Published in final edited form as:

Transfusion. 2010 June ; 50(6): 1303–1311. doi:10.1111/j.1537-2995.2009.02573.x.

DIIa and DIII Type 5 are encoded by the same allele and are associated with altered RHCE*ce alleles: clinical implications

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Abstract

BACKGROUND—The partial D phenotype DIIa was originally reported to be associated with 455A>C in Exon 3, 602C>G in Exon 4, and 667T>G in Exon 5. Other alleles with these changes were subsequently identified and designated DIII Types 5, 6, and 7, as they had additional alterations. The observation that DNA samples associated with the DIIa phenotype had more changes than those originally reported motivated us to reanalyze the DIIa probands (BP and DJ) from the original study. We also studied additional DIIa samples to clarify the *RHD* background and establish the associated *RHCE*.

STUDY DESIGN AND METHODS—Hemagglutination testing was performed by standard methods. *RHD* and *RHCE* were analyzed by combinations of polymerase chain reaction–restriction fragment length polymorphism, exon-specific sequencing, cloning, or direct sequencing of Rh-cDNAs.

RESULTS—The *RHD* alleles from BP, DJ, and 58 additional DIIa samples had the three reported nucleotide changes as well as 186G>T, 410C>T, and 819G>A. The DIIa allele was associated with several altered *RHCE**ce-alleles, the prominent one being ceS (48C, 733G, 1006T).

CONCLUSION—The DIIa phenotype is associated with six *RHD* changes, five of which encode amino acid changes, and partial DIIa and DIII Type 5 are encoded by the same *RHD* allele. In all samples, *RHD**DIIa was inherited with altered *RHCE**ce. Patients with partial DIIa are at risk for production of alloanti-D, but they are also at risk for alloanti-e, -c, or antibodies to high-prevalence Rh antigens if there is no conventional *RHCE**ce in trans. Among 39 patients studied, 16 had alloanti-D and 27 had alloanti-e or anti-hrB.

The D antigen is more complex than many blood group antigens because it represents the presence (D+) or absence (D−) of the RhD polypeptide in the membrane, rather than a single-amino-acid change in a protein. D antigen has numerous epitopes that were originally defined by antibodies from D+ people who made alloanti-D. The altered D antigen designated as the DIII category was reported by Tippett and Sanger in 1962.¹ It was one of six categories (I through VI) used to distinguish the D antigen found in D+ people who made alloanti-D. The authors predicted that the red blood cells (RBCs) from these people lacked some part of normal D, and they had made an antibody to the missing portion.

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CONFLICT OF INTEREST

The authors have no conflicts to disclose.

Whereas most anti-D made by D- people agglutinated RBCs of all six categories, anti-D made by people with D categories gave specific patterns of reactivity when tested against each other. Category DIII RBCs were agglutinated by anti-D made by all other categories, but not by anti-D from other people with DIII RBCs. DIII was subsequently divided into three classes (DIIIa, DIIIb, and DIIIc). DIIIa was only found in blacks and is associated with a R₀ (Dce) haplotype,² and the RBCs express the DAK antigen.³

In 1997, the partial D phenotype DIIIa was reported to be due to the replacement of three *RHD* nucleotides by equivalent nucleotides from *RHCE*, specifically Nucleotide 455A>C located in Exon 3 and predicted to encode a Asp152Thr change in the RhD protein, Nucleotide 602C>G in Exon 4 encoding a Thr201Arg, and Nucleotide 667T>G in Exon 5 encoding Phe223Val.⁴ Following that report, several alleles were identified that had some or all of these replacements, but with additional changes (Table 1), and these were designated DIII Type 4,⁵ Type 5,⁶ Type 6, and Type 7.⁷

The observation that DNA samples from people with RBCs that serologically appeared to be DIIIa had additional changes, along with the three reported, motivated us to reanalyze samples from the DIIIa probands (BP and DJ) studied by Huang and coworkers⁴ and herein referred to as the original DIIIa probands.⁸ We also studied a large number of DIIIa samples and investigated *RHCE*ce* to determine the specific RH haplotypes associated with *RHD*DIIIa*.

MATERIALS AND METHODS

Samples

The study included the two original DIIIa probands, BP and DJ, and 58 additional DIIIa samples. In total, 21 donors and 39 patients were investigated. The samples in this study phenotyped as DAK+ and/or were submitted for investigation to determine the RH genotype.

Serologic testing

Hemagglutination testing of the RBCs and antibody identification were performed in test tubes or gel cards by standard methods.⁹ If fresh pretransfusion RBCs were available, samples were typed for hr^B with the FOR-2E3 monoclonal antibody¹⁰ or polyclonal anti-hr^B from our collections. Some were also tested with polyclonal anti-DAK, -V, -VS, and -V/VS from our collections.

Genomic DNA extraction, amplification, and sequencing

Genomic DNA was extracted from peripheral white blood cells with a commercial kit (QIAamp, Qiagen, Inc., Valencia, CA). DNA was subjected to polymerase chain reaction (PCR) amplification using *RHD*- and *RHCE*-specific primers¹¹ and analyzed by multiplex PCR, PCR-restriction fragment length polymorphism (RFLP), or direct sequencing of specific exons.

Multiplex-PCR, PCR-RFLP, exon-specific PCR, allele-specific PCR, and *RHD* zygosity

Multiplex PCR was performed to detect *RHD* Exon 4, Exon 7, and the inactivating *RHD* pseudogene and for C/c typing.¹² For some samples, *RHD* zygosity was determined by assaying for the presence of the hybrid Rhesus box.¹³

RHD analysis

Exon 2 was amplified and a *Bst*XI-RFLP was designed and used to detect 186G>T. Exon 5 was amplified and a *Hinc*II-RFLP was used to detect 667T>G and a *Taq*I-RFLP to detect 697G>C. Exon 8 was amplified and a *Nla*III-RFLP was used to detect the 1136C>T change. A *Nla*IV-RFLP or allele-specific PCR was used to detect the Exon 3 Nucleotide 455A>C change. Amplification and direct sequencing were done for the remaining *RHD* exons with the exception of those for which Rh-cDNA sequencing was done.

RHCE analysis

Exon 1 was amplified and *Apa*I-RFLP or *Hha*I-RFLP was used to detect Nucleotide 48G>C. Exon 5 was investigated by amplification and sequencing. Additionally a *Bfa*I-RFLP was used to confirm the 733C>G change associated with the V+VS+ phenotype and a *Mnl*I-RFLP to confirm the nucleotide 676C>G polymorphism associated with E/e. Allele-specific PCR used to detect the Exon 7 1006G>T polymorphism associated with a V-VS+ phenotype was confirmed by sequencing, and a *Hph*I-RFLP was used to detect the presence of 1025C>T change. Amplification and sequencing of Exons 3, 5, 6, and 8 were also performed, with the exception of those for which Rh-cDNA sequencing was done.

RNA extraction and Rh-cDNA cloning and sequencing

To confirm the genomic results and ascertain the specific alleles carrying the changes, we analyzed the mRNA transcripts from reticulocyte-enriched RBCs by synthesis and cloning of Rh-cDNAs from 23 samples. RNA was isolated from the reticulocytes (QIAzol, Qiagen, Inc.). For samples analyzed in Philadelphia, reverse transcription was carried out with Superscript II and random hexamers and oligo(dT) primer, according to the manufacturer's instructions (Superscript first-strand synthesis system, Invitrogen, Carlsbad, CA). PCR amplification was carried out for 35 cycles with primers complementary to the 5' and 3' regions of RHCE and RHD cDNAs. PCR products were checked for purity on agarose gels, recovered with gel isolation (QIAquick PCR purification, Qiagen), and cloned into TOPO II (Invitrogen) for sequencing by the University of Pennsylvania DNA sequencing facility. Sequences were aligned, and protein sequence comparisons were performed with ClustalX. For samples analyzed in New York, RNA was isolated from the reticulocytes (TriZol and PureLink Micro-to-Midi total RNA purification system, Invitrogen). Reverse transcription was carried out with gene-specific *RHD* and *RHCE* primers and Superscript III according to manufacturer's instructions (Superscript III first-strand synthesis SuperMix, Invitrogen). PCR amplification was carried out for 35 cycles with primers to amplify Exons 1 to 4 and Exons 5 to 10 in *RHD* and *RHCE*. PCR products were checked for purity on agarose gels and directly sequenced by either the New York Blood Center DNA sequencing facility or GENEWIZ, Inc. (South Plain-field, NJ). Sequences were aligned, and protein sequence comparisons were performed with Sequencher 4.8 (GeneCodes, Ann Arbor, MI).

RESULTS

RHD analysis in original DIIIa probands BP and DJ

The results of *RHD* exon-specific genomic testing on BP and DJ are summarized in Table 1. In addition to the three changes previously reported (Exon 3, 455A>C; Exon 4, 602C>G; and Exon 5, 667 T>G),⁴ both samples had changes in Exon 2, 186G>T (L62F), and Exon 3, 410C>T (A137V), and a 819G>A change in Exon 6 that does not encode an amino acid change in the protein. *RHD* Exons 1, 7, 8, 9, and 10 had no changes, and neither proband had the *RHD*-inactivating pseudogene or the hybrid Rhesus box associated with deletion of *RHD* (not shown). At the *RHD* locus, BP carried DIIIa in trans to the common hybrid found in blacks,¹⁴ which has been referred to as *RHD-CE-D^S* Type 1,¹⁵ or *RHD-CE(4-7)-D* and

herein is termed *RHD*DIIIa-CE(4-7)-D* (see Discussion). DJ had *DIIIa* with a conventional *RHD* in trans.

RHD analysis in other samples

Investigation of *RHD* in additional samples (described under Materials and Methods) with *DIIIa* revealed that all had the identical six nucleotide changes as BP and DJ with the exception of the silent 819G>A change, which was absent in five alleles (designated *DIIIa* [819G]). Table 2 summarizes the serologic results and the RH alleles found in 21 donor samples, and Table 3 summarizes the 39 patient samples. Two donors were homozygous for *RHD*DIIIa* (D1 and D21), seven donors had conventional *RHD* in trans, five had the common hybrid *RHD-CE(4-7)-D* (associated with the (C)ceS haplotype), one had *RHD*DAR*, and six had a deletion of *RHD* (four tested, two presumed). Among the 39 patient samples, five were homozygous for *RHD*DIIIa* (P1, P2, P3, P4, and P5), 16 had the hybrid *RHD-CE(4-7)-D* and the (C)ceS haplotype in trans, eight had conventional *RHD* in trans and one had altered *RHD*D(314V)*,¹⁶ seven had a *RHD* deletion (six tested, one presumed), and two had an inactivating *RHD* pseudogene. Among all 60 samples, seven were homozygous for *RHD*DIIIa*, so there were a total of 67 *DIIIa* alleles.

Many of the patient samples identified as having *RHD*DIIIa* also had serum antibodies with anti-e-like, anti-Ce-like, or anti-hr^B specificities, with or without anti-D (Table 3). This suggested that *RHD*DIIIa* was associated with altered *RHCE*ce*. Therefore, we investigated the V/VS and hr^B phenotypes in those for which nontrans-fused RBC samples were available and determined the associated *RHCE*ce*-alleles in all samples.

Serologic testing

Many of the patients were chronically transfused, and the Rh phenotypes for some are from historical records. All RBCs from partial *DIIIa* homozygotes and those with a single-copy *DIIIa* in trans to deletion of *RHD* were strongly reactive with commercial anti-D by the immediate spin. The strength of reactivity of the C antigen depended on the anti-C reagent used. The reactivity of the e antigen on RBCs of samples homozygous for ceS alleles or with ceS in trans to cE was also weaker than single-dose controls. RBCs from samples with ceS alleles are also positive for VS (but not V), and RBCs with *RHCE*ce(733G)* encoding 245Val express both V and VS antigens (summarized in Reid and Lomas-Francis¹⁷). The expression of V and VS is often weak when present in a single dose. RBCs from the sample (D19) with a new allele, *RHCE*ce(1025T)*, were negative for V/VS and hr^B positive. Expression of the high-prevalence hr^B antigen was absent on RBCs of samples homozygous for ceS alleles or with ceS in trans to cE or deletion alleles. From our testing it is apparent that the Rhce protein with 16Cys and 245Val does not express hr^B, while Rhce with 16Trp and 245Val may express hr^B weakly.

RHCE analysis

Exon 5 analysis for *RHCE*E/e* was consistent with the RBC phenotype and multiplex testing confirmed the presence of *RHCE*c* in all of the samples. Multiplex testing for *RHCE*C* was negative in all but one sample, P28, in which the significantly depressed C expression, denoted as (+), was encoded by the R^N allele. The 21 samples that were C+ by serologic testing (five donors and 16 patients) and negative for *RHCE*C* by multiplex, had a hybrid *RHD*DIIIa-CE(4-7)-D*, which encodes an altered C antigen. This hybrid gene is associated with a *RHCE*ce*, which encodes the V-VS+ phenotype,¹⁴ and together they encode the haplotype referred to as r'S or (C)ce^S.

Table 4 and Fig. 1 summarize the different *RHCE*ce* found with the 67 partial *DIIIa*-encoding alleles and presumed to be in cis to *RHD*DIIIa*. The majority (63 alleles) had the

48G>C change in Exon 1 common in ce alleles in blacks.¹⁸ The 733C>G polymorphism associated with V+VS+ was present in all but two samples, from South Africa. The 1006G>T change, which silences expression of V, was present in 54 alleles. A previously observed 1025C>T change in Exon 7¹⁹ was present in five samples, with (n = 3) or without (n = 2) concurrent 48C and 733G polymorphisms. The majority of the DIIIa alleles (n = 54) were associated with *RHCE*ce* (48C, 733G, 1006T), which encodes 16Cys, 245Val, and 336Cys, referred to as ceS. Five were associated with *RHCE*ce* (48C, 733G), which lacks the 1006 polymorphism, three with *RHCE*ce* (48C, 733G, 1025T), two with *RHCE*ce* (733G) alone, two with *RHCE*ce*(1025T) alone, and one with a hybrid *RHCE*ce* (48C)-D(4-10). No conventional *RHCE*ce* was associated with *RHD*DIIIa*.

DISCUSSION

The original DIIIa probands, BP and DJ, in addition to the three changes described in the original report,⁴ have changes 186T, 410T, and 819A, for a total of six single-nucleotide changes from normal *RHD* (Nucleotides 186, 410, 455, 602, 667, and 819). Five of the six changes encode amino acid changes in RhD, that is, Leu62Phe, Ala137Val, Asn152Thr, Thr201Arg, and Phe223Val, and the sixth is silent. The specific amino acid changes associated with DIIIa were confirmed by analysis of *RHD* in 58 additional samples. The silent 819G>A change was not present in five DIIIa alleles. Importantly, these changes are identical to those associated with the allele designated DIII Type 5, which was so named because it carried some of the same changes reported initially for DIIIa.⁶ Thus, DIII Type 5 is the same as DIIIa. We recommend that the term DIII Type 5 be made obsolete and that this allele be classified as DIIIa to provide important consistency in nomenclature between the DIIIa phenotype and the associated allele (genotype).

Determination of the complete RH genotype revealed that *RHD*DIIIa* is associated with altered *RHCE*ce*. Among the 67 DIIIa alleles, most were linked to *RHCE*ce*(48C, 733G, 1006T). This allele, often referred to as ceS, encodes amino acid changes 16Cys, 245Val, and 336Cys and is associated with a partial e, partial c, hr^B –, V–VS+ phenotype.^{20,21} Of the remaining alleles presumed linked to DIIIa, five had 48C and 733G and two had only the 733G change. The absence of 1006T in these latter alleles is associated with the V+VS+ phenotype. Five were linked to ce-alleles with a 1025C>T, predicted to encode a Thr342Ile amino acid change in Rhce. The 1025T was present alone (two alleles) or in conjunction with both 48C and 733G (three alleles) and are new alleles not previously reported. Although we observed the 1025C>T change previously, in those samples, it was on an allele with a 48C change and was given the designation *RHCE*ceTI*.¹⁹ The two alleles with the single 1025T change were found in two South African donors, suggesting that this polymorphism is not recent and is of African origin. Finally, the hybrid *RHCE*ce*(48C)-D(4-10) presumed linked to DIIIa was previously reported to be associated with a Dc– phenotype.²² In summary, all the DIIIa alleles in this large study were associated with altered *RHCE*ce* with the majority encoding VS, or V/VS, partial e, and probably partial c²¹ and most associated with a hr^B – phenotype.

Several previous studies demonstrated linkage of RHD-alleles encoding partial D with specific altered *RHCE*ce*. These are summarized in Table 5. The allele designated *RHCE*ceTI* was shown to be linked to *RHD*DIVa-2*.¹⁹ *RHCE*ceAR* or *RHCE*ceEK* encoding a hr^S – phenotype are often inherited with *RHD*DAR*.²³ *RHCE*ceMO*, which also encodes an hr^S – phenotype, is frequently found with *RHD*DAU0* in African Americans.²⁴ The hybrid *RHD*DIIIa-CE(4-7)-D*, common in blacks, is linked to ceS.^{14,15}

Many of the patients (n = 16) and several donors (n = 5) had the hybrid *RHD*DIIIa-CE(4-7)-D* linked to *RHCE*ceS*, encoding the haplotype known as (C)ceS or r'S, in trans to

the haplotype with *RHD*DIIIa*. The confluence of these two haplotypes in patients with complex Rh antibodies was also observed in a recent study from Pham and colleagues.¹⁵ In all samples reported here the hybrid has 5' Exons 1, 2, and 3 identical to those found in *RHD*DIIIa*, Exons 4–7 are identical to *RHCE*ce* but with 733G and 1006T (ceS markers), and Exons 8–10 are identical to conventional *RHD*. The *RHD*DIIIa* origin of the 5' exons in this hybrid allele have been noted by others.¹⁵ To distinguish this hybrid from the hybrid that occurs on a conventional *RHD* background, we suggest that the DIIIa origin be reflected in the allele designation as *RHD*DIIIa-CE(4-7)-D*. Although the allele encoding DIVa-2 also shares identity in Exons 1 through 3 with DIIIa, the DIVa allele is not linked to *RHCE*ceS* (unpublished observations); hence, this hybrid very likely originated on a DIIIa rather than the DIVa background. It should be noted that the *NlaIV* PCR-RFLP described as specific for this hybrid¹⁴ and the PCR–sequence-specific primer assay that targets 455A>C²⁵ cannot discriminate between partial *RHD*DIIIa* and the hybrid *RHD*DIIIa-CE(4-7)-D*. Additional gene analysis is required.

Clinical considerations

The partial DIIIa phenotype is recognized as the most common partial allele in blacks.² This partial D goes unrecognized in routine serologic testing because the RBCs type strongly D+ in direct tests. Individuals whose RBCs have the DIIIa phenotype are at risk for production of anti-D after exposure to conventional D antigen via blood transfusion or pregnancy. Sixteen of 37 (43%) of the pregnant or transfused patients in this report had anti-D.

The majority of the patients were referred to our laboratories because of multiple antibodies. Table 3 shows that the specificities were primarily in the Rh system, with anti-K, -Fy^a, -Jk^b, and -S also present in some. Although DIIIa was known to be associated with a R₀ (Dce) haplotype and production of anti-D, a large number of transfused patients, 29 of 37 (78%), were e+ and had anti-e and/or -hr^B. This study found that alleles presumed to be in cis with DIIIa encode partial e antigen and a hr^B – (or hr^B weak) phenotype. The observation that partial DIIIa is linked to partial e is of relevance for transfusion because patients with partial DIIIa are not only at risk for production of alloanti-D, they are also at increased risk for production of alloanti-e (or anti-hr^B) if there is no conventional *RHCE*ce* or *RHCE*Ce* in trans. The presence of anti-e in e+ patients with alleles encoding amino acid changes in Rhce support the fact that these are alloantibodies. The situation is complicated when the antibodies demonstrate cross-reactivity with the patient's own cells. The clinical implications require further study.

Four samples were from women who were pregnant (P3, P13, P15, and P21). P3, P13, and P15 had made anti-E and -hr^B. The antibodies in the serum of P21 were identified as C-like and anti-e (Table 3), which are often called -hr^B depending on the laboratory doing the identification and cells available for testing. P3 was lost to follow-up. P13 was not compliant with prenatal care and had a history of five previous pregnancies. Only two resulted in live births, and hemolytic disease was suspected in the other three. The neonate delivered early by C-section had a 2+ DAT and was E– with mild anemia, but did not require transfusion. P15 and P21 delivered early, and both babies had 2+ DAT and mild anemia at delivery. Two maternal units from these women had been donated before delivery but were not needed. No fetal morbidity or mortality was associated with the anti-e or -hr^B in these pregnancies. Anti-hr^B is not associated with severe hemolytic disease (personal observations from our laboratories and summarized in Reid and Lomas-Francis¹⁷), but a positive DAT with mild anemia is often seen. To our knowledge, no fetal mortality has been reported associated with partial DIIIa and production of anti-D. However, monitoring of the pregnancy is certainly recommended and anemia has been observed.

Acknowledgments

This study was supported in part by an NIH grant HL091030.

We thank Cheng Han-Huang from New York Blood Center for helpful discussions, the staff of the American Red Cross National Reference Laboratory for Blood Group Serology, and of the New York Blood Center Laboratory of Immunohematology for red blood cell typing.

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Fig. 1.

Diagram of *RHD***DIIIa* and the associated *RHCE***ce* found in this study. The 10 *RHD* exons are shown as white boxes and the *RHCE* as gray. The location of the nucleotide changes is indicated as a broad line, and the numbered position and change are given.

TABLE 1

DIII alleles with the nucleotide changes and associated amino acid changes

RHD allele	Nucleotide amino acid						
	186G>T Leu62Phe	410C>T Ala137Val	455A>C Asn152Thr	602C>G Thr201Arg	667T>G Phe223Val	819G>A Silent	
DIIIa (Huang et al. ⁴)			C	G	G		
DIIIa (BP, DJ)	T	T	C	G	G	A*	
DIII type 4	T	T	C	C	T	G	
DIII Type 5	T	T	C	G	G	A	
DIII type 6	G	T	C	G	G	A	
DIII type 7	CE Exon 2 (five nucleotides)					G	A

* Nucleotide 819G>A does not encode an amino acid change and is not present in some DIIIa alleles.

TABLE 2

Donor serologic results and RH alleles

Donor	D	C	E	c	e	V/VS	hr ^B	DAK	RHD alleles	RHCE alleles
D1	+	0	0	+	+		0	+	DIIIa	ceS
D2, D3, D4, D5									DIIIa	ceS
	+	+ ^w	0	+	+	+	0	+	DIIIa	ceS
									Hybrid DIIIa-CE(4-7)-D ⁺	ceS
D6	+	0	0	+	+		0		DIIIa	ceS
D7									deletion	ceS
	+	0	0	+	+ ^w		0 ⁺		DIIIa	ceS
									D	ce(733G)
D8, D9, D10, D11									DIIIa	ceS
	+	0	+	+	+ ^w	+	0	+	D	cE
									DIIIa	ce(48C, 733G, 1025T) [§]
D12	+	0	+	+	+	+			D	cE
D13									DIIIa	ceS
	+	0	0	+	+				D	ce
									DIIIa	ceS
D14, D15, D16									deletion	ce
	+	0	0	+	+	+	1+		DIIIa	ceS
									DIIIa (819G)	ce
D17									deletion assumed	ceS
	+	0	0	+	+	+	+ ^w	+	DIIIa	ce(48C)
									deletion assumed	ce(1025T) [§]
D18	+	+	0	+	+	+		+	DIIIa	ce(1025T) [§]
D19									Hybrid DIIIa-CE(4-7)-D ⁺	ceS
	+	0	0	+	+	0	+	+	DIIIa	ce(1025T) [§]
									deletion assumed	ce
D20									DIIIa	ceS
	+	0	0	+	+		0		DAR	ceAR with 697C>G [§]
									DIIIa	ce(48C, 733G)
D21	+	0	0	+	+	+	1+		DIIIa	ce(733G)

^wWeak reactivity with some antisera.

[†] This hybrid has also been called *RHD-CE-D^S* or *RHD-CE(4-7)-D^{14,15}* (see discussion).

[‡] Human polyclonal reagent used.

[§] New allele.

ceAR = ce(48C, 712G, 733G, 787G, 800A, 916G); ceS = ce(48C, 733G, 1006T).

deletion = tested and found positive for the hybrid box associated with deletion of *RHD*.

TABLE 3

Patient serologic results and RH alleles

Patient	Referral reason	D	C	E	e	V/VS	hr ^B	DAK	RHD alleles	RHCE alleles
P1	Anti-D, -C, -E, -hr ^B , -K	+	0	0	+	+	0	+	DIIa	ceS
P2	Anti-D, -e, -hr ^B , -Hr ^B	+	0	0	+	+			DIIa	ceS
P3	Anti-E, -hr ^B	+	0	0	+	+	0		DIIa	ceS
P4	Anti-D, -C, -E, -e, -S, -K, -Fy ^a , -WA	+	0	0	+	+			DIIa (819G presumed)	ceS
P5	Population study	+	0	0	+	+	0	+	DIIa	ce(48C, 733G)
P6	Anti-e-like	+	^w	0	+	+/0	+	+	DIIa (819G)	ceS
P7	Anti-e-like	+	+	0	+	+	0	+	Hybrid DIIa-CE(4-7)-D	ceS
P8	Anti-D, -E, -hr ^B , -M, WA	+	^w	0	+	+			DIIa	ceS
P9	Anti-E, -hr ^B , -K	+	+	0	+	+	0		Hybrid DIIa-CE(4-7)-D	ceS
P10	Anti-E, -hr ^B , -S, -Jk ^b , -K	+	+	0	+	+	0		DIIa	ceS
P11	Anti-E, -C, -hr ^B	+	+	0	+	+			Hybrid DIIa-CE(4-7)-D	ceS
P12	Anti-D, -E, -hr ^B	+	+	0	+	+	0		DIIa	ceS
P13	Anti-E, -hr ^B	+	+	0	+	+	0		Hybrid DIIa-CE(4-7)-D	ceS
P14	Anti-D, -E, -hr ^B , -WA	+	+	0	+	+	0		Hybrid DIIa-CE(4-7)-D	ceS

Patient	Referral reason	D	C	E	c	e	V/N/S	hr ^B	DAK	RHD alleles	RHCE alleles
P15	Anti-E, -hr ^B	+	+	0	+	+	0			DIIIa	ceS
P16	Anti-D, -E, -hr ^B	+	+	0	+	+	0			Hybrid DIIIa-CE(4-7)-D DIIIa	ceS ceS
P17	Anti-E, -C, -high in Rh	+	+	0	+	+				Hybrid DIIIa-CE(4-7)-D DIIIa	ceS ceS
P18	Anti-E, -C, -e	+	+	0	+	+				Hybrid DIIIa-CE(4-7)-D DIIIa	ceS ceS
P19	Anti-e, -hr ^B	+	+	0	+	+				Hybrid DIIIa-CE(4-7)-D DIIIa	ceS ceS
P20	Anti-D	+	+	0 ^w	+	+	+		+	Hybrid DIIIa-CE(4-7)-D DIIIa	ceS ce(48C, 733G)
P21	Anti-C, -e-like	+	+	0	+	+	0			Hybrid DIIIa-CE(4-7)-D DIIIa	ceS ce(48C)-D(4-10)
P22	Anti-e-like, -E, -M	+	0	0	+	+			+	Hybrid DIIIa-CE(4-7)-D DIIIa	ceS ceS
P23	Anti-e-like	+	0	+	+	+	0		+	D DIIIa	ce(48C, 733G) ceS
P24	Anti-hr ^B , -Jk ^b	+	0	+	+	+	0			DIIIa	cE ceS
P25	No data available	+	0	0	+	+			+	D DIIIa	cE ceS
P26	Anti-C, -E, -hr ^B , -K, -S, -Fy ^a	+	0	0	+	+				D DIIIa	ceCF(48C, 697G, 733G) ceS
P27	Anti-E, -e	+	0	0	+	+				D DIIIa	ce(733G) ceS
P28	Anti-C, -hr ^B , -K, -Fy ^a	+	(+)	0	+	+	0			D DIIIa	ce(48C, 733G) ceS
P29	Possible anti-hr ^S	+	0	0	+	+	0/+ ^w			D DIIIa	R ^N ceS
P30	Anti-e-like	+	0	0	+	+				D DIIIa (819G)	ce ce(733G)

Patient	Referral reason	D	C	E	c	e	V/S	hr ^B	DAK	RHD alleles	RHCE alleles
P31	Anti-D	+	0	0	+	+	+		+	D(314V) DIIa Deletion assumed	ce ceS ce(254G) ²⁶ ce(48C,733G) ce(733G) ceS ce(733G) ceS ce
P32	Anti-D	+	0	0	+	+				DIIa (819G) Deletion	
P33	Anti-D, -C, -E, -e, -K	+	0	0	+	+		0		DIIa Deletion	ceS ce(733G)
P34	Anti-D, -C, -E	+	0	0	+	+				DIIa Deletion	ceS ce(733G)
P35	Anti-D, -C, -E, -Fy ^a , -S	+	0	0	+	+				DIIa Deletion	ce ce(48C,733G,1025T) [*] ce(733G)
P36	Anti-E, -hr ^B	+	0	0	+	+			+	DIIa Deletion	ceS ceS ceS
P37	Anti-C, -E, -K, -hr ^B	+	0	0	+	+				DIIa Deletion	ceS ceS ceS
P38	Anti-D	+	0	0	+	+				DIIa Pseudogene ψ	ceS ce(48C)
P39	Anti-D	+	0	0	+	+				DIIa Pseudogene ψ	ce(48C,733G,1025T) [*] ce

* New allele.

ceS = ce(48C, 733G, 1006T).

deletion = tested and found positive for the hybrid box associated with deletion of *RHD*.

TABLE 4

Summary of RHCE alleles presumed linked to RHD* DIIIa

RHCE allele	48G>C Trp16Cys Exon 1	733C>G Leu245Val Exon 5	1006G>T Gly336Cys Exon 7	1025C>T Thr342Ile Exon 7	Encodes	Number of alleles	
						Patient	Donors
ceS or ce(48C, 733G, 1006T)	C	G	T	C	V- VS+ hr ^B -	36	18
ce(48C, 733G)	C	G	G	C	V+ VS+ hr ^B -	4	1
ce(48C, 733G, 1025T)*	C	G	G	T	V+ VS- hr ^B †	2	1
ce(733G)	G	G	G	C	V+ VS+ hr ^B +w	1	1
ce(1025T)*	G	C	G	T	V- VS- hr ^B +		2
ce(48C)-D(Ex 4-10)	C				V- VS- hr ^B -	1	1

* Alleles not previously reported.

† No informative samples.

TABLE 5

Summary of altered RH alleles demonstrating linkage

<i>RHD</i> *	<i>RHCE</i> *	Previous terminology/reference
<i>DIVa</i>	<i>ceTI</i>	Vege et al. ¹⁹
<i>DAR</i>	<i>ceAR</i>	Hemker et al. ²³
<i>DAR</i>	<i>ceEK</i>	
<i>DAU0</i>	<i>ceMO</i>	Westhoff et al. ²⁴
<i>DIIIa-CE(4-7)-D</i>	<i>ceS</i>	<i>RHD-CE(4-7)-D</i> or (C)ceS Type 1 ^{14,15,21}
<i>D-CE(4-7)-D</i>	<i>ceS</i>	<i>RHD-CE-D^S</i> Type 2 or (C)ceS Type 2 ¹⁵
<i>DIIIa</i>	<i>ceS</i>	This article