

RhD Complexity and clinical relevance

Silvia Ferrari

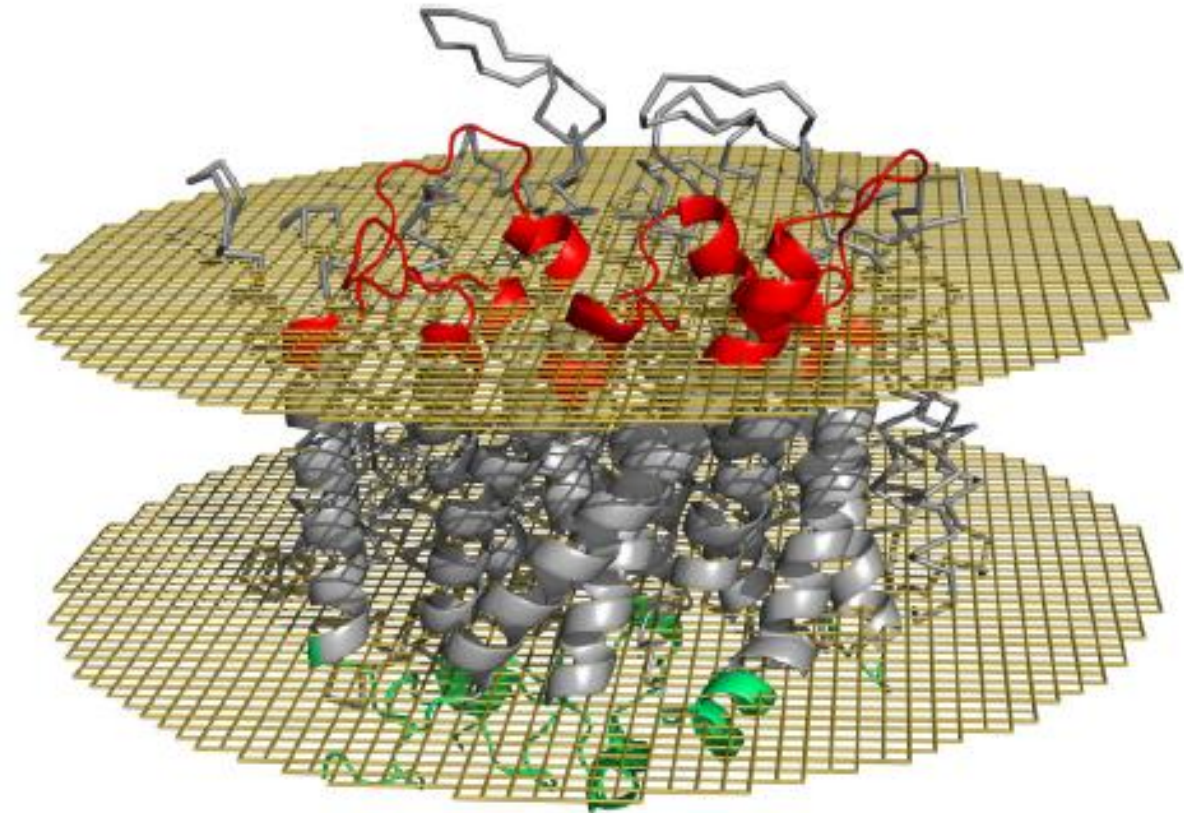
Director Scientific Affairs, Transfusion WW

Reason of complexity

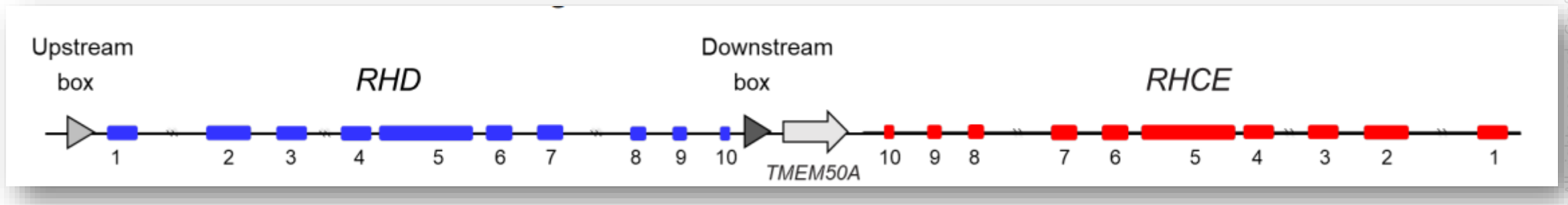
The reason we are talking here today about the RhD lies in its complexity, as this three-dimensional image shows.

Transfusion. 2021;61:1286–1301.

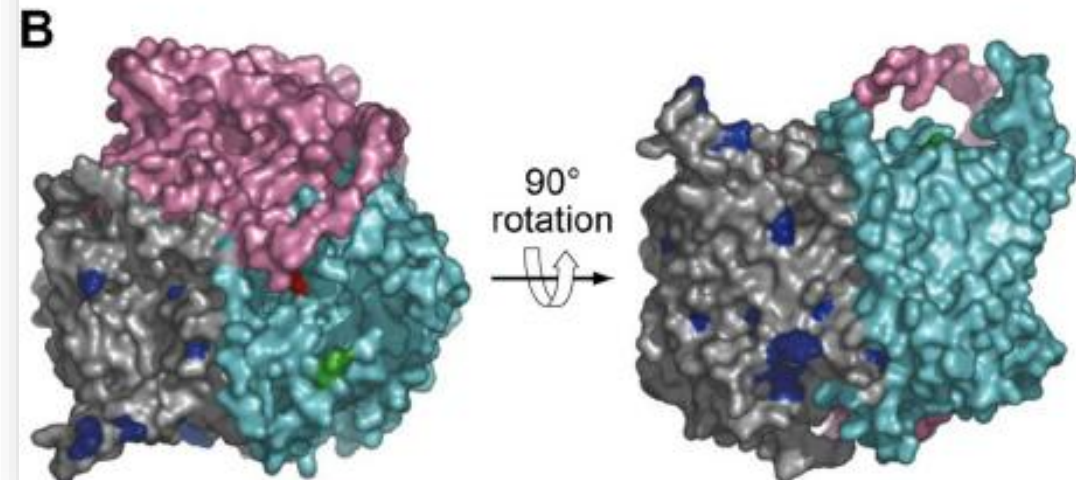
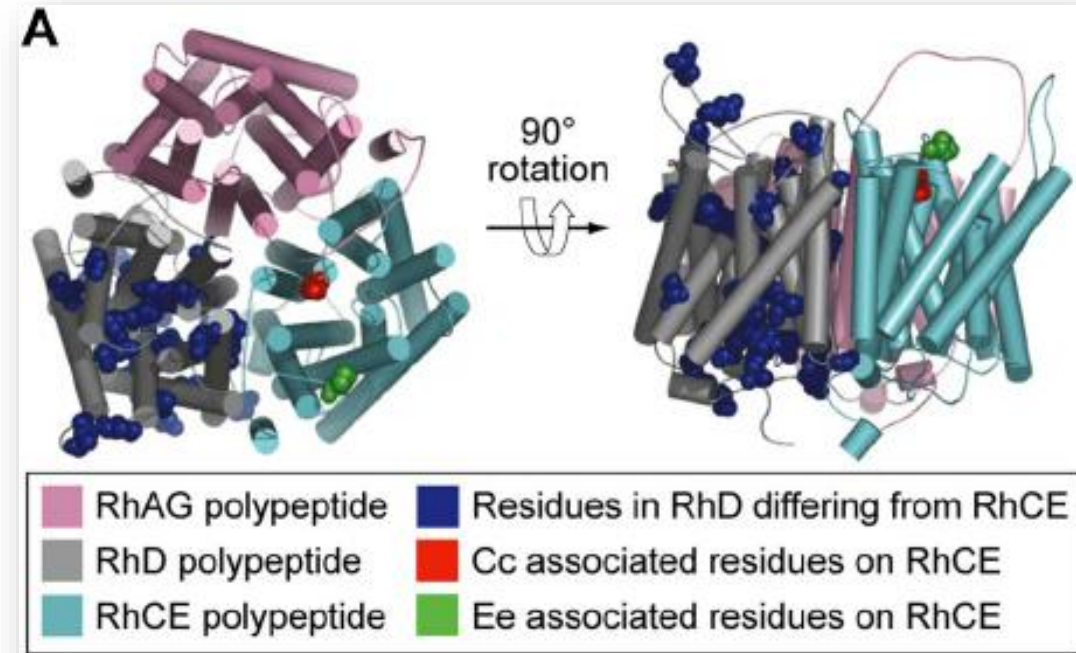
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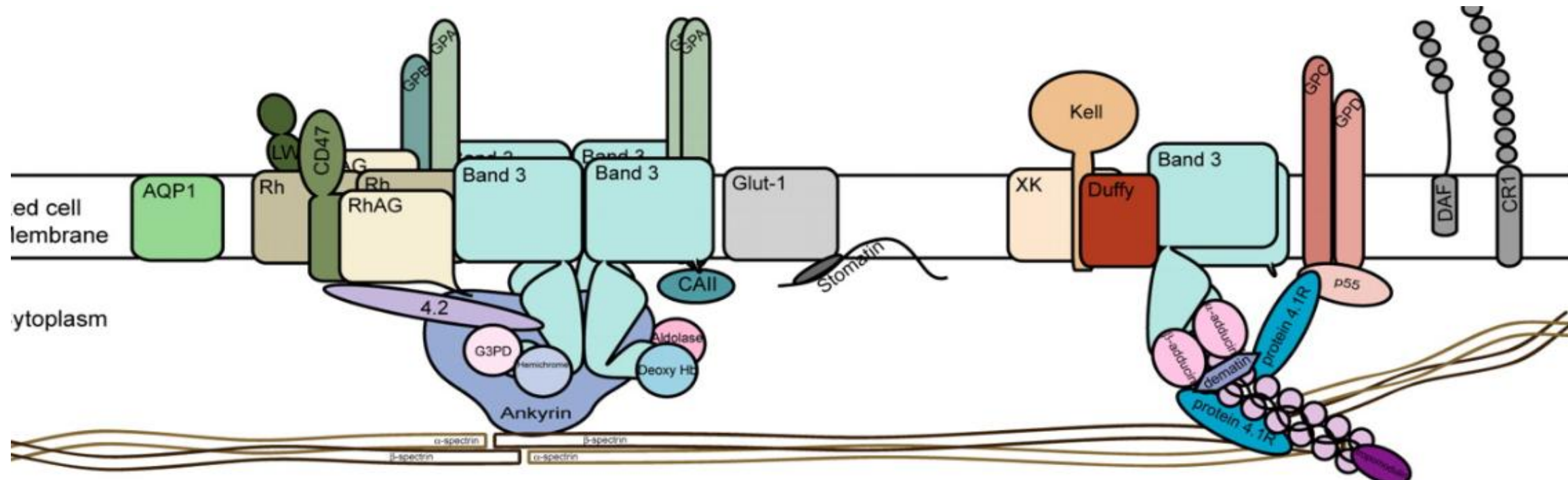


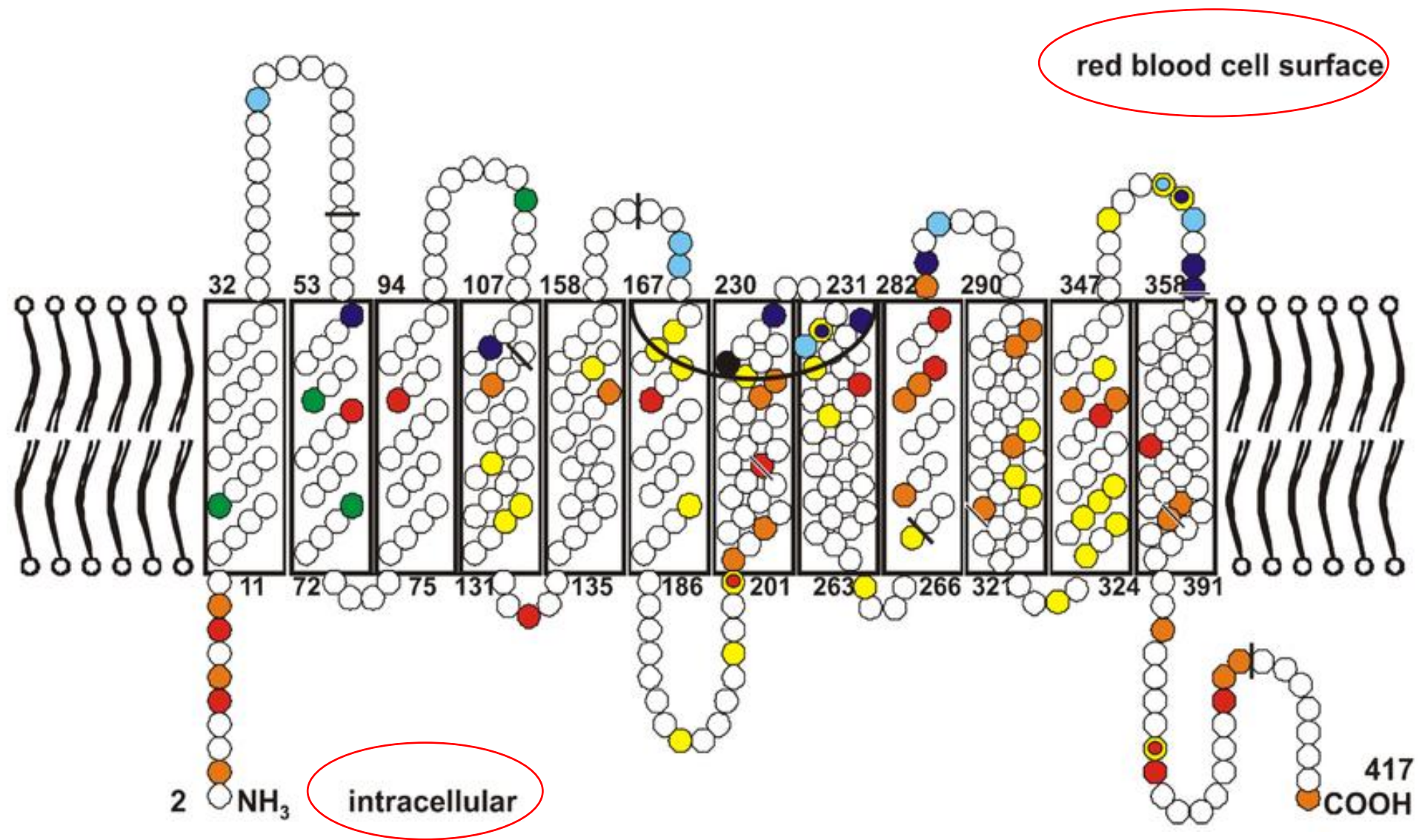
- Genes are located on human chromosome 1
- 10 exons
- 91,7% sequence identity (out of 417aa only 36 are different)
- encoding a 417-amino-acid,transmembrane protein (RhD and RhCE, respectively) exclusively expressed at the surface of erythroid cells in a multiprotein complex



The reason we are talking here today about the RhD lies in its complexity, as this three-dimensional image shows.

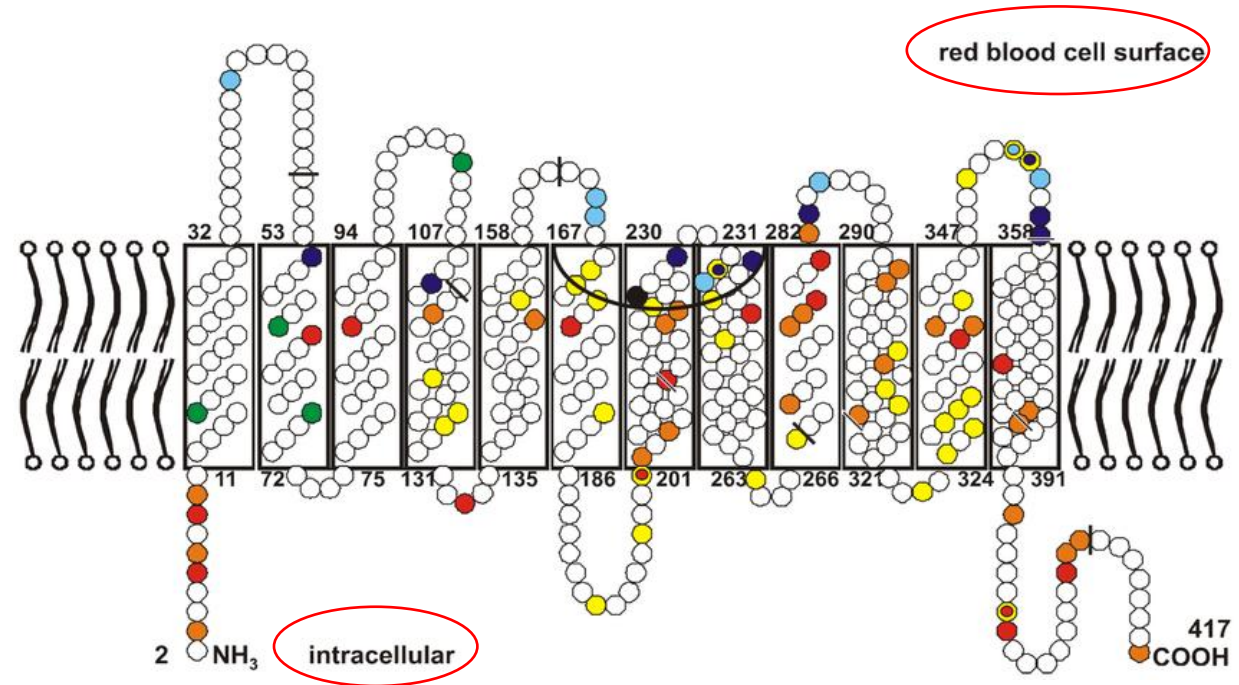






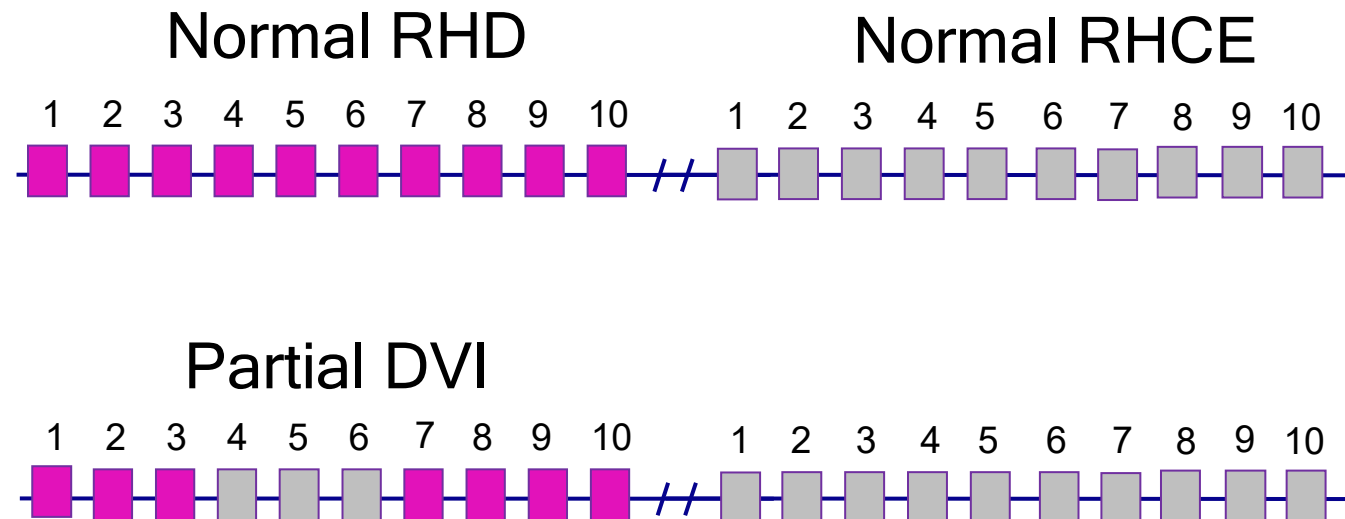
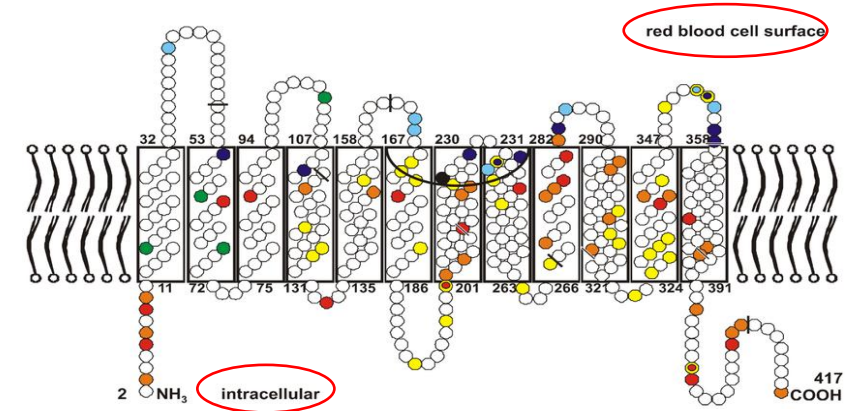
Weak D Polymorphism

- Mutations result in aa substitution in intramembranous portion (>90 SNPs)
- Reduction of D antigen >10 fold
- Expresses all D epitopes
- Weak D 1, 2 and 3 don't form allo anti-D



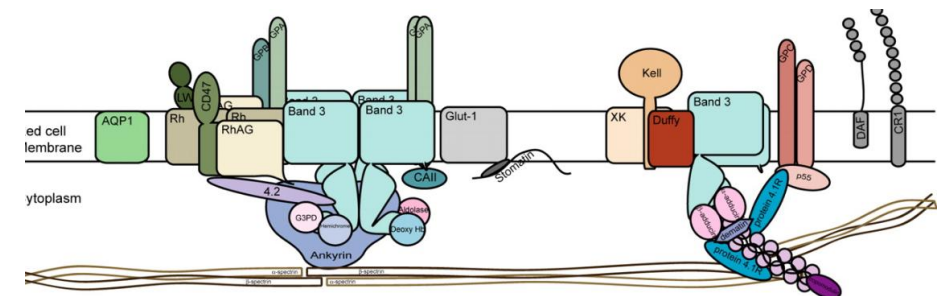
Partial D Polymorphism

- >30 epitopes on the extracellular portion
- Entire portion of some epitopes could be missing
- Patients missing a single Rh epitope can make antibody to the missing piece
- Partial Ds are not necessarily compatible with each other



Lack of *RH* Gene Expression

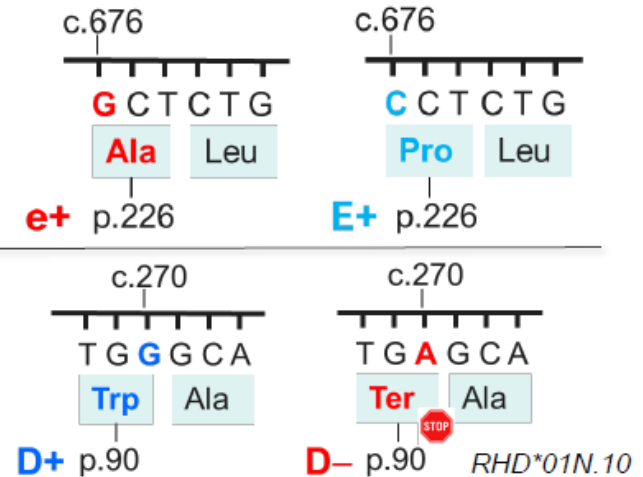
- **Rh null:** No D,Cc or Ee antigens
 - Caused by a mutation in RHAG gene (in the regulator type)
 - Or to the deleted *RHD* paired with silent *RHCE* allele (in the amorph type)
- **D - -:** Lacks Cc,Ee; has enhanced D
 - Mutation in *RHCE* gene
- **Rh negative:** Lacks D antigen
 - Deletion of *RHD* gene, Caucasians ($\approx 15\%$), Tunisia ($\approx 94\%$)
 - African descent: *RHD* pseudogene, & hybrids
 - Asians: Del



Common genetic mechanisms that impact expression

- **Missense mutations – most common**

- Nucleotide substitution that results in different amino acid
- Can result in weak, partial, or “el” phenotypes

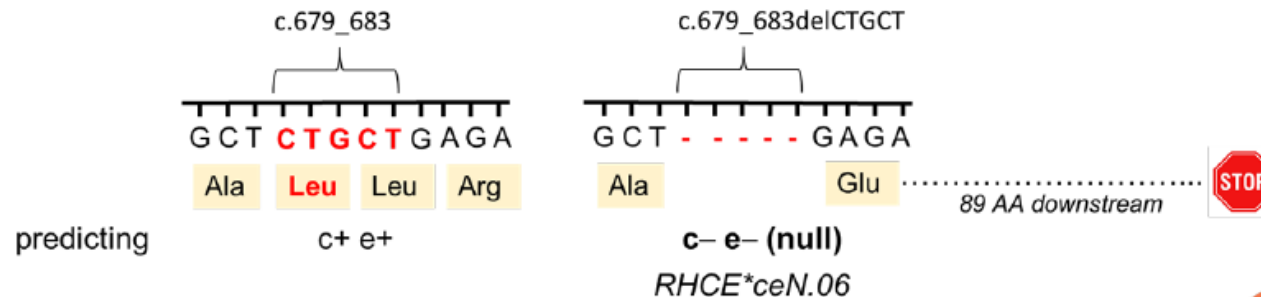


- **Nonsense mutations**

- Nucleotide change that causes immediate stop codon
- Truncated protein, often leads to antigen negative phenotype

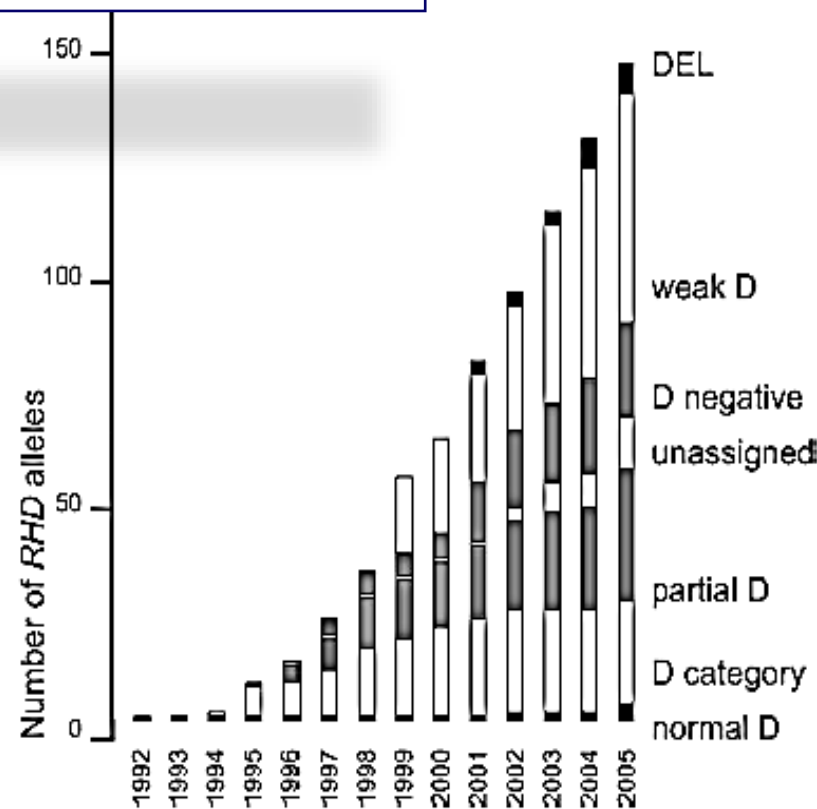
- **Indel:** Insertion (“In”) or deletion (“del”) of 1 or more nucleotides

- Often causes frameshift and premature stop codon
- Truncated protein, often leads to antigen negative phenotype



New York Blood Center Enterprises

From the 1990s to the present, the evolution in the discovery of antigenic variants has been remarkable (although in this photo we can only see up to 2005!)...and we got to 600 variants



Flegel W.A., Trans. Clinique et Biol. 2006

The first examples of weak D and DEL were discovered in 1999 and 2001, respectively

bjh

variants of RHD – current testing and clinical consequences

Full Text:

International Blood Group Antigen Conference: 15th Blood and Transfusion World Congress

Since 1940, all the Rh blood group system is clinically important as it causes haemolytic transfusion reactions and haemolytic disease of the fetus and newborn. Although most people are Rh positive (D+), there is a significant D- population estimated at under 1% in period D-. Some very large molecularly defined and biologically important variations (D1, D2, D3, D4, D5, D6, D7, D8, D9, D10, D11, D12, D13, D14, D15, D16, D17, D18, D19, D20, D21, D22, D23, D24, D25, D26, D27, D28, D29, D30, D31, D32, D33, D34, D35, D36, D37, D38, D39, D40, D41, D42, D43, D44, D45, D46, D47, D48, D49, D50, D51, D52, D53, D54, D55, D56, D57, D58, D59, D60, D61, D62, D63, D64, D65, D66, D67, D68, D69, D70, D71, D72, D73, D74, D75, D76, D77, D78, D79, D80, D81, D82, D83, D84, D85, D86, D87, D88, D89, D90, D91, D92, D93, D94, D95, D96, D97, D98, D99, D100, D101, D102, D103, D104, D105, D106, D107, D108, D109, D110, D111, D112, D113, D114, D115, D116, D117, D118, D119, D120, D121, D122, D123, D124, D125, D126, D127, D128, D129, D130, D131, D132, D133, D134, D135, D136, D137, D138, D139, D140, D141, D142, D143, D144, D145, D146, D147, D148, D149, D150, D151, D152, D153, D154, D155, D156, D157, D158, D159, D160, D161, D162, D163, D164, D165, D166, D167, D168, D169, D170, D171, D172, 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Distribution, prevalence

World map showing the prevalence of RhD status by country. The legend indicates:

- RhD Partial
- RhD Weak

The map shows that the majority of countries, including North America, South America, Europe, Africa, and Australia, are colored dark orange, indicating a partial RhD status. Some countries in Europe and Asia are colored light orange, indicating a weak RhD status.

Region/Ethnicity	Common RhD Variants	Type	Estimated Prevalence	Clinical Relevance
Europe (Caucasian)	Weak D Types 1, 2, 3	Weak D	0.2–1.0%	Usually non-immunogenic
Europe (Caucasian)	DVI	Partial D	0.1–0.5%	Can cause anti-D formation
Africa / African descent	DAR	Partial D	1–2%	High immunogenicity
Africa / African descent	DAU	Partial D	1–2%	High immunogenicity
Africa / African descent	DIIIa	Partial D	1–2%	High immunogenicity
Africa / African descent	DNB	Partial D	1–2%	High immunogenicity
Asia (East Asian)	DEL	Very Weak D	10–30% of RhD-neg	Often missed in serology
Asia (East Asian)	Weak D Type 4.2	Weak D	Rare	Variable immunogenicity
Latin America	Weak D Types 1–3	Mixed	0.5–1.5%	Depends on ancestry
Latin America	DVI	Partial D	0.5–1.5%	Depends on ancestry
Middle East	DVI	Partial D	0.5–1.0%	Risk of alloimmunization
Middle East	DIIIa	Partial D	0.5–1.0%	Risk of alloimmunization

<https://doi.org/10.1309/AJCPSXN9HQ4DELJE>

Weak or Discrepant RhD: International



Korea: 0.01-0.02%
Type 15, DBS1, Type 25

Japan: 0.01%
c.960G>A, Type 15, Type 24

*China and Korea:
Type rare 'D-negatives' for C and E
to seek Asian DELs => manage as D+*

China: 0.01% Type 15, DVI.3, Type 25

Thailand: 0.01%: DVI.3, Type 25

Serology

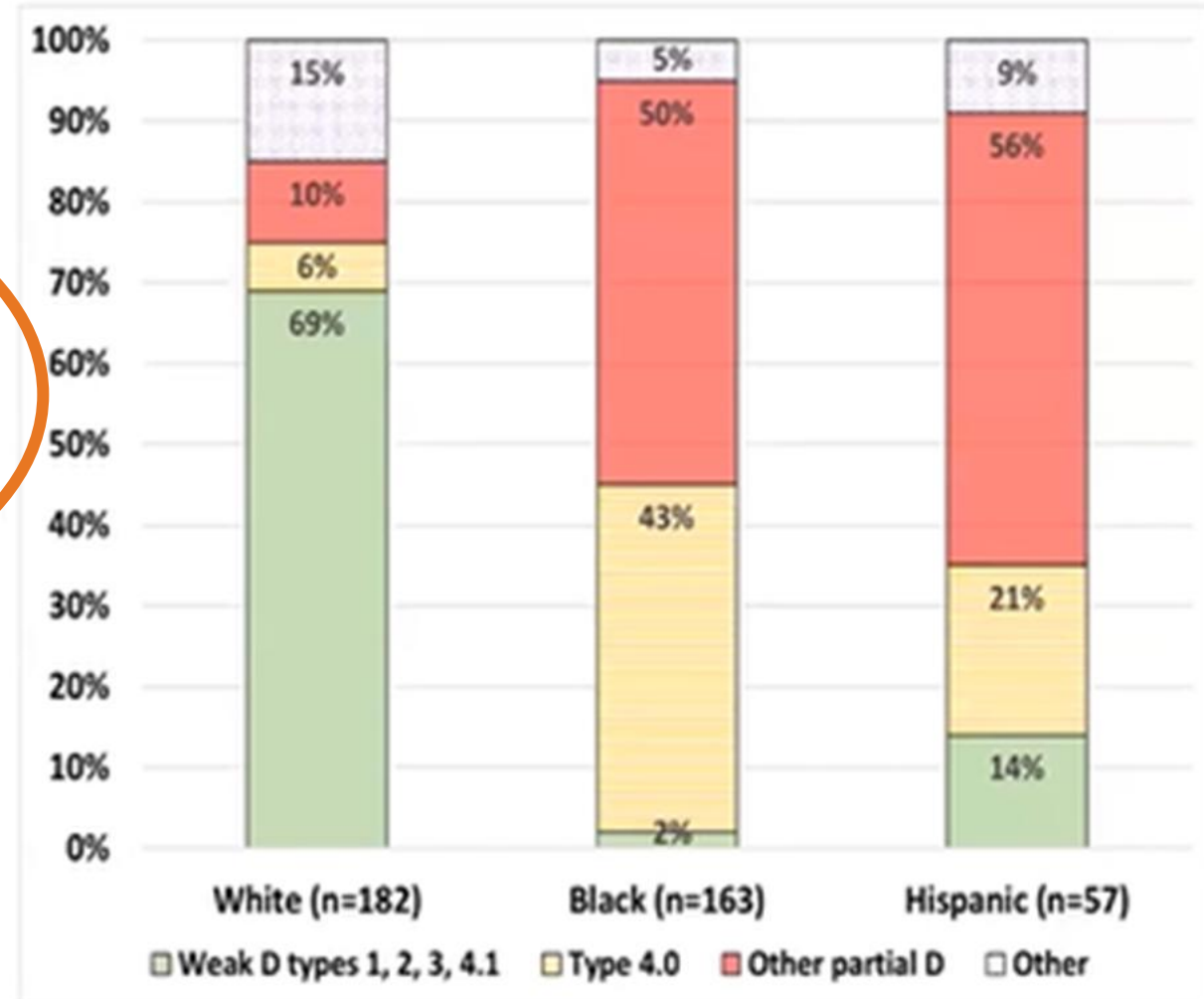
US

Weak or discrepant RhD phenotypes in US: 4 US genotyping studies with race/ethnicity*

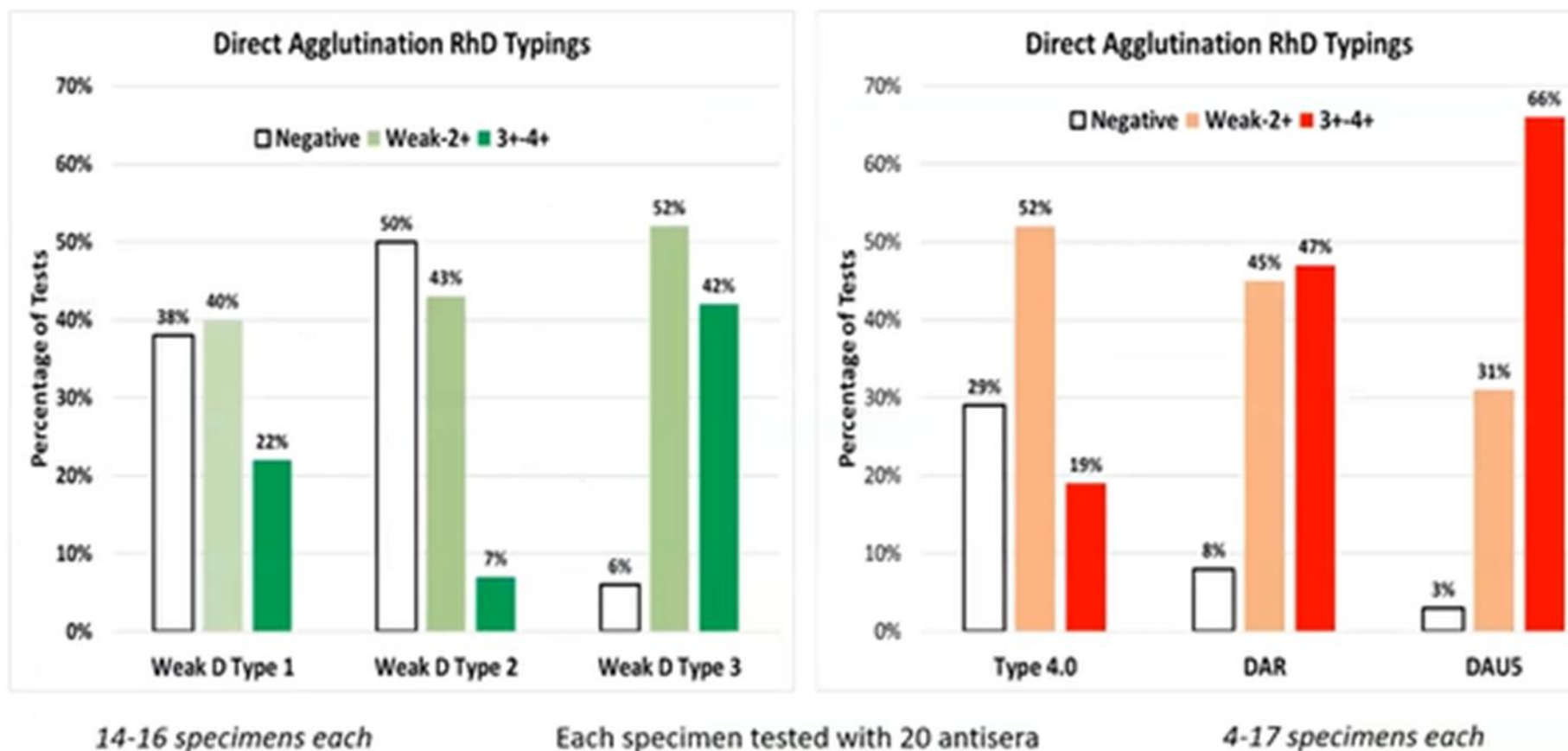
Composite data compiled from:

Boston 2010: Wang et al
New York-US 2021: Vege et al
Chicago 2022: Barriteau et al
Los Angeles 2021: Hudgins et al

*Ramsey G, Barriteau CM
submitted for publication



Study of anti-D reactivity with known *RHD* alleles



Theiler C, Hu Z-G, et al. Weak and partial D phenotyping: a comparison study between molecular and serologic results. *Transfusion* 2022;62:148-49A

Received: 3 August 2016 | Accepted: 23 February 2017

DOI: 10.1002/jcla.22221

RESEARCH ARTICLE

WILEY

RHD alleles among pregnant women with serologic discrepant weak D phenotypes from a multiethnic population and risk of alloimmunization

Carolina Bonet Bub¹  | Maria Giselda Aravechia¹ | Thiago Henrique Costa¹ |
José Mauro Kutner¹ | Lilian Castilho^{1,2}

Brazil

Serologic reagents cannot always discriminate between weak D and partial D, and reactivity patterns often reflect the characteristic of the reagent rather than the D expression on RBCs but, as the genetic basis of most common RhD variant antigens has been determined, D variants have been classified at molecular level.^{6,8}

This recommendation supports the use of *RHD* genotyping in obstetric patients in order to avoid confusion due to discrepant serologic results and to indicate the RhIG. Knowledge of *RHD* variant alleles and risk of alloimmunization in obstetric patients are important for prenatal management.

A total of 104 pregnant women samples with discrepant results or weak reactivity with two monoclonal anti-D reagents in routine diagnostics were tested by hemagglutination with currently used MoAbs in Brazil and by molecular analyses.

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Brazil

RHD allele	Nucleotide changes	Haplotypes	Risk for anti-D
RHD*weak D type 1	809T>G	DCe	No
RHD*weak D type 2	1154G>C	DcE	No
RHD*weak D type 3	8C>G	DCe	No
RHD*weak partial 4.0	602C>G, 607A>G, 667T>G, 744C>T, 957G>A, 1025T>C	Dce	Yes
RHD*weak D type 38	833G>A	DCe	Yes
RHD*weak D type 45	1195G>A	DcE	Yes
RHD*weak D type 67	722C>T	DcE	Yes
RHD*DAR1.00	602C>G, 667T>G, 1025T>C	Dce	Yes
RHD*DVI.1	505A>C, 509T>G, 514A>T, 544T>A, 577G>A, 594A>T, 602C>G, 667T>G, 676G>C, 697G>C, 712G>A, 733G>C, 744C>T, 787G>A, 800A>T (RHCE-like segment of CE-allele encompassing exons 4-5)	DcE	Yes

December 2016 access in <http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/> in blood group allele tables.

TABLE 2 RHD alleles and reactivity with monoclonal antibodies (MoAbs)

Samples (n)	RHD alleles	MoAbs reactivity			
		Tube		Gel Grifols	
		MS201	MS26 IAT	P3X61	P3X290, P3X35, P3X61, P
9	RHD*weak D type 1	2+	4+	2+	2+
12	RHD*weak D type 2	0	2+	0	1+
2	RHD*weak D type 3	2+	3+	3+	4+
51	RHD*weak partial 4.0	3+	4+	4+	2+
6	RHD*weak D type 38	0	(+)	0	0
1	RHD*weak D type 45	0	(+)	0	0
1	RHD*weak D type 67	0	(+)	0	0
12	RHD*DAR	0	3+	0	2+
10	RHD*DVI	0	2+	0	1+

Brazil

tentially at risk for the development of anti-D. If we were to select candidates for RhIG based only on the serological techniques applied in this study, we would not recommend prophylaxis for pregnant women with *RHD*weak partial 4.0* as it was detected with all MoAb selected and, we would unnecessarily recommend the RhIG for the carriers of *RHD*weak D type 2* that showed the same pattern of serologic reactivity of partial D DVI. Our data show that although those variants have different molecular background they can present the same serologic pattern of reactivity. This finding reinforces the importance to perform *RHD* genotyping to identify weak D discrepancies for obstetric pa-

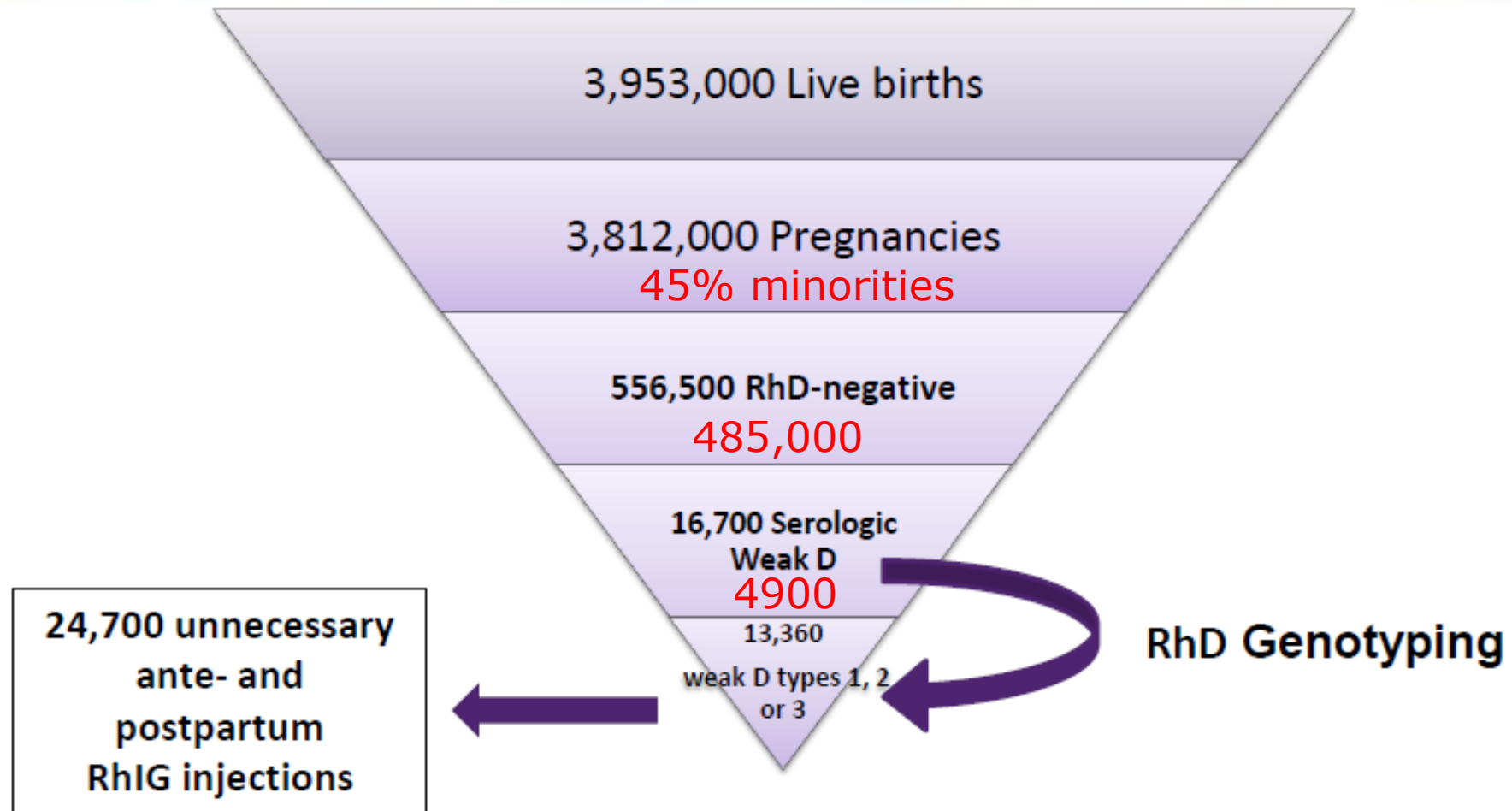
Transfusion

Mar2015;55:680-689

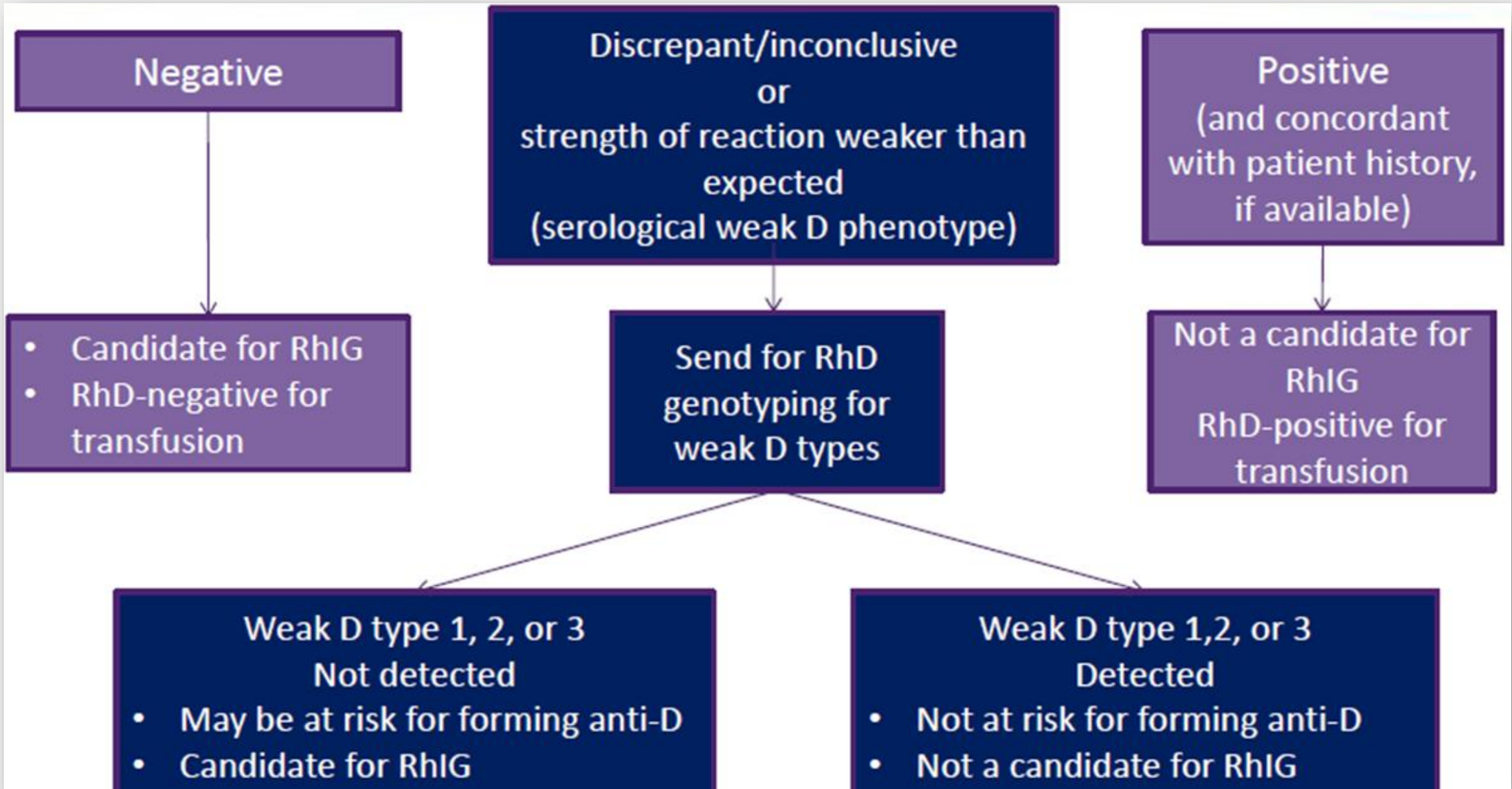
It's time to phase in *RHD* genotyping for patients with a serologic weak D phenotype

S. Gerald Sandler,¹ Willy A. Flegel,² Connie M. Westhoff,³ Gregory A. Denomme,⁴ Meghan Delaney,⁵ Margaret A. Keller,⁶ Susan T. Johnson,⁷ Louis Katz,⁸ John T. Queenan,⁹ Ralph R. Vassallo,¹⁰ and Clayton D. Simon¹¹

RhD working group



In red, re-calculation based on effective incidence in minorities



Case studies

Case 1

On the 4th of March your lab notified Immucor of a discrepancy in RhD typing for samples xxx and xxx belonging to the same patient.

Neo Iris gave a Negative report on the RhD typing (reading values 17.9 on both samples with Anti-D Rapid).

The samples tested on Echo V2 gave an NTD (not determined) result, falling into the "gray area" for equivocal reactions.

- **the patient had a previous RhD positive typing**
- the EFS sent a report for a confirmed RhD typing

Genotyping made by the German Red Cross:

The patient is a PARTIAL RHD: *RHD*DFR1*

THIS NEEDS TO BE REPORTED AS RHD NEGATIVE and treated accordingly for transfusion and immunoprophylaxis purposes.

Case 2

On the night of Friday 04/21/2023, the Neo Iris returned a negative RHD result while a Patient **was known to be RH D pos by her anteriority.**

The Patient with 6 g/dL of hemoglobin having received several transfusions in RhD Negative (2 CGR on 03/21 and 1 CGR on 04/13)

On 04/21, in the clinic's emergency room, a possible double population was not detected in the two RhD wells (anti-D rapid and anti-D Novaclone)

The scores are between 21 and 24.3: therefore, high but negative because below the threshold of 30, results without double-population alarm.

The patient is a PARTIAL RHD: RHD*10.X - DAU

THIS VARIANT MUST BE CONSIDERED AS RHD NEGATIVE and treated accordingly in the obstetric and transfusion context.

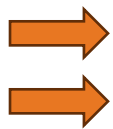
The genotype shows that the Patient's previous RhD phenotype was FALSE and clinically dangerous. Under these conditions it is normal that the double population after RhD negative transfusion is not visible.

Case 3 - Discrepancies study – Italy 2019

Serology – molecular correlation

Mean of anti-D agglutination score obtained from all the donor with the same RhD genotype

RhD genotype	Anti-D typing by gel card (ID AB0, Biorad)			Anti-D typing by solid phase (Capture Ready, Immucor)		
	anti-D at screening IgM (TH-28)+IgG(MS-26)		anti-D IAT IgG(ESD1M, 175-2)	anti-D at screening IgM (RUM-1)		anti-D IAT IgM (D175-2)+IgG(D415-1E4)
	Call	Agglut. score	Agglut. score	Call	Agglut. score (%)	Agglut. score (%)
DD	POS	4+	4+	POS	100	99
weak D type 1	nd	2+	3+	nd	51	99
weak D type 2	nd	1+	2+	NEG	20	99
weak D type 3	POS	3+	3+	nd	64	99
weak D type 4	POS	3+	3+	nd	69	99
weak D type 4.1	nd	2+	2+	nd	46	99
weak D type 4.2	POS	3+	3+	nd	69	99
weak D type 5	NEG	0	2+	NEG	11	88
weak D type 11	NEG	0	0	NEG	15	45
DFR	POS	4+	2+	NEG	14	98
DNB	POS	4+	2+	nd	76	99
D cat. V	nd	2+	3+	NEG	11	87
D cat. VI - type 1	nd	2+	2+	NEG	18	89
weak D 1 / weak D 4	nd	2+	2+	nd	43	99
weak D 2 / weak D 4	NEG	0	2+	NEG	17	78



➤ Different reactivity due to different anti-D reagents.

➤ All 3+ reactivity (usually considered normal) had a D variant

Case 4

Case Study

Patient:
African American
Delivered her 3rd baby
Anti-D, 3+ by PEG IAT in her
plasma at delivery
Autocontrol negative

D typing results indicate her
RBCs are D+ with (slightly)
weakened expression
RBCs also C– E– c+ e+

Results obtained in direct testing

Anti-D reagents IgM + IgG	Reaction with Patient RBCs
# 1	2+
# 2	3+
# 3	3+
# 4	2+

DNA analysis predicts:
Presence of weak D type 4
Associated with 2 amino acid
changes: T201R and F223V
that are predicted to be in the
internal portion of RhD

Case 5

- 77-year-old man
- Malignant neoplasm
- Patient previously has **typed D+ on multiple occasions**
- Over time strength of reaction with Anti-D weakened
- Patient RBCs now type D–

BeadChip array for prediction of common RBC phenotype

Antigen	Results
c	+
C	+
e	+
E	0

- **DNA:**
 - Markers for *RHCE***C*** present
- **Serology:**
 - C– with 2 examples of Anti-C
 - No indication of mixed population
- **D and C in question**

Serology testing

- Initial testing

Anti-D	
IS	0
IAT	0

DAT	IS	5 min
	0	0
POLY	0	0
Control	0	0

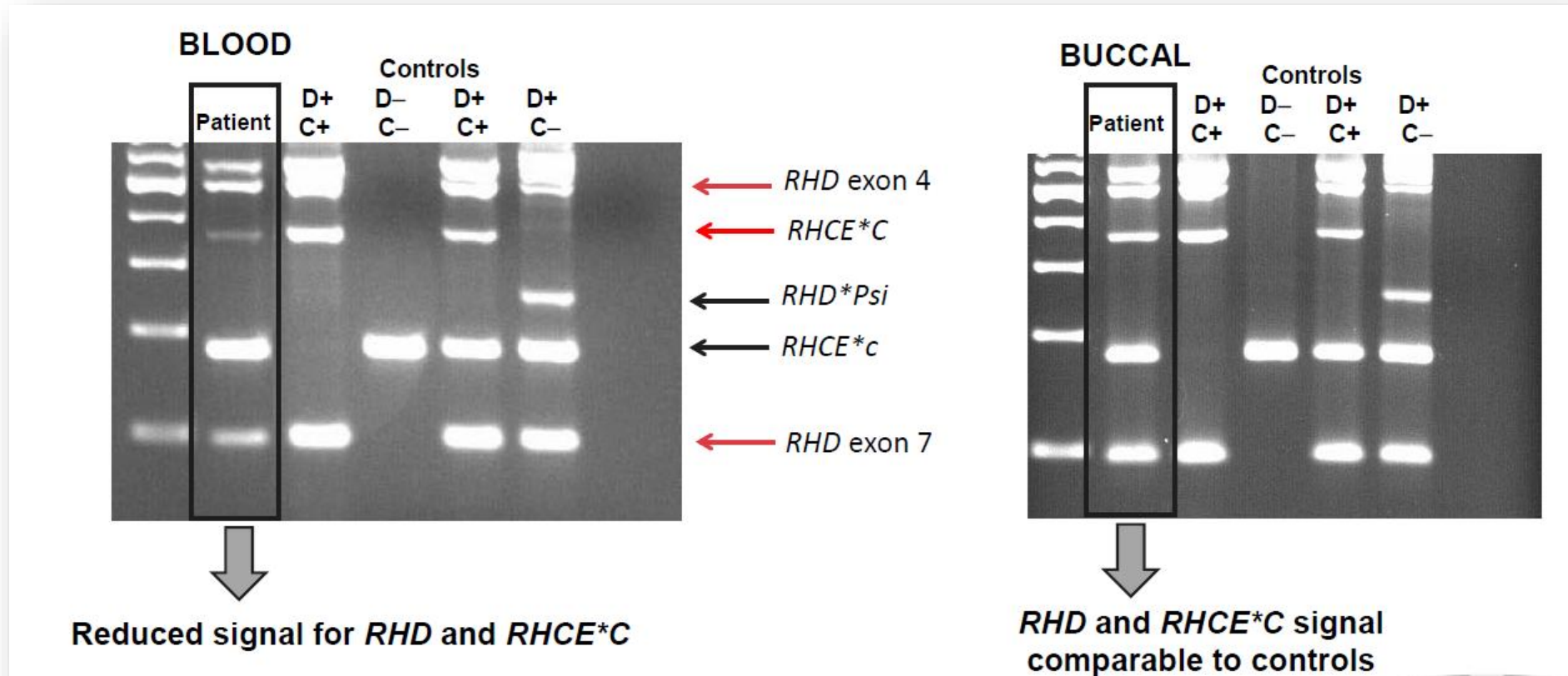
- Testing with multiple reagents

Anti-D	Patient RBCs	
	IS	IAT
# 1 mono	0	0
# 2 mono	0	0
# 3 mono	0	0
# 4 poly	nt	0

- RBCs also type C– E– c+ e+

➤ Patient is an apparent rr

- Adsorption/elution with polyclonal anti-D and anti-C is revealing the D but not the C



Many reports for reduced expression of Rh antigens and Rh mosaicism reported in leukemia, myeloid metaplasia, myelofibrosis, and polycythemia.

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Many reports for reduced expression of Rh antigens and Rh mosaicism reported in leukemia, myeloid metaplasia, myelofibrosis, and polycythemia.

Loss of Rh Antigen Associated With Acquired Rh Antibodies and a Chromosome Translocation in a Patient With Myeloid Metaplasia

By Barry Cooper, Peter V. Tishler, Leonard Atkins,
and W. Roy Breg

Myeloid metaplasia is a clonal disease of the marrow pluripotent stem cell in which a constant cytogenetic abnormality could result in an altered antigenic characteristic of hematopoietic cells. A 57-yr-old man who had acquired myeloid metaplasia at age 37 was noted to be Rh negative, although blood typing at age 33 was Rh positive. His erythrocytes reacted with anti-c and anti-e, typical for an Rh negative individual. Analysis of 11 metaphase bone marrow cells by G-banding revealed 46 chromosomes with a consistent anomaly in 100% of cells involving chromosomes 1 and

13. The changes were consistent with a reciprocal translocation, with break points at approximately 1p32 and 13q22, although break points at 1p13 and 13q14 were also possible. Since previous cytogenetic data have localized the Rh gene to the short arm of chromosome 1, our data indicate the Rh gene lies within the segment 1p13--1p32. Serum of this patient had circulating anti-D and anti-C antibodies. Consequently, this patient lost immunologic tolerance to a "self antigen" after losing the ability to express this antigen.

Blood 1979; 54:642-647

Report of a patient who made anti-D and anti-C after loss of antigens

Case 6

RhD RT typing with

- Anti-D Novaclone
- Anti-D Rapid

Negative

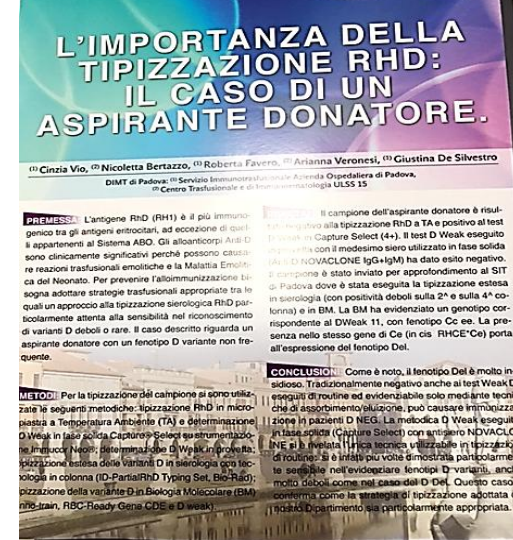
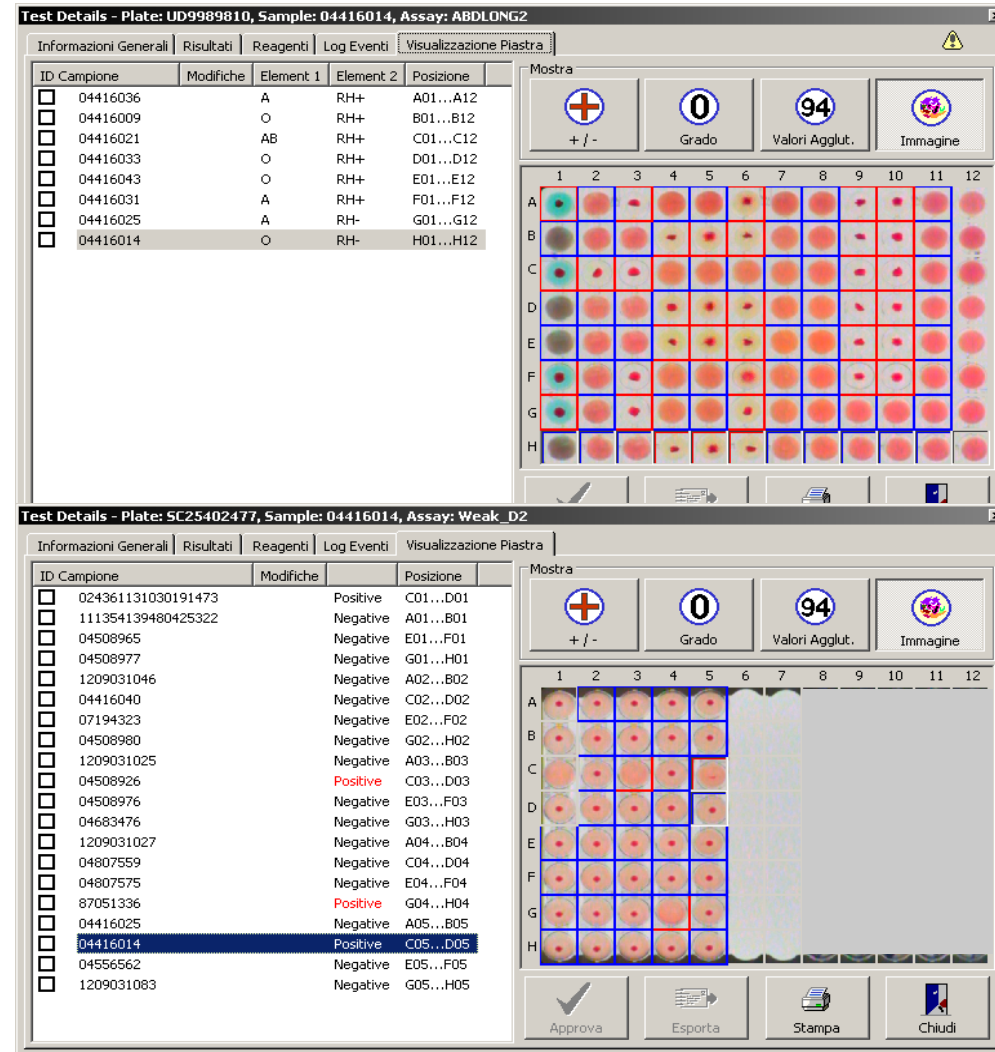
Weak D test in tube

Negative

Weak D test with Solid

Phase on Iris

Positive



© Werfen All rights reserved. Confidential. For internal use only.

- The genotyping revealed a Weak D type 11
- c.885G>T in the RHD gene, resulting in M295I (Methionine to Isoleucine at codon 295)
- Donors need to be recognized and typed accordingly
- Poland: ~1 in 4,457; Southwestern Germany: ~1 in 90,909

RhD alloimmunization

RhD Alloimmunization in Hospitalized Patients

1. Oslo University Hospital Retrospective Study

- Study Period: 2006–2011
- Population: 607 RhD negative patients (mostly cancer and cardiovascular surgery)
- Findings: After receiving 1926 RhD positive units, **22.2%** developed anti-D antibodies. In 19.7%, the antibodies became evanescent over time.
- Strategic RhD positive transfusion can conserve RhD negative units but carries a measurable immunization risk

<https://onlinelibrary.wiley.com/doi/epdf/10.1111/tme.13118>

RhD Alloimmunization in Hospitalized Patients

2. Trauma Patients of Childbearing Age

- Study: University of Pittsburgh, published in Hematology (2021)
- Findings: Among 96 RhD negative trauma patients aged 13–50 who received RhD positive RBCs or low-titer group O whole blood (LTOWB), 42.7% developed anti-D antibodies
- High alloimmunization risk in emergency transfusions, especially relevant for women of childbearing age

<https://www.tandfonline.com/doi/pdf/10.1080/16078454.2021.1905395>

RhD Alloimmunization in Hospitalized Patients

3. BEST Collaborative Study on Alloimmunization Sources

- Scope: 1200 RhD negative patients across 30 institutions in 5 countries
- Findings:
 - 52% of males developed anti-D due to transfusion.
 - 5% of females developed anti-D from transfusion (most from pregnancy).
 - 33% had additional antibodies (anti-C, anti-E)
- Transfusion is a major source of RhD alloimmunization, especially in males.

Transfusion. Published online March 10, 2025. doi:10.1111/trf.18202

In conclusion: where to find information on the variant?

- ISBT: www.isbtweb.org/isbt-working-parties
- Rheference for *RHD*: www.rheference.org
- Rhesus base for *RHD*: www.uni-ulm.de/fileadmin/website_uni_ulm/archiv/wflegel/RH
- NYBCe for *RHCE*: www.nybce.org/national-center-for-blood-group-genomics/rhce-table/
- ErythroGene: www.erythroGene.com/
- Blood Group Antigen FactsBook

Thanks!