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The JAL antigen (RH48) is the result of a change in *RHCE* that encodes Arg114Trp

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Abstract

BACKGROUND—The JAL antigen (Rh48) was discovered more than 30 years ago when it caused hemolytic disease of the fetus and newborn in an African American family. A decade later it was found to cause hemolytic disease of the fetus and newborn in a Caucasian family. The presence of the same low-prevalence antigen in two different ethnic groups is rare, but additional JAL+ in both groups was subsequently identified. This study was undertaken to investigate the RH gene(s) responsible for expression of JAL and to determine the structural relationship between JAL and other Rh antigens.

STUDY DESIGN AND METHODS—Samples from 17 JAL+ people were included: 2 Caucasian, 6 African American, 7 African Brazilian, 1 Caribbean, and 1 Puerto Rican. *RHCE* and *RHD* were investigated at the genomic level, and Rh cDNAs were cloned and sequenced for some samples.

RESULTS—Caucasian JAL+ probands had *RHCE**Ce, while JAL+ probands with African ancestry had *RHCE**ce, but all had a nucleotide 340C>T change in Exon 3 of *RHCE* predicted to encode Arg114Trp. The JAL-encoding *RHCE**ce also had 733C>G (Leu245Val) and was linked to conventional *RHD* or to *RHD**DAU0.

CONCLUSIONS—JAL+ results from a nucleotide 340C>T (Arg114Trp) on either a Ce or ce background. Homology modeling of the JAL+ RhCE protein suggests that the Arg→Trp change eliminates a critical loop-stabilizing H-bond between the side chain of Arg114 and the e-specific amino acid Ala226. Additionally, accommodation of the bulky tryptophan would disrupt the conformation of the extracellular loops containing C/c- and e-specific amino acids, providing a structural hypothesis for the simultaneous altered expression of C/c, e, and V/Vs antigens.

The 50 antigens in the Rh system have been given International Society of Blood Transfusion (ISBT) numbers based on serologic definition. The molecular background responsible for expression of the majority of these has been determined, with a few exceptions, which include JAL (Rh48).¹

The antigen that later became known as JAL was first recognized in 1977 when the red blood cells (RBCs) of the third child of an African American woman had a strongly positive direct antiglobulin test, and the mother's serum sample reacted with the RBCs of her husband. Ten years later, the JAL antigen was again found to be the cause of hemolytic disease of the fetus and newborn, this time in a Swiss family. Over the years, serologists have shared serum samples

from these two probands (S. Allen and J. Pas), so that by 1990 it was recognized that two JAL + (Rh48+) haplotypes existed. One, found in Caucasians, had altered C and e antigens (C)(e), and the other, found in people of African ancestry, had altered c and e antigens (c)(e).^{2,3}

This study was undertaken to determine the molecular basis for JAL expression and to investigate the location and structural alterations in the proteins. JAL+ samples from frozen RBC collections, including the husband of the original producer of anti-JAL, and recent samples referred for investigation were included. The serologic investigation detailing expression of Rh antigens on RBCs from these samples confirmed previous observations of depressed C or c antigen expression and showed that the presence of JAL antigen has not only a quantitative, but also a qualitative, effect on Rh antigens C or c, e, V, VS, hr^B, and hr^S.⁴ We report here that expression of JAL (Rh48) results from a single nucleotide 340C>T change and provide a structural hypothesis for the simultaneous altered expression of C/c, e, and V/VS antigens.

MATERIALS AND METHODS

JAL+ samples

Samples from 17 JAL+ people were included in the study: 2 Caucasian (Swiss and UK), 6 African American, 7 African Brazilian, 1 African Caribbean (UK), and 1 African Puerto Rican. These represent historical JAL+ samples identified in the 1970s and 1980s, including a sample from the index case, and additional samples detected during our extensive investigation to determine the molecular basis of JAL. These samples presented with a diverse array of serologic anomalies, including weak, absent, or discrepant C, c, or e typing and/or production of anti-c or anti-e in an antigen-positive patient or production of an anti-Rh17-like specificity (anti-CEST). A summary of the history, presentation, and serologic characterization of Rh antigen expression, including JAL, on the RBCs of these samples is reported elsewhere.⁴

Testing selected variant RBC samples with anti-JAL

RBCs from selected samples, previously characterized at the molecular level, were tested with anti-JAL (J. Pas serum) by standard tube agglutination in albumin followed by anti-human immunoglobulin G.

Genomic DNA extraction, amplification, and sequencing

Genomic DNA was isolated with a DNA extraction kit (QIAamp DNA blood mini kit, Qiagen, Inc., Valencia, CA) from white blood cells collected in ethylenediaminetetraacetate or from RBC droplets frozen in liquid nitrogen. Polymerase chain reaction (PCR) amplification was performed with RH-specific primers (Invitrogen, Carlsbad, CA), detailed in Table 1, and the products were analyzed by PCR–restriction fragment length polymorphism (RFLP) or direct sequencing by the University of Pennsylvania or the New York Blood Center DNA Sequencing Facility.

RNA extraction and Rh cDNA cloning and sequencing

RNA was isolated from the RBCs of Samples 2 and 5 (QIAzol, Qiagen, Inc.). Reverse transcription was carried out with a first-strand synthesis system and random hexamers and oligo(dT) primer, according to the manufacturer's instructions (Superscript II, Invitrogen). 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 (MiniElute PCR purification, Qiagen, Inc.), and cloned into a TA cloning vector (PCR2.1, TOPO, Invitrogen) for sequencing. Sequences were aligned and protein sequence comparisons were performed with ClustalX.

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

PCR-multiplex analysis was performed to detect *RHD* Exons 4 and 7 and the inactivating *RHD* pseudogene and for C/c typing.⁵ *RHD* zygosity was determined by assaying for the presence of the hybrid Rhesus box and was confirmed for some samples by *Pst*I-RFLP.^{6,7}

RHD was further analyzed by amplification of Exon 5 followed by *Hinc*II-RFLP for the 667T>G polymorphism and *Taq*I-RFLP for the 697G>C change. Exon 8 was amplified and a *Nla*III-RFLP was designed to detect the 1136C>T polymorphism associated with DAU alleles. The specific DAU allele present was further discriminated by amplification and sequencing of Exons 5, 6, and 7. Allele-specific PCR was performed to detect the Exon 3 nucleotide 455A>C associated with DIIIa and the common RHD-CE-D hybrid.

RHCE was analyzed by amplification of Exon 1, followed by *Apa*I-RFLP for 48G>C. After Exon 5 amplification, a *Bfa*I-RFLP was performed to detect the 733C>G polymorphism associated with the V+VS+ phenotype, and a *Mnl*I-RFLP was used to probe the nucleotide 676C>G polymorphism associated with E/e. Allele-specific PCR was performed to detect the Exon 7 1006G>T polymorphism associated with a V-VS+ phenotype. Amplification and sequencing of Exons 3 and 5 were also performed on all samples. Sample 3, from the husband of the original maker of anti-JAL, was further investigated after an additional mutation was found in *RHCE* Exon 5. *RHCE* Exon 5 products were cloned and sequenced to discriminate the two separate alleles. Exons 6 and 8 were also amplified and sequenced.

Homology modeling

The Rh homology model was derived with the use of the *Nitrosomonas* Rh1 crystal structure as template.^{8,9} The human Rh and *Nitrosomonas* Rh1 protein sequences were initially aligned with ClustalX and further adjusted visually. The model was obtained by submitting the alignment to Swiss-Model and viewed with DeepView/Swiss-pdbViewer.^{10,11}

RESULTS

DNA analyses

The serologic typing of the RBCs suggested that JAL was encoded by *RHCE*,⁴ but both *RHCE* and *RHD* were investigated in the samples to predict the haplotypes associated with JAL+ phenotypes. The results of genomic testing are summarized in Table 2.

RHCE analyses

Multiplex testing confirmed the presence of *RHCE**C in all C+ samples, including those with weak C antigen expression (samples 1 and 2), and *RHCE**c in all c+, including those with discrepant or weak c expression (Samples 4, 5, 6, 7, 12, and 13).⁴ All *RHCE**C-positive samples had the characteristic 48C in Exon 1, and this polymorphism was also present in the ce alleles of Samples 3 and 8. This polymorphism, when present in a ce allele, is associated with weakened expression of e antigen.¹² Exon 5 analysis for *RHCE**E/e was consistent with the E/e RBC typing for all samples, including those with discrepant or weak e expression (Samples 2, 8, 10, 11, 12, 13, and 15).⁴ Samples from persons with African ethnicity had the 733C>G polymorphism associated with V+VS+, and six (Samples 11–16) were homozygous 733G/G. None of the samples carried the nucleotide 1006G>T change in Exon 7 associated with V-VS+. Of significance, all JAL+ samples had a nucleotide 340C>T change in Exon 3 of *RHCE*, predicted to encode a Arg114Trp, regardless of the background Ce or ce allele (Table 2). Three individuals, an African American (Sample 12) and two Brazilian siblings (Samples 13 and 15), were homozygous 340T (Table 2).

The index case (Sample 3) had an additional nucleotide 712A>G change, predicted to encode Met238Val, in Exon 5. A 712A>G change has been reported in several variant *RHCE***ce*, specifically *ceBI*, *ceEK*, or *ceAR*. However, these alleles have additional changes in Exons 6 and 8 or in Exon 5. Amplification and sequencing of *RHCE* Exons 5, 6, and 8 revealed no additional changes in Sample 3. Exon 5 *RHCE* products from Sample 3 were also cloned and sequenced, and the 712G change was found on separate fragments from those with 733G, indicating that 712G and 733G reside on different *ce* alleles, that is, in trans.

RHD analyses

Zygoty testing indicated none of the samples had a *RHD* deletion, suggesting they were *RHD* homozygous. (Samples 1 and 17 were insufficient for zygoty testing.) Assays for changes in Exon 5 associated with expression of partial D antigen indicated no change, but 13 of the 17 samples had 1136C>T in Exon 8, predicted to encode Thr379Met, characteristic of *RHD***DAU*.¹³ Analysis of *RHD* Exons 5, 6, and 7 indicated that these samples were *RHD***DAU0* (data not shown).

Testing variant RBC samples with anti-JAL

To determine that JAL+ is independent of the 712G-encoded 238Val present in the index JAL+ proband, and independent of 379Met encoded by *DAU0*, we tested RBCs from samples previously RH genotyped and known to carry these changes, but that lacked the JAL-encoding 340T. Table 3 shows the results of testing RBC with anti-JAL (J. Pas). All did not react with anti-JAL.

RH cDNA analyses

RH exon-specific genomic DNA amplification and sequencing cannot definitively confirm the specific allele on which a nucleotide change is located because two *RHCE* are present. Hybrid rearrangements between *RHD* and *RHCE* can also confound analyses. To definitively confirm the location and molecular basis of JAL, we analyzed the mRNA transcripts from reticulocyte-enriched RBCs by synthesis and cloning of Rh cDNAs from Sample 2, representing the JAL+ RhCe background, and from Sample 5, representing the JAL+ Rhce background. Three different Rh transcripts were found in Sample 2 (Fig. 1A). Consistent with the Rh phenotype, transcripts representing *RHD* and *RHCE***cE* were present, in addition to the *RHCE***Ce* transcripts, which all had the nucleotide 340T change, predicted to encode Arg114Trp. Four different Rh transcripts were identified in Sample 5 (Fig. 1B) representing conventional *RHD*, *RHD***DAU0*, *RHCE***Ce*, and *RHCE***ce* with 340T (Arg114Trp) and 733G (Leu245Val).

RH genotypes in JAL+ samples

Table 4 shows the 11 different presumed RH genotypes present in the 17 JAL+ people investigated. The *RHCE* associated with JAL+, as well as the *RHD* presumed to be in cis are bolded. Three individuals, one African American and two Brazilian sisters (Samples 12, 13, and 15), were homozygous for the JAL-encoding *RHCE***ce*. The JAL-encoding Rhce allele in African ethnic groups is presumed to be linked to a conventional *RHD* in five haplotypes and to *RHD***DAU0* in 13. JAL-encoding alleles were found in trans to several different haplotypes.

Homology model

The homology model of RhCE proteins derived with a *Nitrosomonas* Rh1 template is shown in Fig. 2. The models of RhCe and Rhce are identical in transmembrane helical structure and only the external loop structures differ. The Arg114 residue is located near the exterior surface of transmembrane helix 3 (TM3; Fig. 2A). The hydrophilic nature of the arginine side chain and its proximity to the interface of the plasma membrane and the hydrophilic exterior suggest

that the side chain of arginine would extend toward the membrane surface in a process called “snorkeling,” rather than remain within the hydrophobic phospholipid bilayer. The e-specific residue, Ala226, located on external loop 4, is also shown. In the model, one of the terminal nitrogens of the Arg114 side chain (Fig. 2A, blue) and the carbonyl oxygen (Fig. 2A, red) of Ala226 are close enough to form a hydrogen bond (Fig. 2A, green). Substitution of tryptophan for arginine is shown in Fig. 2B. The side chain of Trp is not as hydrophilic as Arg, so it would be more energetically favorable for Trp to remain within the phospholipid bilayer. However, the large, bulky, aromatic side chain of Trp would be difficult to accommodate within this space without structural displacement. The Trp side chain is also not able to form the hydrogen bond with Ala226. Figures 2C and 2D show the relationship of Arg114Trp with C/c-specific residues on loop 2 (Figs. 2C and 2D, yellow) and the e-specific residue on loop 4 (Figs. 2C and 2D, pink). The TM3 helix, where Arg114 is located, is at the base of external loop 2 where the C/c epitope is located.

DISCUSSION

Investigation of RH in 17 JAL+ samples firmly establishes that expression of the JAL (Rh48) antigen results from a nucleotide 340C>T change (Arg114Trp) on either the RhCe or the Rhce protein. Interestingly, the RHCE*Ce allele encoding the JAL antigen was reported previously and given the designation CeMA by Noizat-Pirenne and colleagues¹⁴ when investigating the cause of weak C antigen expression. Although this allele was not previously recognized to encode JAL, altered or weak C antigen expression is consistent with our serologic results.⁴ The RHCE*ce allele encoding JAL has previously been reported as ce^S(340) and was investigated because of altered or weak e antigen expression.¹⁵ The RHCE*ce^S(340) allele also has the 733C>G polymorphism associated with V+VS+, but was not recognized to encode JAL. However, altered or weak e antigen expression is consistent with our serologic results.⁴ We show here for the first time that the Arg114Trp change, which can be encoded on either a RHCE*Ce or a RHCE*ce allele, is associated with expression of the low-prevalence Rh antigen, JAL. In African black ethnic groups, RHCE*ce (340) was found in cis to conventional *RHD* or to *RHD**DAU0. *RHD**DAU0 is not uncommon in samples from African ethnic groups and has a frequency as high as 18 percent in Mali.¹⁶

This study of 17 individuals suggests that JAL arose by two or possibly three separate genetic events. In Caucasians, a 340C>T change was acquired on *RHCE**Ce, while in African blacks a 340C>T change occurred on *RHCE**ce in cis to conventional *RHD* and in cis to *RHD**DAU0. These may have occurred as separate events or, alternatively, through recombination of the JAL+ allele onto a conventional *RHD*-containing haplotype. JAL remains a low-prevalence antigen as molecular screening of 500 African American samples, from Philadelphia and referred from throughout the United States, for the 340T>C change detected one additional sample. Nevertheless, JAL should be considered in cases of unexplained hemolytic disease of the fetus and newborn in women demonstrating serum antibodies reactive with the paternal RBCs.

The original JAL+ proband was included in this study. Serologic investigation had previously determined that the RBCs express another low-prevalence antigen in addition to JAL.² Indeed, we found an additional polymorphism, a nucleotide 712A>G predicted to encode a Met238Val amino acid change, in the RHCE*ce allele in trans to the JAL-encoding allele. This allele, designated here as RHCE*ce(712), also has the 48C polymorphism and has not been previously reported. This allele may be the molecular basis for expression of an additional low-prevalence antigen and is under further investigation.

Expression of JAL in Caucasians is associated with altered C and e antigens (C)(e), while in people of African ancestry it is accompanied by altered c and e antigens (c)(e).^{2,3} Homology

modeling of JAL-expressing proteins suggests that Arg114 is in a strategic location to affect both the C/c and e epitopes. Arg114 is located near the exterior surface of the TM3 helix, which provides the base of the second external loop of RhCE, where C or c is located. At the same time, one of the terminal nitrogens of the Arg114 side chain forms a hydrogen bond with the e-specific Ala226 in the fourth external loop. Trp is not capable of maintaining this hydrogen bond, so it would be lost when 114Trp is present, as in the JAL variant. In addition, the side chain of Trp is not as hydrophilic as that of Arg, so it would be more energetically favorable for Trp to remain within the phospholipid bilayer. Trp has 12 possible rotamers in the model, and all of them are associated with steric interference between Trp and various amino acids in this region. Therefore, according to the model, the large, bulky, aromatic side chain of Trp cannot be accommodated within this area of the protein without inducing a change in conformation. We hypothesize that both the loss of the hydrogen bond between Arg114 and Ala226 and the conformational modification caused by the Trp side chain result in a change in the serologic detection of e and C/c when JAL is present.

All of the JAL+ individuals of African ancestry also have the Leu245Val change associated with V+VS+, although V and VS antigens are absent or only very weakly expressed on the RBCs in the presence of JAL.⁴ Leu245Val is predicted to be embedded in the membrane in alpha helix 8 of the RhCE protein. Leu and Val are both branched-chain amino acids with highly hydrophobic side chains. However, the side chain of Val has one less methylene group. The absence of the methylene group could lead to cavity formation in this region of the protein, making packing with side chains of adjacent residues less than optimal and leading to structural rearrangements in this region. Indeed, the same modification in alpha helices in other proteins has been shown to have major effects on structure.^{17,18} According to the model, the e-specific Ala226 is a helix-capping residue, which means it is important for maintaining the structure of the helix and, consequently, the structure of the loop. Therefore, loss of the Ala226 hydrogen bond with Arg114 in JAL+ and any subsequent conformational change in this region could lead to a radical change in loop structure, causing the disappearance or weakening of V and VS epitope(s).

In summary, samples from Caucasian JAL+ probands had *RHCE*Ce* and those from Black JAL+ probands had *RHCE*ce*, but all had a nucleotide 340C>T change in Exon 3 of *RHCE*, encoding Arg114Trp. The JAL-encoding *RHCE*ce* allele also has 733C>G (Leu245Val) and is often found in cis to *RHD*DAU0*. Homology modeling of JAL-expressing proteins suggests that the mutation from Arg to Trp eliminates a critical helix-stabilizing H-bond between the side chain of arginine and nearby residues and destabilizes the helix carrying e, C/c, or V/VS providing a structural hypothesis for the simultaneous altered expression of Rh C/c, e, and V/VS antigens.

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A.

B.

Fig. 1.

Diagram of *RHD* and *RHCE* haplotypes deduced from Rh cDNA transcripts isolated from (A) a Caucasian JAL+ (Sample 2) and (B) an African JAL+ (Sample 5). The 10 exons (coding regions) of *RHD* are shown as black boxes, and *RHCE* are white. Exon 2 of *RHCE**Ce is homologous to Exon 2 of *RHD*, so it is also represented by a black box. The location of the JAL 340 T>C change in *RHCE* Exon 3, encoding Arg114Trp, and the accompanying Exon 5 nucleotide 733G>C polymorphism, encoding Leu245Val and present only in the African JAL+ background, are shown as lines.

Fig. 2. Homology model wild-type RhCE and the JAL+ RhCE protein. Close-up views. (A) Wild-type with Arg114. (B) JAL+ 114Trp change. The alpha helical regions are shown in red, and the loops in gray. The amino acid side chains are shown in Corey-Pauling-Koltun (CPK) colors (oxygen = red, nitrogen = blue, carbon = white). The hydrogen bond between the side chain nitrogen of arginine and the carbonyl oxygen of alanine 226 is shown in green. Membrane views. (C) Wild-type, and (D) JAL+. The e-expressing loop is pink, and the C and c-expressing loop is shown as yellow. The models of RhCe and Rhce are identical in transmembrane helical structure, and only the external loop structures would differ.

TABLE 1

Primers used in this study*

Exon	Sequence	Sense/antisense	Region	Location
<i>RHCE</i>				
1	gcacacaggATGAGCTCTAA	Sense	D/CE UTR5'/Exon 1	−9 (I) to 11 (E)
	Agatgggggaatctttctc	Antisense	CE Intron 1	+106 to 126
3	Gcccaacagtgtttgtgaa	Sense	D/CE Intron 2	−107 to −88
	Atattgccaggttcattc	Antisense	CE Intron 3	+299 to 317
5	Gaggttgctgagccaagatcg	Sense	CE Intron 4	−332 to 354
	Tatgtgtgctagtcctgtagac	Antisense	D/CE Intron 5	+34 to 56
6	Gcagtagtgagctggcccacgtgtcca	Sense	CE Intron 5	−194 to −167
	Cctgctggccttcagccaagcagagagca	Antisense	CE Intron 6	+21 to 50
7	Catagtgtgtccgtatg	Sense	CE Intron 6	−256 to −275
	GTGATCTCTCCAAGCAGACC	Antisense	CE Exon 7	1006 to 1025
	GTGATCTCTCCAAGCAGACA	Antisense	CE Exon 7	1006 to 1025
8	Ctggaggctctgagaggttaaa	Sense	CE Intron 7	−348 to −327
	Catagacatccagccacacggca	Antisense	D/CE Intron 8	+66 to 88
<i>RHD</i>				
3	TTGTCGGTGCTGATCTCAGTGGA	Sense	D Exon 3	363 to 383
	GATATTACTGATGACCATCCTCATGG	Antisense	CE Exon 3	480 to 455
	GATATTACTGATGACCATCCTCATGT	Antisense	D Exon 3	480 to 455
5	Taagcacttcacagagcagg	Sense	D Intron 4	+171 to 190
	Tatgtgtgctagtcctgtagac	Antisense	D/CE Intron 5	+34 to 56
6	Gagtgtgatgggtgcctaggtgctgtgcacct	Sense	D/CE Intron 5	−258 to −290
	Cctgctggccttcagccaagcagagagg	Antisense	D Intron 6	+21 to 50
7	cttcatttcaacaactccccga	Sense	D Intron 6	−160 to 182
	gtgataaatccatccaaggtagggccggccagaat	Antisense	D Intron 7	+149 to 184
8	ctggaggctctgagaggttgag	Sense	D Intron 7	−327 to −348
	catagacatccagccacacggca	Antisense	D/CE Intron 8	+66 to 88
RH-cDNA	ATGAGCTCTAAGTACCCGC	Sense	D/CE	1 to 19
	<u>cgacggatcc</u> AAATCCAACAGCCAAAT	Antisense	D/CE	1235 to 1251

* Lower case letters are used for nucleotides in introns and those in exons are in uppercase letters. Nucleotides added to design a restriction enzyme site are underlined.

TABLE 2

RH genomic analyses

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Multiplex C/c	Cc	Cc	cc	Cc	Cc	Cc	Cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	Cc
RHCE																	
Exon 1 48G>C	GC	GC	GC	GC	GC	GC	GC	GC	GC	GC	GC	GC	GC	GC	GC	GC	GC
Exon 3 340C>T JAL	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	TT	TT	CT	TT	CT	CT
Exon 5 676C>G	GG	CG	GG	GG	GG	GG	GG	GG	CG	CG	GG	GG	GG	GG	GG	GG	GG
E/e	ee	Ee	ee	ee	ee	Ee	ee	ee	Ee	Ee	ee	ee	ee	ee	ee	ee	ee
712A>G	AA	AA	AG	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
733C>G V+VS+	CC	CC	CG	CG	CG	CG	CG	CG	CG	CG	GG	GG	GG	GG	GG	GG	CG
Exon 7 1006G>T	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG
RHD																	
Zygosity	NT	D/D	D/D	D/D	D/D	D/D	D/D	D/D	D/D	D/D	D/D	D/D	D/D	D/D	D/D	D/D	NT
Exon 8 1136C>T DAU0	CC	CC	CC	CT	CT	CT	CT	CT	CT	CC	CT	CT	CT	CT	CT	CT	CT

NT = not tested.

TABLE 3

Results of testing RBCs from RH genotyped samples that share some nucleotide changes with some of the JAL+ samples in this study*

RH genotype	Relevant nucleotide change(s)	Anti-JAL (J. Pas)
<i>RHD</i> * <i>DOL-RHCE</i> * <i>ceBI</i> / <i>RHD</i> * <i>DOL-RHCE</i> * <i>ceEK</i>	712A>G, Met238Val	0
<i>RHD</i> * <i>DOL-RHCE</i> * <i>ceBI</i> / <i>RHCE</i> * <i>ce16Cys</i>	712A>G, Met238Val	0
<i>RHD</i> * <i>DAR-RHCE</i> * <i>ceAR</i> / <i>RHCE</i> * <i>ce</i> (r)	712A>G, Met238Val	0
<i>RHD</i> * <i>DAR-RHCE</i> * <i>ceAR</i> / <i>RHCE</i> * <i>R^N</i>	712A>G, Met238Val	0
<i>RHD</i> * <i>DAR-RHCE</i> * <i>ceEK</i> / <i>RHD-CE(3-7)D-RHCE</i> * <i>ce^S</i> (r'S)	712A>G, Met238Val	0
<i>RHD</i> * <i>DAU0-RHCE</i> * <i>ce16Cys</i> / <i>RHD-CE(3-7)D-RHCE</i> * <i>ce^S</i> (r'S)	1136C>T Thr379Met	0
<i>RHD</i> * <i>DAU0-RHCE</i> * <i>ceMO</i> / <i>RHD</i> * <i>DAR-RHCE</i> * <i>ce</i>	1136C>T Thr379Met	0
<i>RHD</i> * <i>DAU0-RHCE</i> * <i>ce16Cys</i> / <i>RHD-RHCE</i> * <i>cE</i> (R ₂)	1136C>T Thr379Met	0

* Alleles carrying the relevant change are in boldface.

TABLE 4

RH genotypes

Sample	Origin/ethnicity	<i>RHCE</i> *	<i>RHD</i> *
1	Caucasian UK	<i>CeMA</i>	<i>D</i>
		<i>ce</i>	
2	Caucasian Switzerland	<i>CeMA</i>	<i>D</i>
		<i>cE</i>	<i>D</i>
3	African American	<i>ce^S(340)</i>	<i>D</i>
		<i>ce(712)</i>	<i>D</i>
4	Caribbean UK	<i>ce^S(340)</i>	<i>DAU0</i>
		<i>Ce</i>	<i>D</i>
5	African American	<i>ce^S(340)</i>	<i>DAU0</i>
		<i>Ce</i>	<i>D</i>
6	African American	<i>ce^S(340)</i>	<i>DAU0</i>
		<i>Ce</i>	<i>D</i>
7	African American	<i>ce^S(340)</i>	<i>DAU0</i>
		<i>Ce</i>	<i>D</i>
8	African Brazilian	<i>ce^S(340)</i>	<i>DAU0</i>
		<i>ce48C</i>	<i>RHD</i>
9	Son of Subject 8	<i>ce^S(340)</i>	<i>DAU0</i>
		<i>cE</i>	<i>D</i>
10	Puerto Rican	<i>ce^S(340)</i>	<i>D</i>
		<i>cE</i>	<i>D</i>
11	African American	<i>ce^S(340)</i>	<i>DAU0</i>
		<i>ce^S</i>	<i>D</i>
12	African American	<i>ce^S(340)</i>	<i>DAU0</i>
		<i>ce^S(340)</i>	<i>D</i>
13	African Brazilian	<i>ce^S(340)</i>	<i>DAU0</i>
		<i>ce^S(340)</i>	<i>D</i>
14	Sister of Subject 13	<i>ce^S(340)</i>	<i>DAU0</i>
		<i>ce^S</i>	<i>D</i>
15	Sister of Subject 13	<i>ce^S(340)</i>	<i>DAU0</i>
		<i>ce^S(340)</i>	<i>D</i>
16	Sister of Subject 13	<i>ce^S(340)</i>	<i>DAU0</i>
		<i>ce^S</i>	<i>D</i>
17	Child of Subject 13	<i>ce^S(340)</i>	<i>DAU0</i>
		<i>Ce</i>	<i>D</i>

ce^S = 16Cys, 245Val (V+/VS+).

ce^S(340) = 114Trp (JAL), 245Val (V-/VS-).