Molecular Testing: What can it do for the Blood Bank

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American Red Cross Southern Region Reference Laboratory
Objectives

- Brief overview of DNA and terminology
- Current technology
- Applications of molecular testing results
DNA to Antigen

Nucleotides: A, G, T, C

DNA - template for duplication to mRNA

mRNA translated to form protein

Protein structure on red cell surface
Molecular Basis of Blood Group Antigens

• Genes encoding 28 of 29 blood group systems
  – Only P1 remains to be solved

• 40 genes; over 1,100 alleles that encode the blood group antigens and phenotypes

• More than 300 antigens
Molecular changes that result in Antigen or Phenotype

- Single nucleotide change-most common
- Deletion or insertion of a nucleotide
- Deletion or duplication of an exon
- Deletion of a gene
- Gene crossover, conversion, recombinant event
- Absence of a required interacting protein
- Presence of a modifying gene
- Unknown

Serology and DNA are complementary

• Serologically defined red cell types used to determine the molecular bases of variant forms of the gene.
• Molecular basis allows DNA analysis to be performed to predict the presence or absence of an antigen (Ag).
• Most antigens are result of SNP change.
  – PCR-based assays identify these changes
PCR: Polymerase Chain Reaction

- Used to amplify a specific region of DNA that contains the nucleotide that encodes the Ag.
- PCR reaction mixture contains primers complementary to the gene of interest.
  - Primer: 18 to 36 base pairs; designed to amplify short DNA segments.
- DNA-PCR reactants in a thermal cycler.
  - Denature the DNA.
  - Combine with primers.
  - Stabilization. Repeat 25 to 35 times to amplify.

Significant Advances in Molecular Testing

- Gene targets have been validated
- High correlations with RBC phenotype
- Discrepancies between serologic and molecular results extensively investigated
- Manual testing replaced by automated platforms
Challenges in Molecular Blood Group Testing

• Numerous polymorphisms
  Ex.: ABO and Rh blood group systems

• Detection of common silencing mutations
  Examples:
  • *GATA* mutation that silences Fy^b^ expression on RBCs
  • Two changes on the *GYPB* silence glycophorin B for Ss expression
Current Applications of DNA testing in the Blood Bank

• Typing multiply transfused patients
• Lack of rare or reliable antisera
  – Ex: Dombrock; Low frequency antigens-V, VS
• Reagent discrepancies
  – Contaminating specificities
  – Weak antigen expression
• Determining zygosity
• Testing fetal DNA
• High volume donor screening
Case 1

- Hospital performs ABO confirmation typing on RBC labeled Group O, Rh Positive

- Anti-A,B reacts 1+

- Donor history checked
  - 1\textsuperscript{st}-time donor
  - Automated typing: Group O, Rh Positive
### Case 1: Serologic Investigation

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Reverse Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1</td>
</tr>
<tr>
<td>A</td>
<td>0/0</td>
</tr>
<tr>
<td>B</td>
<td>0/0</td>
</tr>
<tr>
<td>A, B</td>
<td>1+w/1+</td>
</tr>
<tr>
<td>#1</td>
<td>#2</td>
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</tbody>
</table>

**Readings:** I.S./ 15' RT

#1-Forward typing reagents used by hospital
Case 1: Investigation continues

Repeat forward testing with papain-treated donor cells:

All reagents remain negative except anti-A,B #1

Absorption-elution studies with anti-A and anti-B: No reactivity detected

Investigate donor mix-up: none found

Review of ABO testing: on previous donations by the donor
Case 1: Molecular results

- Two nucleotide deletions
  - position 261 characteristic of O alleles
  - position 467 characteristic of A2 alleles
- Genomic cloning and sequencing: weak A subgroup allele
  - ABO*AwO2 (350C,467T, and 1060 deleted C)

**ABO genotype: AwO2/01**
Case 1 Molecular results cont.

- **AwO2** allele reported

- Expression of A antigen on these cells has only been detected as weak reactivity with reagents containing the ES15 monoclonal antibody.
Case 1 Conclusion

• Donor is group A
  – Antigen not detectable by most reagents
  – Deferred from routine blood donation.

• What if the unit was transfused as group O?
  – No reports of transfusion reactions

• What if this person needs blood?
  – As a patient, classify him as group O
Case 2: Another ABO problem

- 32 y.o. woman with anemia and weakness
- Recently transfused in another state
  - History: group AB with anti-A1
- Strong anti-IH in plasma plus anti-Fya
- ABO results (Galileo): B Positive
  - A1 cells: 2+
  - B cells: 0
## Case 2: Manual ABO testing

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I.S.</td>
<td>3+</td>
<td>4+</td>
<td>2+</td>
</tr>
<tr>
<td>I.S.</td>
<td></td>
<td></td>
<td>2+</td>
</tr>
<tr>
<td>R.T.</td>
<td></td>
<td></td>
<td>2+</td>
</tr>
<tr>
<td>37C</td>
<td></td>
<td></td>
<td>1+</td>
</tr>
</tbody>
</table>
Case 2: Continued

• ABO testing inconclusive
• Group O RBC units transfused over next few weeks
• Anti-IH remained strong
• Molecular testing: Predicted group A\textsuperscript{2}B
  – Genotype: A2/B101
• Group AB units could be crossmatched
Case 3: Rh testing

• 62 y.o. man going to surgery

• Hospital results
  – ABO/Rh: A Positive
  – Antibody ID: Anti-D ??
  – DAT Positive
Case 3: Serologic Investigation

- Anti-D typing: 3+; Rh control: 0
  - Other typings: Normal, no mixed field
- DAT: 3+ (Poly and IgG)
  - Eluate: Anti-D (4+)
- Plasma: Anti-D (2+)
  - Other specificities were excluded

Warm autoantibody with D specificity ??
Case 3: Continued

- Patient RBCs treated with EDTA-glycine to dissociate the IgG – DAT Negative
- Patient plasma tested with his DAT neg. cells
  - Weak reaction – much weaker than other Rh Positive cells; control negative
- What does this mean?
  - Is pt. really Rh Positive?
  - Is he an Rh variant?
Case 3: Molecular results

- PCR results
  - Exons 4 and 7 present
  - Negative for the inactivating *RHD* pseudogene
  - Zygosity determination for hybrid Rhesus box
- Sequencing: Negative for weak D types
- Conclusion: *RHD* heterozygote
  - *Not predicted to make allo anti-D*

*Patient can receive Rh positive blood.*

*Anti-D was most likely autoantibody.*
Case 4: More D abnormalities

• 83 y.o. woman with low H/H

• History:
  – Transfused in past year
    • most recent 2 months ago
  – Reference Lab: O Pos, DAT-Wk +;
    • Warm autoantibody, Allo anti-E

• Current sample: O Positive
  – DAT: 2+ (Poly and IgG)
Case 4: Serologic investigation

<table>
<thead>
<tr>
<th>Cells</th>
<th>Plasma</th>
<th>Eluate</th>
</tr>
</thead>
<tbody>
<tr>
<td>D+ E-</td>
<td>1+</td>
<td>3+</td>
</tr>
<tr>
<td>D+ E+</td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td>D- E-</td>
<td>0</td>
<td>1+</td>
</tr>
</tbody>
</table>
Case 4: Molecular Results

- **RHD** heterozygote
- **RHD** sequencing: nucleotide 520 G>A
  - Predicted to encode an amino acid change associated with **weak D type 33** (Reported in Taiwanese in 2003)
    - Persons not reported to be at risk for production of allo anti-D
    - NRLBGS previously tested another patient with this type who made allo anti-D

**Patient should receive Rh negative blood**
Case 5: Another blood group

- 77 y.o. man with myelodysplastic syndrome

- History provided by hospital:
  - Transfused about 4 weeks ago with 2 RBCs
  - A Pos; Negative antibody screen

- Current test results at hospital:
  - Antibody screen: 2 of 3 cells react 2+ at IAT
  - DAT: 1+ (poly and IgG)
Case 5: Serologic results

- DAT positive (recently transfused)
- Eluate: anti-Jka plus additional weak reactions
- Plasma: anti-Jka (PeG and Gel testing)
- Phenotyping: Cell separation performed – Kidd typings inconclusive
- Jk(a-) crossmatch compatible units sent
Case 5: Molecular results

- **Predicted phenotype:** Jk(a+b+)

  **Review of serology:**
  - Consistent with autoantibody with Jka specificity
  - Jk(a-) blood is not required

- Patient not seen again

- Patients with autoantibody specificities sometimes show panagglutinins when tested again
  - Specificity no longer honored
Case 6: Back to Rh

- 57 y.o. woman with phlebitis
- History provided by hospital
  - Transfused 8 months before
  - O Pos with anti-C, -e and –K identified
- Two red cells ordered; Hgb. 8.4
Case 6: Serologic Findings

- 1st time referred to Reference Lab
- O Pos; DAT negative
- Phenotype: C-, K-, e weak, Jk(b-), S-
- Plasma tested with cells negative for C, e + K
  - Anti-Jkb and anti-S identified
- C-, e-, K-, Jk(b-), S- found in 9 of 10,000 donors-------Two units found and provided
Case 6-Additional Investigation

- Additional e typings:
  - 2 licensed sources
- Results indicate pt. may have weakened or variant form of e antigen
- Is the previously identified anti-e an allo- or auto-antibody?

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<tbody>
<tr>
<td>#1</td>
<td>1+w</td>
<td>3+</td>
</tr>
<tr>
<td>#2</td>
<td>2+</td>
<td>4+</td>
</tr>
</tbody>
</table>
Case 6: Molecular results

- Extensive analysis including sequencing the *RHCE* gene for exons 1 through 8.
- No changes associated with altered or partial e antigen expression and production of allo anti-e.
- If the anti-e shows characteristics of an alloantibody, Rh-cDNA testing can be done to rule out rare or new hybrid alleles.
  - Further serologic evaluation will be performed.
Case 7: Unexpected Benefit

- 31 y.o. pregnant woman
  - African-American

- Hospital results
  - Patient typed A Rh Positive
  - Found apparent anti-D and suspected partial D
  - No history of RhIg administration
Case 7 Serologic Findings

- A, Rh Positive (D typing: 4+)
- DAT negative
- Rh phenotyping: C inconclusive, E-, c+, e+
- Plasma: Anti-D;
  - Apparent allo antibody
  - Does she have partial D?
Case 7: Molecular testing

- Partial **RHD*DIVα-2**: Encodes for low incidence Goα antigen.
- **SURPRISE**...... Both RHCE genes are unusual
  - Altered e, VS+ V+, hrB negative

Predicted phenotype:
- Partial D+, C-E-c+ partial e+, VS+, V+, hrB-
- Transfuse Rh negative, compatible red cells
  .....very conservatively!
Case 8

- 48 y.o. woman with sickle cell anemia

- Hospital reported
  - 4 RBCs transfused 6 weeks ago
  - Anti-Fya previously identified
  - Suspect anti-C in eluate
Case 8: Serologic Findings

- O Positive
- DAT: Weak Positive in Gel (Negative in tube)
- Hypotonic wash performed to obtain autologous red cells
  - C-, K-, Fy(a-b-), Jk(a-)
- Eluate: anti-C
Case 8: Additional Serology

- Plasma: 2+ reactions with all e+ cells
  - All e- cells are non-reactive; could not r/o C, K, Jsa
- Pt. types e positive (4+) and DAT is positive
  - Plasma reactions are stronger than DAT
- Provided e-C- units and recommended molecular testing to determine if anti-e is auto- or allo-antibody
More Case 8 Findings

- Plasma: Most e-, C-, Fy(a-) cells compatible
  - Everything ruled out except K and Jsa
- Why should she make anti-e?
  - Her red cells react 3+ with anti-e
- Perhaps her red cells have a variant form of e
- Cells lacking hr^B and hr^S are reactive
- Report “anti-e like”, anti-C, anti-Fya and unidentified reactivity
Case 8: Molecular Results

- **RHD:** DIIIa/RHD (One partial and one normal D)
- **RHCE:**
  - **RHCE*cs:** Associated with partial e, VS+, V-, hrB- phenotype.
  - **RHCE*ce**
- **Predicted phenotype:**
  D+C- E+ c+ partial e+, VS+, V-, hrB-
  Good news!! Patient can receive R2R2 (e-), Fy(a-) blood
Donor Testing

- As donors are identified with unusual types, the information is added to the rare donor database (ARDP).
- Attempts made to make molecular matches for patients with anti-$hr^B$ or anti-$hr^S$
  - $hr^B$ negative blood is acceptable but a more exact match can be made using molecular results
  - Ex: $RHCE^{*}ce(733G)$ produces one $hr^B$ – phenotype and $RHCE^{*}ce(48C,733G)$ makes another
Study on prevention of alloimmunization in sickle cell patients

- Tournamille C et al. Transfusion 2010;50:13-19
- Studied sickle cell patients who typed C+
- Screened for alleles associated with partial C
- Patient history:
  - Alloantibodies produced
  - Determined the number of C+ transfusions
Study continued

Group 1: Retrospective review

- Clinic patients n=1076
- 2/3 HbSS; others HbSC and S/βThal
- 22% typed C+
- 5% of C+ patients made anti-C
Study continued

Group 2

- 177 patients with sickle cell disease transfused in Paris area (Afro-Caribbeans)
- 49 had the (C)ce^s haplotype without C on the other haplotype-----had partial C antigen
- 37 of 49 received C+ blood

Of this group, 30% produced anti-C
Study continued

• Authors concluded
  – The need to detect partial C within C+ HbSS pts.
  – To prevent immunization in this group

• Study needs to be repeated in the U.S. HbSS patient population

Are our C+ HbSS patients at risk to produce anti-C?
Conclusions

• Serology will not be replaced by molecular testing in the near future

• Molecular testing is
  – a valuable addition to the tools available for solving serologic problems
  – the best way to match blood for patients with antibodies that are serologically difficult to define
References

• A Workshop on Molecular Methods in Immunohematology. Transfusion 2007; 47, July Supplement.

• Immunohematology 2008, Volume 24, No. 4
Happy St. Patrick’s Day