Molecular biology of partial D and weak D

Implications for Blood Bank Practice

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http://www.uni-ulm.de/~wflegel/RH/ 2002
Rhesus molecular biology

- Introduction
  - Structure of $RH$ genes and Rh proteins
- partial D (D category)
- weak D
- Rh negative and $RHD$ heterozygosity
- Rh pos. units in Rh neg. donor pool
Relevance of Rhesus

Protein
- Most important blood group system encoded by proteins
- Major cause of HDN
- Group of Rhesus-like proteins are major constituents of RBC membrane

Gene
- Most polymorph blood group system known
- Gene cluster: duplication and deletion
- Multiple gene conversions and mutations
- Complex model system for genotyping
**RHD** alleles by type of molecular variation

adapted from: The RhesusBase
http://www.uni-ulm.de/~fwagner/RH/RB/
Molecular structure of RH gene locus

Rhesus box

Blood 95(2000)2272
Extended molecular structure of RH gene locus

Human

NPD014
P29

upstream Rhesus box
downstream Rhesus box

RHD

SMP1

RHCE

50,000 bp
Human RH locus compared to mouse genome project data

- **Human**
  - NPD014
  - P29
  - RHD
  - SMP1
  - RHCE
  - 50,000 bp

- **Mouse**
  - NPD014
  - P29
  - SMP1
  - RH

**Legend**
- **NPD014**: Gene symbol
- **P29**: Gene symbol
- **RHD**: Gene symbol
- **SMP1**: Gene symbol
- **RHCE**: Gene symbol
- **upstream Rhesus box**: Promoter region
- **downstream Rhesus box**: Promoter region

**Diagram Description**
- The diagram illustrates the human RH locus compared to the mouse genome project data.
- The human RH locus includes the genes NPD014, P29, RHD, SMP1, and RHCE.
- The mouse genome project data shows a similar arrangement with NPD014, P29, SMP1, and RH.
- The 50,000 bp scale indicates the genomic distance between the genes.
**RHCE**: ancestral position
**RHD** is the duplicated gene

**Human**

**Mouse**

**Blood** 99(2002)2272
Differences between RhD and RhCE

Clin Lab 48(2002)53
Rhesus molecular biology

- Introduction
- Partial D (D category)
  - \textit{RHD/RHCE} hybrid alleles
    - Typically causing "D categories"
    - Subset of partial D
- Weak D
- Rh negative and \textit{RHD} heterozygosity
- Rh pos. units in Rh neg. donor pool
Examples for *RHD-CE-D* hybrid alleles

1. **RHD**
   - DV type 2
   - DVI type 1
     - type 2
     - type 3
   - RHCE
Mechanism of gene conversion

A

B

C

RHD

SMP1

RHCE

D^v_type III

BMC Genet 2(2001)10
## Frequency of aberrant *RHD*

<table>
<thead>
<tr>
<th>Aberrant RhD</th>
<th>Minimal Frequency</th>
<th>Anti-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>DVII</td>
<td>1:900</td>
<td>uncommon</td>
</tr>
<tr>
<td>DVI</td>
<td>1:6,800</td>
<td>common</td>
</tr>
<tr>
<td>DIV</td>
<td>1:10,000</td>
<td>variable</td>
</tr>
<tr>
<td>DV</td>
<td>1:30,000</td>
<td>variable</td>
</tr>
<tr>
<td>DIII-like</td>
<td>1:30,000</td>
<td>uncommon</td>
</tr>
<tr>
<td>DFR</td>
<td>1:60,000</td>
<td>uncommon</td>
</tr>
<tr>
<td>$R_0^{Har}$ (Rh33)</td>
<td>&lt; 1:60,000</td>
<td>uncommon</td>
</tr>
</tbody>
</table>

* Means, serologic screen of > 60,000 donors

Transfus Clin Biol 3(1996)10s
Implications for Blood Bank Practice
Current D typing in Europe differs between patients and blood donors

- patients, pregnant women and newborns
  - two IgM monoclonal type that do not detect D category VI
  - no antiglobulin test
    - DVI is deliberately typed Rh negative

- donors
  - suitable polyclonal or oligoclonal reagents in indirect antiglobulin test (e.g. gel test)
    - DVI, all partial D and very weak D are typed Rh positive

Blood 91(1998)2166
German (since 1996), UK & Dutch guidelines
Rhesus molecular biology

- Introduction
- partial D (D category)
  - Missense mutations
    - If exofacial, typically causing partial D other than D categories
- weak D
  - Missense mutations
    - If non-exofacial, causing weak D types
- Rh negative and RHD heterozygosity
- Rh pos. units in Rh neg. donor pool
Aberrant RHD with single mutations

partial D including D categories

weak D types

weak D type 1

Blood 93(1999)385
weak D type 1

A

exon 6

AGACTTTATGGCACAG

100

weak D type 1

control

B

800 bp

549 bp

339 bp

210/213 bp

type 1

control
Distribution of weak D types

weak D type 1
CDe

weak D type 2
cDE

weak D type 3
CDe

weak D type 4
cDe

weak D type 5 - 16

among 272 weak D samples excluding DVI
Blood 93(1999)385
Quality assurance

- D pos. proband with anti-D
- Registry since 1998
- 60 submissions confirmed
- 13 international submissions
- Several new partial D, like DNB, DOL, DAU-3
- 20 weak D samples:
  - Allo-anti-D among weak D type 4.2 (DAR) & type 15 only
  - No allo-anti-D among prevalent weak D types: they all carried auto-anti-D

Implications for Blood Bank Practice

Does knowledge of partial D and weak D status serve a clinically useful purpose?

- Carriers of most partial D and some weak D types can be anti-D immunized:
  - D typing should avoid their being transfused with Rhesus positive blood

- Carriers of most weak D types cannot be anti-D immunized:
  - transfuse with Rhesus positive blood
  - avoid common practice of wasting Rh neg. blood
Antigen densities of weak D types

- Type 1
- Type 2
- Type 3
- Type 4
- Type 5
- Type 7
- Type 9, 16
- Type 10
- Type 11, 12, 17
- Type 15
- Type 8
Implications for Blood Bank Practice
Quality control by molecularly defined
weak D types

- weak D type 2
  - preferred for quality assurance (sensitivity)
    of anti-D sera and of D typing methods

- weak D types of lower antigen density,
  like type 5 or type 15
  - useful for precise cut-off definition and control

- applicable to
  - D typing methods in clinical lab
  - D typing kits by manufacturers

Rhesus molecular biology

- Introduction
- partial D (D category)
- weak D
- Rh negative and \textit{RHD} heterozygosity
- Rh pos. units in Rh neg. donor pool
D positive and D negative

- **D pos.**
  - RHD → SMP1 → RHCE

- **D neg.**
  - SMP1 → RHCE
  - SMP1 → RHCE
  - RHD → SMP1 → RHCE
RHD deletion

A

B

C

Blood 95(2000)2272
Detection of RHD heterozygous status in fathers

RHD SMP1 RHCE
SMP1 RHCE

5’ 23,130 bp 9,416 bp 6,557 bp
Rhesus molecular biology

- Introduction
- partial D (D category)
- weak D
- Rh negative and *RHD* heterozygosity
- Rh pos. units in Rh neg. donor pool
D+/- chimera causing anti-D immunizations

- Donor carries two RBC populations:
  - 95% Rh neg.
  - 5% Rh pos.
- Total of 13 donations
  - Caused anti-D in the latest 2 eligible transfusion recipients

BMC Genet 2(2001)10
Implications for Blood Bank Practice
Quality control by molecular typing of serologic Rh neg. donors

● among 8,442 Rh neg. donors
  – 1 donor with D+/- chimerism
  – 4 donors with very weak positive antigen D

● If representative
  – about 1 anti-D immunization per 50,000 donations (4 per 200,000 donations in 1 year)
  – cost-efficiency of molecular typing would be proven

● Established practice since 1-1-2002
Implications for Blood Bank Practice

Why *RHD* genotyping?

- **Superior sensitivity**
  - uncover (many?) weak D in the “Rhesus negative“ donor pool of blood centers

- **Superior specificity**
  - as genotyping becomes routine, clinical implications of known and new *RHD* alleles will be recognized
**DNB: a partial D with anti-D frequent in Central Europe**

<table>
<thead>
<tr>
<th>Population</th>
<th>Phenotype frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swiss (Lugano)</td>
<td>1:292</td>
</tr>
<tr>
<td>Swiss (Bern)</td>
<td>1:538</td>
</tr>
<tr>
<td>German (Ulm)</td>
<td>1:1,644</td>
</tr>
<tr>
<td>Dane (Aarhus)</td>
<td>&lt; 1:798 *</td>
</tr>
</tbody>
</table>

* upper limit of 95% confidence interval (Poisson distribution)

most frequent partial D known so far

**Blood 100(2002)2253**
Routine application of PCR

- DNA isolation: 30 min
- PCR set up: 10 min / 45 min
- PCR (cycling): 90 min
- Gel separation: 30 min
- Gel dyeing: 30 min
- Evaluation: 10 min

Total: 3.5 – 4 h

Image shows a gel electrophoresis with bands indicating the presence of specific amplicons in lanes 4 and 8, with a control lane (M).
Recommended *RHD PCR*

**A**

- Control
- Intron 4
- Exon 7

**B**

- Control
- Intron 7
- $RHD(W16X)$
- $RHD\Psi$

<table>
<thead>
<tr>
<th>standard RHD</th>
<th>RHD negative</th>
<th>RHD-CE(8-9)-D</th>
<th>RHD(W16X)</th>
<th>RHD\psi</th>
<th>s</th>
<th>D cat V type II</th>
<th>D cat V type III</th>
</tr>
</thead>
</table>

Positive predictive value $> 0.9999$
## Specificity of *RHD* genotyping: expected rates of false positives in published assays

<table>
<thead>
<tr>
<th>PCR strategy</th>
<th>Rate of false positives</th>
<th>Positive predictive value of positive result</th>
<th>Polymorphism tested (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 10 only</td>
<td>1:1,276</td>
<td>0.999216</td>
<td>1</td>
</tr>
<tr>
<td>Intron 4/exon 7</td>
<td>1:4,081</td>
<td>0.999755</td>
<td>2</td>
</tr>
<tr>
<td>Intron 4/exon 7/<em>RHD Ψ</em></td>
<td>1:4,700</td>
<td>0.999787</td>
<td>3</td>
</tr>
<tr>
<td>Exons 3, 4, 5, 6, 7, 9</td>
<td>1:6,051</td>
<td>0.999835</td>
<td>6</td>
</tr>
<tr>
<td>Exons 2, 3, 4, 5, 6, 7, 9, 10</td>
<td>1:6,051</td>
<td>0.999835</td>
<td>8</td>
</tr>
<tr>
<td>Intron 4/exon 7/<em>intron7/W16X/RHD Ψ</em></td>
<td>1:12,533</td>
<td>0.999920</td>
<td>5</td>
</tr>
</tbody>
</table>
Established indications for blood group genotyping

- First choice in prenatal diagnosis
  - from amniotic fluid or trophoblastic cells
  - from mother’s peripheral blood
- Poly-transfused patients
  - if standard serology failed
- Auto- and allo-immunohemolytic anemia
  - if standard serology failed
- RHD genotyping in fathers
- Weak D types and other aberrant RH alleles
  - for decision on anti-D prophylaxis and anti-D prophylaxis

Implication for Blood Bank Practice

- **Type patients** with two monoclonal anti-D that do not react with DVI
  - no antiglobulin, but sensitive methods
  - no slide tests for Rh neg.
  - patients, including pregnant women & newborns

- **Type donors** with oligoclonal anti-D
  - plus antiglobulin and sensitive methods

- **Use weak D for quality assurance**
  - molecularly defined weak D type 2
Implication for Blood Bank Practice

- Transfuse weak D with Rh positive blood
  - don’t waste Rh negative blood
- Transfuse DVI with Rh negative blood
  - and other partial D, if known
- Utilize $RH$ genotyping for established applications
Current problems in *Rhesus*

- Molecular biology
  - Frequency and types of aberrant *RH* haplotypes in various populations
  - 3D structure of RhD
  - Composition of Rh complex
- Clinical aspects
  - Immunization caused by partial D, weak D & D+/- chimera
  - Immunization in recipients carrying partial D or weak D
- Rendering genotyping practical & cost-efficient in the routine lab