

TRANSFUSION MEDICINE

RH genotype matching for transfusion support in sickle cell disease

Stella T. Chou,¹ Perry Evans,² Sunitha Vege,³ Sarita L. Coleman,¹ David F. Friedman,^{1,4} Margaret Keller,⁵ and Connie M. Westhoff³¹Department of Pediatrics and ²Department of Biomedical and Health Informatics, The Children's Hospital of Philadelphia, Philadelphia, PA; ³Laboratory of Immunohematology and Genomics, New York Blood Center, New York, NY; ⁴Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia, Philadelphia, PA; and ⁵National Molecular Laboratory at the American Red Cross, Philadelphia, PA

KEY POINTS

- **RH genotyping of red cells may improve matching of patients and donors and reduce Rh alloimmunization.**
- **RH genotype matching may improve use of an African American blood donor inventory.**

Rh alloimmunization remains a challenge for patients with sickle cell disease (SCD) despite transfusion of serologic Rh C, E, and K antigen-matched red cells. Inheritance of altered RH alleles contributes to the prevalence of Rh antibodies after blood transfusion in patients with SCD and explains approximately one-third of cases. The remainder seem to be stimulated by altered Rh proteins on African American donor red cells. Matching patients with donors on the basis of RH genotype may mitigate Rh alloimmunization, but the feasibility and resources required are not known. We compared RH allele frequencies between patients with SCD (n = 857) and African American donors (n = 587) and showed that RH allele frequencies are similar. Overall, 29% of RHD and 53% of RHCE alleles are altered in patients and African American donors. We modeled RH genotype matching compared with serologic Rh D, C, and E, along with K antigen matching, and found that approximately twice the number of African American donors would be required for RH genotype vs Rh serologic matching at

our institution. We demonstrated that African American donor recruitment is necessary to maintain an adequate supply of C-, E-, and K-negative donor units to avoid depleting the Rh-negative (RhD⁻) blood supply. Our results suggest that prophylactic RH genetic matching for patients with SCD is feasible with a donor pool comprised primarily of African-Americans and would optimize the use of our existing minority donor inventory. The current cost of RH genotyping all minority donors and management of the data remain limiting factors. (Blood. 2018;132(11):1198-1207)

Introduction

There are 5 common Rh antigens (D, C, c, E, e), but the Rh blood group system is more complex, with more than 50 antigens defined at the serologic level encoded by 2 genes, *RHD* and *RHCE*. *RHD* and *RHCE* are a duplicated gene family with a high level of sequence homology. The close proximity of *RHD* and *RHCE* and chromosomal orientation has resulted in genetic exchange causing single or multiple nucleotide changes or hybrid alleles from gene conversions as well as accumulation of novel single amino acid changes. The degree of *RH* genetic diversity differs depending on ethnic group, with increased diversity in African populations, and hence patients with sickle cell disease (SCD) compared with Europeans who represent the majority of blood donors.¹⁻³ Approximately 85% of African American patients with SCD carry at least 1 *RH* allele that differs from that commonly found in white blood donors.¹ Many variant alleles result in partial Rh antigen expression in which some epitopes are lacking, which leads an individual to recognize the conventional antigen as foreign. We previously reported that antibodies directed against the Rh system remain the most common specificities among patients with SCD despite providing D, C, E, and K antigen-matched red cells from primarily

African American donors.¹ One-third of these antibodies were associated with symptomatic delayed hemolytic transfusion reactions or laboratory evidence of decreased transfused red cell survival (delayed transfusion reactions) and pose challenges for subsequent transfusion.

Transfusion with serologic C, E, and K (CEK) antigen-matched donors is recommended for patients with SCD to minimize alloimmunization.⁴ Recruitment of African American donors is important to identify an adequate supply of CEK-negative units.^{3,5} *RH* genotype matching, which considers specific *RH* gene polymorphisms in addition to the serologic phenotype of donors and patients, may reduce or prevent Rh sensitization. The feasibility, including the number and specific *RH* genotypes of donors needed to serve a comprehensive sickle cell center, has not been determined. Although *RH* genotyping all minority donors remains cost prohibitive, new technologies may provide an alternative method that would be comprehensive and cost-effective.^{6,7} The aims of this study were to determine the *RH* diversity in African American donors compared with patients with SCD and to determine whether prophylactic *RH* genetic matching of donors and patients would be feasible from an inventory supply perspective.

Patients and methods

Study samples

Blood samples were obtained from 857 patients with SCD (558 SS, 207 SC, 86 S β^0 /S β^+ thalassemia, 6 S-variant) under an institutional review board–approved protocol and from 587 donors who self-identified as black or African American. Most patients self-identified as African American; 8 patients self-identified as Hispanic. Additional transfusion history is provided in the supplemental Data (available on the Blood Web site).

Red blood cell extended phenotype and genotype

Genomic DNA was isolated from white blood cells by routine methods (QIAamp; QIAGEN, Valencia, CA) from patients and donors and genotyped with RHD and RHCE BeadChip arrays (Bioarray, Warren, NJ) and polymerase chain reaction (PCR) restriction fragment length polymorphism (PCR-RFLP) assays as described previously.^{1,8} Briefly, the RH DNA arrays target 35 RHD and 25 RHCE single nucleotide variations or insertions, described in detail previously.⁷ PCR-RFLP was performed for RHD exon 8 (c.1136C>T, *DAU), RHCE exon 2 (c.254C>G, RHCE*ceAG), and exon 4 (c.577A>G, RHCE*R^N). RHCE exon 2 and RHD exons 2 and 7 were amplified and Sanger sequenced to distinguish RHCE*ceLOC and RHD*VII and to classify RHD*DAU alleles. RHD zygosity was determined by amplification of a 1507-bp fragment from the region associated with RHD deletion.⁹ RHD and RHCE were assigned on the basis of single or combinations of genetic markers, and allelic or haplotype associations were imputed on the basis of Rh complementary DNA cloning and sequencing and allele frequency.^{1,2} Extended blood group antigen profiles (CcEe, K, Fy, Jk, and Ss) were predicted from DNA-based methods and by serology for all patients and donors.

Donor-patient matching virtual simulations

A custom program was created to perform virtual simulations to identify RH genotype and K-matched donor units with and without extended red cell matching for Fya/Fyb, Jka/Jkb, and S/s antigens, from either African American or white donor pools. The African American virtual inventory was established with 200 initial donor units, and 10 to 300 donors were added each weekday at random from our 587 RH genotyped and extended-antigen-profiled African American donors. An inventory of whites was based on published antigen and RH allele frequencies.¹⁰ A donor unit was available for 21 days from collection, consistent with our institutional practice for patients with hemoglobinopathies. Units were issued based on oldest outdate first, but units with multiple antigen-negative, less common antigen combinations, or infrequent RH genotypes were reserved. An RH genotype match was defined as a genotype that did not encode an Rh protein potentially foreign to the patient. For example, the patient and donor were identical for both RHD and RHCE, or the donor was homozygous for one of the RH haplotypes or alleles found in the patient. A less restrictive genetic match considered RHD*DAU0 and RHCE*ce48C to be equivalent to conventional RHD (RHD*01) and RHCE*ce, because proteins encoded by these alleles have not been shown to lack epitopes, and the clinical significance of antibodies made by patients with these alleles is not clear. Matching simulations were performed using actual transfusion needs for patients with SCD at our institution over 4 years (2013-2016). The outcome measured was percent patient unit demand met per year. Five simulations per variable were performed, and the average was reported.

Results

RHD and RHCE variation in African American blood donors is similar to that of patients with SCD

RH genotypes in the patient cohort were compared with donor genotypes (Table 1). Overall, 30% of RHD and 54% of RHCE alleles in patients were altered. Among African American donors, 25% of RHD and 49% of RHCE alleles were altered. Donors had a similar allele distribution compared with patients with the exception of 3 alleles. RHD*weak partial D 4.0 and RHCE*ce48C,733G were more frequent in patients, and conventional RHD was more frequent in donors ($P < .05$; Fisher's exact test). Two alleles common in Africans that encode single amino acid changes, RHD*DAU0 and RHCE*ce48C, were found in 17% and 19% of patients and 14% and 19% of donors, respectively. Variant alleles found primarily in whites, including weak D type 40, weak D type 1, and RHCE*CeCW were observed in 4 different donors and likely reflect population admixture.

Among patients with SCD, 4.6% ($n = 39$) were D[−]. Among D⁺ patients, 6.2% (51 of 818) express partial D antigens, which are lacking some epitopes, and patients are at risk for immunization against conventional RhD protein if transfused with D⁺ units. An additional 14.7% ($n = 120$) have altered RhD protein encoded by RHD*DAU0, either exclusively or with an allele associated with partial D antigen (Figure 1A), and the risk for D sensitization is not clear. Twenty-one percent of C⁺ patients have partial C antigen (50 of 242) and are at risk for anti-C if transfused with conventional C⁺ units as a result of inheriting the hybrid RHD*DIIIa-CE(4-7)-D ($n = 45$), RHCE*CeRN ($n = 4$), or both alleles as compound heterozygotes ($n = 1$). Partial c and e antigens were expressed on red cells in 19.6% c⁺ (166 of 845) and 18.3% of e⁺ (155 of 847) individuals. An additional 20.7% c⁺ ($n = 175$) and 18.2% e⁺ ($n = 154$) have altered Rhce protein encoded by RHCE*ce48C alone or along with a partial c or e, respectively (Figure 1), and the risk for clinically significant anti-e or anti-c is unclear. E variants were less common among E⁺ individuals (3 [1.9%] of 158).

Rh antibodies

In this cohort of patients with SCD, 64% percent (550 of 857) had received at least 1 transfusion of red cells in their lifetime (median age, 12.5 years), and 24.7% ($n = 212$) had been chronically transfused. Despite transfusion of serologic DCEK-matched units from primarily African American donors, 175 anti-Rh specificities were identified in 105 individuals: 43 anti-D, 52 anti-C, 27 anti-E, 30 anti-e, 6 anti-Go^a, 4 anti-V, 3 anti-VS, 3 anti-hr^B, 1 anti-Hr^B, 2 anti-RH32, 1 anti-f, and 3 anti-C^w (Figure 1B; Table 2). Twenty-two of these Rh antibodies (22 [12.6%] of 175) resulted from transfusion of antigen-positive donor units to antigen-negative patients. This included 4 anti-E after transfusion of E⁺ red blood cells to E[−] individuals because they had made anti-e, and 18 antibodies, including anti-Go^a, anti-V, anti-VS, anti-RH32, and anti-C^w as a result of exposure to donor units with these known low-prevalence Rh antigens.

Six anti-D, 5 anti-C, 1 anti-ce (anti-f), and 12 anti-e were detected in patients who typed positive for the antigen but whose RH genotype predicted expression of partial D, C, and e antigens, respectively (Figure 1B; Table 2). Anti-D was also identified in 4 patients with altered D antigen: 2 patients homozygous for RHD*DAU0 and in 2 patients heterozygous for RHD*DAU0 and a partial RHD (RHD*DAU5 or RHD*weak partial D 4.0). Anti-e was found in 9 patients with altered e antigen: 3 patients homozygous for RHCE*ce48C,

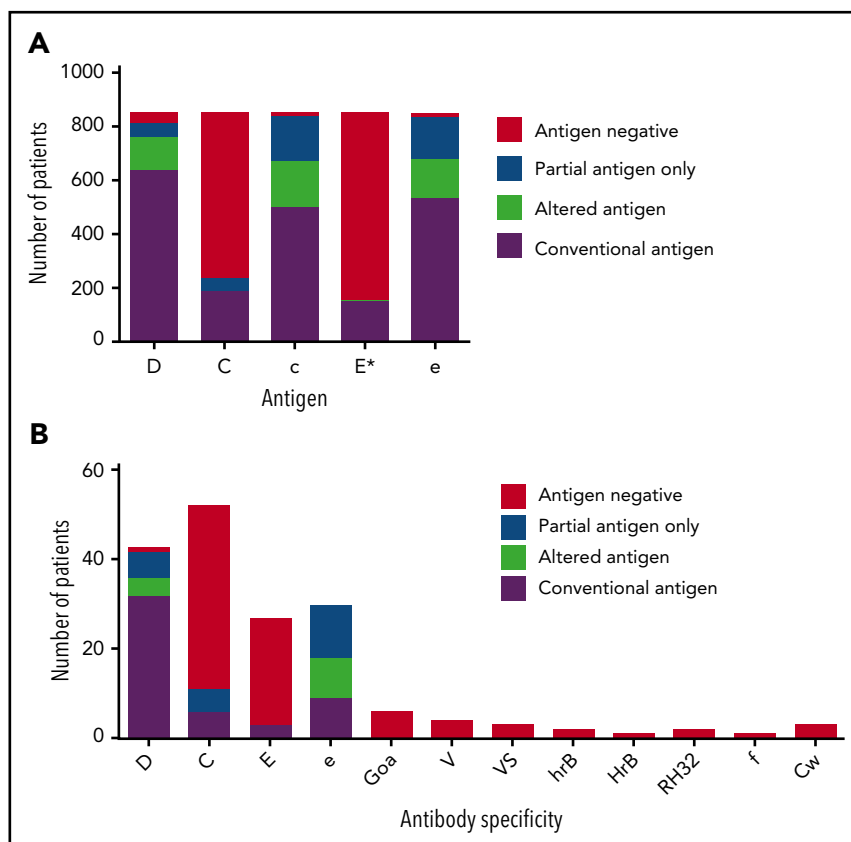


Figure 1. Rh antigen expression predicted by genotype. (A) Rh antigen expression predicted from *RHD* and *RHCE* genotyping that distinguishes individuals whose red cells express variant vs conventional antigens or are antigen negative ($n = 857$ patients). Altered antigen assigned to patients with *RHD**DAU0 or *RHCE**ce48C and no conventional allele in trans. *Three patients expressed variant E antigen only (1.9% E⁺ patients). (B) Predicted Rh antigen expression in immunized individuals with Rh specificities, despite transfusion with Rh matched (D, C, E) red cells from African American donors ($n = 175$ specificities). There were no cases of anti-c detected.

1 with *RHCE**ce48C/*cE, and in 5 heterozygous for *RHCE**ce48C and *RHCE* encoding partial c and e antigens (2 *RHCE**ceS and 1 each *RHCE**ce254G, *RHCE**ce733G, and *RHCE**ceHAR). In addition, 4 anti-hr^B/Hr^B were identified in patients with variant *RHCE* associated with loss of these high-prevalence antigens. Overall, 41 antibodies (41 [23%] of 175) could be explained by homozygous inheritance of partial or altered *RH* alleles. However, 32 anti-D, 6 anti-C, 3 anti-E, and 9 anti-e were present in patients who typed positive for the antigen and had at least 1 corresponding conventional *RHD* or *RHCE* allele (Table 2). Among these, 18% (32 of 175) of the Rh antibodies occurred in patients who had only the corresponding conventional alleles. Finally, 62 antibodies (62 [35%] of 175) were identified in antigen-negative patients who had been transfused with red cells determined by serology to be negative for that antigen and included 1 anti-D, 41 anti-C, and 20 anti-E (Figure 1B; Table 2).

An adequate red cell supply for patients with SCD relies on African American donors

Rh and K phenotype-matched red cells are recommended for patients with SCD who lack C, E, or K antigens,⁴ but maintaining an adequate supply of units lacking these antigens can be challenging for blood centers.^{3,5,11} Comparing the frequency of DCEK antigen phenotypes among the 857 patients and 587 African American donors showed nearly 100% concordance (coefficient of determination, $r^2 = 1$; Figure 2A). In contrast, DCEK antigen phenotype frequency is clearly distinct from that of whites ($r^2 = 0.093$) as expected. The D⁺C⁻E⁻ (R₀R₀ or R₀R) and K⁻ phenotype comprised 53% and 52% of patients with SCD and African American donors, respectively, but less than 3% of whites. The D⁺C⁺E⁻ (R₁R or R₁R₁) and K⁻ phenotype was found in 21.5% of patients as well as African American donors compared with 49.1% of whites. The D⁺C⁻E⁺ (R₂R

or R₂R₂) and K⁻ phenotypes represented 16.1% of patients with SCD, 15.2% of African American donors, and 12.6% of whites.

Provision of extended matched red cells for Fy^a, Fy^b, Jk^a, Jk^b, S, and s antigens in addition to DCEK matching can minimize alloimmunization,^{12,13} but is most often reserved for alloimmunized patients because of cost and lack of availability of extended matched donors. Extended red cell antigen profiles that include Fy^a/Fy^b, Jk^a/Jk^b, and S/s antigen status demonstrated similar frequencies in patients with SCD and African American donors ($r^2 = 0.95$; Figure 2B) but an even wider disparity between patients and whites ($r^2 = 0.023$) than was observed for DCEK. The GATA site mutation in *FY* that results in a lack of Fy^b expression on red cells but not in other tissues was identified in the majority of patients who type Fy(b⁻) (98.9%), and thus these patients are not at risk for anti-Fy^b, and antigen-matched units do not need to be Fy(b⁻). As expected, the most common extended phenotype needed for patients was C⁻, E⁻, K⁻, Fy^a-, Jk^b-, and S-negative (20%), present in 17% of African American donors, but found in <1% of whites.^{10,14}

Matching simulations based on cohort need

Consideration of patient and donor *RH* genotypes may reduce Rh immunization but an estimation of the size of the donor pool required is unknown. To determine whether provision of *RH* genotype matched red cells is possible, we examined the transfusion requirements for patients with SCD at our institution for 4 consecutive years (2013-2016; supplemental Table 1). The average number of units transfused per year was 7045. The number of units issued per weekday ranged from 0 to 79 and averaged 27 units (Figure 3A). Overall, the median number of units per transfusion visit was 4 units (range, 1 to 11 units), but the median was 1 unit for

Table 1. RHD and RHCE frequency in patients (n = 857) and African American donors (n = 587)

RHD frequency				RHCE frequency			
RHD* gene	Patients	African American donors	P	RHCE* gene	Patients	African American donors	P
Deleted D	0.1289	0.1167	.3577	ce conventional	0.2456	0.2726	.1086
RHD ψ	0.0292	0.0383	.2023	ce48C	0.1931	0.1925	1.0
DIIIa-CE(4-7)-D	0.0309	0.0256	.4288	ce733G	0.1324	0.1431	.4403
RHD conventional	0.5449	0.5980	.0053	ce48C,733G	0.0718	0.0273	<.0001
DAU0	0.1651	0.1414	.0848	ce254G	0.0461	0.0426	.7140
Weak partial D 4.0	0.0298	0.0094	.0001	ceS	0.0420	0.0332	.2385
DIVa	0.0181	0.0136	.3736	ceTI	0.0233	0.0187	.4352
DAU3	0.0158	0.0213	.3187	ceMO	0.0093	0.0145	.2153
DIIIa	0.0140	0.0094	.3019	ceCF	0.0035	0.0009	.2524
DAU5	0.0128	0.0077	.2031	ceEK	0.0035	0.0034	1.0
DAR	0.0035	0.0060	.3999	ceAR	0.0023	0.0034	.7226
DOL	0.0018	0.0034	.4523	ceJAL	0.0018	0.0026	.6922
DAU4	0.0012	0.0034	.2316	ceTI type 2	0.0012	0.0009	1.0
DIVa-3	0.0006	0	1.0	ceBI	0.0012	0.0034	.2316
DFR	0.0006	0	1.0	ce733G,1006T	0.0012	0	.5168
DWN	0.0006	0	1.0	ceHAR	0.0006	0	1.0
D(48C)	0.0006	0	1.0	ce48C,254G,733G	0.0006	0	1.0
D(835A)	0.0006	0	1.0	ce254G,733G	0.0006	0	1.0
RHD ψ -like	0.0006	0	1.0	Ce conventional	0.1190	0.1363	.1712
D(667G,800T)	0.0006	0.0009	1.0	CeRN	0.0029	0.0017	.7079
DAU6	0	0.0017	.1652	Ce733G	0.0006	0	1.0
D-CE(3-7)-D	0	0.0017	.1652	CeCW	0	0.0017	.1652
Weak D type 40	0	0.0009	.4065	cE conventional	0.0928	0.1005	.5201
Weak D type 1	0	0.0009	.4065	cE48C	0.0029	0.0009	.4111
				cEIV	0.0018	0	.2560

Bold indicates comparison of allele frequency between patients and donors with $P < .05$ by Fisher's exact test.

RHD*01, conventional RHD.

those episodically transfused (range, 1 to 10 units). Virtual donor-patient matching was performed to identify units by antigen-negative profile and RH genotype. Donor pools were established by using our observed RH allele and antigen frequencies in African American donors (Table 1), and published frequencies for whites¹⁰ (Figure 3B). Donations occurred Monday to Friday, and a unit was available for 21 days from collection to provide units <21 days old. Simulations were performed starting with 10 incoming random blood units (African American only or white) and up to 300 donor units added to the available inventory each weekday. Older units were issued first. Modeling was performed for 4 different matching strategies: serologic DCEK matched, serologic

DCEK with extended antigens (Jk^a, Jk^b, Fy^a, Fy^b, S, and s), RH genotype and K matched, and RH genotype, K, and extended antigen-matched. Unit requests were filled according to actual number of units that would have been required in real time for every patient with SCD transfused from 2013 to 2016 at our institution.

Serologic DCEK and DCEK with extended antigen-matching

We first determined efficiency of serologic matching for D, C, E, and K antigens as recommended for patients with SCD, whereby those lacking the antigen received antigen-negative donor units.

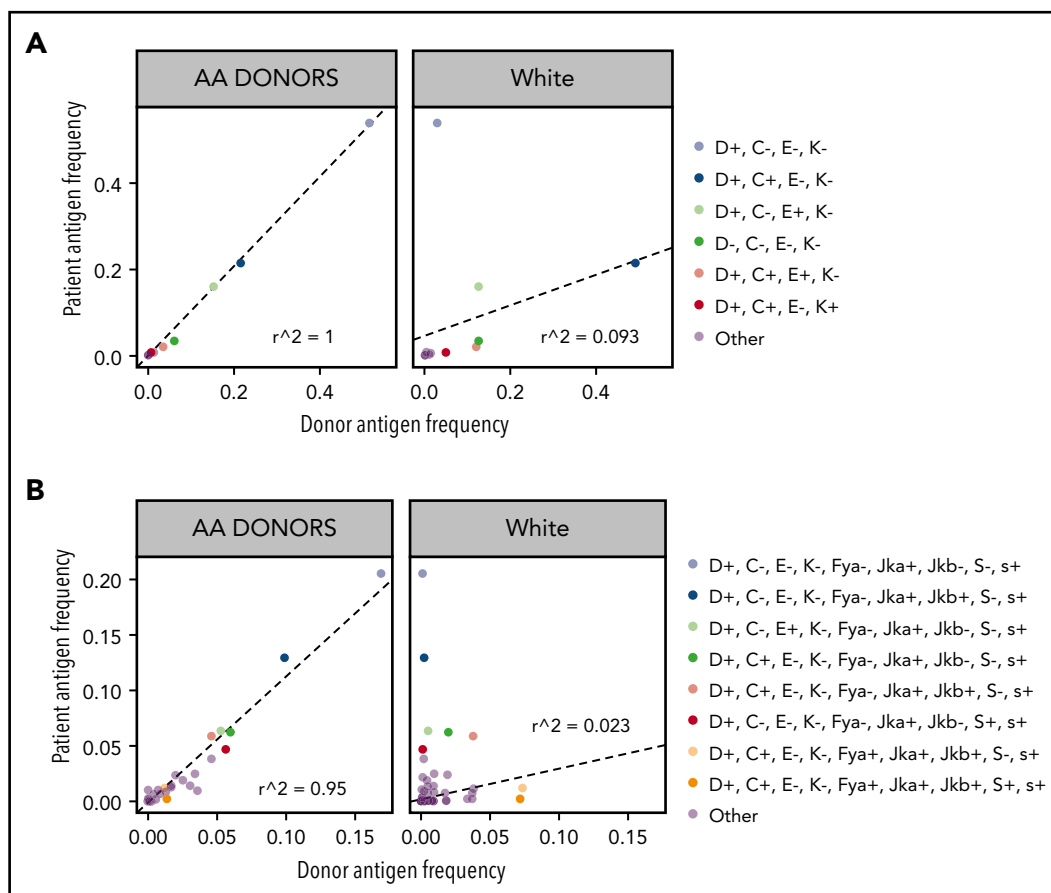


Figure 2. Red blood cell antigen phenotypes in patients with SCD compared with African Americans and whites. (A) Frequency of D, C, E, K, and (B) extended antigen phenotypes of patients with SCD ($n = 857$) compared with African American donors ($n = 587$) or published frequencies for whites. Phenotypes with a frequency of less than 0.05 in all populations are shown in light gray. The r^2 value denotes the coefficient of determination.

To meet 100% of actual patient demand for serologic DCEK-matched units in our cohort (average 27 units per day, range 0-79 units per day), 40 African American or 125 white donors must

enter the donor pool each weekday (Figure 3C, top). However, the 125 white donors require use of D⁻ units for serologic DCEK antigen-matching for D⁺ patients because ~98% of D⁻ units

Table 2. Summary of Rh antigen and *RHD* and *RHCE* allele status in patients transfused with units antigen-matched for CEK but who present with 175 Rh antibodies

Antigen and allele status	Anti-D	Anti-C	Anti-E	Anti-f (anti-ce)	Anti-e	Anti-hr ^B , anti-Hr ^B	Anti-V, anti-VS, anti-Go ^a , anti-Rh32, anti-C ^w	Total Abs
Ag ⁻ received Ag ⁺	0	0	4	0	0	0	18	22
Ag ⁺ with known partial alleles	6	5	0	1	12	4	0	28
Ag ⁺ with altered alleles	4				9			13
Ag ⁺ with 1 conventional and 1 partial or altered allele	11	1	0	0	6	0	0	18
Ag ⁺ with only the conventional allele	21	5	3	0	3	0	0	32
Ag ⁻ receiving Ag ⁻ units	1	41	20	0	0	0	0	62
Total Rh antibodies	43	52	27	1	30	4	18	175

Abs, antibodies; Ag, antigen.

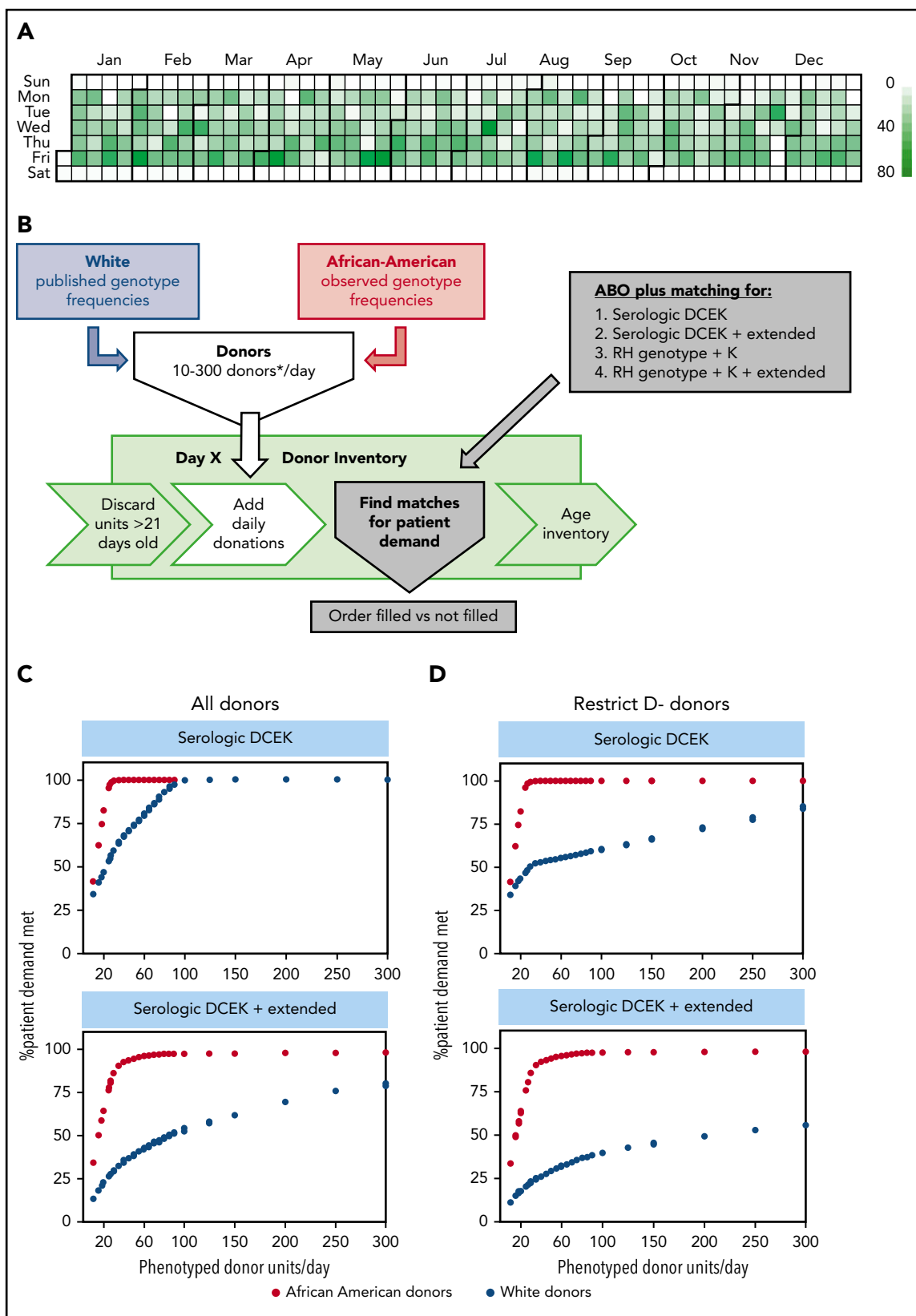


Figure 3. Feasibility of serologic red blood cell matching with African American vs white donors. (A) Actual red cell units issued per day to patients with SCD in a single year at 1 institution. (B) Donor matching simulations with percent of annual patient demand met when using (C) all donors or (D) restricting D⁻ donors to D⁻ patients only. African American or white donor pools were used to match patients with D⁻, C⁻, E⁻, and K-matched units at the resolution of serologic typing with (bottom) or without (top) extended matching for Fy^a, Fy^b, Jk^a, Jk^b, S, and s. Patients negative for an antigen were provided antigen-negative red cells. Antigen-positive patients were issued antigen-positive or antigen-negative red cell units. Data shown are representative from 1 calendar year.

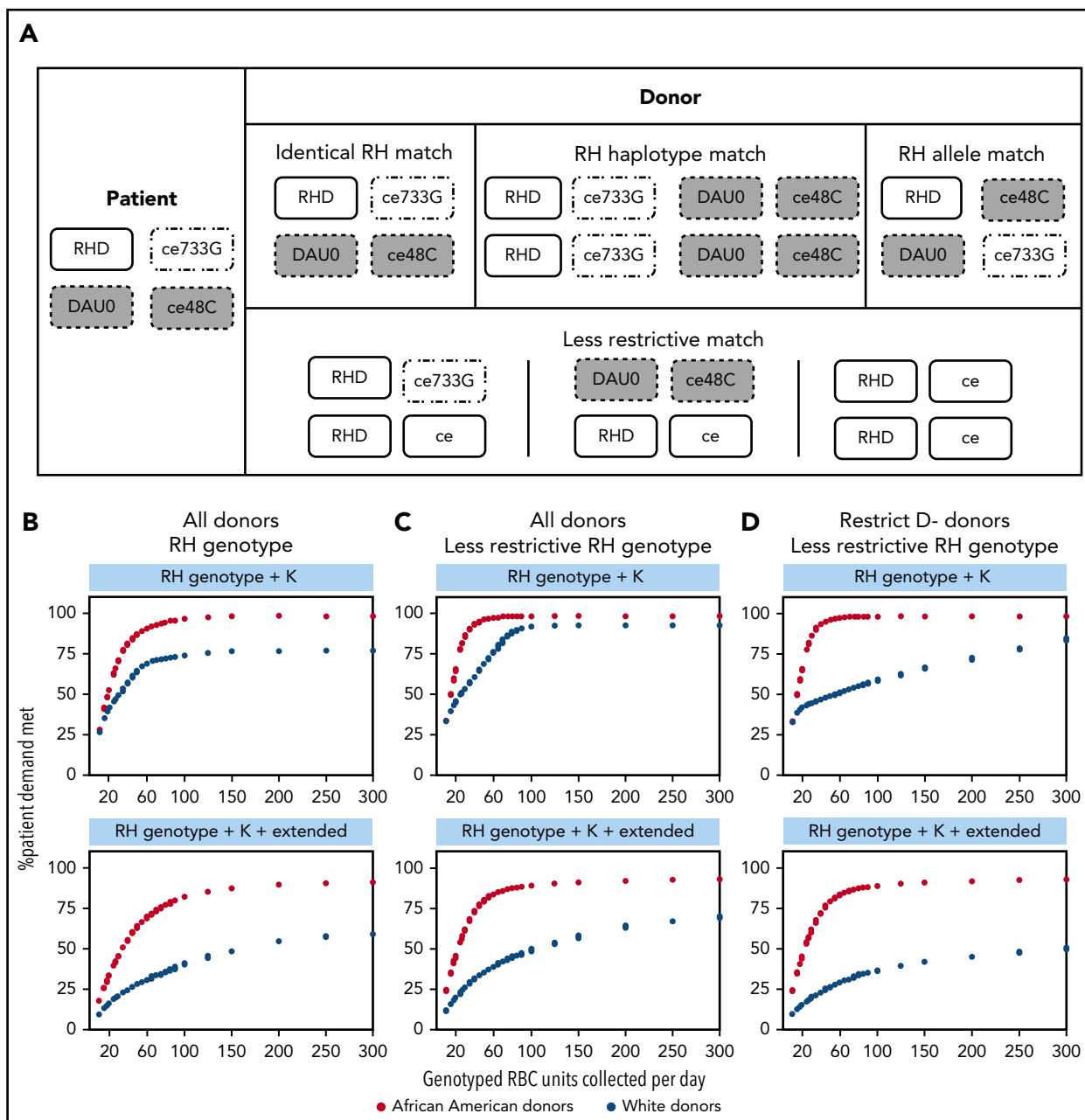


Figure 4. Feasibility of red blood cell matching by RH genotype and K with African American vs white donors. (A) Patient-donor RH matching strategies by identical RH genotype, homozygous RH haplotype, or RH allele match. (B) Donor matching simulations with percent of annual patient demand met when using all donors and RH genotype matching criteria, (C) all donors and a less restrictive RH genotype matching criteria, or (D) restricting D⁻ donors to D⁻ patients only with less restrictive RH genotype matching criteria. African American or white donor pools were used to match patients by ABO, K, and RH genotype-matched units with (bottom) or without (top) extended matching for Fy^a, Fy^b, Jk^a, Jk^b, S, and s. Outside the Rh system, patients negative for an antigen were provided antigen-negative red cells, and antigen-positive patients were issued antigen-positive or antigen-negative red cells. Data shown are representative from 1 calendar year.

from white donors are negative for CEK, but less than 3% of D⁺ whites are negative for CEK. Restricting D⁻ donor units for only D⁻ patients substantially increases the difficulty in finding CEK antigen-matched in the white donor pool but does not have an impact on matching using the African American donor pool (Figure 3D, top, compare with Figure 3C, top). For serologic DCEK and extended antigen-matching (Jk^a, Jk^b, Fy^a, Fy^b, S, and s), 95% of patient need was met with 55 African American donors per day, whereas with 300 white donors per day, ~80% of patient need would be met (Figure 3C, bottom), but again,

would need to rely on D⁻ units from the white donor pool (Figure 3C [bottom] and D).

RH genotype, K, and extended matching

We next examined the feasibility of antigen matching with consideration of patient and donor RH genotypes and K status. Among patients, there were 98 different RHD/RHCE allele combinations. Patients were assigned units from donors with RH genotypes that predicted no foreign Rh protein exposure to the patient. For example, a patient whose genotype was compound

heterozygous for *RHD***RHD*/*RHD***DAU0* and *RHCE***ce733G*/*RHCE***ce48C* was eligible for donor units that had an identical *RH* genotype, homozygous for 1 *RH* haplotype, or an *RH* allele match (Figure 4A). *RH* genotype and K matching reached 95% of patient demand met once 85 African American donors were available per day (Figure 4B, top) and plateaued at 98% demand met with 150 African American donors per day. Recruiting >150 African American donors each day or providing units up to 42 days postcollection (not shown) did not improve the ability to meet demand because several individuals had uncommon or rare *RH* genotypes. *RH* genotype and K matching with white donors reached a maximum of 76% demand met with 150 donors per day and would rely heavily on D⁻ donors (Figure 4B, top).

RHDDAU0* and *RHCE***ce48C* as equivalent to conventional alleles**

Less restrictive *RH* matching criteria were then used in which *RHD***DAU0* and *RHCE***ce48C* alleles were considered equivalent to conventional alleles. These 2 alleles are very frequent in this population (Table 1) and have not been shown to lack Rh epitopes. This strategy decreased the number of *RH* genotyped African American donors required for the matching strategy, with greater than 95% patient demand met with as few as 50 donors per day but plateaued at a maximum of 98% of blood requests filled (Figure 4C, top). The same approach improved matching with white donors. A maximum of 92% of patient demand was met with 125 white donors per day (Figure 4C, top). However, as expected, avoiding use of D⁻ white donations (Figure 4D, top), only 62% of red cell demand was met with 125 donors per day, and increasing recruitment to 300 donors per day found only 83% of demand met. *RH* genotype, K, and extended antigen–matching would be challenging, particularly with white donors (Figure 4B–D, bottom) but may be feasible for select patients.

Unmet needs

Two patients had no genotype matches as a result of homozygosity for uncommon *RHCE***ce* alleles: 1 individual who required chronic transfusions (165 units per year) was homozygous for *RHCE***ceTI*, and 1 individual with *RHCE***ceTI*/*RHCE***ceEK* who required 3 units in 1 year.

Discussion

We report *RH* genotypes of 1444 patients with SCD and healthy African American blood donors. *RH* allele diversity and frequencies in our patient cohort (*n* = 857) are comparable to those present in African American blood donors (*n* = 587) and, as expected, differ from those in white donors.¹⁰ Overall, 29% of *RHD* and 53% of *RHCE* alleles are altered, and allele frequencies do not differ between our patient population and African American donors. Among 857 patients, 287 (33.5%) have only altered *RH* alleles, and 570 (66.5%) have at least 1 conventional allele, among whom 140 (16%) were homozygous for conventional *RH* alleles and not predicted to be at risk for Rh antibodies.

Despite serologic Rh C-, E-, and K-matched units selected primarily from African American donors, 175 antibodies with Rh specificities were identified in 105 of 550 transfused patients in our cohort (19%; Table 2). Not all antibodies were associated with inheritance of altered *RH* alleles; 18% were found in

patients with the corresponding conventional alleles and 35% were identified in patients who were C- or E-negative and had received donor units typed serologically as negative (Table 2). These observations suggest that Rh antibodies are not only a result of inheriting altered *RH* alleles but may also be a result of altered Rh epitopes on African American donor red cells. A patient exposed to donor red cells with variant Rh antigens may recognize these as foreign and form an alloantibody. These are often assumed to be autoantibodies because the patient red cells type as positive for that same antigen, but are in fact alloantibodies. Rh epitopes are conformational dependent and are not always straightforward. Rh mimicking or cross-reactive epitopes have been known for more than 30 years.¹⁵ These include expression of C-like epitopes on RhD, D-like epitopes on Rhce,^{16,17} and E-like epitopes on RhCe proteins.¹⁸ The many anti-C and anti-E observed in antigen-negative patients transfused with antigen-negative donor units are likely the result of mimicking epitopes, because unmatched transfusion outside our institution was monitored. Our data suggest that more than 50% of Rh antibodies in our cohort may result from D-, C-, and E-like cross-reactive epitopes stimulated by African American donor cells.

Providing genetically matched red cells at the *RH* loci may potentially mitigate Rh alloimmunization and improve use of our African American donor inventory while avoiding overuse of D⁻ units. Although overall red cell use has declined significantly in the United States, the proportion of D⁻ units distributed has increased by 9.3%.¹⁹ We modeled actual transfusion requirements (6107 to 7588 units per year) for our patients over a 4-year period to determine whether *RH* genotype matching was feasible within our existing African American donor collections. We found that serologic CEK matching requires 40 African American donations each weekday to support an average 27 units transfused per day at our institution, which serves ~100 patients requiring chronic transfusion, and to avoid use of the D⁻ blood supply. Rh matching based on *RH* genotype and K status was possible for 95% of patient needs if twice as many (ie, 85) African American donors were collected each weekday. Importantly, less restrictive genetic matching, which considers *RHD***DAU0* and *RHCE***ce48C* to be equivalent to conventional alleles, required only 10 additional donations (~50 donors) each weekday than serologic CEK matching. Additional studies are needed to determine which *RH* alleles, including *RHD***DAU0* and *RHCE***ce48C*, are associated with alloimmunization in practice. Extended phenotype matching would require 55 African American donors each day to support our program, and *RH* genotype combined with extended phenotype would not be feasible without a significant increase in African American donations.

Our results confirm that serologic CEK matching, *RH* genetic matching, and extended antigen–matching all require a donor pool comprised primarily of African Americans to avoid depletion of D⁻ donor resources. Although a single-center study suggested that a primarily white donor inventory could provide CEK-matched units for the majority of patients with SCD, or extended antigen–matched products for some, it remained challenging for individuals that required a higher number of units for red cell exchange.⁵ We demonstrate that a white donor base results in a heavy reliance on D⁻ units to provide CEK-matched red cells, with or without extended matching. This is consistent with efforts by donor centers to recruit African Americans to

maintain a sufficient inventory of C-, E-, and K-negative red cells for patients with SCD. One study reported that one-third of 1637 units transfused to D⁺ patients with SCD over 3 years were D⁻ (primarily white donors), despite the availability of D⁺C⁻E⁻K⁻ units in the donor center inventory from large numbers of African American donors (>6000 over 3 years).¹⁴ The authors provide potential explanations, including possible limited daily availability of required D⁺ antigen-matched donor units, or the relative ease by which D⁻ units can be confirmed CEK-negative at the hospital compared with getting D⁺ units shipped from their supplier.

RH genotype matching may also improve use of African American donations. Among this large cohort of African American patients, 6% of D⁺ individuals had partial D, 21% of C⁺ patients had partial C, and 21% were homozygous for altered Rhce antigens such that all are at risk for alloantibody production if prophylactic Rh antigen-matching is based on serologic testing. To prevent Rh alloimmunization, these patients could be better matched with donors by RH genotype. Twenty-one percent of patients with SCD had an R₁ (DCE) or R₁r (DCE/ce) D⁺C⁺E⁻K⁻ phenotype and could potentially receive units from the 21.5% of R₁ or R₁r African American donors. Another 16.1% of patients had R₂R₂ (DcE/DcE) or R₂r (DcE/ce) D⁺C⁺E⁻K⁻ phenotype and could receive R₂R₂ or R₂r units from the 15.2% of African American donors with these same RH alleles. This strategy would relieve the demand on R₀R₀ (Dce) D⁺C⁻E⁻K⁻ units from the African American donor pool and potentially improve inventory management. Currently, D⁺ patients with anti-D receive D⁻ units, but RH genotype matched D⁺ units for these patients would lessen the demand on the D⁻ blood supply. Over a 10-year period, 17.4% of units transfused to our D⁺ patients with SCD were type O/D⁻, primarily because of a history of anti-D immunization. Similarly, C⁺ patients who have made anti-C as a result of inheriting partial C antigen could be matched with C⁺ donors with the same RH genotype and conserve C⁻ units.

Red cell genotyping for extended antigens has increasingly been integrated into donor centers²⁰ and has the potential to increase the practice of antigen-matching of patients with donors for more than ABO and RhD. RH genotyping can inform patient care by providing insight to determine whether Rh antibodies are allo- or autoantibodies, to predict clinical significance, and to aid transfusion decisions. RH genotype-matched red cells is one potential strategy to reduce alloimmunization and improve red cell use. Our study suggests that prophylactic RH genotype matching may be feasible from an inventory perspective in our blood centers,

which collect ~1500 African American donors each month. African American donor recruitment can be challenging, but strategies used by some blood centers have been successful.^{21,22} The cost of RH genotyping is currently prohibitive for matching, but with improved sequencing approaches, we anticipate cost will not be a longstanding challenge. Strategies to improve African American donor recruitment (or tailored for Hispanic or Arabic patients), more cost-effective and comprehensive genotyping technologies, and a data storage and shared information system would be necessary for effective implementation.

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Authorship

Contribution: S.T.C., M.K., and C.M.W. designed the study, analyzed results, and wrote the manuscript; P.E., S.V., and D.F.F. conducted research, analyzed results, and edited the manuscript; and S.L.C. obtained informed consent and maintained all clinical data in a research database.

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ORCID profile: P.E., 0000-0001-9391-2452.

Correspondence: Stella T. Chou, The Children's Hospital of Philadelphia, 3615 Civic Center Blvd, Abramson Research Center, Room 316D, Philadelphia, PA 19104; e-mail: chous@email.chop.edu.

Footnotes

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