Molecular Typing of Red Cell Antigens
(When, Why and How?)

Transfusion Practice for Sickle Cell Disease
Education Session VIII - ASFA & AABB Joint Session
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Genomics Revolution

• Impacting all areas of laboratory testing
  • Identification of microbes
  • Tumor diagnosis
  • Coagulation therapy

• Transfusion Medicine
  – Genes encoding blood group antigens cloned in 1990’s
  – DNA-based approach to “predict” red cell & platelet antigens
  – Most antigens are encode by single nucleotide polymorphisms
    • “SNP-typing”
  – >300 blood group antigens
    • 18-30 are routine problems for transfusion
  – “extended antigen profile”
  – Not for routine ABO and Rh typing
When and Why? DNA-based antigen typing

- **Type multiply transfused patients**
  - avoid interference from circulating transfused donor RBCs
  - cell separations labor intensive and can be inaccurate

- **Type RBCs coated with immunoglobulin (+DAT)**
  - alternative – chemical treatment (AET, DTT)
  - labor intensive; destroy or weaken some antigens

- **Type clinically significant blood groups for which there are no commercial reagents**
  - Do(a/b), Hy, Jo(a), Js(a/b), Co(a), Yt(a), VVS, U, etc.
DNA-based antigen testing: Strengths

• Do not need a RBC sample
  – buccal swab - bone marrow transplant patients
  – fetal amniocytes

• Determine fetal risk for HDFN (antibodies to RBCs)
  NAIT (antibodies to platelet antigens)
  - Paternal testing to determine risk
  - gene copy number (zygosity: RhD and HPA)

• Test for numerous minor antigens in a single assay
  – improved accuracy
    • antigen typing
    • antibody ID
  – find uncommon combinations of antigens in donor inventory
  – provide higher level of care
>15 years experience: methods have evolved

Manual
RFLP/SSP

Semi-Automated
Real-time PCR

Automated
DNA probes on miniaturized beads on silicone chip
BioArray/Immucor

PCR

DNA probes on colored beads
Luminex
Progenika

Gel Electrophoresis

Automated readout

8 samples

96 samples

automated interpretation expands use

New York Blood Center
ENCODED BEADS IN RANDOM ARRAY

Markers for 35 antigens/chip

20-30 beads each probe

96 samples = 3,360 antigens

8 samples = 280 antigens

8 different samples

Probe N
128 colors

FYA

FYB
Human Erythrocyte Antigen (HEA) Phenotyping by DNA Analysis Report

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Phenotype read-out
+ = positive
0 = negative

35 antigens

+ HgbS

V/VS
## ID CORE-XT

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*1: according to ISBT terminology

*2: Weak antigen expression

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37 antigens
Laboratory Environment for Testing

• **3 separate laboratory areas**
  – Sample DNA extraction
  – Pre-PCR set-up (“clean”)
  – Post-PCR analysis (“contaminated”)

• **Power of PCR to amplify contamination from environment**
  – Sterile-like techniques
  – Hood with UV or positive pressure room
  – Dedicated equipment and supplies
  – Gloves
  – Filter tips for pipets

**Challenge:**
Methods and equipment not common in hospital blood banks
Laboratory Pre-transfusion Testing
Routine: ABO, RhD type and antibody screen

2016: 36 Blood group systems (352 antigens)

Approach has not changed in >60 years

1945: Introduction of Indirect Antiglobulin Test

- Carbohydrate
- Single-pass membrane protein
- Multi-pass membrane protein
- GPI-linked protein
- Adsorbed from plasma

Cover Transfusion, Reid et al.
Why interest in more than ABO and D?

ALLOIMMUNIZATION

~3% transfused patients make antibodies (alloimmunized) to foreign red cell antigens

35% or more of chronic transfused patients
- increase costs of each subsequent transfusion
- delay in providing transfusion
- life-threatening in emergency

11.6 M transfusions in U.S./year
32,000 transfusions / day

Is this level of complication acceptable medical practice today?
65% of antibodies drop to undetectable levels in 6 months

- patient at risk for transfusion reaction
- can be life-threatening
  - 90% anti-Jk\textsuperscript{a} disappeared
- all had disappeared by 10 years
- only anti-D was very stable

The persistence and evanescence of blood group alloantibodies in men. Tormey CA, Stack G. *Transfusion* 2009, 49:505-12

What is the value of antibody screen and crossmatch for detecting compatibility?
Why interest in more than ABO and Rh?

Females: pregnancy complications

**hemolytic disease of fetus and newborn**

1960’s Rh disease prevention with **Rh immune globulin injection**
- prevents maternal antibody production

**K antigen** – 10% potentially exposed
  - Anti-K 1/100 pregnancies, 40% K+ babies severe anemia

**c antigen** – 18% potentially exposed
  - Anti-c – 32 fetal deaths in England and Wales (1977-90)
Prevention of Alloimmunization - Western World

The most common antibody specificities: Rh C, E, c; K (Kell)

- **Germany**
  - Majority get CcEe & K matched

- **Netherlands**
  - Females <45yr c E K
  - SCD/Thal CEK, Fya, Jkb, Ss

- **Finland**
  - Females CcEe & K matched

- **UK**
  - Females get K-

- **Switzerland**
  - Females CcEe & K
  - Chronic transfused or alloantibody - match for CcEe K Fy, Jk, Ss

- **U.S.**
  - Matching for CEK for SCD is common but not universal (due to cost)

- **Australia**
  - Females and children K-
Higher Level of Patient Care

• Blood transfusions have declined significantly over the last five years
  – advances in surgical techniques
  – patient blood management (PBM) programs

• Lower hgb threshold for patients (7.0 gm/dl) and limited transfusion
  – Optimal RBC survival more important than ever

• Health Care Landscape
  – focus on outcomes – improved patient care
  – personalized medicine with Genomics
When to apply genomics to transfusion therapy?

- **Patients with warm autoantibodies when compatibility cannot be demonstrated by routine methods**
  - Genotyping allows extended patient antigen profile
  - Select “antigen matched” (antigen negative) RBCs for transfusion [Rh CcEe, K, Fya/b, Jka/b, Ss]
  - Avoids the use of term “least incompatible” units
  - Allows transfusion of units “antigen-matched for clinically significant blood group antigens”

- **Improve patient care AND turn-around time**
  - eliminate frequent repeat adsorptions to remove the autoantibodies
  - to rule out new underlying RBC alloantibodies.
When to apply genomics to transfusion therapy?

• **Patients receiving monoclonal anti-CD38 therapy**
  – Multiple myeloma
    • CD38 highly expressed on plasma cells
      – more applications in clinical trials
    • Additional monoclonals in trials
      – anti-CD47

**Problem:**
– CD38 and CD47 also expressed on RBCs
  • Antibody circulating in patient serum/plasma
    – antibody screen – positive
    – crossmatch – positive
    – compatibility cannot be demonstrated

Anti-CD38 (DARA; Daratumumab; Darzalex™)
When to apply genomics to transfusion therapy?

- **Patients receiving monoclonal anti-CD38 therapy**
  - Use 0.2M DTT to treat test RBCs
    - denatures CD38 on RBCs
    - can detect underlying alloantibodies
  - Problem: DTT denatures other RBC proteins
    - Kell, Dombrock, Yt, LW, Gerbich, Cromer
    - Miss detecting these antibodies
  - DTT not routinely used in hospital laboratory
  - DTT treated cells not commercially available

- **Patients receiving monoclonal anti-CD47 therapy**
  - interferes with ABO/Rh typing, crossmatch, antibody screen
    - high level expression on RBCs
    - cannot remove CD47 from the RBC
When to apply genomics to transfusion therapy?

• **Rh typing for D**
  
  – Altered expression of RhD in approximately
    • 2% of Caucasians; 1% of Asians; 4% of Black and Hispanic
  
  “Weak D” or “Partial D” - routine typing cannot distinguish
  • “Partial D” – lack RhD epitopes; at risk for anti-D
  • “Weak D”- do not lack RhD epitopes; not at risk
  
  – Of clinical importance for
    • women of child-bearing age
    • to avoid pregnancy complications
    • consider transfusion with D negative units
    • determine who is candidate for Rh immune globulin (RhIG)
When to apply genomics to transfusion therapy?

- **Transfusion dependent patients** who make antibodies or any patient who has made one alloantibody
  - increase risk (20 fold) for additional antibodies

- **Sickle Cell Disease and alloimmunization**
  - U.S. >100,000 patients (~12,000 in NY)
    - ~50% are chronically transfused
  - 1/12 (8%) of African-Americans carry the gene
    - 1/500 African American births HgbS/S sickle cell anemia
    - 1/35,000 Hispanic births
      - over 1,000 affected newborns each year
Mobility, migration and SCD

Number of migrants with HbS increased over other migrants

WHO number of international migrants
1960 - 92.6 million, 1.6M with HbS
2000 - 165.2 million, 3.6M with HbS

Population movements create a long-term burden on health-care systems

Mobility, migration and SCD

- HbS prevalence due to protection from malaria in endemic regions
  - Global population movements having a substantial effect on the distribution of the HbS gene

Random sampling of data points for presence of HbS gene

Piel et al. Lancet 2013:381:142-51
Rh Antigens - defined by serology

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Serologic Rh typing – 5 principal antigens
- found in all ethnic groups

RH genotyping
- > 495 different RHD alleles
- > 155 different RHCE alleles (majority in Blacks)

red = associated primarily with Black African ethnicity
**RH Locus – 2 Genes**

Rh “positive”

- **RHD**
  - 3’ → 5’
  - D antigen

- **RHCE**
  - 5’ → 3’
  - C/c and E/e

35 amino acid differences

Rh “negative”

- **RHCE**
  - C/c and E/e

Gene deletion, majority

- XXXXXXXXX

>85 others due to mutations

Serologic typing reagents detect 5 principal antigens: D, C/c, E/e
**RH alleles clinically relevant to date**

Gene

- **RHD**
  - 10 exons
  - 5' to 3' 30 kb
  - > 495 RHD alleles
  - > 495 different RhD proteins

- **RHCE**
  - 10 exons
  - 5' to 3'
  - > 155 RHCE alleles
  - > 155 different RHCE proteins

RBC’s

- RhD
- RhCE

C/c  E/e
**RHD hybrid alleles = “Partial D antigen”**

RHD exons replaced with RHCE exons

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RBCs type as RhD+, but patients can make anti-D
**RHCE allele variation in African Blacks**

**RHD**

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**RHCE**

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- **ce**
  - ce
  - ceS
  - hrB-
  - ce
  - ce (JAL)
  - ceMO
  - hrS-hrB-
  - ceTI
  - hrS-
  - ceEK
  - hrS-
  - ceAR
  - hrS-
  - ceBI
  - hrS-
RHCE genotyping needed for C antigen-matching

RBCs type as C+, but patients can make anti-C

- Frequency estimated to be 8-22% of C+ African Blacks
- Patients better served with C− donor units

**RHD**

- D/Ce/D hybrid
- Does not encode D
- Encodes altered C+

**RHCE**

- encodes altered e

- N-linked glycosylation site

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- W16C
- L245V
- G336C

Ce<sup>s</sup>

VS+

hr<sup>B−</sup>
when serology isn’t enough

– 4 year old (born 2000) Sickle Cell Disease
  – Primary Treatment – hydroxyurea
  • Transfusion protocol
  – antigen-match for C, E, K

• Pre-transfusion typing
  – O positive (D+C+c+E-e+)
    – extended phenotype K-, Jk(a+b-), Fy(a-b-), S+s-, M+N+

  – Transfused with E- and K- units
Transfusion History

Pt: O pos D+C+c+E-e+

- 2000 (5 months of age) – 1 unit
- 2001 – 2 units
- 2004 – 2 units
- ~4 months after last transfusion
  - pain and fever & enlarged spleen
    - 4 gm/dl hgb
  - surgery for splenectomy
    - 2+ antibody Screen
    - 2+ DAT
  - E-K- units were incompatible
    - Anti-Jk(b)
    - Anti-C and –e (-hrB)
• **RHD** = not at risk for anti-D

• **Hybrid - altered expression of C antigen**
  – explains anti-C in patient whose RBCs type as C+
    • In U.S. ~35% of African Blacks who are C+ RBCs
    • **should receive C- units to prevent anti-C**

• **RHCE - encodes altered expression of e antigen**
  – Explains production of anti-e in patient whose RBCs type e+
Options for Transfusion

O Pos  D+C+E-c+e+

• **Units negative for C and e (DcE/DcE; R2R2)**
  - 2% of donors
  - Patient at significant risk for anti-E
    • Save for emergency transfusion

• **RH genotype matched donor**
  - Family members ?
  - One younger sibling HgbS/S
    • Parents are HgbS+
  - Aunt - O positive, K-, E-, Jk(b-), HgbS-

• **American Rare Donor Registry**
  - 2004 – 4 eligible donors K-, E-, Jk(b-), HgbS- and serology hrB-
    - 1 - California
    - 1 - Florida
    - 1 - Wisconsin
    - 1 - Louisiana
RH Genotype Donor Matching

• Aunt

Normal D

RHD

D-CE-D

hybrid

D- C+

RHCE

ce

Normal

ceS

VS+ V-

- Incompatible - one allele encoding conventional e antigen

• ARDP - 3 donors

- Compatible – homozygous for one of his RH haplotypes

D-CE-D

hybrid

D- C+

RHD

D-CE-D

hybrid

D- C+

RHCE

ceS

VS+ V-

ceS

VS+ V-
Transfusion

- 3 units
  - 1 unit transfused pre-surgery
  - 1 unit transfused subsequent tonsillectomy
  - 1 stored for future use

- Maintained on hydroxyurea therapy

- Has received 10 genotyped units
  - 2004-2016

- No additional antibodies

Future
Genomics Revolution

• Whole genome sequence data will be available
• Especially for patients with chronic disease

• Will need to “read” i.e. translate the information

• “Sequence Once; Read Often”

45 RBC genes
-346 antigens

6 platelet genes
-33 antigens

*Ilumina HiSeq* - 30X coverage

William J. Lane, MD, PhD
Promise Genotyping

Transfusion Medicine decision making through Bioinformatics
Thank You!

New York Blood Center