Molecular Immunohematology: The European Perspective

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Molecular blood group typing
The European Perspective

- Current diagnostic applications of blood group genotyping
- Blood donor’s molecular typing
- Prospects and challenges for the near future
The BST Center in Barcelona

- The Banc de Sang i Teixits (BST) is a public company belonging to the Catalan Public Health Service.
- It is the blood bank that supplies blood components to all the hospitals in Catalonia.
- It is fully responsible for transfusion services in hospitals of the Catalan Public Healthcare Network.
- It participates in hospital transfusion committees.
- It has the Immunohematology Reference Laboratory.
Disclaimer

- All blood group genotyping tests commercially available in the US and Canada are for research use only. Not for use in diagnostic procedures.
Current Diagnostic Applications of BG Genotyping

- Identification of RhD variants
- Fetal blood group typing
- Patients typing
- Resolution of complex Immunohematology cases
- HPA typing
Identification of RhD variants

- Complexity of the Rh system
- Discrepant or weak results in serological RhD typing
- \textit{RHD} Molecular typing allows the identification of the allelic variant $\rightarrow$ Partial D/ Weak D
- Serological distinction between these two entities is not straightforward.
Altered RhD antigen expression

- Patients requiring RBC transfusion
- Pregnant women

The genetic identification allows improved decision making in the selection of RBCs for transfusion and in RhIG administration and prenatal care.
Molecular identification of RhD variants

- *RHD* genotyping is currently performed in diagnostic immunohematology laboratories with the aim to:

  → Confirm or discard the most common weak D types.

  → Identify less frequent D variants (known partial D, rare variants from other populations, etc)

  → Discriminate alloanti-D vs autoanti-D
Molecular identification of RhD variants

- **PCR-SSP kits available - CE marked**
Molecular identification of RhD variants

- **BLOODchip® Reference Genotyping Platform** – 128 SNPs

**Covers 237 allelic variants**
Of the blood group systems: ABO, RhD/RhCE, MNS, Kell, Kidd, Duffy, Diego, Dombrock, Colton and Lutheran, plus HPA.

Extensive *RHD* genotyping: 173 different alleles
- Weak D variants
- Partial D variants
- Del variants
- D negative variants

- **27 Systems installed in Europe**
- **24 BLOODchip service users**
Bloodgen Consortium

Amsterdam, Netherlands
Rotterdam, Netherlands
Bristol, UK (UWE and BITS)
Derio, Spain
Lund, Sweden
Ulm, Germany
Prague, Czech Republic

Banc de Sang i Teixits
RHD variants identified

Samples submitted to BST for decision making

- Weak D Type 2: 35%
- Weak D Type 1: 33%
- Weak D Type 4.0/4.1: 10%
- Weak D Type 11: 6%
- Other Partial D: 3%
- Weak D Type 4.2/DAR: 3%
- DVI Type 1: 2%
- DVI Type 4: 2%
- Weak D Type 59: 1%
- Silent RHD alleles: 1%
Fetal Blood Group Typing

- Prior to 2001 the usual source of fetal DNA was a sample of amniotic fluid or chorionic villi.

- Cell-free fetal DNA is detectable in the blood of pregnant women → amount increasing throughout gestation.

- Fetal RhD type can be predicted reliably from the fetal DNA in the plasma of D neg pregnant women from beginning of 2nd trimester.
Non-invasive fetal blood group typing from maternal plasma cell-free fetal DNA is now a clinical reality.

Offered by many laboratories in Europe, to identify the fetus **not** at risk of HDFN.

Different assays are currently used for reliable genotyping of D, C, c, E and K by quantitative real-time PCR techniques.
Fetal Blood Group Typing at BST

- Service offered since 2004 → 930 samples referred.
Fetal RHD typing: Application to all D neg pregnant women

- Trials of high-throughput methods have demonstrated that accurate fetal D testing in all D- pregnant women is feasible.
- Fetal RHD typing to target antenatal anti-D prophylaxis already introduced in Denmark (2010) and the Netherlands (2011).

Original Article

Report of the first nationally implemented clinical routine screening for fetal RHD in D- pregnant women to ascertain the requirement for antenatal RhD prophylaxis

Frederik Banch Clausen, Mette Christiansen, Rudi Steffensen, Steffen Jørgensen, Christian Nielsen, Marianne Antonius Jakobsen, Rikke Dyhrberg Madsen, Karina Jensen, Grethe Risum Krog, Klaus Rieneck, Ulrik Sprogøe, Keld Mikkelsen Homburg, Niels Grunnet, and Morten Hanefeld Dziegiet
Blood Group Genotyping of Patients

- Multiple/ recently transfused patients
- AIHA patients
- Patients with unusual serological results
- Extensive genotyping of patients with SCD, thalassemia, myelodysplastic syndromes or other pathologies requiring chronic transfusions.

BST $\Rightarrow$ 260 extensively genotyped patients
Blood Group Genotyping of Patients

- Labor-intensive molecular biology techniques and laboratory-specific “in house” protocols have been applied for many years.

- These have evolved into more comprehensive tests based on new technologies → multiple molecular assays can be performed simultaneously.
Comprehensive Blood Group Genotyping Platforms

- **BLOODchip® Reference Platform**

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<tr>
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Comprehensive Blood Group Genotyping Platforms

- *BLOODchip ID Platforms* ⇒ *based on the xMAP® technology.*
Comprehensive Blood Group Genotyping Platforms

- **BLOODchip ID Platforms – Installed at BST in April 2011**

- **ID-CORE +**: Genetic identification of 33 RBC antigens
  - Flexible
  - Medium/high-throughput
  - Short processing time
  - LIS connection
  - CE marked
HPA typing – Clinical Application

Alloantibodies against Platelet Antigens may induce:

- Fetal/neonatal alloimmune thrombocytopenia (FNAIT)
- Post-transfusion purpura (PTP)
- Platelet transfusion refractoriness (PR)

Rapid and accurate HPA typing of patients is critical for confirmation of the diagnosis ⇒ Good clinical management, specially when HPA-matched platelets are required.
HPA typing with ID-HPA
HPA Genotyping of Donors

- Such high-throughput genotyping platforms analyzing HPA polymorphisms are being used for large-scale blood or platelet apheresis donor typing.

- Regular HPA genotyping of apheresis donors has facilitated the identification of HPA-1a negative platelet donors.

- BST: Aprox. 70 HPA-1a negative platelet donors <40 ⇒ regular programmed donations.
Blood Donors Molecular Typing at the BST

Focused on specific groups of donors:

- Extensive genotyping of blood donors from immigrant populations

  ⇒ Aim: Identify blood donors expressing low-incidence antigens (Di\textsuperscript{a}, Js\textsuperscript{a}, Mi\textsuperscript{a}, Co\textsuperscript{b}, Kp\textsuperscript{a}) or donors with rare phenotypes: Di(a+b), Co(a-b+), Fy(a-b-), HPA-1(a-b+)

  ⇒ Utility:

  - For transfusion (rare phenotype blood units)
  - RBC panel units (Antibody identification)
  - Diagnostic (reagent cells)
Blood Donors Molecular Typing at the BST

- **RHD Genotyping of D negative blood donors with RhC and/or RhE positive**
  
  ⇒ Utility:
  - Quality control of D negative units
  - Estimate the real incidence of Del units ⇒ 1.6%

- Used the BLOODchip Reference platform
  
  ⇒ Total of 2,200 blood donors extensively genotyped
Blood Donors Molecular Typing

- Finnish Red Cross Blood Service experience

Routine genotyping of blood donors with ID-Core+ since September 2012

Criteria
- blood group A or O, K neg
- previous donation within a year
- rare donors, ethnic minorities
Blood Donors Molecular Typing

- Results automatically transferred from BIDS
  - laboratory information management system (LabVantage Sapphire /Software Point)
    - genotypes
    - predicted phenotypes
    - notes
  - blood bank information management system (eProgesa /MAK-system)
    - phenotypes
    - comparison of results
Current availability of reference gDNA reagents for blood group genotyping internal quality controls

Reference: http://www.nibsc.ac.uk/products/biological_reference_materials
Prospects and Challenges for the near future

- Mass screening of blood donors to increase antigen-negative inventory
- Relevant blood group antigens with recently identified molecular basis
  ⇒ DNA-based typing
- Further implementation of non-invasive fetal RHD genotyping programs in D neg pregnant women
  ⇒ rational administration of anti-D Ig prophylaxis
Thank you for your attention