Review Article

RH genetic variation and the impact for typing and personalized transfusion strategies: a narrative review

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Background and Objective: This work reviews *RHD* alleles expressing variant antigens that can cause typing discrepancies and *RHCE* alleles that are associated with loss of high prevalence antigens and alloimmunization. Strategies to use molecular methods to identify the *RH* alleles and accurately assess alloimmunization risk and personalize clinical care in the setting of pregnancy and transfusion medicine are discussed. Genetic variation in *RHD* can result in alterations in expression levels as well as loss or gain of epitope(s) that can result in variant antigens. These variants may make it difficult to accurately phenotype red cells using anti-D reagents and serologic techniques. Some RhD variants are associated with risk of alloimmunization following transfusion of D+ red blood cells (RBCs) or pregnancy with a D+ fetus. In addition, the impact of polymorphisms in the *RHCE* gene will be discussed, focusing on alleles common in individuals of African descent that encode partial antigens and/or loss of high prevalence antigens.

Methods: Peer-reviewed literature published in English in PubMed from 1960 to January 2022, AABB guidance documents and AABB standards as well as ISBT Working Party for Red Cell Immunogenetics and Blood Group Terminology.

Key Content and Findings: A brief history of the terminology and approach used to manage individuals with non-binary (positive or negative) RhD antigen status is reviewed and compared to findings in other countries. First, *RHD* alleles identified in US patients with a weak or discrepant RhD type is reviewed. Second, strategies to identify patients who may be at risk of allo-anti-D are discussed along with how *RHD* genotyping can provide critical information to assess that risk. Third, a strategy will be presented for selecting donor RBC units for patients who have made alloantibodies in the RH system, including patients with sickle cell disease. This process of "*RH* allele selection" has been instrumental in providing transfusion support to alloimmunized patients, including those with sickle cell disease. It is based on the premise that a patient with partial Rh antigens and allo-antibodies to the conventional antigens would be predicted to be compatible with red cells from a donor who expresses the same or similar partial antigens.

Conclusions: This work presents the genetics and serology of the RH blood group system that can lead to antigen discrepancies and alloimmunization due to expression of partial antigens or loss of high prevalence antigens. This work presents strategies used to identify those at risk of allo-anti-D and describes *RH* allele matching for selection of red cells for patients who have produced allo-antibodies to high prevalence antigens such as RH19 (hr^S) and RH31 (hr^B).

Keywords: RH; alloimmunization; serologic weak D phenotype; *RH* allele selection

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Introduction

Rationale

The purpose of this narrative review is to provide the reader with updated guidance on the clinical utility identifying patients at risk for allo-anti-D formation as well as strategies that can be used to determine candidacy for Rh immune globulin in pregnant women. This narrative also reviews the genetic complexity behind phenotypes lacking high prevalence antigens encoded by the *RHCE* gene and describes an effective strategy that has been used for more than a decade in the US to personalize donor unit selection in Rh alloimmunized patients based on *RH* allele information. This strategy can be used in alloimmunized patients in whom transfusion has become seemingly impossible. The objective is to provide the reader with information that can be used to reduce Rh alloimmunization and increase personalized patient care related to red cell alloimmunization. I present the following article in accordance with the Narrative Review reporting checklist (available at https://aob.amegroups.com/article/view/10.21037/aob-22-6/rc).

Methods

Peer-reviewed literatures published in English and listed in PubMed from 1960 to January 2022 were reviewed while compiling this work. In addition, AABB guidance documents and AABB standards as well as guidelines published by the American Society of Hematology and the British Society of Haematology were reviewed and cited where appropriate. The allele tables on the website of the ISBT Working Party for Red Cell Immunogenetics and Blood Group Terminology were also reviewed and referenced. Relevant peer-reviewed abstracts by the author were reviewed and compiled data from such work is included where appropriate (see *Table 1*).

The RH blood group system

The RH blood group system includes two genes (*RHD* and *RHCE*), both with 10 exons, that encode a total of 56 antigens. While the *RHD* gene encodes one common antigen, RhD or RH1, the *RHCE* gene encodes two sets of antithetical common antigens, big C (RH2) and little c (RH4) and big E (RH3) and little e (RH5). Expression of RhD and RhCE antigens requires co-expression with the RhAG protein in a multimeric protein complex.

RH locus

The *RH* locus, located on Chromosome 1, includes the highly homologous and highly polymorphic *RHD* and *RHCE* genes. The RhD and Rhce polypeptides are multipass transmembrane proteins that are part of a multiprotein complex on the red cell surface. The RhD polypeptide carries the RhD antigen with multiple epitopes and can express neoepitopes such as Go^{a} (RH30) or DAK (RH54). The *RHCE* gene has four normal allele types (*RHCE*ce*, *RHCE*Ce*, *RHCE*cE* and *RHCE*CE*) that encode Rhce polypeptides expressing antithetical antigens c or C and e or E.

The RHD gene

The *RHD* gene (NG_007494) includes 10 exons and encompasses ~58,000 base pairs of DNA on chromosome 1. It is polymorphic with variants including deletions, gene conversions, and non-synonymous single nucleotide variants (1).

There are several mechanisms and more than 80 *RHD* alleles that are associated with a null or RhD negative phenotype (see *Table 2*). *RHD*01N.01*, deletion of the gene's 10 exons is the most common RhD negative allele in Caucasians while *RHD*08N.01*, the silenced *RHD* allele is the most common RhD negative allele in individuals of African descent. There are several *RHD* alleles (*RHD*01N.02* – *RHD*01N.05*) that do not express the D antigen due to gene conversion involving replacement of multiple exons with those from the *RHCE* gene. In addition, several *RHD* alleles involve gene conversion that abolishes RhD expression and gains expression of a variant of the C antigen, with *RHD*DIIIa-CE(4-7)-D* or *RHD*03N.01* the most common in African Americans. Finally, there are *RHD* alleles in which splicing defects abolish RhD expression (e.g., *RHD*01N.24*), those with insertion/deletion polymorphisms that lead to frameshifts and premature termination (ex. *RHD*01N.11*) and those with nonsense mutations (ex. *RHD*01N.18*) as well as those with nonsynonymous mutations where the mechanism is less clear (ex. *RHD*01N.80*).

RhD antigen

The RhD polypeptide expresses the RhD or RH1 antigen. *RHD* alleles that encode RhD antigens have historically been categorized on one of three groups (weak, partial or DEL). Partial RhD antigens are defined as antigens in

Table 1 The search strategy summary

Items	Specification
Date range of searches	November 2021 to January 2022
Databases and other sources searched	PubMed; peer-reviewed abstracts published or co-published by the author; service requests received from the American Red Cross National Molecular Laboratory from July 2018 to June 2021; the ISBT Working Party on Red Cell Immunogenetics and Blood Group Terminology *RHD* and *RHCE* allele tables
Terms used to search PubMed	"RhD"[MeSH] AND "discrepan*"[MeSH]
	"RhD"[MeSH] AND "alloimmun*"[MeSH]
	"Rh"[MeSH] AND "matching"[MeSH]
	"RhD"[MeSH] AND "weak"[MeSH]
	"RhD"[MeSH] AND "partial"[MeSH]
	"sickle"[MeSH] AND "prophylactic"[MeSH] AND "antigen"[MeSH}
	"American Society of Hematology"[MeSH] AND "sickle"[MeSH] AND "guidance"[MeSH]
	"British Society of Hematology"[MeSH] AND "sickle"[MeSH] AND "guidance"[MeSH]
	"American Rare Donor Program"[MeSH]
Timeframe	1960–January 2022
Inclusion and exclusion criteria	Focus was placed on original papers and reviews in English about the RH blood group system, with specific focus on Rh variants, serologic discrepancies and population studies.
Selection process	It was conducted independently by the author

Table 2 Demographics of 879 US patients submitted to American Red Cross National Molecular Laboratory for *RHD* genotyping

Race/Ethnicity	n (%)
AA	363 (41.3)
Cauc	366 (41.6)
Other	98 (11.1)
Hisp	28 (3.2)
NP	10 (1.1)
MR	6 (0.7)
API	5 (0.6)
AIAN	3 (0.3)

AA, African American; Cauc, Caucasian; Hisp, Hispanic; NP, not provided; MR, mixed race; API, Asian Pacific Islander; AIAN, American Indian Alaska Native.

which one or more epitopes recognized by monoclonal antibodies is lacking.

As is the case with some other blood group antigens, including other antigens in the RH blood group system, expression is not binary, meaning that classifying the phenotype as positive or negative is incomplete and inaccurate. Though the red cells may test positive with an anti-D reagent, if the *RHD* gene sequence differs from the consensus sequence the antigen may be different. These differences may impact copy number, gain or loss of antigen epitopes or both as well as reactivity with human or reagent anti-D. Panels of monoclonal antibodies have been used to investigate the epitope loss in RhD variants (2) and flow cytometry has been used to assess D antigen sites per red blood cell (RBC) in subjects expressing RhD variants (3).

Changing terminology

In 1946, the weak D phenotype was initially described and defined as red cells that agglutinate with some but not all anti-D reagents and was given the term D^U (4). When anti-D sensitizes the RBCs but they do not directly agglutinate, visualization can be enhanced by the use of antiglobulin sera. Subsequently, the US put in place policies requiring blood donor RBCs that type D- to be retested using antiglobulin ("weak D test") and if agglutination was seen, to be classified as D+ (5). This additional testing is not required of pregnant women who type D-; instead, they are

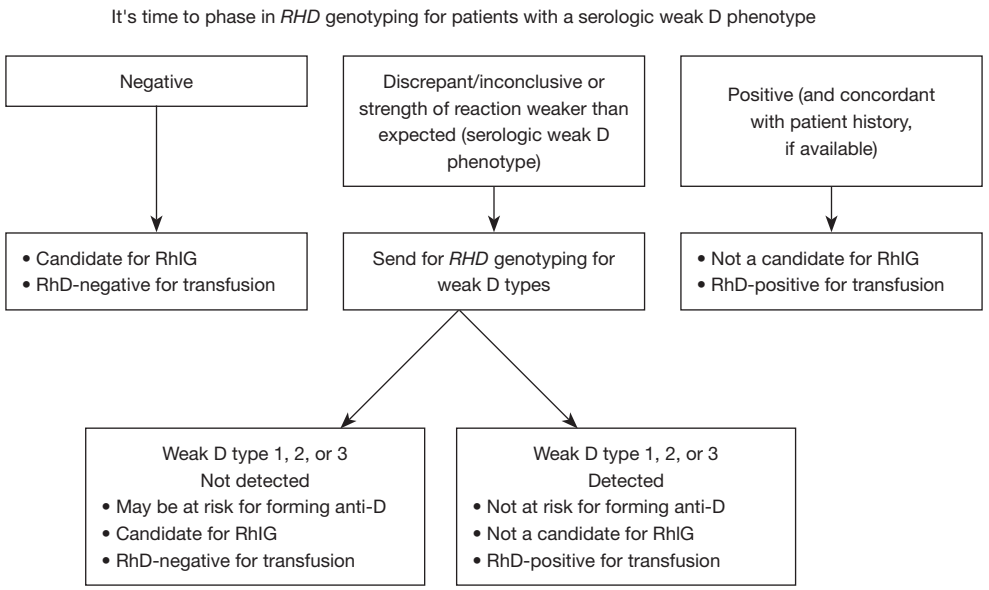

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Figure 1 Algorithm for resolving serologic weak D phenotype test results by *RHD* genotyping to determine candidacy for RhIG and RhD type for transfusions. RhIG, Rh immunoglobulin.

given Rh immunoglobulin (RhIg). The term "D mosaic" was coined in 1962 to describe red cells that express RhD antigen that is missing one or more epitopes defined by reactivity with monoclonal anti-D antisera (6). While initially the D^U term was used to define a red cell with a quantitative but not qualitative difference in the D antigen, this terminology was fraught with challenges. First because there is no D^U antigen or anti-D^U antibody. Second because some D^U+ individuals, initially thought to carry little risk of allo-anti-D formation, were found to make allo-anti-D. The term D^U was replaced with "weak D" in 1992 (7).

Changing guidance

Since the 1990s, our understanding of the complexity of *RHD* genetic variation and its impact on the RhD antigen has grown significantly. In 2013, Geoffrey Daniels proposed using the term "D variant" to avoid confusion with antigens that can type weakly D+, yet are associated with epitope loss and allo-anti-D production (8). In 2014, Sandler *et al.* presented results of a College of American Pathology (CAP) survey of 3,100 US hospitals about policies and procedures around testing individuals with weak D phenotypes and administration of RhIg which highlighted the lack of standard practice in the US (9). In 2015, an inter-organizational work group published a commentary (10) with two key take aways, both illustrated in *Figure 1*.

(I) A serologic weak D phenotype was redefined as reactivity of RBCs with an anti-D reagent giving no or weak (≤2+) reactivity in initial testing, but agglutinating moderately or strongly with antihuman globulin.

(II) The RhD phenotype of individuals with a serologic weak D phenotype should be resolved by using *RHD* genotyping to identify the *RHD* alleles carried by the patient. In this way, individuals found to carry *RHD* variant alleles weak D type 1, 2 or 3 (*RHD*01W.1*, *RHD*01W.2*, *RHD*01W.3*) are not considered at risk for allo-anti-D (10), and would avoid RhIg administration.

Based on the number of live births and transfusion recipients and ethnic background of the US population, this strategy was estimated to identify more than 13,000 women of child-bearing age in whom more than 24,000 doses of RhIg would not be needed. The recommendation to "phase-in" *RHD* genotyping, specifically of women of child-bearing age with a serologic phenotype was endorsed by the AABB and CAP (11). Financial modeling suggested that this approach could add clinical benefit without additional costs (12).

Strategies for identifying those at risk

Since the release of the joint statement (11), there has been a growing appreciation for the ability of molecular methods to assess a patient's risk of alloimmunization to RhD more precisely. However, besides the flow chart from Sandler *et al.* (10) reproduced in *Figure 1* and a review (13), there is no specific guidance for how a hospital blood bank would identify patients that may benefit from *RHD* genotyping. Further, many hospital blood banks have automated antigen typing platforms, whether solid phase or gel technology and these platforms may or may not give the user the ability to easily review results of specimens demonstrating weaker reactivities. Several healthcare systems have evaluated algorithms to select samples for *RHD* genotyping. A repeating theme is the use of differential anti-D reactivity between two methods or reagents to identify those who likely carry *RHD* variant alleles and express RhD variant antigens. It is important to emphasize that serologic testing cannot accurately distinguish between RhD variant antigens that put the patient at risk of alloimmunization or not. *RHD* genotyping of these individuals can identify the specific alleles and that information can be used to assess risk of alloimmunization. This strategy can focus genotyping efforts on patients who would benefit the most, since it is not currently cost-effective to perform *RHD* genotyping of all patients and blood donors.

There have been several publications that describe the use of anti-D reactivity differences to identify patients who could benefit from *RHD* genotyping (14-19) (see *Table 3*). These studies share a two reagent and/or two-method strategy and are highly effective at identifying samples for which *RHD* genotyping predicts expression of RhD variants. Importantly, monoclonal anti-D panels are now recognized as being ineffective at differentiating patients at risk of allo-anti-D while *RHD* genotyping can differentiate samples carrying weak D types 1, 2 and 3 from samples carrying partial D alleles. The two-test approach does not require additional testing in laboratories accredited by AABB, since these organizations already require two determinations of ABO/Rh for pre-transfusion testing for allogeneic transfusion (31).

Benefit of RHD genotyping: avoiding overtreatment

Among Caucasians, in whom a serologic weak D phenotype is found at a prevalence of 0.2–1% (15,32), 73–80% of these will be found to carry *RHD* alleles weak D type 1, 2 or 3 (*RHD*01W.1*, *RHD*01W.2*, *RHD*01W.3*) (10,15). Thus, in a primarily Caucasian population, the main benefit of implementing a process to identify patients with serologic weak D phenotype for *RHD* genotyping will be identification of those women of childbearing age who do not require RhIg prophylaxis. Importantly, the benefits and the outcome of such an approach may be different in other ethnic groups.

Benefit of RHD genotyping: identifying those at risk who are hiding in plain sight

Among non-Caucasians, in whom a serologic weak D phenotype is more often associated with inheritance of a partial D allele, implementing an *RHD* genotyping strategy can identify patients who may have typed D+ on an automated testing instrument and who may not have been identified as at risk of allo-anti-D otherwise. Though some monoclonal anti-D reagents will be non-reactive with red cells expressing a partial D phenotype, it is common for such cells to react strongly with some anti-D reagents typically used on automated platforms in the US yet weakly or not at all by manual tube agglutination method. Implementing a process to test women of child-bearing age by two different methods and/or anti-D reagents will help to identify women who may express a partial D phenotype and who could benefit from *RHD* genotyping to accurately assess candidacy for RhIg. A prerequisite for this approach is prenatal care with access to laboratory testing in a timeframe that allows for appropriate RhIg administration during and shortly after delivery.

Findings of a large national US reference laboratory

Keller *et al.* (20) described *RHD* genotyping of 879 patient samples referred to a large national molecular laboratory from across the US in calendar year 2020. These specimens were identified by hospitals based on their own criteria, which were not provided. The race/ethnicity of the cohort was 41% African American (AA), 41% Caucasian (CAUC), 11.1% "other", 3.2% Hispanic, 1.1% race not provided, 0.7% mixed race, 0.6% Asian and 0.3% Native American. *RHD* genotyping predicted 63.5% to express a D variant with 31.5% hemizygous or homozygous for Weak D Types 1, 2 and 3. Among Caucasian patients, the majority (61.7%) carried Weak D Types 1, 2 and 3. Among African American patients, 58% were predicted to express a partial

Table 3 Summary of *RHD* variant alleles as percent of individuals tested in multiple studies, by country

First author (reference)	Subject No.	Patient or donor	*RHD* variant alleles	% of total	Weak Type 1, 2, or 3	% of variants	Weak Type 4.0	% of variants	Distinct alleles found
USA									
Keller (20)	879	P	558	63	277	50	87	16	19
Wang (21)	11	P	10	91	1	10	4	40	4
Hudgins (19)	80	P	52	65	10	19	20	38	15
Canada									
Denomme (14)	55	P	53	96	25	47	6	11	12
Brazil									
Campos (22)	129	P	98	76	83	85	14	14	6
Bub (23)	104	P	104	100	23	22	51	49	9
Dezan (24)	58	P	58	100	29	50	0	0	4
Iran									
Oodi (25)	100	P&D	100	100	15	15	0	0	18
Thailand									
Thongbut (26)	51	D	38	75	0	0	0	0	13
Korea									
Chung (27)	23	P	21	91	0	0	0	0	10
Egypt									
Bakry (28)	50	P&D	45	90	15	33	11	24	10
China									
Yan (29)	32	D	32	100	0	0	0	0	9
Tunisia									
Ouchari (30)	67	D	64	96	2	3	60	94	5

D phenotype with 16 different alleles identified, of which the most common *RHD* variant allele was weak D type 4.0 (*Table 2*). The *RHD* alleles identified in this large US cohort are listed in *Table 4*. The breakdown of patients by race/ethnicity that were found to carry Weak D Types 1, 2 or 3 or other *RHD* alleles encoding partial D are listed in *Table 5* and *Table 6*. This study is the largest of several published studies (*Table 3*). Approximately two-thirds (63%) of subjects were found to carry a variant RhD antigen. This percentage is lower than the other studies where subjects were identified using a specific algorithm, such as that used in Luo *et al.* or Horn *et al.* (17,18). Interestingly, when the large study (20) is compared to Hudgins *et al.* (19), in which one-tenth the number of subjects were tested, a similar percent of *RHD* variants were identified (65% *vs.* 63%) and though the sample size of Keller *et al.* was more than ten-fold larger than that of Hudgins *et al.*, it identified 19 distinct *RHD* variant alleles while Hudgins identified 15 alleles. This may suggest that the number of alleles involved in typing discrepancies in the US population is small and mostly known.

Phasing out serologic weak D result

In 2020, Flegel *et al.* published a commentary (33) that suggested that blood banks and Immunohematology reference laboratories should stop releasing reports with "weak D+" or "serologic weak D+" interpretations as a final result. Instead, the authors suggest that such cases should be referred to molecular reference laboratories where

Table 4 Number and percent of patients with *RHD* alleles identified in the cohort of 879 US patients

RHD allele[#]	All patients (n=879)		Caucasians (n=366)		African Americans (n=363)		Other (n=150)	
	n	%	n	%	n	%	n	%
*RHD*01*	245	27.9	94	25.7	104	29	47	31.3
*RHD*01W.01 (weak D Type 1)*	149	17.0	123	33.6	4	1.1	22	14.7
*RHD*01W.02 (weak D Type 2)*	100	11.4	77	21.0	6	1.7	17	11.3
*RHD*weak D Type 4.0*	87	9.9	12	3.3	67	18.5	8	5.3
*RHD*01W.03 (weak D Type 3)*	30	3.4	28	7.7	1	0.3	1	0.7
*RHD*DAR*	71	8.1	2	0.5	54	14.9	15	10
*RHD*DIIIa*	17	1.9	0	0	17	4.7	0	0
*RHD*DIVa*	12	1.4	0	0	11	3.0	1	0.7
*RHD*08N.01 (RHD*Psi)*	5	0.6	0	0	5	1.4	0	0
*RHD*DOL*	4	0.5	1	0.3	2	0.6	1	0.7
*RHD*DAU5*	4	0.5	0	0	3	0.8	1	0.7
*RHD*DCS or DFV*	3	0.3	2	0.5	0	0	1	0.7
*RHD*DFR*	3	0.3	1	0.3	0	0	2	1.3
*RHD*DAU0*	2	0.2	0	0	2	0.6	0	0
*RHD*DWN*	2	0.2	0	0	2	0.6	0	0
*RHD*DAU4*	1	0.1	0	0	1	0.3	0	0
*RHD*DUC*	1	0.1	1	0.3	0	0	0	0
*RHD*DIIIc*	1	0.1	0	0	0	0	1	0.7
*RHD*DVI*	1	0.1	0	0	0	0	1	0.7
*RHD*DNB*	1	0.1	1	0.3	0	0	0	0
*RHD*DSPM*	1	0.1	0	0	1	0.3	0	0
*RHD*667G,809G*	1	0.1	1	0.3	0	0	0	0

[#], Homozygosity and hemizygosity due to *RHD*01N.01* were not differentiated. *RHD*03N.01* alleles cannot be quantified since allele assignments in many cases were equivocal [ex. *DIIIa/DIIIa* or *DIIIa/DIIIa-CE(4-7)-D*].

Table 5 Number and percent of cohort of 879 US patients predicted to be at risk for allo-anti-D

RHD allele	Risk of allo-anti-D	All patients		Caucasian		African American		Other	
		n	%	n	%	n	%	n	%
*RHD*01*	N	281	32	97	26.5	130	35.8	27	27.6
Weak D Type 1, 2 or 3	N	277	31.5	226	61.7	11	3	30	30.6
Weak D Type 4	??	87	9.9	12	3.3	67	18.5	4	4.1
Various partial D alleles	Y	194	22.1	11	3	141	39.7	31	31.6
*RHD*01N.01*	Y	40	4.6	20	5.5	11	3	6	6.1

N, no; Y, yes; ??, no consensus.

Table 6 Number (n) & percent (%) of cohort of 879 US patients that carry weak D Types 1, 2 or 3, by race/ethnicity

RHD alleles	All patients		Caucasian		AA (n)	API (n)	Hisp (n)	AIAN (n)	Other (n)	Mixed (n)	NP (n)
	n	%	n	%							
Weak D Type 1, 2 or 3	277	31.5	226	61.7	11	1	3	2	30	0	1
Type 1	147	16.7	121	33.1	4	1	3	0	17	0	1
Type 2	98	11.1	75	20.5	6	0	0	2	12	0	0
Type 3	30	3.4	28	7.7	1	0	0	0	1	0	0
Type 1/Type 2	2	0.2	0	N/A	0	0	0	0	0	0	0

AA, African American; API, Asian Pacific Islander; Hisp, Hispanic; AIAN, American Indian Alaska Native; NP, not provided; N/A, not applicable.

genotyping can be used to identify the *RHD* alleles. With knowledge of the *RHD* alleles that are responsible for the weak D phenotype, the risk of alloimmunization can be more accurately assessed. In pregnant women and women of child-bearing age, this information is critical to determine candidacy for RhIG; in all patients, the information can be used to determine which require RhD negative blood products. This process offers a genome-informed and personalized approach to RhD alloimmunization risk assessment. Furthermore, since *RHD* alleles associated with partial D phenotypes are often co-inherited with *RHCE* alleles encoding partial antigens or lacking high prevalence antigens, this information may uncover additional Rh alloimmunization risk. The American Red Cross National Molecular Laboratory saw a 6.3% increase in service requests for *RHD* genotyping of patients from FY19 (July 2018 to June 2019) to FY2020 (July 2019 to June 2020) followed by a 23.8% increase from FY2020 to FY2021 (July 2020 to June 2021). The later increase may have been influenced by the Flegel *et al.* publication (33) which was released electronically in March 2020.

Personalized transfusion support for patients lacking high prevalence antigens in the RH system

In patient populations where chronic transfusion is a therapeutic modality (ex. sickle cell anemia and thalassemia), RBC phenotype matching has been used for more than twenty-five years (34-37). More recently, prophylactic red cell antigen matching for common RBC antigens has become commonplace (38,39). Matching for C, E and K is recommended by the British Society of Haematology (40). The American Society of Hematology (ASH) recently recommended matching for C, E and K as the standard of care for all patients with sickle cell disease, optimally before the first transfusion (41). The ASH guidelines also recommend that when *RH* genotyping identifies a partial C antigen (either by *RHD*03N.01* or *RHCE*Ce.10*) in a patient without a conventional *RHCE* allele expressing the C antigen, the patient should receive C- red cell products to avoid allo-anti-C. In addition, genotyping for red cell antigens is preferred over serologic phenotyping due to increased accuracy and the ability to predict phenotypes for antigens for which commercial reagents are not available or not reliable (41-43).

Personalized transfusion support for patients with sickle cell disease

In patients with SCD, it has been appreciated for some time that since this population is predominantly of African descent, and since in the USA the blood donor base is primarily Caucasian, there is a high likelihood of mismatches in what had been termed "minor" blood group antigens (i.e., Fy^a, Fy^b, Jk^a, Jk^b, S, s). In the US, the desire to "extend" the match from ABO/Rh and C and E antigens to these additional antigens resulted in a variety of donor recruitment programs aimed at collecting RBC units from African American donors (44). However, individuals of African descent have extensive genetic variation in the *RH* locus, with not only partial RhD phenotypes but loss of high prevalence antigens such as hr^B, hr^S and Hr (45). While RBC units from Caucasians with normal Rh antigens may put patients of African descent with partial Rh antigens at risk of alloantibodies in the RH system, RBC units from African descent donors expressing neoepitopes such as Go^a in individuals with *RHD*DIVa* can alloimmunize patients of African descent who do not express the same RhD variant antigen(s) (46). A study of Brazilian patients

with SCD found that those who typed D+ with unexpected anti-D carried *RHD* alleles encoding partial D antigens (47). Additionally, patients of African descent who type C+ with anti-C and/or e+ with anti-e often carry variant *RHCE* alleles encoding partial antigens. In the case of the C+ patient with anti-C, they may not express the C antigen but instead a hybrid *RHD-RHCE* allele that encodes a partial C antigen (48).

There has been increasing interest in molecular matching for patients with SCD, including matching at the allele level for *RH* variants (49). In 2017, Fasano *et al.* (50) noted that more studies would be needed to determine if use of *RH*-allele matched RBC units would be a feasible approach to prevent Rh alloimmunization. The feasibility of such an approach was later examined using virtual simulations (51). The study involved use of identical matches, haplotype matches and less restrictive matches that classified *RHD*DAU0* as equivalent to *RHD*01* and *RHCE*ce48C* equivalent to *RHCE*ce*. A recent modeling study provides evidence that some RhD variants may be "benign" based on impact of amino acid substitutions on tertiary structure of the RhD molecule (52). Chou *et al.* (51) noted that the success of prophylactic *RH* allele matching protocol would be dependent on improved recruitment of African American blood donors, an issue also highlighted by Karafin *et al.* (53).

Identifying patients lacking hr^B (RH31) and/or hr^S (RH19)

There are multiple *RHCE* alleles that predict loss of high-prevalence antigens hr^B (RH31) and/or hr^S (RH19) (42) (*Table* 7). This is an important distinction from most other blood group antigens and should be taken into account when designing a process for selection of RBC units based on *RH* alleles and their predicted phenotypes. In addition, there are no commercial reagents to screen for donors lacking either of these antigens such that molecular screening is the primary means of donor identification. While many of the *RHCE*ce* alleles that encode Rhce molecules lacking hr^B include the single nucleotide variant (SNV) C>G at coding position 733 in the *RHCE* gene (c.733C>G), not all alleles carrying c.733G are associated with an hr^B- phenotype and for those that do, most but not all will express both V and VS, since the V is lost when the c.733G is coinherited with c.1006T. And there are *RHCE*ce* alleles such as *RHCE*ceAG* that do not carry c.733G yet encode an hr^B- phenotype. The *RHCE*ce* alleles that encode hr^S- phenotype are varied in the SNVs they carry; several carry c.712 and some gain expression of the low prevalence antigen RH49 (STEM). This heterogeneity requires, at minimum, medium-resolution *RHCE* genotype information for selection of donor units based on *RH* alleles of the donor and patient. While the commonly used commercial red cell genotyping kits are low-resolution, containing a small number of markers that are insufficient to assign alleles with confidence, additional testing and/or medium-resolution assays include additional SNVs that allow allele assignments with increased accuracy (54). High-resolution methodologies such as Sanger sequencing of genomic DNA or cDNA or Next Generation sequencing provide the greatest accuracy, but analysis and interpretation are labor-intensive and complex (55).

Personalized transfusion support for alloimmunized patients using RH allele selection

The American Rare Donor Program (ARDP) aids in identifying rare blood products for alloimmunized patients (56). These products include units lacking high prevalence antigens hr^B and hr^S (defined by genotype) in the RH blood group system. In a review of ARDP activities from 2005 and 2010 (56), there were 10 requests for hr^B- or hr^S-units in 2005, and since 2010 these requests are filled with RH genotype-selected units, when possible (56,57). In 2013, the process or *RH* allele selection used by the ARDP was formalized (58) and additional reports of its use have been presented (59). In 2020, the ARDP received 126 requests for *RH* allele selected red cell units for 53 patients. An analysis of these requests (60) showed that 49% of these patients carried at least one *RHCE*ce733G* allele. Though the ISBT Working Party on Red Cell Immunogenetics and Blood Group Terminology currently classifies this allele as having an hr^B+^{vw}/- phenotype, allo-anti-hr^B has been reported (61,62). Therefore, ARDP considers this allele to be associated with risk of allo-anti-hr^B and provides *RH* allele selected units upon request.

As Chou *et al.* noted (51), large numbers of well characterized AA donors would be needed to put into practice routine selection of donor units based on *RH* alleles. To that end, the American Red Cross National Molecular Laboratory developed high-throughput assays to test E-, K-, hemoglobin (Hgb) S negative donors of African descent for both *RHCE* (63) and *RHD* (64) variants. In an analysis of more than 6,000 predominantly African American donors tested by HemoID DQS red cell genotyping panel (Agena Bioscience), 564 donors predicted to be E-, K-, HgbS- and predicted to be hr^B+^{vw}/- or hr^B- or hr^S negative or E-e+^{w}

Table 7 *RHCE* alleles lacking high prevalence antigens with the positive (pos), negative (neg), weak or partial expression of Rh antigens

RHCE allele (ISBT)	*RHCE* allele (alias)	RH31 (hr^B)	RH19 (hr^S)	RH18 (Hr)	RH10 (V)	RH20 (VS)	RH5 (e)	RH4 (c)	RH43 (Crawford)	RH49 (STEM)	RH58 (CELO)	RH59 (CEAG)	RH61 (CEVF)	*RHD* alleles often linked
*RHCE*ce.04.01*	*RHCE*ceAR*	pos	neg	neg	weak	neg	Partial	Partial	neg	neg	pos	pos	pos	*RHD*DAR*
*RHCE*ce.05.01*	*RHCE*ceEK*	pos	neg	neg	neg	neg	Partial	Partial	neg	neg	pos	pos	pos	*RHD*01N.01*
*RHCE*ce.06.01*	*RHCE*ceAG*	neg	pos	pos	neg	neg	Partial	pos	neg	neg	pos	neg	pos	*RHD*01N.01*
*RHCE*ce.07.01*	*RHCE*ceMO.01*	neg	neg	pos	neg	neg	Partial	Partial	neg	neg	pos	pos	neg	*RHD*DAU*
*RHCE*ce.07.02*	*RHCE*ceMO.02*	neg	neg	pos	neg	neg	Partial	Partial	neg	neg	pos	pos	neg	*RHD*DAU*
*RHCE*ce.08*	*RHCE*ceBI*	pos	neg	neg	neg	neg	Partial	pos	neg	pos	pos	pos	pos	*RHD*DOL*
*RHCE*ce.09*	*RHCE*ceSM*	pos	neg	neg	neg	neg	Weak	pos	neg	pos	pos	pos	pos	*RHD*DOL*
*RHCE*ce.20.01*	*RHCE*ce733G*	vw/ neg	pos	pos	pos	pos	Partial	Partial	neg	neg	pos	pos	pos	*RHD*01*
*RHCE*ce. 20.02.01*	*RHCE*48C, 733G*	neg	pos	pos	pos	pos	Partial	Partial	neg	neg	pos	pos	pos	*RHD*weak D type 4.0*
*RHCE*ce.20.03*	*RHCE*ce48C, 733G,1006T*	neg	pos	pos	neg	pos	Partial	Partial	neg	neg	pos	pos	pos	*RHD*DIIIa* or *RHD*DIIIa-CE(4-7)-D*
*RHCE*ce.20.04*	*RHCE*ce48C, 733G,1025T*	neg	pos	pos	pos	pos	Partial	pos	neg	neg	pos	pos	pos	*RHD*weak D type 4.0* or *RHD*DIIIa*
*RHCE*ce.20.05*	*RHCE*ce733G, 1006T*	neg	pos	pos	neg	pos	Partial	pos	neg	neg	pos	pos	pos	*RHD*DIIIa-CE(4-7)-D*
*RHCE*ce.20.06*	*RHCE*ceCF*	neg	neg	pos	unk	pos	Partial	Partial	pos	neg	neg	pos	pos	*RHD*weak D type 4.0* or *RHD*01N.01*
*RHCE*ce.20.08*	*RHCE*48C, 733G,748A*	neg	pos	pos	pos	pos	Weak	pos	neg	neg	pos	pos	pos	*RHD*weak D type 4.0*
*RHCE*ce.33*	*RHCE*506C*	neg	pos	pos	neg	neg	Partial	pos	neg	neg	pos	pos	pos	*RHD*weak D type 4.0*

ISBT, International Society of Blood Transfusion.

were selected for further characterization. Genotyping was performed in 96-well format with custom *RHCE* (10 SNVs) and *RHD* (18 SNVs) oligonucleotide extension assays designed for MALDI-TOF. *Table 8* shows that 82 (14.5%) of those characterized were predicted to be homozygous for *RHCE*ce733G* and predicted to be hr^B+^{vw}/-. All of these carried a normal *RHD*01* allele. While 5% were predicted to be hr^B- and only 0.5% predicted to be hr^S-. It has not been well appreciated that the E- hr^S- phenotype is at least an order of magnitude rarer than the E- hr^B- phenotype. Also of note, nearly half of the hr^B- and hr^s- donors were predicted to express a partial D phenotype. There is a strong association between loss of the high prevalence antigens hr^B and/or hr^S and a partial D phenotype. In addition, individuals who are found to carry the *RHCE*ce48C,733G,1006T* allele may carry the *RHD*DIIIa-CE(4-7)-D* allele and together are associated with the r's phenotype. This phenotype is associated with expression of a partial C antigen and in patients who do not express a normal C antigen, with risk of allo-anti-C. Another important factor derived from *RHD* and *RHCE* co-inheritance data is the finding that most *RHCE*ce733G* alleles are linked to a normal *RHD*01* allele encoding a D+ phenotype.

The RH allele selection process

Importantly, precise *RH* allele selection requires genotype information for *RHCE* SNVs not interrogated by commonly

Table 8 Predicted phenotypes of predominantly African American Blood Donors tested by RBC genotyping panel, *RH* genotyping panels

Test or phenotype	N	Of all donors tested	Of those selected for *RH* characterization
Red blood cell (RBC) genotyping panel	6,392	–	–
Custom *RHCE* and *RHD* assays	564	8.8%	–
Predicted to type $hr^{B+^{VW}}$/-	82	1.3%	14.5%
Predicted to type hr^{B}-	28	0.4%	5.0%
r's homozygous	7	0.1%	1.2%
Predicted to be hr^{S}-	3	0.05%	0.5%
Predicted to express altered C	44	–	7.8%
Predicted to express partial D	268	–	47.5%

RBC, red blood cell.

Table 9 Description of Tiers for *RH* allele selection

Tier	*RHCE*	*RHD*
1	Probable alleles of the donor are the same as the probable alleles of the patient	Probable alleles of the donor are the same as the probable alleles of the patient, or the donor does not express RhD antigen
2	The donor is homozygous for one of the probable alleles of the patient	The donor is hemizygous or homozygous for one of the probable alleles of the patient
3	The donor carries one or two alleles that are not the same but have a similar phenotype as the alleles of the patient	The donor may carry an *RHD* allele predicted to express a "benign" variant while the patient is predicted to express a normal RhD
4	The donor carries one or two alleles that are not the same and have a different phenotype than the alleles of the patient. This could be due to gain of low prevalence antigen(s) such as V or VS	The donor carries one or two alleles that are not the same and have a different phenotype than the alleles of the patient. This could be due to gain of low prevalence antigen(s) such as Go(a) or DAK

used low-resolution, commercially-available red cell genotyping panels. The ARDP requires *RHCE* c.48G>C, c.340C>T, c.697C>G, c.712A>G, c.733C>G, c.1006G>T and c.1025C>T genotype results for submission of a rare donor based on hr^{B}- phenotype and *RHCE* c.48G>C, c.667G>T, c.712A>G, 818C>T and 916A>G for submission of a rare donor based on hr^{S}- phenotype. Since most laboratories are not performing high-resolution testing, the alleles assigned to the patient are considered the "probable alleles" to convey that there is the potential that the patient carries other variants not interrogated that may change the allele assignment and, in some cases, the predicted phenotype. It is also noteworthy that with more laboratories performing high-resolution methods like full gene or exon sequencing of *RHD* and/or *RHCE* alleles, additional *RH* alleles are likely to be identified and the allele assignments made based on medium resolution genotyping methods may need to be updated. A Punnett square is generated for each patient for whom RH allele selected units are requested, based on the *RH* alleles they carry. Though a Punnett square could be generated with all known *RHD* and *RHCE* alleles, those that are Tier 1, 2 and 3 and for which there are donors in the American Rare Donor Program are typically included. This approach could be modified based on the population and the RH characterization of rare donors.

The use of a Punnett square allows donors with the same or similar alleles to be assigned to Tiers.

Table 9 defines the tiers and how they are assigned. Notably, with *RHD*, zygosity plays a role as well as an appreciation that some *RHD* variant alleles such as *RHD*DIIIa-CE(4-7)-D* do not express RhD but instead express an partial C antigen. *RHCE* alleles "drive" allele selection since matching is typically performed due to anti-e, -e-like, $-hr^{B}$, $-hr^{S}$ or -Hr antibodies. Importantly patients who carry two different *RHCE* alleles generally have more potentially compatible donors since the list of donors would

Table 10 A Punnett square is provided for a patient with *RHCE*ce* and *RHCE*Ce* alleles and c+ C+ e+ E- phenotype to illustrate how donors are assigned Tiers when their *RH* alleles are compared to the alleles of the patient

RHCE alleles	*RHCE*ce*	*RHCE*Ce*	*RHCE*cE*	*RHCE*CE*
*RHCE*ce*	Tier 2	Tier 1	Tier 4	Tier 4
*RHCE*Ce*		Tier 2	Tier 4	Tier 4
*RHCE*cE*			Tier 4	Tier 4
*RHCE*CE*				Tier 4

The Punnett Square includes the four normal *RHCE* alleles. The donor homozygous for *RHCE*ce* is seen in the top left box assigned Tier 2 since they are homozygous for one of the alleles of the patient and are not predicted to express antigens not expressed by the patient. The donor with one *RHCE*ce* allele and one *RHCE*Ce* allele is seen in the next box assigned Tier 1 since both the alleles of the donor match both alleles of the patient. The donor homozygous for *RHCE*Ce* is assigned Tier 2 since they are homozygous for one of the alleles of the patient and are not predicted to express antigens not expressed by the patient. Donors with *RHCE*cE* or *RHCE*CE* in any combination are assigned Tier 4 since they would express E antigen that the patient does not; these are included for illustration purposes only. *Tables 11,12* show examples of *RHCE* Punnett squares and *Tables 13,14* show examples of *RHD* Punnett squares for patients carrying variant Rh antigens. Both *RHCE* and *RHD* Punnett squares are generated for each patient case and the larger of the Tier number is assigned to the donor. Red Cell units from donors ranked Tier 1 or Tier 2 are preferred over donors ranked Tier 3, and Tier 3 is preferred over Tier 4 units. There is no evidence that Tier 1 RBCs are superior to Tier 2 RBCs.

Table 11 Punnett square is shown for a patient who is homozygous for *RHCE*ce733G* with partial c+ C- partial e+ E- V+ VS+ $hr^{B}+^{vw}$/- phenotype

RHCE alleles	*RHCE*ce733G*	*RHCE*ce48C,733G*	*RHCE*ce48C,733G,1006T*	*RHCE*ce48C*	*RHCE*ce*
*RHCE*ce733G*	Tier 1	Tier 3	Tier 3	Tier 4	Tier 4
*RHCE*ce48C,733G*		Tier 3	Tier 3	Tier 4	Tier 4
*RHCE*ce48C,733G,1006T*			Tier 3	Tier 4	Tier 4
*RHCE*ce48C*				Tier 4	Tier 4
*RHCE*ce*					Tier 4

A donor who is homozygous for the *RHCE*ce733G* allele is assigned to Tier 1 since they have the same alleles as the patient. Donors with other *RHCE*ce* alleles with hr^{B}- phenotype are expected to be compatible but since not identical are assigned Tier 3. Neither the consensus allele *RHCE*ce* nor the common variant *RHCE*ce48C* lack the high-prevalence antigen hr^{B} and therefore are not expected to be compatible with the patient who has made allo-anti-e or-hr^{B}, therefore they are assigned Tier 4.

include donors carrying the same combination of alleles as the patient (Tier 1) as well as those homozygous for either allele (Tier 2) or those carrying different alleles predicted to encode a similar phenotype.

A clinical case example of RH allele selection

The ARDP received a request for blood from a blood center outside of the US for an 18-year old female with sickle cell disease. The patient was A positive with anti-C, -e, -Wr^{a} and an antibody to a high-prevalence antigen in the Rh system. This antibody was found to be anti-hr^{B}. *RHCE* genotyping predicted that the patient to carry two different *RHCE* alleles: *RHCE*ceVS.03* [also known as *RHCE*ce48C*,*733G*,*1006T*] and *RHCE*ceVS.03* [also known as *RHCE*ce733G*]. *RHD* genotyping predicted the patient to carry two different *RHD* alleles: *RHD*03N.01* [also known as *RHD*DIIIa-CE(4-7)-D*] and *RHD*01*. Based on this information, the patient has a predicted phenotype of D+ partial C+ E- partial e+ partial c+ V+ VS+ hr^{S}+ and $hr^{B}+^{vw}$/-. The patient's plasma was non-reactive at PEG IgG-AGT phase with RBCs from two Tier 2 donors carrying *RHD*01* (homozygous or hemizygous) and homozygous for *RHCE*ce733G*. *Tables 10-15* illustrates the use of a Punnett Square of *RHCE* or *RHD* alleles in *RH* allele selection of donors based on a patient's *RH* alleles, with *Table 10* showing the use of a Punnett Square using *RHCE* common alleles for illustrative purposes only. The *RHCE* Punnett square in *Table 13* and *RHD* Punnett square in *Table 14* are applicable to the clinical case example. *Table 16* lists the

Table 12 Punnett square for patient homozygous for *RHCE*ce48C,733G,1006T* allele with partial c+ C- partial e+ E- V- VS+ including loss of hr^B antigen

RHCE alleles	*RHCE*ce48C,733G,1006T*	*RHCE*ce48C,733G*	*RHCE*ce733G*	*RHCE*ce48C*	*RHCE*ce*
*RHCE*ce48C,733G,1006T*	Tier 1	Tier 3	Tier 3	Tier 4	Tier 4
*RHCE*ce48C,733G*		Tier 3	Tier 3	Tier 4	Tier 4
*RHCE*ce733G*			Tier 3	Tier 4	Tier 4
*RHCE*ce48C*				Tier 4	Tier 4
*RHCE*ce*					Tier 4

The Punnett square includes the allele carried by the patient as well as the *RHCE*ce48C,733G* allele that also lacks hr^B and the *RHCE*ce733G* allele with hr^B+^{vw}/- phenotype. Since these two alleles are not identical and also express V, they are assigned Tier 3. Alleles that would not be considered include *RHCE*ce* or *RHCE*ce48C* because they do not lack the hr^B antigen; these are assigned Tier 4.

Table 13 Punnett Square for patient with *RHCE*ce48C,733G,1006T* and *RHCE*ce733G* alleles and partial c+ C- partial e+ E- V+ VS+ hr^B+^{vw}/- phenotype

RHCE alleles	*RHCE*ce48C,733G,1006T*	*RHCE*ce733G*	*RHCE*ce48C,733G*	*RHCE*ce48C*	*RHCE*ce*
*RHCE*ce48C,733G,1006T*	Tier 2	Tier 1	Tier 3	Tier 4	Tier 4
*RHCE*ce733G*		Tier 2	Tier 3	Tier 4	Tier 4
*RHCE*ce48C,733G*			Tier 3	Tier 4	Tier 4
*RHCE*ce48C*				Tier 4	Tier 4
*RHCE*ce*					Tier 4

The Punnett square includes the allele carried by the patient as well as the *RHCE*ce48C,733G* allele that also lacks hr^B and the *RHCE*ce733G* allele with hr^B+^{vw}/- phenotype. Since these two alleles are not identical and also express V, they are assigned Tier 3. Alleles that would not be considered include *RHCE*ce* or *RHCE*ce48C* because they do not lack the hr^B antigen; these are assigned Tier 4 for illustrative purposes.

Table 14 Punnett Square for patient with *RHD*DIIIa-CE(4-7)-D* and *RHD*01* alleles and predicted phenotype of D+ altered C+

RHD alleles	*RHD*DIIIa-CE(4-7)-D*	*RHD*01*	*RHD*01N.01*	*RHD*08N.01*	*RHD*DAU0*	*RHD*weak D type 4.0*
*RHD*DIIIa-CE(4-7)-D*	Tier 2	Tier 1	Tier 2	Tier 2	Tier 3	Tier 3
*RHD*01*		Tier 2	Tier 2	Tier 2	Tier 3	Tier 3
*RHD*01N.01*			Tier 1	Tier 1	Tier 3	Tier 3
*RHD*08N.01*				Tier 1	Tier 3	Tier 3
*RHD*DAU0*					Tier 3	Tier 3
*RHD*weak D type 4.0*						Tier 3

This Punnett square includes donors who carry one or two *RHD*DIIIa-CE(4-7)-D* alleles predicted to express an altered C antigen, or two conventional *RHD*01* alleles, or one of each. The donor who is homozygous for one of the two distinct alleles carried by the patient are ranked Tier 2, while donors with both alleles are ranked Tier 1. Also included as options are the non-functional *RHD*08N.01* or deleted *RHD*01N.01*, both of which have a D- phenotype and would be ranked Tier 1. Also included are alleles that encode what are considered "benign variants" *RHD*DAU0* and *RHD*Weak D Type 4.0*; these are ranked Tier 3. When *RHD* and *RHCE* tiers are determined for a donor, that donor's tier rank is the higher value. For example, if the donor carries identical *RHD* alleles (Tier 1) but is homozygous for one *RHCE* allele in a patient with two distinct alleles (Tier 2), the overall Tier for that donor would be Tier 2

Table 15 Punnett Square for patient homozygous for *RHD*DIIIa-CE(4-7)-D* and predicted phenotype of D- altered C+

RHD alleles	*RHD*DIIIa-CE(4-7)-D*	*RHD*01N.01*	*RHD*08N.01*
*RHD*DIIIa-CE(4-7)-D*	Tier 1	Tier 1	Tier 1
*RHD*01N.01*		Tier 1	Tier 1
*RHD*08N.01*			Tier 1

This Punnett square includes donors who carry one or two *RHD*DIIIa-CE(4-7)-D* alleles predicted to express an altered C antigen. No donors with conventional *RHD*01* or benign variant alleles such as *RHD*DAU0* or *RHD* alleles encoding partial D antigens are included since these would be D+ and therefore not compatible with the patient. There are fewer options for patients who do not express RhD antigen or who carry a hybrid gene that expresses altered C antigen. Donors that have non-functional *RHD* allele(s) (e.g., *RHD*08N.01*) or have *RHD* gene(s) deleted (*RHD*01N.01*), though they carry different alleles than the patient, are ranked Tier 1 since they do not encode a protein product nor any antigens. Any donors with *RHD* alleles that express normal, weak or partial RhD antigens would be ranked Tier 4.

Table 16 Number of *RH* allele selected donors by *RHD* and *RHCE* allele combinations with Tier assignment based on clinical case example

RH Haplotype		Donors	
Probable *RHD* alleles	Probable *RHCE* alleles	N	Tier
*RHD*01/RHD-CE(4-7)-D*	*RHCE*ce733G/RHCE*ce48C,733G,1006T*	102	1
*RHD*01* (homoz/hemiz)	*RHCE*ce733G/RHCE*ce48C,733G,1006T*	4	2
RHD-CE(4-7)-D (homoz/hemiz)	*RHCE*ce48C,733G,1006T* homozygous	36	2
*RHD*01/RHD-CE(4-7)-D*	*RHCE*ce48C,733G,1006T* homozygous	7	2
*RHD*01* (homoz/hemiz)	*RHCE*ce48C,733G,1006T/RHCE*ce733G*	6	2
RHD-CE(4-7)-D (homoz/hemiz)	*RHCE*ce48C,733G,1006T/RHCE*ce733G*	5	2
*RHD*01* (homoz/hemiz)	*RHCE*ce733G* homozygous	88	2
*RHD*01/RHD-CE(4-7)-D*	*RHCE*ce733G* homozygous	1	2
*RHD*01N.01* homozygous	*RHCE*ce733G* homozygous	1	2
RHD not tested	*RHCE*ce733G* homozygous	357	2
*RHD*01* (homoz/hemiz)	*RHCE*ce48C,733G/RHCE*ce733G*	2	3
*RHD*01* (homoz/hemiz)	*RHCE*ce48C,733G* homozygous	7	3
*RHD*01* (homoz/hemiz)	*RHCE*ce733G/RHCE*ce48C,733G,1006T*	4	3
*RHD*01/RHD-CE(4-7)-D*	*RHCE*ce48C,733G/RHCE*ce48C,733G,1006T*	3	3
*RHD*01/ RHD*DAU0*	*RHCE*ce733G/RHCE*ce48C,733G*	2	3
*RHD*01/ RHD*DAU0*	*RHCE*ce48C,733G* homozygous	1	3
*RHD*01/ RHD*DAU0*	*RHCE*ce733G* homozygous	1	3
RHD not tested	*RHCE*ce48C,733G* homozygous	1	3
RHD not tested	*RHCE*ce48C,733G/RHCE*ce48C,733G,1006T*	12	3
RHD not tested	*RHCE*ce733G/RHCE*ce48C,733G,1006T*	50	3
RHD not tested	*RHCE*ce733G/RHCE*ce48C,733G*	5	3

homoz/hemiz, homozygous or hemizygous.

allele combinations and number of ABO/Rh compatible donors carrying *RH* alleles predicted to have the same or similar phenotype as the patient. This case was initially presented at ISBT in 2019 (62). A frozen RBC unit from a Tier 2 donor was shipped. The unit was stored and was reported to have been thawed at a later date and transfused to the patient without incident.

A Review of ARDP activities involving RH allele selection

A review of ARDP cases from the year 2020 involving *RH* allele selected donor units showed that of the 1,045 requests for rare blood, 126 (12%) requests involving 53 patients were for *RH* allele selected units. Nearly half (26 of 53) of these patients carried the *RHCE*ce733G* allele, with 11 (42%) reported to have anti-hr^B and 5 (19%) allo-anti-e or allo-anti-e-like antibody with 10 (38%) having warm reactive autoantibodies, 3 (12%) having cold reactive autoantibodies and 11 (42%) have multiple alloantibodies. At that time, the ARDP database included 1,979 donors that carried the *RHCE*ce733G* allele with 982 (50%) being homozygous. Of the requests for allele-selected units for patients with an *RHCE*ce733G* allele, 6 (8%) were unable to be filled; these 6 requests required the units to also be negative for C, E, K, S, Fy^a with three requiring Fy(b-), two requiring N- and two requiring Jk(b-) units. This analysis illustrates that the ability to fill *RH* allele selected unit requests is impacted not only by the number of donors with *RH* allele information but also by the presence of multiple alloantibodies in the patient. ABO type compatibility also plays a role in the number of donors whose units could fill such requests (65).

Challenges to RH allele selection: ambiguous phenotypes

A factor that complicates using *RH* allele selected donor units for Rh alloimmunized patients with partial Rh antigens is ambiguous phenotypes. These include the hr^B+^{vw}/- phenotype assigned to red cells carrying the *RHCE*ce733G* allele. This phenotype is confusing to clinicians who need to be able to interpret alloimmunization risk, to manufacturers of genotyping kits that include algorithms to assign predicted phenotypes as well as to reference laboratories asked to provide transfusion recommendations based on molecular reports. Ambiguity also lies in the alloimmunization risk for anti-D in patients carrying the *RHD*DAU0* and *RHD*weak D type 4.0* alleles (52). This is also true of the *RHCE*ce48C* allele found in more than 10% of African American blood donors (66) for which ISBT Working Party on Red Cell Immunogenetics and Blood Group Terminology (1) assigns a phenotype of $e+^{weak}$ yet allo-anti-e has been reported in individuals homozygous for this allele (67). Finally, transfusion medicine professionals are trained to provide antigen negative blood products for alloimmunized patients. However, an *RH* allele selected donor unit may type positive for one or more of the Rh antigens for which a patient may have antibodies, yet the unit would be predicted to be compatible since both the donor and the patient express the same or similar partial antigen(s). Finally, neither hospital computer systems nor blood establishment computer systems are likely equipped to store *RH* allele information such that it can be used by transfusion medicine professionals in the selection of blood products. Due to the many *RHCE* alleles expressing partial e antigens, some with incomplete information regarding clinically relevant Rh antigen phenotype classifications, it would be beneficial for the transfusion medicine community to classify this group of antigens e^{VAR}; this approach would be similar to that taken with U^{VAR} (68) and would allow databases to use this "new" antigen to overcome limitations of the current binary status (e+ or e–) (69).

Discussion/summary

Though there are limitations to our current understanding the genetics of the RH blood group system and the implications of the many variant alleles and resulting antigens on alloimmunization risk, the benefits of using *RH* allele information for clinical care are clear. Multiple studies, many of which were reviewed here, have demonstrated clear benefit to the use of *RHD* genotyping to provide allele information that can be used to assess alloimmunization risk. The value of this methodology is most obvious in women of child-bearing age to determine who would benefit from Rh immune prophylaxis and who does not require it. Also, in patients with variant *RHCE* alleles, selection of RBC units from donors with the same or similar alleles holds great promise to deliver personalized transfusion medicine, especially in patient populations where chronic transfusion is associated with high rates of alloimmunization. Whereas the use of *RH* allele selected donor units is currently limited to patients with Rh alloantibodies, if donor centers can attract and retain donors of African descent and can identify those with variant *RH* alleles lacking high prevalence antigens, there would be the potential to use this approach

prophylactically.

Both in reducing risk of allo-anti-D formation and in identification and selection of compatible blood products for patients who have made antibodies to high prevalence antigens, molecular methods can be instrumental in developing a personalized approach to patient care.

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References

1. Available online: https://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/
2. Cartron JP, Rouillac C, Le Van Kim C, et al. Tentative model for the mapping of D epitopes on the RhD polypeptide. Transfus Clin Biol 1996;3:497-503.
3. Flegel WA, Wagner FF. RHD epitope density profiles of RHD variant red cells analyzed by flow cytometry. Transfus Clin Biol 1996;3:429-31.
4. STRATTON F. A new Rh allelomorph. Nature 1946;158:25.
5. Scientific Committee of the Joint Blood Council & Standards Committee of the American Association of Blood Banks. Standards for a Blood Transfusion Service. Washington, DC: Joint Blood Council Inc., 1958.
6. Tippett P, Sanger R. Observations on subdivisions of the Rh antigen D. Vox Sang 1962;7:9-13.
7. Agre PC, Davies DM, Issitt PD, et al. A proposal to standardize terminology for weak D antigen. Transfusion 1992;32:86-7.
8. Daniels G. Variants of RhD--current testing and clinical consequences. Br J Haematol 2013;161:461-70.
9. Sandler SG, Roseff SD, Domen RE, et al. Policies and procedures related to testing for weak D phenotypes and administration of Rh immune globulin: results and recommendations related to supplemental questions in the Comprehensive Transfusion Medicine survey of the
1. College of American Pathologists. Arch Pathol Lab Med 2014;138:620-5.
10. Sandler SG, Flegel WA, Westhoff CM, et al. It's time to phase in RHD genotyping for patients with a serologic weak D phenotype. College of American Pathologists Transfusion Medicine Resource Committee Work Group. Transfusion 2015;55:680-9.
11. Joint Statement on Phasing-In RHD Genotyping for Pregnant Women and Other Females of Childbearing Potential with a Serologic Weak D Phenotype. AABB Advocacy Statement 2015. Available online: https://www.aabb.org/docs/default-source/default-document-library/positions/statement150722.pdf?sfvrsn=f4f44c30_6
12. Kacker S, Vassallo R, Keller MA, et al. Financial

implications of RHD genotyping of pregnant women with a serologic weak D phenotype. Transfusion 2015;55:2095-103.
13. Sandler SG, Chen LN, Flegel WA. Serological weak D phenotypes: a review and guidance for interpreting the RhD blood type using the RHD genotype. Br J Haematol 2017;179:10-9.
14. Denomme GA, Wagner FF, Fernandes BJ, et al. Partial D, weak D types, and novel RHD alleles among 33,864 multiethnic patients: implications for anti-D alloimmunization and prevention. Transfusion 2005;45:1554-60.
15. Noizat-Pirenne F, Verdier M, Lejealle A, et al. Weak D phenotypes and transfusion safety: where do we stand in daily practice? Transfusion 2007;47:1616-20.
16. Denomme GA, Dake LR, Vilensky D, et al. Rh discrepancies caused by variable reactivity of partial and weak D types with different serologic techniques. Transfusion 2008;48:473-8.
17. Luo X, Keller MA, James I, et al. Strategies to identify candidates for D variant genotyping. Blood Transfus 2018;16:293-301.
18. Horn TN, Keller J, Keller MA, et al. Identifying obstetrics patients in whom RHD genotyping can be used to assess risk of D alloimmunization. Immunohematology 2020;36:146-51.
19. Hudgins JP, Matsushita C, Tuma CW, et al. Identification of RHD allelic variants discovered by atypical typing results on the NEO/Echo platforms. Immunohematology 2021;37:165-70.
20. Keller M, Lindquist M, Smith A, et al. Analysis of RHD alleles in patients in the USA. Vox Sanguinis 2021;116:38.
21. Wang D, Lane C, Quillen K. Prevalence of RhD variants, confirmed by molecular genotyping, in a multiethnic prenatal population. Am J Clin Pathol 2010;134:438-42.
22. Campos FC, Mota MA, Aravechia MG, et al. Variant RHD Types in Brazilians With Discrepancies in RhD Typing. J Clin Lab Anal 2016;30:845-8.
23. Bub CB, Aravechia MG, Costa TH, et al. RHD alleles among pregnant women with serologic discrepant weak D phenotypes from a multiethnic population and risk of alloimmunization. J Clin Lab Anal 2018;32:e22221.
24. Dezan MR, Oliveira VB, Gomes ÇN, et al. High frequency of variant RHD genotypes among donors and patients of mixed origin with serologic weak-D phenotype. J Clin Lab Anal 2018;32:e22596.
25. Oodi A, Daneshvar Z, Goudarzi S, et al. RHD genotyping of serological weak D phenotypes in the Iranian blood donors and patients. Transfus Apher Sci 2020;59:102870.
26. Thongbut J, Laengsri V, Raud L, et al. Nation-wide investigation of RHD variants in Thai blood donors: Impact for molecular diagnostics. Transfusion 2021;61:931-8.
27. Chung YN, Kim TY, Yu H, et al. Molecular basis of serological weak D phenotypes and RhD typing discrepancies identified in the Korean population. Blood Transfus 2021;19:327-34.
28. Bakry RM, Nasreldin E, Hassaballa AE, et al. Evaluation of molecular typing and serological methods in solving discrepant results of weak and partial D (Rh) in South Egypt. Asian J Transfus Sci 2019;13:110-4.
29. Yan L, Wu J, Zhu F, et al. Molecular basis of D variants in Chinese persons. Transfusion 2007;47:471-7.
30. Ouchari M, Srivastava K, Romdhane H, et al. Transfusion strategy for weak D Type 4.0 based on RHD alleles and RH haplotypes in Tunisia. Transfusion 2018;58:306-12.
31. Standard for Blood Banks and Transfusion Services, 32nd Edition, AABB 2020.
32. Wagner FF, Kasulke D, Kerowgan M, et al. Frequencies of the blood groups ABO, Rhesus, D category VI, Kell, and of clinically relevant high-frequency antigens in south-western Germany. Infusionsther Transfusionsmed 1995;22:285-90.
33. Flegel WA, Denomme GA, Queenan JT, et al. It's time to phase out "serologic weak D phenotype" and resolve D types with RHD genotyping including weak D type 4. Transfusion 2020;60:855-9.
34. Tahhan HR, Holbrook CT, Braddy LR, et al. Antigen-matched donor blood in the transfusion management of patients with sickle cell disease. Transfusion 1994;34:562-9.
35. Vichinsky EP, Luban NL, Wright E, et al. Prospective RBC phenotype matching in a stroke-prevention trial in sickle cell anemia: a multicenter transfusion trial. Transfusion 2001;41:1086-92.
36. Castro O, Sandler SG, Houston-Yu P, et al. Predicting the effect of transfusing only phenotype-matched RBCs to patients with sickle cell disease: theoretical and practical implications. Transfusion 2002;42:684-90.
37. Lasalle-Williams M, Nuss R, Le T, et al. Extended red blood cell antigen matching for transfusions in sickle cell disease: a review of a 14-year experience from a single center (CME). Transfusion 2011;51:1732-9.
38. Ribeiro KR, Guarnieri MH, da Costa DC, et al. DNA array analysis for red blood cell antigens facilitates the

transfusion support with antigen-matched blood in patients with sickle cell disease. Vox Sang 2009;97:147-52.
39. Wilkinson K, Harris S, Gaur P, et al. Molecular blood typing augments serologic testing and allows for enhanced matching of red blood cells for transfusion in patients with sickle cell disease. Transfusion 2012;52:381-8.
40. Davis BA, Allard S, Qureshi A, et al. Guidelines on red cell transfusion in sickle cell disease. Part I: principles and laboratory aspects. Br J Haematol 2017;176:179-91.
41. Chou ST, Alsawas M, Fasano RM, et al. American Society of Hematology 2020 guidelines for sickle cell disease: transfusion support. Blood Adv 2020;4:327-55.
42. Klapper E, Zhang Y, Figueroa P, et al. Toward extended phenotype matching: a new operational paradigm for the transfusion service. Transfusion 2010;50:536-46.
43. Castilho L, Dinardo CL. Optimized Antigen-Matched in Sickle Cell Disease Patients: Chances and Challenges in Molecular Times - the Brazilian Way. Transfus Med Hemother 2018;45:258-62.
44. Sesok-Pizzini DA, Friedman DF, Smith-Whitley K, et al. Transfusion support of patients with sickle cell disease at the Children's Hospital of Philadelphia. Immunohematology 2006;22:121-5.
45. Pham BN, Peyrard T, Juszczak G, et al. Heterogeneous molecular background of the weak C, VS+, hr B-, Hr B- phenotype in black persons. Transfusion 2009;49:495-504.
46. Chou ST, Jackson T, Vege S, et al. High prevalence of red blood cell alloimmunization in sickle cell disease despite transfusion from Rh-matched minority donors. Blood 2013;122:1062-71.
47. Dinardo CL, Kelly S, Dezan MR, et al. Diversity of RH and transfusion support in Brazilian sickle cell disease patients with unexplained Rh antibodies. Transfusion 2019;59:3228-35.
48. Faas BH, Beckers EA, Wildoer P, et al. Molecular background of VS and weak C expression in blacks. Transfusion 1997;37:38-44.
49. Nance ST, Keller MA. Comments on: molecular matching of red blood cells is superior to serological matching in sickle cell disease patients. Rev Bras Hematol Hemoter 2013;35:9-11.
50. Fasano RM, Sullivan HC, Bray RA, et al. Genotyping Applications for Transplantation and Transfusion Management: The Emory Experience. Arch Pathol Lab Med 2017;141:329-40.
51. Chou ST, Evans P, Vege S, et al. RH genotype matching for transfusion support in sickle cell disease. Blood 2018;132:1198-207.
52. Floch A, Pirenne F, Barrault A, et al. Insights into anti-D formation in carriers of RhD variants through studies of 3D intraprotein interactions. Transfusion 2021;61:1286-301.
53. Karafin MS, Field JJ, Gottschall JL, et al. Barriers to using molecularly typed minority red blood cell donors in support of chronically transfused adult patients with sickle cell disease. Transfusion 2015;55:1399-406.
54. Crowley JA, Horn T, Keller MA. RHCE Characterization requires both commercial genotyping arrays and lab-developed tests. Transfusion 2013;53:172A.
55. Montemayor C, Brunker PAR, Keller MA. Banking with precision: transfusion medicine as a potential universal application in clinical genomics. Curr Opin Hematol 2019;26:480-7.
56. Meny GM, Flickinger C, Marcucci C. The American Rare Donor Program. J Crit Care 2013;28:110.e9-110.e18.
57. Castilho L, Flickinger C, Horn T, et al. The Importance of molecular testing to Identify and provide compatible donor units for patients with anti-hrB and anti-hrS. Vox Sanguinis 2011;101:269.
58. Keller M, Horn T, Crowley JA, et al. Genotype Compatibility Tables for Matching Patients and Donors for Rh Variants. Transfusion 2013;53:174A.
59. Keller MA, Horn T, Fludd DR, et al. RH Allele Matching for Rh Alloimmunized Patients: Personalized Medicine to the Nth Degree. Transfusion 2019;59:143A.
60. Keller MA, Kavitsky V, Maurer JL, et al. RHCE*ce733G, Alloimmunization and Meeting the Blood Needs of Alloimmunized Patients. Transfusion 2021;61:145A
61. Bhoj V, Blair P, Friedman D, et al. An Anti-hrB-like Antibody in a Sickle Cell Patient with a Variant e Confirmed by Molecular Testing. Transfusion 2013;53:121A.
62. Keller MA, Mansfield P, Maurer J, et al. Identifying compatible blood for Rh alloimmunized patients: An international case. Vox Sanguinis 2019;114:85.
63. Keller MA, Keller J, Thomas D, et al. Genotyping Panel designed to identify blood donors lacking high prevalence Rh antigens. Vox Sanguinis 2018;113:252.
64. Thomas DE, Keller MA. High Throughput RH Genotyping of More than 500 African American Blood Donors. Transfusion 2021;61:132A.
65. Maurer JL, Nance ST, Kavitsky V, et al. High Prevalence Antigen Negative Unit Requests: What If It Includes Being O Negative? Transfusion 2021;60:159A.
66. Keller MA, Crowley J, Horn T, et al. Characterization of RH locus in African American Blood Donors. Transfusion

2013;53:28A.
67. Lapadat R, Meyer E, Josephson C, et al. Exon Sequencing Improves Red Blood Cell Phenotype Matching in a Patient Homozygous for the RHCE*ce48C Allele. 2015. Transfusion 2015;55:152A.
68. Storry JR, Reid ME, Fetics S, et al. Mutations in GYPB exon 5 drive the S–s–U+var phenotype in persons of African descent: implications for transfusion. 2003. Transfusion 2003;43:1738-47.
69. Nance SN, Mansfield P. Use of eVAR and anti-eVAR as interim terms. Immunohematology 2022. (In press). doi: 10.21307/immunohematology-2022-058.

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