The Scientific Basis of Red Cell Antigen Genotyping and its Application to the Transfusion Laboratory

Maryse St-Louis, Ph.D
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GIVE BLOOD. GIVE LIFE.
Scientific Basis of Red Cell Antigen Genotyping
ISBT and Blood Groups

- Number of blood groups: 30
  (31 Forsmann, 32 Langereis and 33 Junior pending)
- Number of antigens: 328 + 3 (284 - 3)
- Mostly the result of SNPs
- More than 1200 different alleles
# Blood Groups and Antigens

<table>
<thead>
<tr>
<th>Group</th>
<th>Ags</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
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<td>ABO</td>
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<tr>
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<td>GYP A, GYP B, GYP E</td>
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<td>RHD, RHCE</td>
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<tr>
<td>CO</td>
<td>4</td>
<td>AQP1</td>
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</table>

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<tr>
<td>RHAG</td>
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</tbody>
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*Give Blood. Give Life.*
Forms and Functions

Phenotype vs Genotype

**Phenotype**
- Antigens
- Reagents (antibodies)
- Agglutination

**Genotype**
- Genes
- Amplification + SNPs analysis and/or sequencing
Description

- Nucleic acid-based analysis
- In a blood bank setting: RBC, ptls, neutro, granulo, HLA
- Only minute amount of blood needed
- Performed once
1. Cell lysis
2. Column purification
3. Wash
4. Elution
Genotyping Use

- Prenatal assays
- Polytransfused/recently transfused patients
- Positive DAT
- RHD zygosity
- Reagents availability
- Genetic variants
- Rare blood donors
- Red blood cell panels
Antigens to Genotype

• The most immunogenic
• Antigens with known molecular basis
• Antigens for which reagents are lacking (Js\textsuperscript{a}, VS, V, Do\textsuperscript{a}, Do\textsuperscript{b}, Lu\textsuperscript{b}, Yt\textsuperscript{a}, Sc1, LW)
• High prevalence (to find negatives)
• Low prevalence (to find positives, R&D)
• Specific to cultural minorities
• However, patience needed for others (PEL, Vel, etc.)
Variant Alleles

- More than 1200 alleles described
- Known variants in every blood group
- Assays developed for the most frequent
- New variants are missed
- Additional analyses required
Application to the Transfusion Laboratory
Examples

- Recently transfused patient
- Serology reagents lacking
- Complex RH cases
- \textit{RHD} zygosity
Recently Transfused Patient

- 75 years old AB+ Caucasian man
- Transfused < 3 months
- Antibody identification: anti-E, -K and –S
- Patient’s sample was genotyped: $RHCE^*Ee, KEL^*01/KEL^*02, MNS^*03/MNS^*04$
  $RHCE^*e/RHCE^*e, KEL^*02/KEL^*02, MNS^*04/MNS^*04$
- Found compatible blood (E-, K-, S-)
Serology Reagents Lacking

- Chronically transfused patients
  - Three patients with anti-$\text{Do}^a$, -Js$^a$, -VS and -V
  - Three patients with anti-Js$^a$, -VS and -V
  - One patient with anti-Co$^b$
Serology Reagents Lacking

• > 3000 units genotyped for DO*01/DO*02
• > 900 units genotyped for DO*01/DO*02, KEL*06/KEL*07, RHCE*RH20, RHCE*RH10, DO*04 and DO*05
• 134 units genotyped for KEL*06/KEL*07 only
• As for CO*01/CO*02, none for now
Complex RH Cases

• Case 1

35 years old O- Black female
Anti-C and –e
Her phenotype:
C+E-c+e+, K-, Fy(a-b-), Jk(a+b+), S-s-, U+var

*RHD* sequence: DIIla
*RHCE* sequence: 733C>G + 1006G>T
(C) E- (c) (e) VS+ V- hrB-

Conclusion: anti-C and anti-hrB

No donors available for her
Complex RH Cases

• Case 2

27 years old, O+ Moroccan Female
Anti-C and -e
Her phenotype:
C-E-c+e+, K-, Fy(a-b-), Jk(a+b+), S-s+

RHCE sequence: 733C>G
C-E-(c)(e) VS+ V+ hr^{Bw/-}

Conclusion: anti-C and anti-hr^{B}

Several donors available for her
**RHD Zygosity**

- D- woman + D+ father
- 1\textsuperscript{st} pregnancy: D+ baby, anti-D, mother received large doses of RhIg
- 2\textsuperscript{nd} pregnancy 18 months later: miscarriage at week 7, anti-D titer = 2,000, now reduced to 500
- They are planning another pregnancy
- \textit{RHD} zygosity of father: DD
Pros and Cons

• Rare alleles missed
• Other techniques required such as sequencing
• In some instances, several SNPs needed to predict a phenotype ($FY$)
• New polymorphisms = phenotype?
Summary

- Blood group genotyping is a great tool!
- Complement to serology
- Serology is still the gold standard
- Rigorous interpretation is crucial
- Ethnic background useful
- Good communication between the medical team, hospital’s blood bank personnel and the blood provider
Acknowledgements

- Organizing committee
- Héma-Québec’s IRL
- My team: Josée Perreault and Josée Lavoie
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