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^a, Do^a, Do^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 *Fy*^{b}

- A nucleotide change in *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
- 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-Fy^{b} and only rarely make anti-Fy3 or –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)***

EW: Is it allo or auto-anti-D?

- 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+

Is it allo or auto-anti-D? continued

- 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?

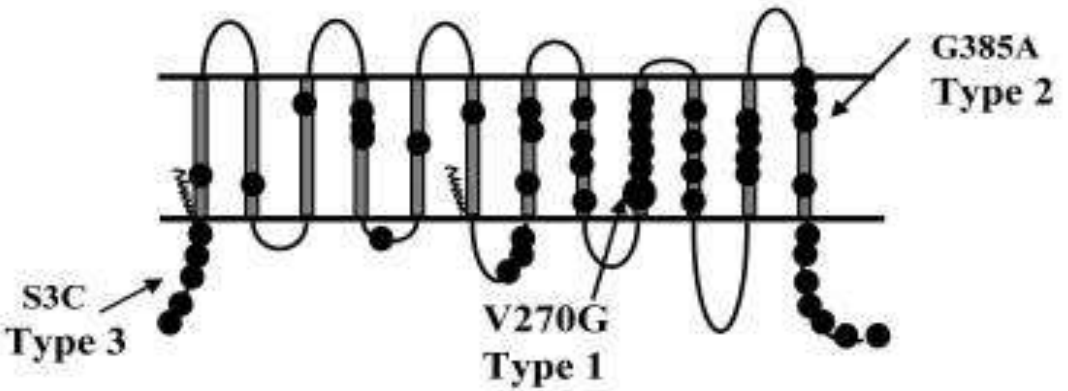
- *RHD alleles:*
  - *RHD*DAR:* encodes **partial** D, associated with production of allo anti-D
  - Inactive *RHD allele:* does not encode D
- RHCE alleles:
  - *RHCE****ce****AR:* associated with VS-V+, hr^{S}-
  - *RHCE****ce****(48C): associated with weak e expression.*

EW: What does it mean?

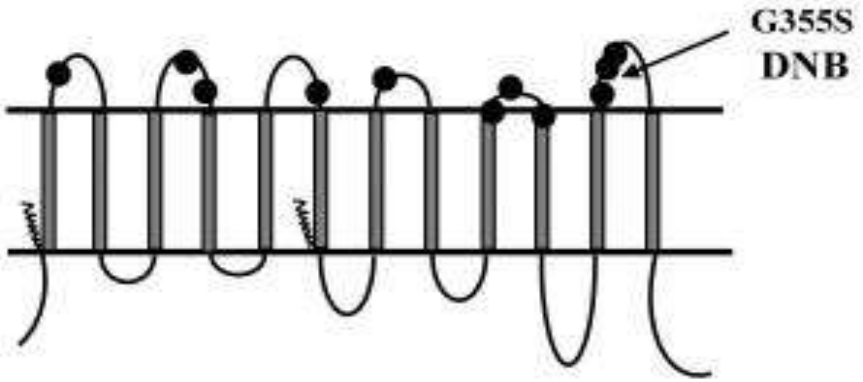
- ***D:*** *Molecular testing provided evidence that EW inherited a partial D gene. The other RHD allele is inactive.*
- ***ce:*** *One allele for partial e (*hr^{s}); One for weak e expression.
- *Original phenotype:* ***D+C-E-c+e+ or RoRo***
- *Phenotype predicted by genotype:*

  partial D+C-E-c+e+ or Ro ***variant /r*** ***variant***
- *Need to consider possibility of anti-e or-f(ce) in future work-ups. Give D-, C-, Js(a-) units*

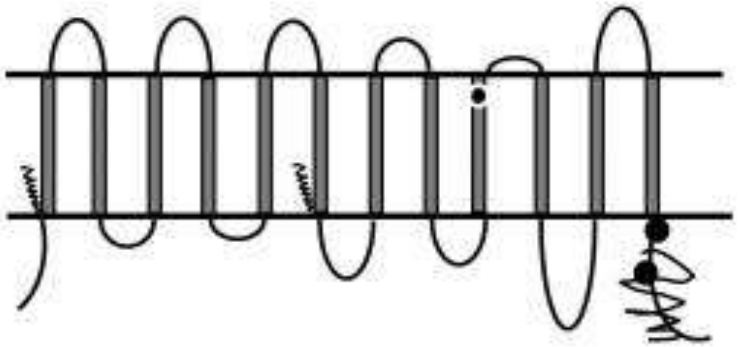
A. Weak D



B. Partial D

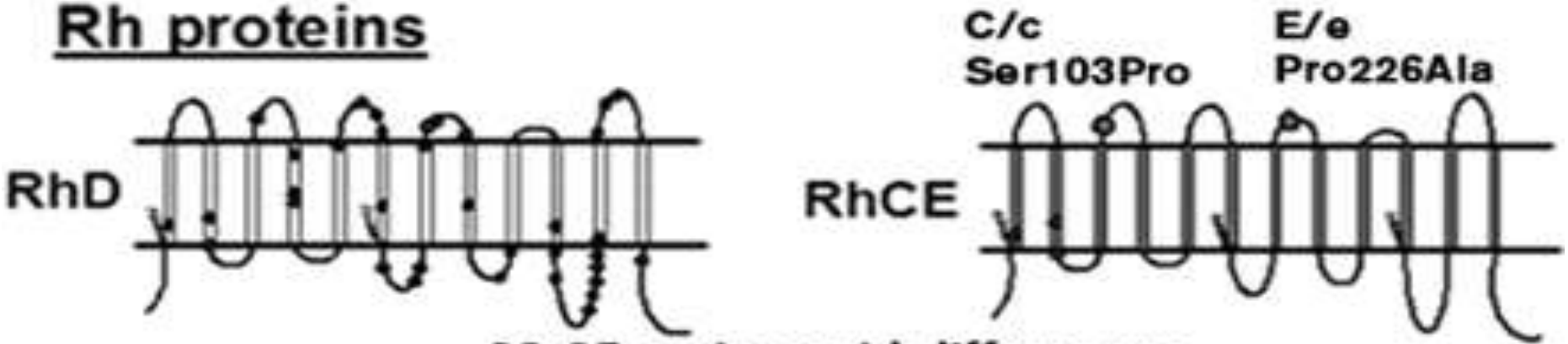


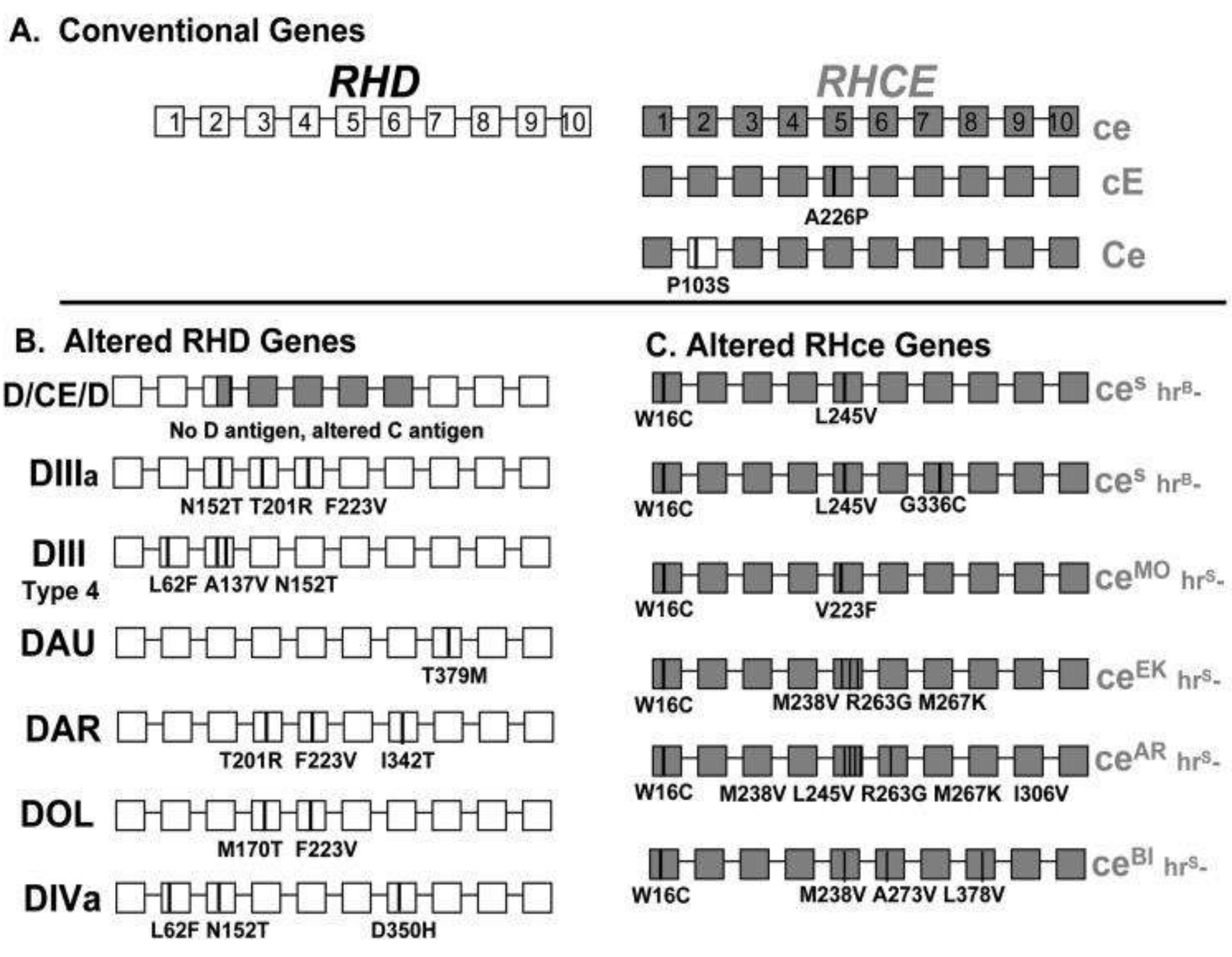
C. D_{el}



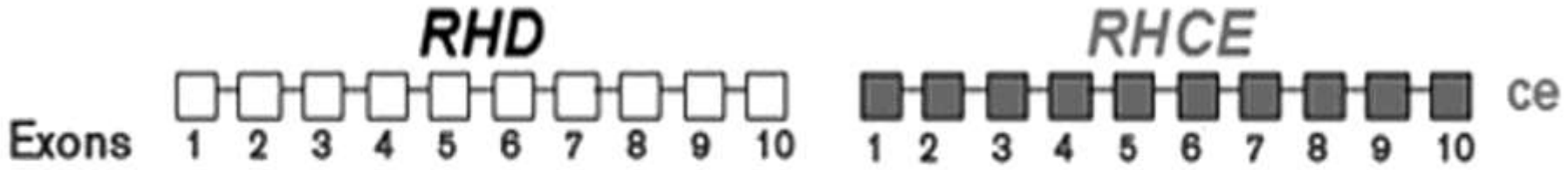
RH LOCUS

Rh proteins

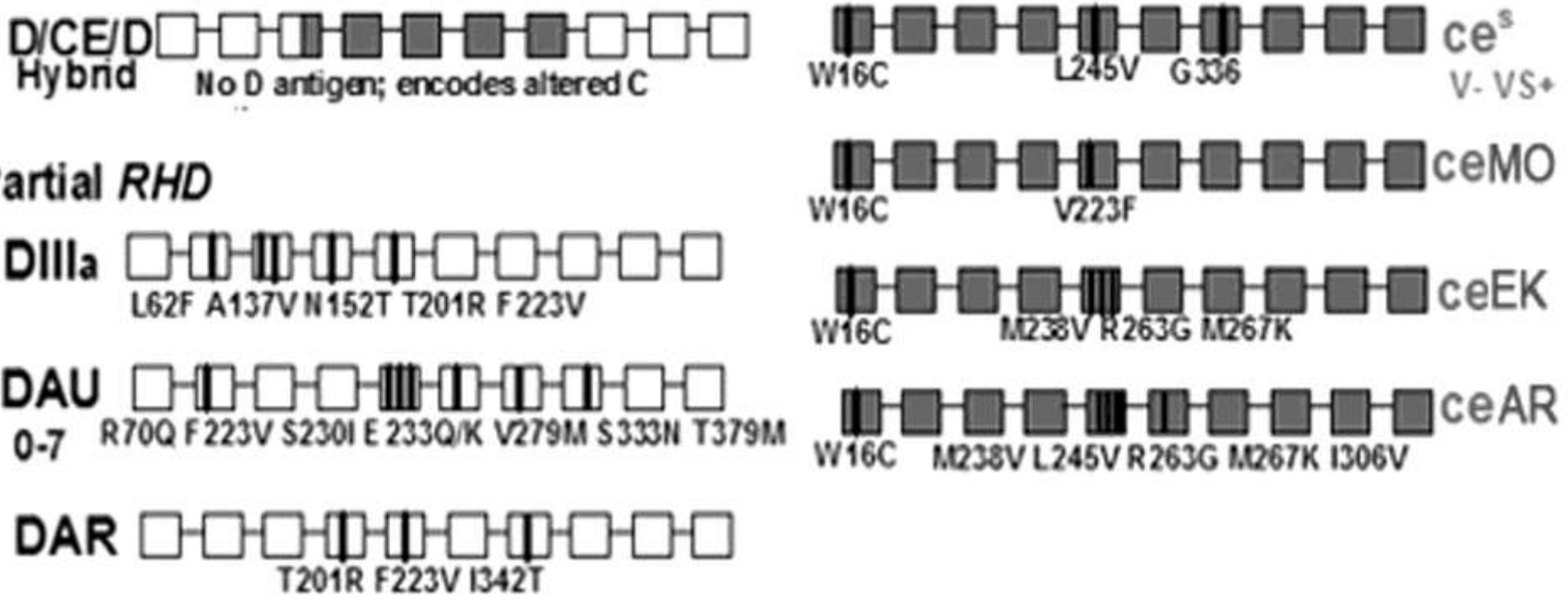



A. Conventional Genes
RHD
1 2 3 4 5 6 7 8 9 10
RHCE
1 2 3 4 5 6 7 8 9 10
ce
cE
A226P
Ce
P103S
B. Altered RHD Genes
D/CE/D
No D antigen, altered C antigen
DIIIa
N152T T201R F223V
DIII
Type 4
L62F A137V N152T
DAU
T379M
DAR
T201R F223V I342T
DOL
M170T F223V
DIVa
L62F N152T D350H
C. Altered RHce Genes
ceS hrB-
W16C L245V
ceS hrB-
W16C L245V G336C
ceMO hrS-
W16C V223F
ceEK hrS-
W16C M238V R263G M267K
ceAR hrS-
W16C M238V L245V R263G M267K I306V
ceBI hrS-
W16C M238V A273V L378V

Conventional RH genes



Altered/Variant RH genes



Stella T. Chou and Connie M. Westhoff. **Molecular biology of the Rh system: clinical considerations for transfusion in sickle cell disease.**
Hematology, Jan 2009; 2009: 178 - 184.

- 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.

Reports *continued*

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.

Reports *continued*

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 hr^{B}- phenotype and production of allo anti-e, -hr^{B} or –ce(f).

Finding hr^{B} negative donors

- Currently at least 50 genetic backgrounds for hr^{B} negative status
- 1st choice: e negative (when pt is E+)
- 2nd choice: molecular match by American Rare Donor Program
  - (C)ceS / (C)ceS
  - DAU0-ce / (C)ceS
  - DIIIa ceS/(C)ceS
  - (C)ceS/ Y ce16C
- 3rd 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^{s} **negative** phenotype.
- Molecular results confirm the serologic results.
- Transfuse with D-, hr^{s} **negative** blood –an extemely 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}, $Jk^{\mathbf{b}}$, $Le^{\mathbf{a}}$, 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*ce^{s}*:
  - 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
  - *DIIIa-ce^{s} / 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 / $r^{\mathbf{variant}}$
- Predicted phenotype: D+,C- E- c+ e+, $hr^{\mathbf{B}}$+
- 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**$\mathbf{ce^s}$*:* associated with partial e and with VS+V-, hr^B- phenotype
  - *RHCE**$\mathbf{cE}$*:* a normal gene (e negative)
  - **Patient is predicted to be hr^B negative**
- *RHD alleles:* homozygote D positive
  - *RHD*DIIIa:* partial D
  - *RHD: no changes associated with partial D; appears normal*
  - **Not expected to produce allo anti-D**

RT: What does it mean?

- Original phenotype: D+ C- E+ c+ e+ or R_oR_2
- Rh genotype: *Ro* ***variant*** */ R_2*
  - Or *partial DIIIa-ce^s / DcE*
- *Predicted phenotype:*
  - D+ *C*- E+ c+ partial e+, VS+ V- hr^B-
- Patient can be transfused with R_2R_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