Received: 2 November 2021 | Revised: 8 August 2022 | Accepted: 21 August 2022

DOI: 10.1111/trf.17145

BRIEF REPORT

TRANSFUSION

RHD genotyping to resolve weak and discrepant RhD patient phenotypes

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Abstract

Background: We instituted *RHD* genotyping in our transfusion service for obstetrical patients and transfusion candidates. We sought to examine how *RHD* genotyping resolved weak or discrepant automated microplate direct agglutination (MDA) RhD phenotypings and impacted needs for Rh Immune Globulin (RhIG) and D-negative RBCs.

Study Design and Methods: We investigated RhD phenotypes with equivocal or reagent-discrepant automated MDA (Immucor, Norcross, GA), weak-2+ immediate-spin tube typings, historically discrepant RhD typings, or D+ typings with anti-D. We performed microarray *RHD* genotyping (RHD BeadChip, Immucor BioArray Solutions, Warren, NJ). Patients were managed as D+ with weak-D types 1, 2, and 3, and as D-negative with all other results.

Results: Our weak-D prevalence was 0.14%. Among 138 patients (73 obstetrics, 65 transfusion candidates), 38% had weak-D types 1, 2 or 3, 25% weak partial type 4.0, 21% other partial-D variant alleles, and 15% no variant detected. One novel allele with weak partial type 4.0 variants plus c.150T>C (Val50Val) was discovered. Weak D types 1, 2 or 3 were identified in 66% (48/73) of Whites versus 3% (2/62) of diverse ethnic patients ($p < .0001$). *RHD* genotyping changed RhD management in 60 patients (43%) (49 to D+, 11 to D-negative), resulting in net conservation of D-negative RBCs (98 avoided, 14 given) and RhIG (8 avoided, 3 given).

Conclusion: In our patient population, equivocal or reagent-discrepant MDA RhD phenotypes were highly specific for weak-D or partial-D *RHD* genotypes. Resolution of *RHD* genotype status reduced our use of D-negative RBCs and RhIG.

Abbreviations: ?, equivocal reaction in MDA; AGT, antiglobulin test; D4, anti-D Series 4 (Immucor); D5, anti-D Series 5 (Immucor); DNA, deoxyribonucleic acid; DΨ, D-negative pseudogene; IS, immediate spin; MDA, microplate direct agglutination; NVD, no variant detected; OB, obstetrical; RBC, red blood cells; RhIG, Rh Immune Globulin.

KEYWORDS
blood group genomics, immunohematology (RBC serology, blood groups)

1 | INTRODUCTION

In patients with weak or discrepant RhD phenotypes, *RHD* genotyping is recommended in obstetrics to distinguish patients with or without risk of anti-D alloimmunization and determine candidacy for Rh Immune Globulin (RhIG).[1,2] However, in automated RhD typing systems with diverse methods and reagents, there is limited disparate information on how to efficiently define weak D phenotypes needing *RHD* allele determination.[3–9] 9 Furthermore, previously United States and Canadian studies have not prospectively examined the clinical impact of *RHD* genotyping on ensuing needs for D-negative RBC transfusions. We sought to examine these issues after initiating *RHD* genotyping in our transfusion service.

2 | METHODS

2.1 | Patients

We implemented *RHD* genotyping for obstetrical patients and RBC transfusion candidates with weak or discrepant RhD phenotypes at Northwestern Memorial Hospital, an academic medical center in Chicago, Illinois. In this prospective quality improvement project, we excluded patients transfused in the previous 3 months, obscuring serological phenotyping.

2.2 | Rh serological phenotyping

We performed all testing per manufacturer directions. Routine RhD typing was by automated microplate direct agglutination (MDA) employing two monoclonal IgM reagents in Series 4 (D4, clone MS201) and Series 5 (D5, TH28) (Echo Lumena or Galileo NEO, Immucor, Norcross, GA). First-time patients often had confirmatory manual RhD typings (Gamma-clone, Immucor, IgM GAMA401, IgG F8D8).

We identified most patients when MDA gave equivocal reactions or reactive/non-reactive discrepancies between reagents. Equivocal reactions were defined in manufacturer directions as greater than the negative cutoff and less than the positive cutoff, and scaled as <1+ with Echo Lumena and ≤2+ with Galileo NEO.[10,11] Some patients were investigated for weak-2+ tube immediate-spin (IS) confirmatory typings, discrepancies with historical typings in our laboratory or elsewhere, or D+ typing with anti-D. For this project, after qualification for genotyping, we typed specimens by tube method for RhD for comparison, and for RhCcEe where shown (Gamma-clone, Immucor). Specimens negative or equivocal in MDA underwent automated antiglobulin-test (AGT) RhD typing (IgG MS26).

2.3 | *RHD* genotyping

Genomic DNA was isolated from peripheral white blood cells by routine methods (QIAamp, QIAGEN, Inc., Valencia, CA). We employed a DNA-array assay, which identified *RHD* variant alleles and *RHCE*ceHAR* (RHD BeadChip, Immucor BioArray Solutions, Warren, NJ).[12–14] For alleles with two possible *RHD* variant allele calls, the more likely allele was inferred based on race/ethnicity, frequencies, and RhC phenotypes.[15] Zygosity was undetermined unless heterozygous variants were detected. Reference *RHD* analysis (New York Blood Center[16]) was obtained in one case with exon-specific low signals. One case underwent DNA-based RBC phenotyping (HEA BeadChip, Immucor BioArray Solutions, Warren, NJ).

2.4 | Clinical interpretations and outcomes

We treated weak-D-phenotype patients as D-negative pending *RHD* genotyping. Patients with weak D types 1, 2, and 3 were considered D+ for RhIG eligibility and RBC transfusions.[1,2] Patients with partial D genotypes[17] were managed as D-negative. Patients with no variant detected (NVD) were considered D-negative in case of uninterrogated partial-D alleles. For patients with changed RhD management, we reviewed RhIG needs, RBC transfusions, and antibody screens (automated solid phase red cell adherence, Immucor) after transfusion of D+ RBCs.

2.5 | Statistics

Categorical statistical comparisons utilized Fisher's two-tailed exact test for $p < .05$ (GraphPad Software, San

TABLE 1 *RHD* variants and RhD management in 138 patients with weak or discrepant RhD phenotypes

RHD variants	OB	Non-OB	Sum (%)	RhD management changes (%)
Weak D type 1	16	10	26	25
Weak D type 2	0	2	2	2
Weak D type 3	12	12	24	22
Weak D Types 1, 2, and 3	28	24	52 (37.7%)	49 to D+ (35.5%)
Weak partial D type 4.0[a] or 4.3:	20	14	34 (24.6%)	
Type 4.0 or 4.3	12	13	25	3
Type 4.0 or 4.3/DΨ	4	1	5	
Type 4.0 or 4.3/DIIIa or DIIIa-CE(4-7)-D[a] (C+)	4	0	4	
Other partial D:	15	14	29 (21.0%)	
DAR	7	6	13	1
DAR/DΨ	3	2	5	
DOL1 or DOL2	4	2	6	1
DAU4[a] or DV type 5 (C−)	1	0	1	1
DNB	0	1	1	1
DIIIa	0	1	1	1
DIIIa[a] or DIIIa-CE(4-7)-D/Type 4.0[a] or 4.3 (C−)	0	1	1	
DIVa[b] (C−)	0	1	1	
No variant detected (NVD)	8	13	21 (15.2%)	3
No *RHD*	2[c]	0	2 (1.4%)	
All other than Types 1, 2, and 3	45	41	86 (62.3%)	11 to D− (8.0%)
Totals	73 (53%)	65 (47%)	138	60 (43.48%)

Note: RhD management changes: *RHD* genotyping changed RhIG eligibility or RhD type of RBC transfusions. DΨ: D-negative pseudogene. Non-OB: transfusion candidates. OB: obstetrics.

[a]More likely variant (Section 2.3); RhC typings shown.

[b]RHD-BeadChip possible alternative DIVa/DIIIa-CE(4-7)-D was ruled out because RBCs were C-negative and HEA BeadChip genotyping performed because of anti-C predicted VS-negative phenotype.

[c]Possible *RHCE* variants (Section 3.5).

Diego, CA). Percentages may not sum to 100% due to rounding.

3 | RESULTS

3.1 | Weak-D-phenotype patients

In 2.5 years (2019–2021) we identified 138 patients with weak or discrepant RhD phenotypes included here. We had 73 obstetrical cases (53%): 35 women during pregnancy or fertility planning, 36 women at delivery or end of pregnancy, and two fathers (one during his partner's pregnancy and one after a weak-D-phenotype newborn); and 65 patients (47%) with current or potential transfusion need the following: 25 undergoing surgery, 27 with cancer, and 13 with other medical conditions. Our prevalence of serological weak D phenotypes was 0.14% of all type-and-screen specimens.

3.2 | *RHD* variants

Fifty-two patients (38%) had weak D types 1, 2 or 3; 34 (25%) had weak D types 4.0 or 4.3; 29 (21%) had other partial D variant alleles; 21 (15%) had NVD; and 2 cases (1%) had no *RHD* or *RHCE*ceHAR* detected (Table 1).

3.3 | Race and ethnicity

Our patients' race and ethnicity are shown in Table 2. Weak D types 1, 2, and 3 were identified in 48/73 (66%) Whites compared to 2/62 (3%) ethnically diverse patients ($p < .0001$). In our obstetrical service, an unrelated study provided recent overall race/ethnicity distributions[18] for comparison with our weak-D-phenotype obstetrical patients. Blacks comprised 9% (1050/11,617) of our overall obstetrical population and 41% (29/71) of our weak-D-phenotype obstetrical cohort. Using these data,

TABLE 2 Race/ethnicity and *RHD* variants

Race/ethnicity	Weak D Type 1	Weak D Type 2	Weak D Type 3	Weak partial D Type 4.0	Partial D DAR	Partial D DOL	Partial D DAU4	Partial D DNB	Partial D DIIIa	Partial D DIVa	NVD	No *RHD*	All
White	25	2	21	8				1			14	2	73
Black				23	15	4	1		1	1	3		48
Hispanic			2	2	3	2			1		2		12
Asian											1		1
Native American											1		1
Unknown	1		1	1									3
Totals	26	2	24	34	18	6	1	1	2	1	21	2	138

Abbreviation: NVD, no variant detected.

the estimated weak-D prevalence in our Black obstetrical patients was 0.64% compared to 0.09%–0.11% in Hispanics and Whites.

3.4 | RhD serological phenotypes

MDA reactions were equivocal ($n = 101$) or reagent-discrepant ($n = 7$) in 108/137 cases (79%) (Table S1). Direct agglutination was negative or equivocal in 62% (85/137) of D4 and 91% (125/137) of D5 typings. Eighteen specimens (13%) nonreactive by MDA were investigated because of reactive IS reactions in confirmatory typings ($n = 9$) or discrepant historical typings ($n = 9$). Eleven cases (8%) with ≥1+ MDA reactions were investigated because of weak-2+ IS reactions ($n = 6$) or anti-D ($n = 5$). One case had 1+ IS typing but MDA typings were unavailable.

Among 108 equivocal or reagent-discrepant RhD typings in MDA, 91 cases had an *RHD* variant identified or no *RHD* detected (84% specificity). Specimens with only negative or equivocal reactions in MDA were more likely to have weak D types 1, 2, or 3 (50/84, 60%) than those with stronger reactions (2/53, 4%, $p < .0001$). In tube IS typings, 8% (4/49) of types 1, 2, and 3 specimens reacted 3–4+, compared to 35% (12/34) of weak partial type 4.0 cases and 56% (15/27) of other partial D specimens. In specimens with only negative or equivocal D4/D5 reactions, 64/65 specimens typed in automated AGT (98%) were reactive.

3.5 | Selected cases

A White obstetrical patient's RBC typings were 3+ by D4, equivocal by D5, 2+ by tube IS, and Dce in phenotyping. The DNA assay reported weak D type 4.0 or 4.3 but displayed "low-signal" for exon 2 analytes. Reference testing detected the *RHD*-deletion hybrid box, and exon-2 sequencing revealed the changes in *RHD*weak D type 4.0* plus silent c.150T>C (p.Val50Val). c.150T>C does not affect splicing,[19] but may have prevented BeadChip exon-2 primer binding as with *RHD*DAR6*.[20] A weak partial D type 4.0 variant allele plus c.150C is not in current *RHD* allele rosters.[15,17,21]

Two specimens with no *RHD* or *RHCE*ceHAR* detected by RHD BeadChip reacted 3–4+ with D4, negative with D5, and IS- and AGT-negative in Gamma-clone tube typing (Crawford-negative).[22] *RHCE* variants carrying clone-dependent D-like antigenicity were suspected.

3.6 | Patients with plasma anti-D antibody reactivity

Six patients had past or current anti-D with DOL1 or DOL2 (2), DNB, DIIIa, DIVa, and weak D type 1. Four were medical or gynecological patients. Two were evaluated at pregnancy. One patient with normal RhD phenotype and anti-D (IgG titer 1) had DOL1 or DOL2. Her newborn had normal IS D+ typing and negative anti-IgG direct antiglobulin test (DAT) (not genotyped). The other obstetrical patient had a history of anti-D with no recent RhIG in a past infertility evaluation. We identified her weak D phenotype at a subsequent miscarriage when genotyping revealed weak D type 1. We recommended future RhIG coverage as a precaution, but based on reported experience she likely had auto-anti-D.[2]

3.7 | RhD management

Fifty-one patients with types 1, 2, and 3 (37%) were managed as D+ and 87 were managed as D-negative (63%).

RHD genotyping changed RhD management in 60 cases (43.48%) from D-negative to D+ ($n = 49$, 35.5%) or D+ to D-negative ($n = 11$, 8.0%). Eight patients switched to D+ management and subsequently received 98 D+ RBC units. Six had follow-up antibody screening for 1–15 months. None developed anti-D. Eight women avoided RhIG at delivery or subsequent pregnancies. Among those who switched to D-negative management, 3 received 14 D-negative RBCs, and 3 received RhIG.

We cautiously managed our obstetrical and sporadically transfused patients with weak partial D type 4.0 in this cohort as D-negative, since anti-D has occurred many times (including with a positive monocyte monolayer assay).[23,24] However, if necessary we would issue D+ RBCs to type 4.0 patients with difficult antibodies or high transfusion needs.[2,25]

4 | CONCLUSION

We successfully implemented *RHD* genotyping in our transfusion service to resolve the clinical management of patients with serological weak-D phenotypes. Equivocal or discrepant IgM anti-D typing reactions in automated MDA tests were highly specific for weak and partial D genotypes. Notably, 56% (15/27) of our patients with partial D variants had 3–4+ IS tube typings and would have been missed in some genotyping decision protocols.[4,7,8] *RHD* genotyping results changed RhIG or RBC transfusion management in 43% of our cohort. A high proportion of ethnically diverse patients and over one-third of White patients with weak or discrepant D phenotypes had *RHD* genotypes warranting D-negative management. However, our post-genotyping net ratio of D-negative RBCs spared to D-negative RBCs needed was 98:14.

ACKNOWLEDGMENTS

We thank the staff of Northwestern Memorial Hospital Blood Bank for their dedication and the Laboratory of Immunohematology and Genomics, New York Blood Center, for performing reference *RHD* genomic testing.

CONFLICT OF INTEREST

Glenn Ramsey has received lecture honoraria from Immucor, Inc.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Barriteau CM, Lindholm PF, Hartman K, Sumugod RD, Ramsey G. *RHD* genotyping to resolve weak and discrepant RhD patient phenotypes. Transfusion. 2022;62(11):2194–9. https://doi.org/10.1111/trf.17145