Advances in molecular diagnostic

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Genoa, Italy

www.humanvariomeproject.org/GG2020
Diagnosis of beta-thalassemia

We observe on day-1 fresh blood in EDTA:

* absence of Hb A₂ (<0.5%)
* presence of elevated percentages of Hb F
* possible presence of Hb variants

In this condition:

The correct quantification of Hb A is very important for a presumptive or a conclusive diagnosis at birth
## Normal Subject

### At birth

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Calibrated Area %</th>
<th>Area %</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>83.5*</td>
<td>1.21</td>
<td>3682834</td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>0.3*</td>
<td>3.62</td>
<td>13520</td>
<td></td>
</tr>
</tbody>
</table>

*Values outside of expected ranges

Analysis comments:

### After 3 weeks

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Calibrated Area %</th>
<th>Area %</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>73.4*</td>
<td>1.19</td>
<td>3681146</td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>0.5*</td>
<td>3.0</td>
<td>28062</td>
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</tbody>
</table>

*Values outside of expected ranges

Analysis comments:

### After 5 weeks

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Calibrated Area %</th>
<th>Area %</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>64.0*</td>
<td>1.23</td>
<td>1533925</td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>1.3*</td>
<td>3.0</td>
<td>34355</td>
<td></td>
</tr>
</tbody>
</table>

*Values outside of expected ranges

Analysis comments:
21 weeks, heterozygous fetus  
\( \beta^\circ \text{Thalassemia (cod 39)} \)

Heterozygous newborn  
\( \beta^\circ \text{Thalassemia (cod 39)} \)

Heterozygous newborn  
\( \beta^+ \text{Thalassemia (IVSI-110)} \)

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<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Calibrated Area 1</th>
<th>Area 1</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>102.4*</td>
<td>---</td>
<td>1.22</td>
<td>2414370</td>
</tr>
<tr>
<td>Ao</td>
<td>---</td>
<td>3.4</td>
<td>2.34</td>
<td>812610</td>
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</tbody>
</table>

*Values outside of expected ranges*  

Analysis comments:

F Concentration = 102.4* %  
A2 Concentration = %  

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<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Calibrated Area 1</th>
<th>Area 1</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
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<tbody>
<tr>
<td>Unknown</td>
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<td>0.7</td>
<td>0.95</td>
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<tr>
<td>F</td>
<td>95.3*</td>
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<td>1.21</td>
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<tr>
<td>Ao</td>
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<td>10.4</td>
<td>2.61</td>
<td>369964</td>
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<tr>
<td>A2</td>
<td>0.2*</td>
<td>---</td>
<td>3.06</td>
<td>9584</td>
</tr>
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</table>

*Values outside of expected ranges*  

Analysis comments:

F Concentration = 95.3* %  
A2 Concentration = 0.3* %  

---

<table>
<thead>
<tr>
<th>Peak Name</th>
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<th>Area 1</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
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<td>P1</td>
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<td>5.5</td>
<td>0.83</td>
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<tr>
<td>F</td>
<td>90.2*</td>
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<td>1.19</td>
<td>1532232</td>
</tr>
<tr>
<td>Ao</td>
<td>---</td>
<td>13.3</td>
<td>2.31</td>
<td>2995580</td>
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</table>

*Values outside of expected ranges*  

Analysis comments:

F Concentration = 90.2* %  
A2 Concentration = %  

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The Human Variome Project  
an NGO official partner of UNESCO  
Global Gobi 2020 Challenge  

www.humanvariomeproject.org/GG2020
Hematological and DNA studies

>78
>27
A
A₂

<78
<27
MCV fL
MCH pg

<78
<27
A
A₂ > 3.5%
F 0.1%–7%
Normal iron

Hb variants
Hb electrophoresis
HPLC
Mass spectroscopy

Sickling positive
Other known variants
RDB
Sequencing

Hb S
Hb C
Hb D
Hb E
Hb O Arab
Hb Lepore

Further investigations required

Characterization of undefined mutations
DGGE
dHPLC
Sequencing

DNA analysis for common β-point mutations
ARMS
RDB

DNA analysis for α-globin mutations
GAP-PCR
MLPA
Sequencing

DNA analysis for β-gene deletion mutations
GAP-PCR
MLPA

Other known variants
RDB
Sequencing

Hb S
Hb C
Hb D
Hb E
Hb O Arab
Hb Lepore

Further investigations required

A. Cao and Y.W. Kan
Decline of birth rate of thalassemia major in Sardinia.

A. Cao and Y.W. Kan

Cite this article as Cold Spring Harb Perspect Med 2013;3:a011775

www.humanvariomeproject.org/GG2020
Chromosome 11
β-LCR
5 4 3 2 1
ε Gγ Aγ ψβ δ β

Cell type
- Megaloblast
- Macrocye
- Normocyte

Site of erythropoiesis
- Liver
- Bone marrow
- Yolk sac
- Spleen

Percentage of total globin synthesis
- α
- β
- γ

Post-conceptual age (weeks)
- Birth
- Postnatal age (weeks)

Chromosome 16
HS-40
5
ζ2 ψζ1 ψα2 ψα1 α2 α1 θ

www.humanvariomeproject.org/GG2020
The diagram illustrates the genetic arrangement of hemoglobin (Hb) genes on chromosomes 11 and 16. The diagram highlights the following:

- **Cromosoma 11**
  - **β-LCR**
  - **ψ1, ψ2, α1, α2, δ, β**
  - **ζ2 ε2** (Hb Gower I)
  - **α2 ε2** (Hb Gower II)
  - **ζ2 γ2** (Hb Portland)
  - **α2 γ2** (HbF)

- **Cromosoma 16**
  - **ψ1, ψα2, ψα1, α2, α1, θ**

The diagram also indicates the expression of different hemoglobin variants in different stages of development:

- **Nell’embrione**
- **Nel feto**
- **Nell’adulto**

The website mentioned at the bottom of the image is: [www.humanvariomeproject.org/GG2020](http://www.humanvariomeproject.org/GG2020)
EMQN Best Practice Guidelines for molecular and haematology methods for carrier identification and prenatal diagnosis of the haemoglobinopathies

Joanne Traeger-Synodinos, Cornelis L Harteveld, John M Old, Mary Petrou, Renzo Galanello, Piero Giordano, Michael Angastioniots, Barbara De la Salle, Shirley Henderson and Alison May on behalf of contributors to the EMQN haemoglobinopathies best practice meeting

Haemoglobinopathies constitute the commonest recessive monogenic disorders worldwide, and the treatment of affected individuals presents a substantial global disease burden. Carrier identification and prenatal diagnosis represent valuable procedures that identify couples at risk for having affected children, so that they can be offered options to have healthy offspring. Molecular diagnosis facilitates prenatal diagnosis and definitive diagnosis of carriers and patients (especially ‘atypical’ cases who often have complex genotype interactions). However, the haemoglobin disorders are unique among all genetic diseases in that identification of carriers is preferable by haematological (biochemical) tests rather than DNA analysis. These Best Practice guidelines offer an overview of recommended strategies and methods for carrier identification and prenatal diagnosis of haemoglobinopathies, and emphasize the importance of appropriately applying and interpreting haematological tests in supporting the optimum application and evaluation of globin gene DNA analysis.

### HbVar: Database of Human Hemoglobin Variants and Thalassemias

http://globin.bx.psu.edu/hbvar/menu.html

<table>
<thead>
<tr>
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<th>Count of results</th>
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<tr>
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<tr>
<td>Total hemoglobin variant entries</td>
<td>1270</td>
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<tr>
<td>Total thalasemia entries</td>
<td>486</td>
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<td>Total entries in both variant and thalasemia categories</td>
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<tr>
<td>Entries involving the alpha1 gene</td>
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<tr>
<td>Entries involving the alpha2 gene</td>
<td>431</td>
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<tr>
<td>Entries involving the beta gene</td>
<td>894</td>
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<tr>
<td>Entries involving the delta gene</td>
<td>117</td>
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<tr>
<td>Entries involving the Agamma gene</td>
<td>58</td>
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<tr>
<td>Entries involving the Ggamma gene</td>
<td>73</td>
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<tr>
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<td>Entries with a fusion gene mutation</td>
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<tr>
<td>Unstable hemoglobins</td>
<td>147</td>
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<td>Methemoglobins</td>
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<thead>
<tr>
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<tbody>
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<td>1774</td>
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<tr>
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<tr>
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<tr>
<td>Entries involving the alpha2 gene</td>
<td>442</td>
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<tr>
<td>Entries involving the beta gene</td>
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<td>Entries involving the delta gene</td>
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<tr>
<td>Entries with an insertion mutation</td>
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<td>Entries with a substitution mutation</td>
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<td>Hemoglobins with low oxygen affinity</td>
<td>48</td>
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<td>Unstable hemoglobins</td>
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<tr>
<td>Methemoglobins</td>
<td>13</td>
</tr>
</tbody>
</table>

10.5.2017  
www.humanvariomeproject.org/GG2020  

03.05.2018
Advances in Molecular Diagnostics

In the last years technology applied to DNA analysis has dramatically changed the molecular diagnosis approach in the genetic field.

Three main factors have been crucial for these changes:
1) evolution in sequencing technology (NGS);
2) amount of data produced and their interpretation by bioinformatics (new pipelines);
3) laboratory management and quality system (certification, accreditation, external quality control).
These changes require a new vision in providing genetic services at population level, taking into account the economical sustainability of the NHS.

- Automation of DNA extraction and analysis

- Merging small laboratories into few larger ones

- Increasing the number of bioinformaticians and of clinical geneticists for the interpretation of results
To fully understand the recent advances in molecular diagnostics, two additional aspects need to be considered:

1) sharing data among professionals worldwide (international databases, national registries)

2) patients empowerment and their involvement in the entire process from diagnosis to therapy.
How investigate genotype complexity?

Three pillars:

1) Clinical characterization (Clinical databases)

2) Genome analysis (exome, genome sequencing)

3) Repository of Biological material (Biobank)
1) Clinical characterization

a) Clinical databases, disease specific:
- Nationals ones ........................................
- Internationals: e.g. EuroBlood net or

b) Sharing phenotypes and genotypes:
  [ClinVar] https://www.phenomecentral.org
  [LOVD] http://www.lovd.nl/3.0/home
Matchmaker Exchange

Connected Nodes

https://www.matchmakerexchange.org/#Supporters
2) Genome analysis

a) Gene sequencing (Sanger seq.)
   - Pathogenetic genes

b) Gene panels (NGS) 50-200 genes
   - Known modifiers/additional genes

c) Clinical Exome 4,000-6,000 genes
   - New relationships among already known genes
2) Genome analysis

d) Full exome, around 25,000 genes (about 2% of genome)
   - New unrelated genes

e) Full genome sequencing
   - New regulatory elements/transcription factors, non-coding RNA, miRNA, ...
2) Genome analysis

New portable lab for on site cheap and very fast first level diagnosis DNA analysis

POINT OF CARE

www.humanvariomeproject.org/GG2020
3) Repository of Biological material (Biobank)

a) In vitro model of genetic diseases

b) Functional studies

c) Personalized target therapy

d) Precision medicine with biological-drugs
PERCORSO RACCOLTA CAMPIONI e DATI

CAMPIONI

- 2 provette in EDTA (4-8 ml)
- 1 provetta in EPARINA (4-8 ml)

MODULISTICA

modulo invio angioedema

C.I. test genetico richieste

C.I. biobanca

CONSERVAZIONE

DISTRIBUTUZIONE

DIAGNOSTICA
E.O. Ospedali Galliera Genova
LABORATORIO GENETICA UMANA

GGB

BIOBANCA
E.O. Ospedali Galliera Genova
"Galliera Genetic Bank"

www.humanvariomeproject.eu/GG2020
A paradigm for best practice in data sharing in genomic studies?

1) Clinical characterization

- Consent
- Sample
- Phenotypic record

Diagnosis

- Phenotypic data standards

Online analysis

BBMRI-LPC clinician/researcher

- Combined genome-phenome database

- Standardized alignment, calling & annotation

- Infrastructure for data sharing

- Sequencing

- Biobank

Sample can be reused for further research

- Infrastructure for sample sharing

- Data can be reused for further research

EGA
Standardizing phenotype-genotype exchange

Global Alliance for Genomics & Health

- Genes
- Environment
- Phenotypes

Emerging GA4GH standard

Standard spec submission – planned for Oct 2018

http://phenopackets.org
Thank you from Galliera hospital in Genoa