</td>
<td>145</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>20-24</td>
<td>187</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>25-29</td>
<td>458</td>
<td>14.2</td>
<td></td>
</tr>
<tr>
<td>30-49</td>
<td>2231</td>
<td>68.7</td>
<td></td>
</tr>
<tr>
<td>Total (0-65)</td>
<td>3241</td>
<td>100</td>
<td>Prevalence: 37.2</td>
</tr>
</tbody>
</table>
Counselling Dilemmas

- The borderline results
  - Need for analysis of both $\alpha$- and $\beta$-
- Thalassemia Intermedia vs. Thalassemia Major
  - Multifactorial
- Thalassemia Intermedia
  - Prognosis for Complication-Free-Survival: Good early in life but Guarded Long-term
  - Extreme Heterogeneity
- Future Therapies
  - Unknown how prognosis will change
  - Will the system be able to support new therapies
- The role of the possibility for BMT
Conclusions

• Controlling births of affected patients is essential in areas with high gene frequency

• The β-TM and SCD prevention program in Greece is a model of successful, large-scale intervention achieving >90% reduction in new affected births.
  • Reduction of annual birth rate by > 90%
  • Prevention of birth of > 3,000 affected fetuses

• Despite the impressive results, there is room for improvement, mainly:
  • in groups of non-Greek origin
  • in carrier identification and
  • in offering specialized genetic counseling

• Pitfalls in accurate phenotyping prediction
Thank you for your attention