Review Article

Impact of *RHCE* variability and complexity in transfusion medicine: a narrative review

Emilia Sippert[1#], Carine Prisco Arnoni[2#], Maria Rios[1]

[1]Office of Blood Research and Review (OBRR), Center for Biologics Evaluation and Research (CBER), U.S. Food and Drug Administration, Silver Spring, MD, USA; [2]Technical Scientific Department, Colsan-Associação Beneficente de Coleta de Sangue, Indianópolis, São Paulo, SP, Brazil

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[#]These authors contributed equally to this work.

Correspondence to: Dr. Emilia Sippert. Office of Blood Research and Review (OBRR), Center for Biologics Evaluation and Research (CBER), U.S. Food and Drug Administration, 10903 New Hampshire Ave, Silver Spring, MD 20993, USA. Email: Emilia.Sippert@fda.hhs.gov.

Background and Objective: Rh is one of the most clinically important blood group systems. It comprises five major antigens (D, C, c, E, and e) in addition to several low and high prevalence antigens. RhD and RhCE proteins are encoded by two adjacent genes namely *RHD* and *RHCE*. These genes are highly homologous and polymorphic resulting in numerous variant alleles that encode variant antigens. In this review, we will discuss *RHCE* genetic diversity, frequency of *RHCE* variant alleles and their clinical aspects, complexities, and the importance of *RH* genotyping to prevent alloimmunization.

Methods: A search restricted to English language was performed using the PubMed electronic database to retrieve publications until June 2021, on *RHCE* variability and complexity. The Medical Subject Headings and free terms used were Rh-Hr Blood-Group System/genetics, sickle cell disease (SCD), *RHCE*, *RHCE* variants, and Rh antibodies. Other relevant articles were found by checking the reference list of the articles collected in the initial search.

Key Content and Findings: Common RhCE antigens are highly immunogenic, and their alloantibodies have been involved in delayed hemolytic transfusion reactions (DHTRs) and hemolytic disease of the fetus and newborn (HDFN). Because the distribution of C/c and E/e is different among ethnic groups, several treatment centers adopt prophylactic CE matching protocols to prevent alloimmunization in chronically transfused patients. Despite measures, alloantibody formation against RhCE antigens is still a problem due to numerous variant phenotypes, mainly in individuals of African origin. Elucidation of molecular basis for *RHCE* variants has allowed to determine the frequency of these alleles in patients and donors of African descent, however, the clinical significance of the Rh alloantibodies elicited by most *RHCE* variants is still unclear.

Conclusions: Molecular assays allowed considerable progress in the identification of genetic basis and characterization of *RHCE* variant alleles in patients with SCD and blood donors. Advances in molecular techniques may enable the screening of rare RhCE donors and improve the support for patients carrying variant RhCE phenotype. However, some questions remain to be answered, mainly regarding the clinical significance of the *RHCE* variants.

Keywords: Rh blood group system; *RHCE* variants; Rh antibody; RhCE protein; sickle cell disease (SCD)

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Introduction

Rh is the second most clinically important of all blood group systems after ABO. It encompasses 56 antigens including five major antigens (D, C, c, E, and e) in addition to low and high prevalence antigens identified by their corresponding antibodies (1,2). *RHD* and *RHCE* genes, which encode Rh antigens, are highly homologous and polymorphic with more than 700 *RHD* and 200 *RHCE* alleles reported to date (1,3-5). Many described *RH* alleles have been identified in individuals of African origin, and it is estimated that 87% of patients with sickle cell disease (SCD) and African descent blood donors carry at least one variant *RH* allele (6). This diversity creates clinical challenges and causes significant rates of Rh alloimmunization (7).

Rh antibodies are the most common antibodies identified in transfused patients, and despite the serological matching for D, C, and E antigens and racially matched blood transfusions (6), Rh alloimmunization persists due to variant Rh antigens present either in patients or blood donors (6-8). Importantly, some variant *RH* alleles have been associated with development of clinically significant alloantibodies causing delayed hemolytic transfusion reactions (DHTRs) or hemolytic disease of the fetus and newborn (HDFN) (6,7,9-11). Ideally, patients carrying Rh variants with an antibody to a high-prevalence antigen or with multiple common antibodies need compatible red blood cell (RBC) units. However, providing such compatible units is often a challenge because *RH* genotyping, required to identify the Rh variants, is expensive and is not a routine method; consequently, partial antigens are usually recognized once alloantibodies have already been formed or when a transfusion reaction has occurred (12). In this review, we will discuss the molecular basis for altered RhCE phenotypes, frequency of variant *RHCE* alleles, clinical significance of alloantibodies, SCD and *RHCE* complexities, and the importance of *RH* genotyping to prevent alloimmunization. We present the following article in accordance with the Narrative Review checklist (available at https://aob.amegroups.com/article/view/10.21037/aob-21-76/rc).

Methods

The literature search strategy summary and the detailed search strategy used in PubMed database to retrieve publications on *RHCE* variability and complexity are shown in *Table 1* and Table S1, respectively. In addition, the *RHCE* allele frequency was collected from the Erythrogene database in the 1000 Genomes dataset (13) and the nucleotide changes confirmed by checking the Blood Group Terminology Table for *RHCE* at the International Society of Blood Transfusion (ISBT) website (1).

Overview on Rh blood group system

The Rh blood group system encompasses two highly homologous genes, *RHD* and *RHCE*, closely located on the short arm of chromosome 1 (1p36.11), which encode RhD and RhCE proteins differing in only 32–35 of 417 amino acids (14-17). These genes are inherent together as a haplotype, each composed of ten exons, and have more than 90% sequence similarity (18). The *RHD* gene encodes the RhD protein, carrying the D antigen (RH1) while the *RHCE* gene encodes the RhCE protein, carrying C (RH2) or c (RH4) and E (RH3) or e (RH5) antigens. The RhD and RhCE proteins are integral to the red cell membrane and form a complex with the RhAG protein, a chaperone required for Rh antigens expression, encoded by *RHAG* gene on chromosome 6. The absence of RhAG protein causes lack of RhD and RhCE protein expression leading to a rare Rh_{null} phenotype, named regulator Rh_{null}. Individuals with this phenotype can readily form alloantibodies on exposure of Rh antigens (3,15,19).

RhCE antigens

The four main *RHCE* alleles encode the Ce, CE, ce, and cE antigen combinations (3) and changes to the *RHCE* gene can alter their antigen expression and/or generate new antigens (20). The C and c antigens specificity are determined by 4 non-synonymous substitution, c.48G>C (p.Trp16Cys), c.178C>A (p.Leu60Ile), c.203G>A (p.Asn68Ser), and c.307T>C (p.Pro103Ser); and 2 synonymous substitution c.150C>T and c.201A>G (1,21). Among these changes, only the p.Pro103Ser substitution, predicted to reside on the second extracellular loop of the RhCE protein, is associated with the C/c immunogenicity (15,21). As amino acids encoded by exon 2 of the *RHCe* allele are identical to those encoded by exon 2 of the *RHD* gene (22), next-generation sequencing data strongly support that a hybrid allele *RHCE*CE-D(2)-CE* is causal for the C+ antigen expression (22,23). The molecular basis for E and e specificities are determined by the nucleotide change c.676G>C in exon 5, resulting in the amino acid substitution, p.Ala226Pro, located on the fourth extracellular loop of the RhCE protein (2).

Table 1 Search strategy summary

Items	Specification
Date of search	July 01, 2021
Databases and other sources searched	PubMed and reference lists of articles identified in the search
MeSH and free terms used	Rh-Hr Blood-Group System/genetics (MeSH), sickle cell disease (MeSH), RHCE, RHCE variants, Rh antibodies
Timeframe	The limit of database until June 30, 2021
Inclusion and exclusion criteria	Articles restricted to English language. No restrictions on publication type
Selection process	Independent literature search was performed by the authors (ES and CPA) using MeSH and free terms separately and in combination. Titles/abstracts of retrieved articles were checked for relevance and selected for further review if addressed *RHCE* genetics, frequency of *RHCE* variant alleles, clinical significance of variant *RHCE* alleles, impact of variant RhCE on patients with SCD, or molecular characterization of variant *RHCE* alleles. Additional relevant papers were identified by manual searching of reference lists of articles identified in the initial search

SCD, sickle cell disease.

Molecular basis of *RHCE* variants

Molecular mechanisms responsible for altered or null Rh phenotypes have been revealed through molecular typing of patients and donors from different ethnic backgrounds and over 200 *RHCE* alleles have been described to date (1,4,24-28). The genetic diversity of the *RHCE* gene is generated by at least four molecular mechanisms: (I) single nucleotide variations (SNVs), (II) insertions, (III) deletions, and (IV) gene rearrangements (gene conversion), that may cause weaken and/or partial expression of C, c, E, and e, induce expression of low-prevalence antigens, and/or loss of expression of high-frequency antigens. Furthermore, some genetic alterations in the *RHCE* may result in a non-functional RhCE protein (e.g., D– –, DC^{w}–, Dc– and D••). The inheritance of non-functional *RHCE* alleles in conjunction with deleted *RHD* results in the lack of any Rh proteins on the red cells membrane giving rise to the amorph Rh$_{null}$ phenotype (2,15).

SNV is the main molecular mechanism responsible for RhCE protein alterations. SNVs are often associated with weak RhCE antigen expression when located in the coding regions inducing an amino acid change in the transmembrane or intracellular regions (29). In contrast, an amino acid change in extracellular regions or a change in a transmembrane or intracellular region causing conformational alterations, can alter epitopes and produce partial phenotypes (e.g., partial "e" and "c" due to c. 733C>G) which are prone to immunization when exposed to the normal antigens. Because antigen expression is not always reduced in partial Rh phenotypes and serological methods cannot distinguish *RH* variants, partial antigens are usually recognized after alloantibodies against missing epitopes are formed or when a transfusion reaction has occurred (9). *RHCE* variants may also cause both weak and partial antigen expression; for example, the *RHCE*ceAG* allele is associated with a weak and partial "e" due to an amino acid substitution (p.Ala85Gly) caused by the SNV c.254C>G in exon 2 (30). In addition, SNVs can also produce stop codons that prematurely terminate protein synthesis, generating null phenotypes [e.g., "E–" and "c–" due to c.221G>A (p.Trp74Ter)] (28).

Single amino acid substitutions in the RhCE protein also can generate new epitopes. These new epitopes are called "low prevalence antigens", occur in less than 1% of the population and are not routinely typed for, but are potently immunogenic. Examples include C^{w} (31), C^{x} (31), Crawford (32), E^{w} (33), JAL (34-36), V and VS (37-39). However, in individuals of African origin V and VS antigen are reasonably common (37). Both of them are associated with the SNV c.733C>G (p.Leu245Val) predicted to be located in the eight transmembrane segment of Rhce protein which causes a conformation change within the ce polypeptide leading to partial c and partial e antigens (38,39). The subsequent loss of V expression results from the c.1006G>T (p.Gly336Cys) change on this background arising the V–VS+ phenotype (39).

Insertions and deletions are less frequent and generally result in a frameshift and a premature stop codon. For

instance, the *RHCE*ceN.01* allele associated with deletion of five nucleotides at positions 80-84 (c.80_84delTCTTC) introduce a frameshift after p.Leu26 (CTC) causing a premature stop codon. As a result, Rhce antigen expression is completely abolished from the RBCs (40).

Gene rearrangement between *RHD* and *RHCE* is common and associated with the formation of hybrid alleles that is favored by the proximity, homology and tail-to-tail orientation of RH genes (41). For example, the most frequent mechanism associated with partial C in individuals of African origin are the hybrid *RHCE*Ce-D(4)-ce (RHCE*CeRN)* identified in R^N individuals, and the *RHD*DIIIa-CEVS(4-7)-D* and *RHD*D-CEVS(4-7)-D* alleles that are part of (C)ce^S haplotype 1 and type 2, respectively (9,39,42-45).

*RHCE*CeRN* encodes weak and partial expression of C and e antigens, absence of the high-prevalence Sec antigen, and expression of low-prevalence R^N and DAK antigens. In addition, R^N erythrocytes slightly overexpress the D antigen (46,47). The R^N haplotype has been described in people of African origin and found to be responsible for partial C antigen expression in 11.8% of C+ patients with SCD in France (48), although in other countries its frequency is lower or absent (12,49,50).

*RHD*DIIIa-CEVS(4-7)-D* and *RHD*D-CEVS(4-7)-D* alleles are linked to *RHCE*ceVS.03* (ce48C, 733G, 1006T) composing (C)ce^S haplotypes, which do not encode the D antigen, instead, they encode partial C, c and e antigens, and loss of highly prevalent hr^B and Hr^B antigens (9,39,43,51). The partial C encoded by (C)ce^S haplotype 1 may have variable expression and, in many cases, go undetected until alloimmunization occurs (38,39,42). In Caucasians, weak C and weak e have been associated with diverse molecular events, for example, *RHCE*CeMA* allele result from the SNV c.340C>T in exon 3 which also cause expression of the low-prevalence Rh antigen, JAL (34,36,52) while *RHCE*CeVA* result from the hybrid *RHCE-D(5)-CE* allele (53).

Overall, altered forms of e and/or c antigens have been associated with numerous *RHCE*ce* variant alleles in people of African origin (*Table 2*). Importantly, individuals with some homozygous *RHCE*ce* variant alleles may also have a loss of high prevalence antigens on the red cells increasing the alloimmunization risk, for instance, hr^S– (RH–18) in *RHCE*ceAR*, *RHCE*ceEK*, *RHCE*ceBI*, *RHCE*ceMO* and *RHCE*ceSM* carriers; hr^B– (RH–31) in *RHCE*ceAG*, *RHCE*ceMO*, *RHCE*ceS*, *RHCE*ceCF*, and *RHCE*ceVS.02.01* carriers (30,55,56,62). These variants should be well-characterized for transfusion purposes because their carriers are at risk of alloimmunization. Moreover, finding compatible blood for patients carrying these variants in both alleles can be a challenge since the molecular background of each variant is distinct and they are also often inherited along with *RHD* variant alleles, consequently, rare antigen-negative RBCs will be required for transfusion (37).

E antigen variants are rare and associated with diverse molecular mechanisms encoding weak or partial E antigen and have been mostly identified in Caucasians (29,63,64). One of the most important alleles reported in association with E variants is *RHCE*cEEW* previously reported as E Variant I. This allele is characterized by the amino acid substitution p.Met167Lys (c.500T>A) located at the third extracellular loop of the RhcE protein leading to an E+ partial, weak or negative phenotype (64). In addition, the p.Met167Lys substitution is also the molecular basis for the rare E^w (RH11) antigen (<0.1% in Caucasians) first described in 1955 (65) and associated with few cases of HDFN (33,63,65,66).

RHCE variant alleles can be inherited in combination with specific *RHD* variant alleles creating an additional degree of complexity and a challenge for transfusion. Some combinations are much higher than expected to occur by chance, indicating linkage of *RHD* alleles encoding partial D with specific altered *RHCE*ce*; for example, *RHCE*ceAR* and *RHCE*ceEK* are often in linkage to *RHD*DAR* (54,55), *RHCE*ce*^{S} is linked to *RHD*DIIIa* (61), *RHCE*ceTI* is linked to *RHD*DIVa-2* (25), and *RHCE*ceMO* is often found with *RHD*DAU0* (56). Frequent *RH* alleles presenting linkage are listed in *Table 2*.

Frequency of *RHCE* variants

RHCE variants are more frequent in African descendants and people with mixed ancestry than in Caucasians and Asians (45,49,50,67). Studies on the diversity and frequency of *RH* alleles in blood donors, and/or patients with SCD who are at high risk of alloimmunization have been conducted using both in-house and commercial genotyping assays (45,49,50,60,67-76). The reported frequencies of *RHCE* variant alleles are inconsistent among reports likely due to several reasons, including differences in study design, molecular strategies used for *RHCE* characterization, and population ethnicity. Additional bias includes lack of consensus on the clinical significance of *RHCE* variants resulting from c.48G>C and c.733C>G [*RHCE*ce.01* (ce48C), *RHCE*ceVS.01* (ce733G), and *RHCE*ceVS.02*

Table 2 Summary of the most frequent variant *RHCE* alleles

RHCE allele [common name (1)]	*RHCE* allele [ISBT name (1)]	References	Nucleotide change	Predicted phenotype			Often linked to *RHD* allele	Population frequency[†]
				HFA	LFA	PA		
*RHCE*ceTI*	*RHCE*01.02.01*	(25)	c.48G>C, c.1025C>T			e[‡], c[‡]	*RHD*DIVa-2*	Africa: 2.27%, America: 0.43%, East Asia: 0%, Europe: 0%
*RHCE*ceAR*	*RHCE*01.04.01*	(54)	c.48G>C, c.712A>G, c.733C>G, c.787A>G, c.800T>A, c.916A>G	Hr–, hr^{S}–	V+[‡], VS–	e[‡], c	*RHD*DAR*	NR
*RHCE*ceEK*	*RHCE*01.05.01*	(55)	c.48G>C, c.712A>G, c.787A>G, c.800T>A	Hr–, hr^{S}–		e[‡], c	*RHD*DAR*	NR
*RHCE*ceAG*	*RHCE*01.06.01*	(30)	c.254C>G	hr^{B}–, CEAG–		e[‡]	*RHD deletion*	Africa: 5.60%, America: 0.72%, East Asia: 0%, Europe: 0%
*RHCE*ceMO*	*RHCE*01.07.01*	(56)	c.48G>C, c.667G>T	hr^{S}–, hr^{B}–, CEVF–		e[‡], c	*RHD*DAU0*	Africa: 1.44%, America: 0.43%, East Asia: 0.20%, Europe: 0.10%
*RHCE*ceBI*	*RHCE*01.08*	(57-59)	c.48G>C, c.712A>G, c.818C>T, c.1132C>G	Hr–, hr^{S}–	STEM+	e[‡]	*RHD*DOL1* or *RHD*DOL2*	Africa: 0.08%, America: 0%, East Asia: 0%, Europe: 0%
*RHCE*ceSM*	*RHCE*01.09*	(57)	c.48G>C, c.712A>G, c.818C>T	Hr–, hr^{S}–	STEM+[‡]		*RHD*DOL1* or *RHD*DOL2*	NR
*RHCE*ceVS.01*	*RHCE*01.20.01*	(38)	c.733C>G	hr^{B}+[‡]	V+, VS+	e, c		Africa: 15.28%, America: 2.31%, East Asia: 0%, Europe: 0.30%
*RHCE*ceVS.02.01*	*RHCE*01.20.02.01*	(38,60)	c.48G>C, c.733C>G	hrB–	V+, VS+	e, c	*RHD*DAR3*	Africa: 2.87%, America: 0%, East Asia: 0%, Europe: 0%
*RHCE*ceS*	*RHCE*01.20.03*	(38,51,61)	c.48G>C, c.733C>G, c.1006G>T	hr^{B}–	V–, VS+	e, c	*D-CE(4-7)-D* or *DIIIa-CE(4-7)-D*	Africa: 4.46%, America: 0.29%, East Asia: 0%, Europe: 0%
*RHCE*ceCF*	*RHCE*01.20.06*	(32,62)	c.48G>C, c.697C>G, c.733C>G	hrS–, hrB–, CELO–	Crawford+, VS+	e, c		Africa: 0.08%, America: 0%, East Asia: 0%, Europe: 0%, South Asia: 0%
*RHCE*CeRN*	*RHCE*02.10.01*	(46,47)	c.505C>A, c.509G>T, c.514T>A, c.544A>T, c.577A>G, c.594T>A, c.602G>C	Sec–	DAK+	C[‡], e[‡]		NR

[†], from Erythrogene database (13); [‡], weakened expression. HFA, high frequency antigen; LFA, low frequency antigen; PA, partial antigen; NR, not reported.

(ce48C, 733G)].

In a study from France, including blood donors and patients of African origin, showed that among individuals with altered expression of RhCE antigens and/or with anti-RhCE alloantibodies in the presence of the corresponding antigen 83% had variant *RHCE* alleles and *RH* haplotypes, and the most frequent were R^N and (C)ce^s haplotypes, *RHCE*ceMO*, and *RHCE*ceAR* alleles (75). The same group reported in a later study, in French blood donors of African origin that 14.2% of that population had a variant *RHCE* allele, being (C)ce^s type 1 haplotype the most frequent followed by *RHCE*ceTI*, *RHCE*ceMO*, and *RHCE*ceAR* alleles (45); suggesting that systematic screening of donors for *RHCE* increases the chances of finding rare *RHCE* variants and may help to fulfill the transfusion needs of patients requiring an *RHCE* genotype matching in France (45).

In the US, approximately 85% of patients with SCD carry at least one variant *RH* allele (6). Independent studies reported similar frequencies of *RHCE* variants in African-American blood donors and patients with SCD, where *RHCE*ce.01* (ce48C), *RHCE*ceVS.01* (ce733G), *RHCE*ceTI*, *RHCE*ceAG*, and *RHCE*ceMO* were the most frequent *RHCE* alleles reported; indicating that they probably would be able to provide transfusion support to patients with SCD, although a large number of genotyped blood donors would be needed (37,49).

In Brazil, where the population is highly admixed, the presence of at least one clinically relevant *RHCE* variant allele has been found in approximately 45% of patients with SCD (12,50) and 53% of blood donors self-declared as of African origin (12,60). However, the frequency of homozygous *RHCE* variant alleles or compound heterozygous in patients with SCD and donors who self-declared as of African origin in Brazil is considerably lower, ranging from 1.4% to 16.9% (12,50,67). Although *RHCE* variant alleles between patients and donors are similar to those found in the US, Brazilian patients with SCD and self-declared African origin donors have a high frequency of R1r phenotype which demonstrates the genetic influence of Caucasian origin (12,50). Nevertheless, characterization of donors self-declared as of African origin is the best choice for finding compatible blood for patients with SCD, since both groups have similar frequencies of RhCE phenotypes and *RHCE* variant alleles (12,77).

Clinical aspects

Alloimmunization is a major adverse effect of blood transfusion, increases the risk of DHTRs and reduces the availability of compatible RBC units. Alloimmunization rates vary depending on antigen profile disparity between blood donors and patient, level of antigen immunogenicity, patient age, medical conditions, and frequency of transfusion events (52,78-80).

Rh antigens are highly immunogenic and can induce not only alloantibodies but also autoantibodies. Rh alloantibodies are the most frequent antibodies in chronically transfused patients (52,78,80). Epidemiological study performed using the "Recipient Epidemiology and Donor Evaluation Study-III" (RED-III) database showed that antibodies against RhCE antigens comprised 47.5% of the clinically significant antibodies detected in the 6597 alloimmunized patients (80). From these, 61.3% were anti-E, 18.5% anti-C, 13.2% anti-c, and 2.5% anti-e (80). To prevent alloimmunization, it has been recommended the use of prophylactic Rh (C/c, E/e) and K antigen matched transfusion for chronically transfused patients, especially for patients with SCD who are at high risk of alloimmunization and have the highest rates of Rh antibodies (81,82). Implementation of this practice has demonstrated to reduce alloimmunization rate and DHTRs (78,83,84).

In warm autoimmune hemolytic anemia, about 80% of patients have in their serum autoantibodies that react optimally at 37 ℃ (2). Although most of these autoantibodies appear to be "nonspecific", many of them have a specificity, and anti-e is the most common followed by anti-c, -E, -D, and -C. Noteworthy, when investigating an apparent autoantibody with Rh specificity or unexplained Rh antibodies, RhCE variants should be considered since they can result in partial antigens that elicit alloantibody formation.

The most common *RHCE* variant alleles found among African descent individuals are *RHCE*ce.01* (ce48C), *RHCE*ceVS.01* (ce733G), and *RHCE*ceVS.02* (ce48C, 733G) but the clinical impact of these variants is questionable. Although the Rhce protein encoded by *RHCE*ce.01* (ce48C) allele does not lack epitopes and the e antigen is not recognized as partial (85), there are studies reporting clinical significance of anti-e in patients who carry *RHCE*ce.01* (ce48C) allele (6,7,86). Analysis of hemoglobin (Hb) and hemoglobin S (HbS) levels in pre- and post-transfusion events showed a reduction of Hb and an increase in HbS levels in a SCD patient carrying anti-e and *RHCE*ce.01/(C)ce^s* genotype who was transfused with RBC e+ (7); and an improved response to transfusion was observed in another SCD patient homozygous for

*RHCE*ce.01* with anti-e, who received a genotyped-matched transfusion (86). Despite these findings, the clinical significance of this variant is still controversial and unclear, and it is currently recognized that patients with these alleles seem to have a lower risk of Rh alloimmunization than patients with other altered alleles (49,85).

*RHCE*ceVS.01* (ce733G) and *RHCE*ceVS.02* (ce48C, 733G) also have questioned clinical significance although both alleles are predicted to generate partial c and e antigens and have also been associated with alloantibodies (6,7,50,87). For example, a study including 16 e+ SCD patients with anti-e, reported that 12 of them (75%) presented *RHCE*ce.01*, *RHCE*ceVS.01* or *RHCE*ceVS.02* alleles, and 4 of those 12 (33%) had evidence of DHTR due to anti-e (6). In contrast, some experts do not consider that *RHCE*ceVS.01* (ce733G) and *RHCE*ceVS.02* (ce48C, 733G) encode partial antigens because in their experience the anti-e antibodies associated with these molecular backgrounds are auto-antibodies (48).

The clinical importance for most antibodies formed in patients carrying variant RhCE phenotypes is not easy to establish because the individual variability to alloimmunization remains poorly understood and the reports are often incomplete, particularly regarding serology data (88,89). Furthermore, the classification of allo- or auto-antibody and the role of the antibodies in a DHTR may be difficult to ascertain because allo- as well as auto-antibodies can lead to DHTRs (11,90-92). Ideally, to discriminate allo- and auto-antibody an auto-adsorption assay should be performed. Nevertheless, auto-adsorption assay cannot be performed in a recently transfused patient and results may be inconclusive for very weakly expressed antigens (93).

Besides partial e, partial c also arises from variant *RHCE* alleles, but fewer cases are reported compared with anti-e. Alteration on c antigen expression is rarely found in variant phenotypes probably because the structure of c epitope(s) involves two adjacent proline residues that might form a more stable structure resistant to perturbations induced by changes in upstream or downstream (94). Nevertheless, some cases of partial c involving different variant *RHCE* alleles have been reported; for example, *RHCE*ceAR* (95,96), *RHCE*ceMO* (55), *RHCE*ceJAL* (35), *RHCE*ceCF* (62), *RHCE*ceEK* (10), *RHCE*ceTI* (25) and also (C)ce^{s} haplotype (51).

Anti-C elicited by partial C expression is also commonly reported, mostly in patients with SCD. Studies in a cohort of SCD patients showed that 20–30% of patients with C+ phenotype have partial C, mostly as a result of (C)ce^{S} and R^{N} haplotypes, and have a high risk of anti-C alloimmunization if transfused with conventional C+ units (9,48,49). Analysis of the clinical significance of this antibody showed heterogeneous results; however, some reports revealed DHTR after transfusion with C+ RBCs (6,9).

Patients carrying variant *RHCE* alleles with lack of expression of high-frequency Rh antigens including Hr^{B} (RH34), hr^{B} (RH31), Hr (RH18), and/or hr^{S} (RH19) are at risk for alloimmunization. Anti-Hr^{B} (RH34) and anti-hr^{B} (RH31) are found mainly in African descent individuals carrying (C)ce^{s} haplotypes (97,98), and anti-Hr and anti-hrS are commonly found in patients carrying *RHCE*ceAR*, *RHCE*ceEK* and *RHCE*ceBI*. *Anti-HrB* and anti-Hr react with all RBCs of common RhCE phenotype, but react strongly with e+ RBCs (98), while anti-hr^{B} and anti-hr^{S} react with e+ RBCs, preferentially with Ce and ce haplotypes, respectively, but do not react with e– (DccEE) RBCs (97). Hence, when Hr^{B}– individuals have an antibody reacting against all RBCs of common Rh phenotype, identification of anti-Hr^{B} associated with anti-hrB is possible through adsorption studies with e– (DccEE) RBCs. Anti-Hr^{B} is adsorbed on e– RBCs and the remaining reactivity in the serum is an anti-hr^{B}. Similarly, when anti-Hr is adsorbed on e– RBCs, the remaining reactivity in the serum is an anti-hr^{S} (55,99,100). The clinical significance of anti-Hr^{B} and anti-Hr is well established for both RBC transfusion and HDFN (55,94,96), and some studies have reported an association of anti-hr^{S} and anti-hr^{B} with adverse effects on the fetus or DHTR (6,7,9,10,35,56,101). Once anti-hr^{B} or anti-hr^{S} is identified, compatible transfusion can be achieved by providing e– RBCs. However, these patients may develop anti-E (if E–), and anti-Hr^{B} or -Hr which may lead to complications in antibody identification and provision of suitable blood.

The mechanism for loss of expression of hr^{S} and hr^{B} has not been fully elucidated, and specific epitope(s) and residues involved have not been definitively localized on the Rh proteins (30,56). The inconsistency in serologic results and the lack of antisera support the use of *RH* genotyping for classification of RBCs with altered Rh antigens. In addition, multiple molecular backgrounds encode similar phenotype as shown in *Table 2*; however, patients with antibodies elicited by those variants are not always compatible with donor's RBCs with the same RhCE phenotype but different molecular background (37,96). For example, anti-c developed by a patient carrying *RHCE*ceAR/Ce* reacts with RBCs with *RHCE*ceEK* and *RHCE*ceBI*, but not with *RHCE*ceMO* and *RHCE*ceJAL*, suggesting that

the c antigen encoded by *RHCE*ceAR* allele is different than that encoded by *RHCE*ceEK* and *RHCE*ceBI* and may express common epitopes with the c antigens encoded by *RHCE*ceMO* and *RHCE*ceJAL* (96). Therefore, for efficient and safe blood transfusion, *RH* genotyping and molecular matching is recommended.

SCD and *RHCE* complexities

Patients with SCD are chronically transfused and usually highly immunized for Rh antigens. The difference in frequency of RhCE antigens among ethnic groups greatly contributes to alloimmunization because in many countries blood donors are mostly of European descent, while SCD is prevalent in African descendants (102-104). Selection of blood donors self-declared as African descent for patients with SCD is a good transfusion strategy adopted by several centers to provide a more similar phenotypic profile and avoid RBC alloimmunization without overuse of D– RBC units, since in individuals from African origin the haplotype Dce is more frequent, while in Caucasians DCe haplotype is more common (49).

A common strategy for reducing the alloimmunization risk in patients with SCD has been to provide prophylactic matching RBC units for C, E, and K antigens (105,106). Regardless of this strategy, alloimmunization against Rh antigens continues to occur due to Rh complexities. The presence of variant *RHCE* alleles in the SCD population has been shown to range from 27% to 58% (6,12,37,67) and patients with SCD carrying variant alleles have a higher risk for alloimmunization (7,48). In the US, 13% of patients with SCD who developed RhCE-alloantibodies were carriers of partial antigen (49), and in France the presence of anti-C was detected in 14.3–30% of patients with partial C antigen (9,88).

Recent evidence about the impact of variant RhCE on patients with SCD has expanded the application of *RH* genotyping since the variants are not distinguished by serological techniques. Special attention to RhCE phenotyping may indicate an altered antigen expression, and further genotyping can inform if the patient is at risk for alloimmunization or to provide insight to determine if Rh antibodies are allo- or auto-antibodies, predict clinical significance, and aid in transfusion decisions (49).

RH genotyping is a great strategy to provide superior matching, reduce alloimmunization and improve red cell utilization (107). Genotype-matching can be achieved by high-throughput genotyping, which offers significant cost savings in both labor and reagents compared with antigen typing by serologic methods, and expands testing to detect genetic variation of antigen expression (107). However, genotyping is still of high cost, fact that leads to the development and use of selection strategies to screen donors with rare RhCE phenotypes, to increase the probability to find them at an affordable cost. The recruitment of donors self-declared as African descent to provide *RH* genetic matching has also been the best choice, as indicated by reports that showed similar frequency of *RHCE* variant alleles among African descendant donors and patients with SCD, even in countries with ethnic admixture (12,49,77). Patient classification in responders and non-responders could be important to restrict molecular matching to patients with higher chance to develop alloantibody (48,105). Centralization of genotyping tests in larger centers has been suggested as alternative that would give support to the smaller centers that frequently transfuse patients with SCD (105). Alternatively, prioritizing some conventional molecular tests (allele-specific PCR or PCR-RFLP) targeting specific SNVs, such as c.733G>C, c.254C>G and c.667G>T to screen the most common variants can also be a useful strategy (108). Additionally, selection of donors with Fy(a–b–) or weak D phenotype may increase the chances of identifying donors lacking high-frequency RhCE antigens (12,77).

Conclusions

RhCE antigens have a significant role in transfusion medicine due to their high immunogenicity and the hemolytic power of their antibodies. In the last two decades, studies have been conducted to elucidate the presence of unexplained RhCE antibodies. Molecular assays allowed considerable progress in the identification of genetic basis and characterization of *RHCE* variant alleles in patients with SCD and blood donors. Current knowledge about variant allele frequencies in different ethnicities allows the blood centers to define target variants for screening aiming to provide matched RBCs to most patients. However, some questions remain to be answered, mainly regarding the clinical significance of the *RHCE* variants. Except for anti-Hr^B and anti-Hr, the clinical importance of antibodies elicited by variant phenotypes is unclear. The interpretations of events that define clinical significance are inconsistent and require comprehensive studies with extensive serological tests and clear association with clinical observations. Additionally, although more than one genetic

variant can predict the same phenotype some of those can be incompatible for transfusion. Therefore, studies that evaluate cross-matching among genetic variants predicting similar phenotype but with different genetic backgrounds are paramount to use genotyping for expanding the availability of donors.

Finally, transfusion of patients carrying variant RhCE phenotype is still a significant challenge in transfusion medicine. Although genotyping revolutionized the knowledge of RhCE variants, its application in routine immunohematology, for donor screening and molecular-matching, is still cost-prohibitive. We expect that technological advances, such as next-generation sequencing or large-scale genotyping microarray platforms, allow screening of rare RhCE donors to improve transfusion care for patients with variant RhCE phenotype.

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Footnote

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Supplementary

Table S1 Detailed search strategy used in PubMed database

Search	Query	Results
#7	Filters: English language, publication dates from 1000/1/1[¥]-2021/6/30	325
#6	#4 OR #5	352
#5	#2 AND #3	43
#4	#1 AND #2	336
#3	cell disease, sickle[MeSH Terms]	24,283
#2	(“RHCE”) OR (“ RHCE variants”) OR (“Rh antibodies”)	959
#1	Rh-Hr Blood-Group System/genetics*[MeSH Terms]	1,647

[¥], corresponds to the limit of the database.

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