BLOOD GROUP GENOMICS

Applications for Transfusion Medicine

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Age of Molecular Medicine

• Genomics revolution
  – Advent of Polymerase Chain Reaction (PCR) in 1985 by Kary Mullis
    • Ability to amplify portion of gene of interest
    • Basis of almost all DNA technologies

• Impacting all areas of laboratory testing
  – Cancer research
  – Personalized medicine and information
    • treatment based on genetics
    • search for gene variations relevant for therapy
    • 23andme.com
    • 2015 Precision Medicine Initiative by Obama

• Transfusion Medicine
  – Genes encoding blood group antigens known
  – Predict red cell, platelet, neutrophil antigen expression
Advantages of DNA testing

- **Type multiply transfused patients**
  - no interference from transfused cells
  - driven by assay design

- **Type RBCs coated with immunoglobulin (+DAT)**
  - alternative – chemical treatment
  - labor intensive

- **Type when no commercial reagents**
  - Dombrock Do^a^/b^, Hy, Jo(a), Kell system Js^a^/b^, V/VS, U, etc.

- **Determine RHD zygosity** (one copy or two copies of D)
  - Test paternal sample when OB patient has anti-D to predict fetal risk for HDN
  - Typing fetus (amniocytes or maternal plasma)
Can do some things serology cannot

- Distinguish weak D from partial D antigen
  - OB patient - determine if candidate for Rh immune globulin
  - Conserved D- blood supply

- RH genotype – patients with sickle cell disease
  - Many have partial D, partial C, or partial e
  - Have complex Rh antibodies
  - Key for antibody identification and selection of donor units

- Determine allo or auto antibody
  - patient RBCs type positive for the antigen
  - has the corresponding antibody in the serum
  - Example: Jk(a+) with anti-Jka

- ABO genotyping
  - Determination of A2 subtype for kidney transplants
  - ABO subgroups
Sample requirements

• Whole blood, buccal swab, amniocytes
  – Any nucleated cell
  – Cannot use leukoreduced samples; not sufficient amount of white cells

• Sample volumes can be low
  – ~1/2 ml or even less

• No sample age requirement

• Can be post-transfusion
DNA technologies

• Targeting a single nucleotide polymorphism (SNP) or a few for one gene
  – Manual assays
  – PCR and electrophoresis
  – Manual interpretations

• Targeting many genes and multiple SNPs
  – DNA arrays
  – Immucor HEA panel – FDA licensed
  – Grifols
  – Interpretations performed by software

• Targeting numerous SNPs in one region of a specific gene
  – DNA sequencing
DARA (Daratumumab; Darzalex™) anti-CD38

- To treat multiple myeloma
  - CD38 highly expressed on plasma cells
- More diseases in clinical trials
  - B cell lymphoma
- New monoclonals being developed

Problem:
- CD38 also expressed on RBCs
- Excess antibody is circulating free in patient serum/plasma
  - antibody screen – positive in IAT
  - crossmatch – positive IAT
  - DAT – usually negative/weakly positive
DARA does not bind to DTT-treated RBCs

+ antibody screen

- antibody screen
Considerations/Challenges to DTT testing

- **DTT treated reagent RBCs not commercially available**
  - Must treat reagent RBCs in the blood bank laboratory
  - Write procedures
  - Train staff
  - Validation

- **QC to confirm performance**
  - positive and negative controls

- **Expiration of DTT-treated cells?**
  - establish laboratory storage limits
  - 1 week anecdotal
# DTT sensitive blood groups – antigens destroyed

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Antigens</th>
<th>Transfusion Reaction Potential</th>
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<tbody>
<tr>
<td>Kell</td>
<td>K, k, Kpa, Kpb, Js, Js</td>
<td>Immediate to delayed, mild to severe</td>
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<td>Knops</td>
<td>Kn, Knb</td>
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<tr>
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<tr>
<td>Raph</td>
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<td>No to moderate</td>
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<tr>
<td>YT</td>
<td>Yta, Ytb</td>
<td>Delayed (rare); Mild</td>
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<tr>
<td>Dombrock</td>
<td>Do, Do, Hy, Jo</td>
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<tr>
<td>Indian (IN)</td>
<td>In, Inb</td>
<td>Decrease cell survival</td>
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<tr>
<td>JMH</td>
<td></td>
<td>Delayed (rare)</td>
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</table>

Some options:

- transfuse K− RBCs
- use Trypsin treated cells to rule out Kell, LW, Yt antibodies
- use cord cells that are antigen typed
- do extended antigen typing on the patient to determine antibody risk
- consider extended matching with donor
Example of HEA/PreciseType panel

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<td>Hemoglobin S</td>
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</table>

24 polymorphisms associated with 35 antigens in single assay

C/c, E/e, V/Vs, K/k, Jsa/b, Kpa/b, Jka/b, Fya/b, MN, SsU, U, U+var, Doa/b, Hy/Jo, Coa/b, Dia/b, LWa/b, Lu/a/b, Sc1/2

(No ABO or RhD)

Assay time: ~5 hours
Not including DNA extraction
AABB recommendations

• For patients going on anti-CD38 therapy (DARA):

  1. Pre-Testing **before** a patient is placed on drug
     obtain sample and perform antibody screening

  2. If transfusion is required, perform extended
     antigen phenotyping/genotyping

  • Know which antibodies the patient can make
  • Have option to use extended antigen matching if
    transfusion is required
Genotyping for extended RBC antigens

• Immucor PreciseType HEA profile - includes 35 antigens
  – For multiple myeloma patients going on DARA therapy the HEA genotyping panel has been given status as a medical indication
  – CPT code 81403
  – Z code and IC10 coding is specific for the HEA FDA licensed test only
  – Most transfusions for multiple myeloma patients are done as out-patient
  – CMS reimbursement as out-patient
Case of Kell’s Fury

- 38 year old Caucasian female
- Pregnant with anti-K; titer 512

- Paternal sample was submitted for genotyping to predict fetal risk for hemolytic disease due to K
  - Had not been phenotyped by sending institution
  - Kk typings traditionally done by serology
  - Some confusion among genetic counselors

- Even though submitted for genotyping, NYBC practice to phenotype when possible
  - RBCs typed K+k–
  - Predicts all children will be K+
DNA results

• HEA Results:
  – *KEL*01/*02; predicted phenotype: K+k+

• Serologic phenotype: K+k– (*KEL*01/01)
  – k typing repeated by different technologist and with additional reagents
  – confirmed RBCs k–

• Manual PCR-RFLP for *KEL* performed:
  • *KEL*01/*02; K+k+

*Suggests weakened or silenced KEL*02 (k) allele
**KEL Sequence Results**

- *KEL* exons 1 to 19 sequenced
- Exon 6:
  - 578T/C; *KEL*01/*02
  - confirmed the HEA and PCR-RFLP results of *KEL*01/*02
- Exon 17:
  - 1790T/T; predicts Js(a–b+)
- Exon 8:
  - 841C/C; predict Kp(a–b+)
  - **Heterozygous for intron 8 +1g>t splice site mutation.**
  - Change was previously reported on *KEL*02 (k) background as a basis for the K₀ phenotype: ISBT# *KEL*02N.13

![Diagram showing exon sequences and mutations](image-url)
Paternal conclusions

• Paternal genotype:
  – *KEL*01/*02N.13
  – Predicts K+k– and explains the serology results

• Children have **50%** chance of being K+, not 100% as would have been concluded if serology alone performed

• The inability to detected silenced alleles is usually mentioned as a limitation of genotyping
  – **BUT** this case highlights the ability to detect the presence of two alleles (K and k) in the paternal sample by genotyping and enable the accurate prediction of risk for HDFN
Amniocentesis testing

• Amniocentesis performed

• DNA extracted and tested by HEA BeadChip and by manual PCR-RFLP
  – Genotyped *KEL*01/*02
  – RBCs of the fetus are predicted to be K+k+

• Fetus unfortunately inherited paternal *KEL*01

• Received 4 interuterine transfusions during pregnancy; last one 2 weeks prior to delivery
  – Baby delivered with slight anemia and jaundice.
  – Clean bill of health by 3 months of age
Summary

• $K_0$ phenotype is very rare but chance of carrying one null (or mod) allele is higher
  – From European studies: ~3.5 to 7.5% of KEL:1,–2 had one KEL*02 null or mod allele

• Case stresses that paternal samples with KEL:1,–2 phenotype, should be genotyped.
  – If one silenced (or mod) allele, fetus will only have 50%, not 100% risk to be affected by maternal anti-K.

• For similar scenarios, genotyping (and phenotyping) is important for accurate prediction of fetal risk to maternal antibody.
### Rh antigens diversity

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**Challenge:** Reagents detect only 5 principal antigens others prevalent in ethnic groups with sickle cell disease
**RH gene diversity**

- **Point mutations**
- **Gene conversion:**
  - Portions of *RHCE* into *RHD*
  - Portions of *RHD* into *RHCE*
  - No reciprocal crossover
  - Generated genetic diversity

- **RHD**: >495 alleles
  - Weak D
  - Partial D
  - Del

- **RHCE**: >110 alleles
  - ce, including hrB−, hrS−
  - Ce
  - cE
  - CE
Case history

- 74 year old African-American female
- Anemia; Hct of 18%
- 19 days earlier received 1 unit of RBCs
- Hospital had identified anti-E, -K, and -Jk^b
- Request for 2 units of RBCs
- Urgent!
# DAT result

Limited selected panel

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**Presence of anti-E, -K, -Jk\(^b\) confirmed**

(Panel not shown)

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</table>

**Some free RBCs seen with anti-C**

But the patient was transfused 1 unit
More testing

Patient’s plasma:
• Anti-E, -K and -Jk\textsuperscript{b} confirmed
• However, all E– K– Jk(b–) RBCs reacted
• Autocontrol was negative
• Antibody to a high prevalence antigen suspected

Patient’s RBCs:
• D+ C+ E– c+ e+; S–; K–; Fy(a–b–); Jk(b–)
• Typing with antibodies to high prevalence antigens showed them to be Js(b–) and hr\textsuperscript{B–}
Patient results continued

- Anti-Js\textsuperscript{b} identified in the patient’s plasma
- Patient urgently needed blood
- Tested RBCs from 4 “phenotype matched” donors with patient’s plasma
  - Js(b–), D+C+E–c+e+, K–, S–, Fy(a–b–), Jk(b–)

<table>
<thead>
<tr>
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\textsuperscript{**} some free RBCs seen with anti-C
But the patient was transfused a month ago

All were 3+ incompatible!
Case Study Continued

• Anti-C identified
• C– E–, K–, S–, Fy(a–b–), Jk(b–), and Js(b–) RBCs were compatible

BUT...

• Patient’s RBCs had been typed as C+
  – 2+ with GammaClone anti-C
  – No evidence of the previously transfused RBCs
  – No evidence of autoantibody

• What are the possibilities and what testing can be done to resolve this?
DNA testing

• Arrays performed:
  – HEA – common red cell; 2 variant RHCE markers
  – RHD – targets many weak, partials, hybrids alleles
  – RHCE – targets many variant c, e, C, and E alleles

• Manual assays:
  – Multiplex PCR: targets C/c as well as RHD exons 4 and 7, inactive RHD pseudogene
  – RHD exon 8 for 1136C>T (DAU alleles) – not on RHD array
  – RHCE exon 2 for 254C>G (e variant) – not on RHCE array
  – Sequencing as needed
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<th>Antigen</th>
<th>Result</th>
<th>Comments</th>
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</table>

**LEGEND**

(+)*: Possible hybrid allele. Additional serological testing recommended for Big C.

(0)*: GATA silencing mutation present

CV (Coefficient of Variation): CV of intensities above recommended maximum

HB (High Background): Signal intensity above recommended maximum

IC (Indeterminate Call): Algorithm unable to confidently predict result

LS (Low Signal): Signal intensity below recommended minimum

NTD (No Typing Determined): Typing was not able to be determined

Var: U variant detected

w: Weak expression
DNA Results

• HEA Array:

<table>
<thead>
<tr>
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<th>+</th>
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</thead>
<tbody>
<tr>
<td>C</td>
<td>(+)*</td>
</tr>
<tr>
<td>e</td>
<td>+</td>
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<tr>
<td>E</td>
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</table>

Possible hybrid

C–  OR  C+(partial)

• HEA array predicts (+)*; targets 2 markers on the RHCE*ceS (C-).
  – This allele commonly linked to hybrid RHD allele and could be C+.

Hybrid \textit{RHD*DIIIa-CE(4-7)-D}

C+ (partial)
DNA Testing and Results

• Results summary:
  – Hybrid $RHD^{*}D_{IIIa-CE(4-7)-D}$: partial C; confirms allo anti-C
  – $RHD^{*}D_{IIIa}$: partial D phenotype and at risk for allo anti-D
  – Homozygous $RHCE^{*}ceS$: partial c and e (and f), VS+V−, hrB−, and at risk for allo anti-e, -c, -f, and/or -hrB
**r'S or (C)ce'S haplotype**

**Hybrid** \( RHD^{DIIIa-CE(4-7)-D} \)

- Does not encode D
- Protein that is made reacts strongly with most anti-C reagents
- Associated with production of allo anti-C
- *Up to 1 in 3 C+ African Americans have this partial C*

**RHCE^{ceS}**

- Partial c and e (and f) antigens
- \( hr^B-, V-VS^+ \)
Transfusion

• The anti-C made by the patient is an alloantibody

• RBCs that are C– should be used for transfusion

• If the patient later made an anti-hr^B and needed rare blood, RH genotype matched units may be needed but some these would be r'S and therefore, C+.

  – Would you accept C+ (same variant) units?

  – Could you accept C+ (same variant) units?
Case history

- 20 yo pregnant female with Sickle Cell Disease
- Pain episode
- First pregnancy, gestational age 17 wks
- On chronic exchange transfusion protocol

- ABO/Rh: B negative
- Antibody screen: Positive

<table>
<thead>
<tr>
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<td>+</td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Anti-D**

- **Auto**: 0

---

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New York Blood Center
Differential for Anti-D in D- pregnant woman

1. **Active anti-D – possible high risk pregnancy**
   - RhD type father by serology
   - if D+: RHD gene zygosity testing to determine gene copy number
     - If two copies of RHD, all children expected to be D+
     - If 1 copy of RHD, 50% chance children can be D–
   - follow maternal antibody titers
   - type fetus from amniocentesis (or maternal plasma if available)

2. **Passive anti-D – rule out patient received RhIG**
   - anti-D not usually seen in first pregnancy
   - titer of passive antibody is less than 1:8
**RH LOCUS**

"Rh positive"  

- **RHD**  
  - D antigen

"Rh negative"  

- **RHCE**  
  - ce, Ce, cE, or CE

- **X Deleted X**  
  - Europeans: 15-17%
  - ce
Paternal *RHD* zygosity: one copy or two?

Two assays for presence of deletion region:

- Father D+/D-
- Possibility that baby could be D negative
Paternal results

- Hybrid box present
  - Indicates at least one deleted D

- Consistent with RHD hemizygote

- Paternal sample results (RBCs typed D+)
  - RHD hemizygote
  - 50% probability that offspring are D–
Additional Testing

<table>
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<tr>
<th>Anti-D</th>
<th>RT</th>
<th>AHG</th>
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<tr>
<td>Gammaclone</td>
<td>0</td>
<td>WP/MF</td>
</tr>
<tr>
<td>Immucor D4</td>
<td>0</td>
<td>1+/MF</td>
</tr>
<tr>
<td>Immucor D5</td>
<td>0</td>
<td>1+/MF</td>
</tr>
</tbody>
</table>

**D typing:**
- positive in AHG phase
  - not D–
  - possible weak D?
  - MF = mixed cell population

MF = mixed cell population
- fetal D+ bleed?
- transfused D+?

**KB - fetal hgb = negative (D typing not due to fetal cells)**

**anti-D titer: 4** probable passive anti-D

- Presented to another Emergency Department 2 weeks ago
- B neg, Antibody screen: negative
- Concern for fetal-maternal hemorrhage
- Received RhIG

**Conclusion: passive anti-D**
Additional history

- Historically B+ at home institution where she receives her exchange transfusions

- History of anti-D detected 8/07

- Anti-D has not demonstrated since 12/07

- Since 2007 on D– transfusion protocol

- Last transfusion 2 months ago
  - 3 units group B, D– PRBC
Hmmm….

• What could be the reason for the mixed field reactivity seen at AHG phase?

• Is the patient D+ or D–?

• Could the patient have a weak D or partial D?
Variation in RhD antigen expression

- **Weak D**
  - require antiglobulin phase for detection (or react weaker than conventional)
  - Have single amino acid changes in RhD
    - Majority (>90%) are Type 1 (V270G), Type 2 (G385A), Type 3 (S3C)
    - Most Caucasian
    - NOT at risk for anti-D
    - Rare exceptions (Type 11, 15, 21, 33) have made anti-D
      - NOT associated with hemolytic disease

  NOT CANDIDATES FOR Rh IMMUNE GLOBULIN

- **Partial D**
  - Many react 3+ : so go undetected by serology **BUT** there are some partials that type weakly D+
  - AT RISK for anti-D because lack some RhD epitopes
    - Partial DVI – (Caucasians) - fatal hemolytic disease reported
    - Partial DIVa and DIIla (African American) – 3+ initial spin

CANDIDATES FOR Rh IMMUNE GLOBIN
Patient’s RHD Genotype

- When first reported as **weak D 4.0** as it did not appear to lack D epitopes
- Now called **weak partial D 4.0** as number of patients have made anti-D
- The risk for anti-D in pregnancy is unknown but appears to be far less than for other partial D phenotypes.
- Patients with this allele are at risk for anti-D
It’s time to phase in *RHD* genotyping for patients with a serologic weak D phenotype


**TRANSFUSION** Volume 55, March 2015

Persons whose *RHD* genotype is weak D type 1, 2, or 3 should be managed as RhD-positive with regard to administration of RhIG and/or selection of blood components for transfusion.

For women with a serologic weak D phenotype associated with *RHD* genotypes other than weak D type 1, 2, or 3, the Work Group recommends that these women receive conventional prophylaxis with RhIG, including postpartum RhIG if the newborn is RhD-positive or has a serologic weak D phenotype.
OB patients RHD genotyping results

- To guide RhIG prophylaxis and selection of blood for transfusion
  - **OB women with D typing discrepancies**
    - positive previously and now negative: or the reverse
    - Rh type from physician office different than hospital
  - **D typing weaker than expected**

<table>
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<tr>
<th>$RHD^*$</th>
<th>weak D type 1</th>
<th>weak D type 2</th>
<th>weak D type 3</th>
<th>weak D type 4.0</th>
<th>Partial DAR</th>
<th>No RHD $RHCE^*ceCF$</th>
<th>New alleles</th>
<th>Total</th>
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<td>16</td>
<td>9</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>36</td>
</tr>
<tr>
<td>% of total tested</td>
<td>44%</td>
<td>25%</td>
<td>5.5%</td>
<td>5.5%</td>
<td>11%</td>
<td>2.8%</td>
<td>5.5%</td>
<td>100%</td>
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<tr>
<td>Risk for anti-D</td>
<td>NO</td>
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<td>YES</td>
<td>YES</td>
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<td>Candidate for RhIG</td>
<td>Candidate for RhIG</td>
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</table>

75% 25%

NYBC experience – since July 2016

• 85 OB patients referred with weak D antigen expression
  – 43 (51%) weak D type 1, 2, or 3
  – 39 (46%) partial D
  – 2 uncommon weak D alleles
  – 1 new allele
Goals of DNA-based testing

• RBC DNA testing performed on all patients
  – Only needs to be done once (assuming no BMT etc.)
  – Results become part of the patient’s electronic medical record
• Minimize (prevent) alloimmunization
  – Provide more precise matched blood versus “least incompatible” when patients have warm autoantibodies
• Reduce cost
  – Decrease patient work-ups:
    • EGA treatment; hypotonic wash
    • Adsorption/elution
  – Locating rare blood
  – These costs have not been previously calculated but offer significant savings.
Reducing or preventing alloimmunization for...

- **Patients with sickle cell disease**
  - C, E, K negative
  - RH genotype for improved Rh matching (D, C)

- **Patients with warm autoantibodies**
  - Decrease number of complex allo and auto adsorptions
  - Eliminate “least incompatible” terminology

- **Patients who have made one alloantibody**
  - 20X increase risk for additional antibodies

- **Females of child-bearing age**
  - Avoid anti-K and anti-c (done in Europe)
    - Kell – 10% potentially exposed
      - Anti-Kell - 1/100 pregnancies; 40% K+ babies have anemia
    - c – 18% potentially exposed
      - Anti-c associated with 32 fetal deaths in England and Wales (1977-1990)
What’s to come…

• Next generation sequencing (NGS)
  – Sequencing of the whole genome
  – Generate mass amounts of data
  – Still few months to results but getting shorter all the time

• Exome targeted sequencing

• Bioinformatics field crucial for interpretation of the data

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Comprehensive red blood cell and platelet antigen prediction from whole genome sequencing: proof of principle

TRANSFUSION 2016;56;743–754

William J. Lane,¹,² Connie M. Westhoff,³ Jon Michael Uy,¹ Maria Aguad,¹
Robin Smeland-Wagman,¹ Richard M. Kaufman,¹ Heidi L. Rehm,¹,²,⁴,⁵ Robert C. Green,²,⁵,⁶ and
Leslie E. Silberstein⁷ for the MedSeq Project*