Partners in Transfusion Medicine:

*Serology and DNA*

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Objectives

- Review applications for genotyping
- Discuss limitations of genetic testing
- Review common Rh genotypes
- Become familiar with information provided by genotyping reports
- Discuss implications and use of genotyping results
Molecular testing applications for patient testing

- Predict phenotype of recently transfused patients and those with WAIHA
- Distinguish alloantibody from autoantibody
- Resolve phenotyping discrepancies
- Detect weakly expressed antigens
- Determine zygosity, particularly RHD
- Identify molecular base of unusual serologic results
And for donors........

- Determine antigen status when antibody is weak or not available
  - Examples: VS, V, Js\textsuperscript{a}, Do\textsuperscript{a}, Do\textsuperscript{b}
- Screening to provide antigen matched units
- Confirm serologic results for reagent cells
  - Efficient; provides large amount of information
- Resolve typing discrepancies
  - e.g. A, B, D, C, e
Why not perform ABO and D testing of donors by DNA?

**ABO:**
- 4 phenotypes: A, B, AB, O; >100 alleles known
- Current hemagglutination tests work well
- Naturally occurring anti-A/-B; a built-in check
- History check for repeat patients or donors
- DNA helpful to distinguish inherited from acquired antigens

**D typing:**
- 1 antigen; ~200 alleles known
- Current hemagglutination tests work well
Limitations of DNA analysis

- DNA and serologic results may not agree:
  - Allogeneic stem cell transplantation
  - Natural chimerism
  - Surrogate mother; after artificial insemination

- DNA results from somatic cells and from WBCs may not agree
  - Allogeneic stem cell transplantation
  - Natural chimerism

Accurate medical history is critical
Another limitation: Genotype does not always equal phenotype

DNA-based assays may detect a normal gene that is not expressed due to presence of a silencing gene

- Person could be falsely identified as antigen-positive
- An antibody could be considered to be auto rather than allo-antibody

It is important to correlate serologic with the molecular results!
Example: *RHD* pseudogene silences expression of the *D* gene

- When present, the person is predicted to be Rh positive based on genotyping only.
- This silencing gene (RHΨ) results in no D antigen production.
- However, serologic testing would show that the person is Rh negative.

*Genotypic*: Rh Positive

*Phenotypic*: Rh negative and capable of producing anti-D.
**RHD Zygosity testing**

- Important to:
  - know the race of the parents
  - test both parents at the same time
  - consider possibility of *RhD* silencing gene

- Examples:
  - 10% of Japanese who type Rh neg have the Del phenotype (*D* ag. detected by absorption/elution)
  - ~25% of Blacks have *RHD* pseudogene (non-functional gene)----no D antigen produced
  - Blacks may have *RHD-CE-D* hybrid: phenotype as *r's*

- Testing now offered by ARC Molecular Lab
GATA mutation silences expression of $\text{Fy}^b$

- A nucleotide change in $\text{DARC}$, the Duffy gene
- Results in disruption of the red cell binding site and prevents expression of the gene
- Duffy glycoprotein - present in many cell lines
- $\text{Fy}(a-b-)$ persons of African descent lack Duffy protein on red cells but not in other tissues
  - Explains why they do not make anti-$\text{Fy}^b$ and only rarely make anti-$\text{Fy}3$ or $-\text{Fy5}$. 
Why is GATA important?

- Tells us which patients are capable of producing anti-Fy\(^b\) or –Fy3
- For alloimmunized patients who benefit from phenotyped matched red cells and who phenotype Fy(a-b-), the genetic information is useful to determine when Fy(b-) blood is needed.

Example: Patient has antibodies to:

C, e, K, Fy\(^a\), Jk\(^b\), S: 4 in 1000 Black donors
C, e, K, Fy\(^a\), **Fy\(^b\)**, Jk\(^b\), S: 1 in 2-3,000 Bl. donors
Other examples of DNA pitfalls

- Nucleotide change in *KEL* gene:
  - Phenotype: **K+w k+**
  - DNA prediction: **K- k+**

- Silenced gene:
  - Phenotype: **Jk(a- b+)**
  - DNA prediction: **Jk(a+ b+)**

- Testing can include changes - How much is feasible or practical?
Rh gene theories and nomenclature refresher

- Fisher-Race: 3 closely linked genes
  - C/c, E/e and D
  - Example: Dce

- Weiner: a single gene encoding several factors
  - Example: R1 (DCe)

- Tippett: Correctly proposed two genes
  - $RHD$ and $RHCE$
    - In close proximity on Chromosome 1, encoding 416 AA proteins; 97% identical
More nomenclature of RH alleles

- **RHD** denotes the normal D allele
  - Additional information to describe partial or variant alleles is added
  
  *Ex.: RHD*DVI* denotes the allele for partial D cat. VI*

- For CE alleles, **RHCE** is followed by the notation for the protein they encode
  - Proteins: *ce, Ce, Ec* or *CE*

  *Example: RHCE*ce* denotes the allele encoding c and e proteins. For variants, the number for the nucleotide substitution is provided in parentheses, RHCE*ce*(733G)*
43 y/o male with chronic renal failure and GI bleeding

Transfused twice in past 2 months

Typed as …AB Neg by hospital

… AB Pos by Reference Lab

Received Rh Neg red cells

DAT: weak positive with IgG and C3

Eluate: Reactive with all cells

D+ cells react 3+; D- cells react 1+
Plasma reacts with all cells positive for D and/or C plus Js(a+) cells

Cell separation performed to phenotype
  - DAT on autologous cells is negative
  - Pt types negative for C, E, K, Fy\(^a\), S, and Js\(^a\)

Additional D+, C- cells tested----all positive
  - Could it be anti-G?

*Pt is bleeding!* D-, C-, Js(a-) RBC provided
EW: What does DNA tell us?

- **RHD alleles:**
  - *RHD*DAR: encodes partial D, associated with production of allo anti-D
  - Inactive *RHD allele*: does not encode D

- **RHCE alleles:**
  - *RHCE*ceAR: associated with VS-V+, hr$^S$-
  - *RHCE*ce(48C): associated with weak e expression.
**D:** Molecular testing provided evidence that EW inherited a partial D gene. The other RHD allele is inactive.

**ce:** One allele for partial e (hrS); One for weak e expression.

**Original phenotype:**  \( D+C-E-c+e^+ \) or \( RoRo \)

**Phenotype predicted by genotype:**

\[ \text{partial } D+C-E-c+e^+ \text{ or } Ro^{\text{variant/r variant}} \]

**Need to consider possibility of anti-e or f(ce) in future work-ups.** Give \( D-, C-, Js(a-) \) units
**RH LOCUS**

- **Rh positive**
  - RHD
  - ce, Ce, cE, or CE

- **Rh negative**
  - deleted RHD
  - ce

**Rh proteins**

- RhD
- RhCE

32-35 amino acid differences

**Variants**
- C/c Ser103Pro
- E/e Pro226Ala
A. Conventional Genes

RHD

1 2 3 4 5 6 7 8 9 10

RHCE

1 2 3 4 5 6 7 8 9 10 ce cE

A226P P103S

B. Altered RHD Genes

D/CE/D

No D antigen, altered C antigen

DIIIa

N152T T201R F223V

DIII

Type 4 L62F A137V N152T

DAU

T379M

DAR

T201R F223V I342T

DOL

M170T F223V

DIVa

L62F N152T D350H

C. Altered RHce Genes

ce^S hr^B-

W16C L245V

W16C L245V G336C

W16C V223F

W16C M238V R263G M267K

W16C M238V L245V R263G M267K I306V

W16C M238V A273V L378V

W16C L245V G336C
Conventional RH genes

RHD

Exons 1 2 3 4 5 6 7 8 9 10

RHCE

Exons 1 2 3 4 5 6 7 8 9 10

Altered/Variant RH genes

D/CE/D

Hybrid No D antigen; encodes altered C

W16C L245V G336 ceS V- VS+

DIIIa

L62F A137V N152T T201R F223V

W16C V223F ceMO

DAU

R70Q F223V S230I E233Q/K V279M S333N T379M

W16C M238V R263G M267K

DAR

T201R F223V I342T

M238V L245V R263G M267K I306V

● Try
● seeing
● things
● from
● a different
● perspective.
Information provided in reports on DNA analysis

- Methods used are provided
  - Ex.: PCR-multiplex analysis
- What was tested?
  - Ex.: inactivating *RHD* pseudogene; zygosity by hybrid box detection.; the exons analyzed
- Results: Exons 4 and 7 are present. Genetic markers for c and e are present. Negative for the inactivating *RHD* pseudogene.
**RHD: Two alleles will be listed.**

*Example:* Rh Positive patient with anti-D reactivity in plasma.

- **RHD*DIIIa:** denotes category D IIIa. The report may also include the specific amino acid changes (62Phe, 137 Val) detected that characterize the variant allele found.

- **RHD** with no changes associated with common partial D.

**Conclusion:** Patient has one partial D and one normal D gene; not expected to make alloanti-D.

**Need to re-evaluate the anti-D reactivity.** Is the anti-D autoantibody? If yes, patient can receive Rh Positive blood.
Example: Serology indicates pt. has anti-e like ab.
Rh typings: D+ C- E- c+ e+  Predict: Dce/Dce

RHCE*ce (48C, 733G)
-48C encodes 16Cys; 733G encodes 245Val
-associated with partial e, and VS+V+, hrB-

RHCE*ce (733G)
- 733G encodes 245Val
- associated with partial e, and VS+V+, hrB-

Conclusion: Patient has two altered CE alleles associated with a hrB- phenotype and production of allo anti-e, -hrB or –ce(f).
Finding hr^B negative donors

- Currently at least 50 genetic backgrounds for hr^B negative status
- 1\(^{st}\) choice: e negative (when pt is E+)
- 2\(^{nd}\) choice: molecular match by American Rare Donor Program
  - (C)ceS / (C)ceS
  - DAU0-ce / (C)ceS
  - DIIIa ceS/(C)ceS
  - (C)ceS/ Y ce16C
- 3\(^{rd}\) choice: crossmatch compatible
TO: Confirmation of rare type

- 35 y/o woman with sickle cell disease
- History of anti-hr$^S$ (partial e) from 1995
  - 2002: anti-D and warm auto detected---Is anti-D auto or allo?
  - 2006: DAT-negative; anti-D reacts like alloantibody
  - 2010: referred for molecular testing
- $RHD^*DAR$: homozygote; partial D
  - Associated with altered ce-allele, $RHCE^*ceAR$, encodes for the hr$^S$ negative
TO: what does it mean?

- Patient inherited two very rare genes that encode for partial D and the hr\textsuperscript{S} \textbf{negative} phenotype.

- Molecular results confirm the serologic results.

- Transfuse with D-, hr\textsuperscript{S} \textbf{negative} blood – an extremely rare phenotype.
BB—Another Rh surprise

- 57 y/o woman with sickle cell disease
- Multiply transfused; Referred to ARC RL numerous times since 2005 from two facilities
- O Positive with anti-D
- Plus antibodies to C, E, Fy^a, Jk^b, Le^a, Le^b, M and ‘high titer-low avidity’ reactivity
- Increasingly difficult to work-up
- Referred for RHD variant genotyping
BB: Genotyping results

- **RHD** homozygote: two D genes present
  - **RHD*DIIIa**— partial D
  - **RHD** type 4.0— weak partial D
  - Report includes the amino acid changes

*Patient inherited two partial D genes!*
**BB: RHCE genotyping results**

- **RHCE*ceS:**
  - Encodes partial c; associated with partial e with VS+V-, hrB- phenotype

- **RHCE*ce(48C,733G):**
  - Encodes partial c; associated with partial e and with VS+V+, hrB- phenotype

*Two variant alleles----partial c and e; f (ce) antigen may also be affected.*
BB’s Rh phenotype

- Original phenotype in 2005:
  - D+ C- E- c+ e+ or RoRo

- Genotype:
  - Ro variant / Ro variant
  - $D_{IIIa-c}$ / Weak Partial RHD type 4.0-ce (48C, 733G)

- Predicted phenotype:
  - Partial D+, C-E-, partial c+, partial e+, VS+ V+, hrB-
BB---How to manage her transfusion needs?

- Partial D with allo anti-D
- Predicted to be hr^B- and could produce allo anti-hr^B, -e or –f (ce)
  - Future work-ups need to consider
- D-, hrB- units are extremely rare
- Inform her physician of this possibility and advise to transfuse as little as possible.
- Test her siblings!
MB: is it allo or auto?

- 51 y/o male with myelodysplastic syndrome
- Numerous transfusions in December 2010; Autoantibody and probable anti-C identified late December
  - Unable to phenotype due to disease and transfusions
  - Allogeneic absorptions required
- One week later: anti-e plus autoantibody
**MB: Genotyping results**

- **RHCE*ce(48C):** associated with weak e expression; encodes amino acid 16Cys
- **RHCE*ce:** no changes associated with common e variants
- ce alleles: one variant and one normal
- **RH genotype:** Ro / r\textsuperscript{variant}
- Predicted phenotype: D+, C- E- c+ e+, hr\textsuperscript{B+}
- Anti-e is most likely autoantibody; e negative blood is not indicated.
MB conclusions

- Negative for ce alleles associated with partial e, hr\(^B\) negative or hr\(^S\) negative phenotypes.
- The ce allele encoding 16Cys has been associated with production of anti-\(e\) or –f (ce).
  - Present in trans to a conventional ce allele. MB is not expected to make anti-\(e\).
- Review of serologic results: Consistent with warm autoantibody with e specificity. MB does not need e negative blood.
RT: another Rh dilemma

- 49 y/o woman with sickle cell disease
- History of anti-C, -Fya and e-like antibody
  - Unable to confirm e antibody as alloimmune
- Transfused with units negative for C, e and Fya for past year
- Referred for molecular testing to investigate of e variant alleles
RT: What does genotyping tell us?

- **RHCE alleles**
  - RHCE\(^{*}\)ce\(^{s}\): associated with partial e and with VS+V-, hr\(^{B}\)- phenotype
  - RHCE\(^{*}\)cE: a normal gene (e negative)
  - Patient is predicted to be hr\(^{B}\) negative

- **RHD alleles**: homozygote D positive
  - RHD\(^{*}\)DIII\(a\): partial D
  - RHD: no changes associated with partial D; appears normal
  - Not expected to produce allo anti-D
Original phenotype: D+ C- E+ c+ e+ or $R_o R_2$

Rh genotype: $Ro^{variant} / R_2$
  - Or $partial Dlll a-ces / DcE$

Predicted phenotype:
  - D+ C- E+ c+ partial e+, VS+ V- hr$^B$-

Patient can be transfused with $R_2 R_2$, Fy(a-)
Conclusions

- DNA testing results are invaluable for resolution of complex antibody problems, especially when Rh antibodies are involved.
- Correlation of serologic and molecular testing results is essential to avoid misinterpretation of results.
- The field of red cell genotyping continues to grow as new alleles are identified and new technologies evolve.