RH DIVERSITY IN MALI: Characterization of a new haplotype *RHD*DIVa/RHCE*ceTI(D2)*

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ABBREVIATIONS
SCD = sickle cell disease;
SNP(s) = single-nucleotide polymorphism(s).

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ABSTRACT

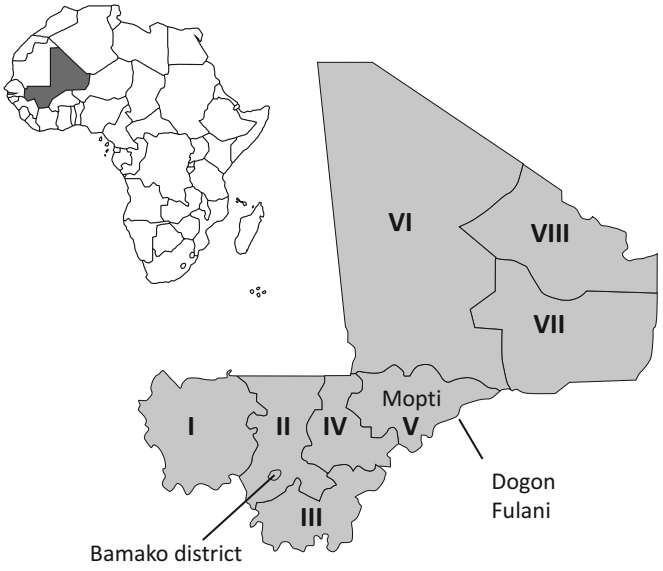
BACKGROUND
Knowledge of RH variants in African populations is critical to improving transfusion safety in countries with populations of African ancestry and to providing valuable information and direction for future development of transfusion in Africa. The purpose of this report is to describe RH diversity in individuals from Mali.

STUDY DESIGN AND METHODS
Blood samples collected from 147 individuals self-identified as Dogon and Fulani were analyzed for Rh antigens and alleles.

RESULTS
The most common *RHD* allele variant was *RHD*DAU0*. Five predicted partial-D phenotypes were attributed to *RHD*DAU3 or RHD*DIVa*. Neither *RHD*DAR* nor *RHD*DIIIa* was found. Investigation of *RHCE* revealed three predicted partial-e antigens encoded by *RHCE*ce(254G)* in trans to *RHCE*cE*. Regarding C antigen, 28 Fulani typed as C1 and 16 of 28 harbored at least one *RHCE*Ce-D(4)-ce*, two being homozygous and predicted to show a rare RH:32,246 phenotype. A new *RHCE*ceTI* with replacement of Exon 2 by *RHD (RHCE*ceTI(D2))* was identified in Dogon and was identified by inheritance study to be in cis to *RHD*DIVa*. These samples typed C– with anti-C polyclonal antibody and monoclonal antibodies (MoAbs) MS24, P3X25513681MS24, and MS273, but positive with anti-RhCe MoAb-BS58. The same pattern was observed in sample with *RHD*DIVa/RHCE*ceTI*.

CONCLUSION
Our survey indicated an uneven distribution of RH variant alleles between Dogon and Fulani, suggesting that study in well-documented cohorts is warranted. A high incidence of predicted partial-C phenotype encoded by *RHCE*Ce-D(4)-ce* was found in Fulani. Further study will also be needed to clarify the clinical significance of the new *DIVa/ceTI(D2)* haplotype encoding partial D and variant ce antigens.

Figure 1: Geographic localization of Mali.
The eight administrative regions (I to VIII) and Bamako district are shown. Dogon and Fulani were from Region V (Mopti).



The RH blood group is one of the most polymorphic and immunogenic blood group systems. Antibodies directed against Rh antigens have been implicated in hemolytic disease of the fetus and newborn, hemolytic transfusion reactions, and autoimmune hemolytic anemia. Rh antigens are encoded by the homologous *RHD* and *RHCE* genes that share 93.8% homology over all introns and coding exons. These two genes consist of 10 exons each and are closely linked in opposite orientation on chromosome 1p36.11.[1]

The complexity of the RH blood group is related to the high diversity of Rh antigens (n 5 54) and RH variant alleles.[2] RH alleles originate from a variety of molecular mechanisms including single-nucleotide polymorphism (SNP), genetic conversion, crossing over, and insertion- deletion. Most RH allele variants have been encountered in people of African ancestry. Some variants such as *RHD*DAU3*, *RHCE*ceTI*, and *RHCE*Ce-D(4)-ce* (also known as *RHCE*CeRN*) encode proteins considered as partial due to their association with antibody production.[3-5] Other variants are referred to as weak since no immunization has been described. Interlinkage between *RHD* and *RHCE* can lead to phenotypes with both partial D and c and/or e antigens as observed for *RHD*DIIIa/RHCE*ce(1025T),4 RHD*DIVa/RHCE*ceTI,4* and *RHD*DOL/RHCE*ceBI* haplotype.[6]

Amino acid changes encoded by DNA polymorphisms can induce expression of low-prevalence antigens or lack of expression of high-prevalence antigens. Accordingly, Rh proteins encoded by *RHD*DIIIa*, *RHD*DOL*, or *RHCE*Ce-D(4)-ce* express the low-prevalence RH:54 antigen that is presumed to be of clinical significance.[2] Several rare phenotypes have been observed. RH:218 is encountered in individuals who are apparent homozygous for RHD*DAR in cis to *RHCE*ceAR* or **ceEK* (or compounds heterozygous). RH:234 has been linked to homozygosity for the *(C)ceS Type 1, (C)ceS Type 2*, or *RHD*DIIIa/RHCE*- ce(733G,1006T)* haplotypes. RH:46 is lacking in individuals who are homozygous for RHCE*Ce-D(4)-ce allele.[7-9] Production of anti-RH18, anti-RH34, or anti-RH46 can be clinically significant requiring special precautions for pregnancy and transfusion, for example, use of equivalent rare antigen-negative red blood cells (RBCs).[7]

In a recent editorial in TRANSFUSION, it was suggested testing of large cohorts of selected ethnic groups to assess allele and phenotype prevalence is essential to allow future evidence-based decisions[10] to optimize transfusion safety, especially for sickle cell disease (SCD) patients who undergo chronic transfusions. The purpose of this report is to describe genetic diversity observed by sequencing *RHD* and *RHCE* alleles in a random survey of individuals from two ethnic groups in Mali.

MATERIALS AND METHODS

Serologic study

Ethylenediaminetetraacetate (EDTA) blood samples were collected from 147 individuals self-identified as Dogon (n 5 101) and Fulani (n = 46) in Region V of Mali (Mopti; Fig. 1). Participants provided written informed consent. Study and consent protocols were approved by the Comité d'Ethique Institutionnel de la Faculté de Médecine, de Pharmacie et d'Odontostomatologie in Mali.

All blood samples were phenotyped for D, C, E, c, and e antigens using the gel column agglutination method (Ortho Clinical Diagnostics, Illkirch, France) with the following monoclonal antibodies (MoAbs): D7B8 for anti-D, MS24 for anti-C, C2 for anti-E, MS42 for anti-c, and *MS16 + MS21 + MS63* for anti-e. Additional testing for C expression was performed on one sample with *RHD*DIVa/ RHCE*ceTI(D2)* haplotype and samples with the *RHD*DIVa/ RHCE*ceTI* haplotype. Direct agglutination test (DAT) in gel matrix, test tube, and microplate was performed using anti- C MoAbs MS24 (Ortho Clinical Diagnostics), MS273 (Eurobio, Courtaboeuf, France), P3X2551368+MS24 (Diagast, Loos, France), respectively. Indirect antiglobulin tests (IATs) were performed with anti-Ce MoAb BS58.[11]

Sequencing of RHD and RHCE

Genomic DNA was isolated from 200 µl of whole blood using a blood DNA mini-kit (QIAmp, Qiagen, Courtaboeuf, France) according to the manufacturer's instructions. Polymerase chain reaction (PCR) assay was performed on the 10 exons of the *RHD* and *RHCE* genes as previously described.[12] Briefly, amplification was performed with 100 ng of genomic DNA in a final volume of 50 µL containing PCR buffer, 2 mmol/L $MgCl_2$, 80 ng/µL bovine serum albumin, 0.2 mmol/L of each dNTP, 0.05 unit of *Taq* DNA polymerase, and 200 nmol/L of each primer. Touchdown PCR included a 5 minute denaturation step at 94 °C followed by amplification cycles consisting of 30 seconds at 94 °C, 30 seconds at the annealing temperature, and 1 minute at 72 °C. Hybridization temperature was lowered 1 °C every 2 cycles from 66 to 61 °C and then a 30-cycle annealing step was performed at 60 °C. Determination of the presence or absence of each exon of *RHD* and *RHCE* genes was performed on 2% agarose gel and visualized using DNA stain (Sight, Euromedex, Strasbourg, France). PCR products were sequenced using the Sanger technique (GATC Biotech, Konstanz, Germany). Sequence alignment to identify polymorphisms was performed using computer software (SeqMan Pro, DNASTAR, Inc., Madison, WI). The presence of the *RHCE*Ce-D(4)-ce* allele was confirmed by allelic discrimination using probes (TaqMan, Life Technologies, Carlsbad, CA) as previously described.[5]

Allele frequencies were determined by counting. Since no *RHD* zygosity determination was performed, results were based on equencing data. We assumed that any sample with heterozygous polymorphism (SNP or STR in coding or noncoding sequence) was dizygous. When no information on zygosity was available (apparent homozygous), *RHD* allele frequency was calculated in the two extreme conditions (hemizygous and dizygous).

Table 1: Rh phenotypes in Dogon and Fulani

Rh phenotype	Dogon (n = 101)		Fulani (n = 46)		Total (n = 147)		Frequency in Africa[2] (%)
	Number	Frequency (%)	Number	Frequency (%)	Number	Frequency (%)	
D+C-c+E-e+	61	60.4	16	34.8	77	52.4	45.8
D+C+c+E-e+	14*	13.9	21	45.7	35	23.8	21.0
D+C-c+E+e+	17	16.8	1	2.2	18	12.2	18.6
D-C-c+E-e+	4	4.0	2	4.3	6	4.1	6.8
D+C+c+E+e+	3*	3.0	1	2.2	4	2.7	4.0
D+C+c-E-e+	0	0	5	10.9	5	3.4	2.0
D+C-c+E+e-	0	0	0	0	0	0.7	0.2
D-C+c+E-e+	2*	2.0	0	0	2	1.4	Rare
D-C-c+E+e+	0	0	0	0	0	0	Rare

* C+ phenotype was due to (C)ceS Type 1 haplotype in two samples with D+C+c+E-e+ phenotype, one sample with D+C+c+E+e+ phe- notype, and two samples with D-C+c+E-e+ phenotype.

Any polymorphism compared to conventional sequence was considered as defining a variant allele independently of clinical relevance (Table 2). Partial predicted c and e phenotypes were deduced from published data showing anti-e alloimmunization in e+ patient or anti-c in c+ patient.

RESULTS

D and CE phenotypes

A total of 147 blood samples collected from two ethnic groups (Dogon and Fulani) in Mali were phenotyped for D, C, E, c, and e antigens. Analysis of phenotype frequency in the overall cohort indicated a typical African profile with a predominance of D+C-c+E-e+ (52.4%) and D+C+c+E-e+ (23.8%; Table 1). Comparison of Dogon and Fulani demonstrated a number of differences. The D+C+c-E-e+ phenotype was absent in Dogon and frequent in Fulani (10.9%) while the D+C-c+E+e+ phenotype was uncommon in Fulani (2.2%) but frequent in Dogon (16.8%).

RHD allele variants

Eight samples, that is, six Dogon and two Fulani, were typed as D-. This phenotype was linked to homozygous *RHD* deletion in four Dogon and one Fulani or the presence of *RHD* pseudogene *(RHD*DPsi)* and/or *(C)ceS Type 1* haplotype. One Fulani was homo- or hemizygous for *RHD*DPsi*. One Dogon had *(C)ceS Type 1* in trans to *RHD* deletion and another was heterozygous for *RHD*DPsi* and *(C)ceS Type 1.*

No variant *RHD* allele was observed in 35 Dogon and 34 Fulani. In the remaining 78 samples, a variety of variant alleles were found (Table 2). The most common *RHD* allele variant was *RHD*DAU0* with a frequency of 0.217 to 0.247 in Dogon and 0.076 in Fulani. The *RHD*DAU0.1* allele was identified in six Dogon. Four *RHD* alleles encoding D partial phenotype were found with the most frequent being *RHD*DIVa* in Dogon (frequency, 0.029) and *RHD*DAU3* in Fulani (frequency, 0.043). Four samples with *RHD*DIVa* or *RHD*DAU3* allele homo- or hemizygous (or in trans to silent allele) were predicted to express a partial D phenotypes, that is, three in Dogon (2.9%) and one in Fulani (2.2%). All samples with predicted partial D phenotypes were typed D1 by hemagglutination.

RHCE allele variants

A total of 11 RHCE*ce variant alleles and one *RHCE*Ce* variant allele were identified in this population study. In addition, two samples carried a RHCE*cE allele with 48G>C transversion. The most frequent RHCE*ce variant allele was *RHCE*ce(48C)* with a frequency of 0.227 in Dogon and 0.152 in Fulani. Four alleles encoding partial e antigen, that is, *RHCE*ce(48C,254G), RHCE*ce(254G), RHCE*- ceMO*, and RHCE*ceTI, were identified. The most frequent allele encoding partial e antigen was *RHCE*ce(254G)*, occurred in 20 Dogon and 11 Fulani. One Dogon sample exhibited *RHCE*ceTI* characterized by a 48G>C and 1025C>T transitions and five Dogon samples exhibited a new allele (see below). Altogether, three samples were predicted to be partial for e antigen.

Twenty-eight of the 47 Fulani in this cohort were typed as C1 including 16 bearing at least one *RHCE*Ce- D(4)-ce* allele. Two amples were predicted to exhibit a rare RH:32,246 phenotype featuring a *RHCE*Ce-D(4)-ce* allele in homozygous state. Altogether, five Dogon and 15 Fulani exhibited a predicted partial C phenotype based on the presence of either (C)ceS Type 1 haplotype in Dogon or *RHCE*Ce-D(4)-ce* in Fulani.

Comparison of phenotype and genotype revealed that three out of five samples with the new allele as well as two samples with *RHCE*Ce-D(4)-ce* allele were C-. No other discrepancies were observed.

New RHCE*ceTI(D2) allele

Sequencing of genomic DNA revealed a new *RHCE* allele in five Dogon. This allele displayed 48C, 150T, 178A, 201G, 203G, 307T (Table 3), and 1025T. The 48C and 1025T SNPs are characteristic of the *RHCE*ceTI* allele. The remaining are common to Exon 2 of the *RHD* gene and *RHCE*C* allele. Since the new allele did not exhibit the 109-bp insertion characteristic feature of the *RHCE*C* allele (data not shown), we assume that the new allele was a *RHCE*ce* variant with Exon 2 being replaced by its *RHD* counterpart. The presence of heterozygous SNPs in exons and introns ascertains the amplification of two alleles for *RHCE* Exons 1 to 3. Two samples (Table 3, Samples 1 and 5) showed 186T in Exon 2 of *RHD* because of *RHD*DIVa* in trans to *RHD*DIIIa-CE(3-7)-D*. Therefore the Exon 2 with 150T, 178A, 201G, 203G, and 307T amplified during *RHCE* analysis is not carried by RHD locus. Altogether, DNA sequencing showed that the new RHCE allele was a *RHCE*ce-D(2)-ce* hybrid allele with 48C and 1025T, which is thereafter referred to as *RHCE*ceTI(D2)*.

Table 2: Distribution of RHD and RHCE variants identified in Dogon and Fulani

Allele and haplotype variant	Predicted Rh partial antigen*	Number of alleles		Allele frequency		Published frequency			
		Dogon (n=202)	Fulani (n=92)	Dogon (n=202)	Fulani (n=92)	Sub-Saharan Africa[12]	French Fy(a–b–) donors[20]	SCD patient (France)[17]	SCD patient (US)[16]
RHD		84-102	51-78	0.416-0.0500	0.554-0.848	NT	NA		NA
*RHD*DIVa*	D	6	0	0.029		0.009	0.019	0.018	0.009
*RHD*DAU3*	D	4-5	4-5	0.019-0.024	0.043-0.054	0.030-0.034	0.041	0.021	0.018
*RHD*DAU5*	D	2	0	0.009		0.016	0.019	0.015	0.020
*RHD*DFR-2*	D	1	0	0.004			NA		
*RHD*weak Type 4.0*		5	0	0.024		0.018-0.020	0.038	0.022	0.049
*RHD*DAU0*		44-50	7	0.217-0.247	0.076	0.177-0.261	0.022	0.187	0.164
*RHD*DAU0.1*		6-8	0	0.029-0.039		NT	NT	NT	NT
*RHD*DIIIa-CE(4-7)-D†*	C	5	0	0.024		0.027	0.032	0.026	0.046
*RHD*DPsi*		7	1-2	0.034	0.010-0.020	0.050-0.057	0.107	0.028	0.027
RHD deletion‡		8	2	0.199	0.147	NT	NA	NT	NT
*RHCE*cE(48C)*		2	0	0.009					
RHCE ce(48C)*		47	14	0.227	0.152	0.252	NA	0.186	0.192
*RHCE*ce(48C,105T)*		2	0	0.009		NT	NT		
*RHCE*ce(48C,254G)*	e	1	0	0.004		NT	NT		
*RHCE*ce(254G)§*	e	20	11	0.099	0.119	NT	NT	0.047	0.058
*RHCE*ceTI(D2)*		5	0	0.024			NT		
*RHCE*ceTI§*	c, e	1	0	0.004		0.020	0.032	0.015	0.033
*RHCE*ceMO§*	c, e	1	0	0.004		0.030	0.028	0.012	0.015
*RHCE*ce(733G)§*	c, e	16	14	0.074	0.152	0.182	NA	0.224	0.197
*RHCE*ce(48C,733G)§*	c, e	21	5	0.108	0.054				
*RHCE*ce(733G,1006T)†§*	c, e	1	0	0.004			NA	0.006	0.006
*RHCE*ce(48C,733G,1006T)†§*	c, e	4	0	0.019		0.030	0.016		
*RHCE*Ce-D(4)-ce§*	C	0	18		0.195		0.030	0.006	0.002

* Partial predicted phenotypes were deduced from published data showing anti-e alloimmunization in e1 patient or anti-c in c1 patient.
† Part of (C)ceS Type 1 haplotype.
‡ Frequency of RHD deletion was calculated based on homozygous samples.
§ Alleles encoding protein that lacks expression of high-prevalence antigen(s). NA 5 not available; NT 5 not tested.

Table 3: Relevant nucleotide polymorphisms in samples with RHD*DIVa/RHCE*ceTI(D2) haplotype*

		RHD		RHCE	
Sample	Genotype	Intron1/Exon 2	Exon 1	Intron 1/Exon 2	Intron 2/Exon 3
1	*DIVa ceTI(D2)*	*IVS1-485g/a*	48C	*IVS1-204c/t*	*IVS2-91a/g*
	(C)ceS Type 1	*IVS-376g*		*IVS1-29g/c*	*IVS2-32c/t*
		186T		*IVS1-20a/g*	
				150C/T	
				178C/A	
				201A/G	
				203A/G	
				307C/T	
2	*DIVa ceTI(D2)*	*IVS1-376a/g*	48G/C	*IVS1-204c/t*	*IVS2-91a/g*
	Weak D Type 4.0 ce	*IVS-29c*		*IVS1-20a/g*	*IVS2-32c/t*
		186G/T		*150C/T*	
				178C/A	
				201A/G	
				203A/G	
				307C/T	
3	*DIVa ceTI(D2)*	*IVS1-376a/g*	48G/C	*IVS1-204c/t*	*IVS2-91a/g*
	DAU0.1 ce(254G)	*186G/T*		*IVS1-20a/g*	*IVS2-32c/t*
				150C/T	
				178C/A	
				201A/G	
				203A/G	
				254C/G	
				307C/T	
4	*DIVa ceTI(D2)*	*IVS1-766g/a*	48C	*IVS1-204c/t*	*IVS2-91a/g*
	DAU0 ce(48C)	*IVS1-376a/g*		*IVS1-20a/g*	*IVS2-32c/t*
		186G/T		*150C/T*	
				178C/A	
				201A/G	
				203A/G	
				307C/T	
5	*DIVa ceTI(D2)*	*IVS1-485g/a*	48C	*IVS1-204c/t*	*IVS2-91a/g*
	(C)ceS Type 1	*IVS1-376a/g*		*IVS1-20a/g*	*IVS2-32c/t*
		IVS1-29c		*150C/T*	
		186T		*178C/A*	
				201A/G	
				203A/G	
				307C/T	

* Heterozygous SNPs are in italics.

Study of intron sequence also revealed that, like *RHCE*ceTI*, this allele also bore three polymorphisms in Introns 2 and 6, that is, IVS2-91a>g, IVS2-32C>T, and IVS6 1 52C>T.

To support the molecular basis of the new allele, an inheritance test was carried out to study transmission in two families. As shown in Figure 2, the *RHCE*ceTI(D2)* allele is in cis to the *RHD*DIVa* allele and the haplotype *RHD*DIVa/RHCE*ceTI(D2)* was integrally transmitted.

Based on the nucleotide sequence observed in coding regions, samples bearing *RHCE*ceTI(D2)* might express all or part of C antigen. Thus, hemagglutination to detect C expression was performed in one sample bearing *RHD*DIVa/RHCE*ceTI(D2)* and in samples with *RHD*DIVa/RHCE*ceTI*. In all cases, DAT in gel matrix, tube, and microplate using anti-C MoAbs MS24, MS273, and P3X2551368+MS24, respectively consistently showed a C- phenotype (Table 4). As previously reported,[11] MoAb BS58 showed no reactivity on C-c+E+e- sample, a weak reactivity on C-c+E-e+ sample, and a strong one on C+c-E-e+ sample. IAT with anti-Ce MoAb BS58 showed reactivity similar to that observed in the C+c-E-e+ control sample and sample with *RHD*DIVa/RHCE*ceTI* haplotype (Fig. S1, available as supporting information in the online version of this paper). Unfortunately, because of paucity of blood sample neither RNA analysis nor further serologic investigations using a larger anti-C panel were achieved.

DISCUSSION

Antibodies against Rh antigens have been implicated in transfusion reactions and hemolytic disease of the fetus and newborn. The high incidence of variant RHD or RHCE alleles in African black persons has been deduced from clinical experience and screening for certain variant RH alleles.12-17 However, data on large cohorts of selected ethnic groups are lacking. Only one study investigating RHD variants has been performed in Mali in blood donors.[13] The purpose of this report is to describe a comprehensive study of RHD and RHCE variants in Dogon and Fulani in Mali. This approach was used because Mali is a multiethnic country and some RH variants repeatedly occur in a single ethnic group.[8]

Phenotyping demonstrated a profile comparable to those reported previously in African populations.[2] However, a number of frequency differences were observed between the two ethnic groups. Ethnic differences have already been noted in Nigeria.[18,19]

Figure 2: Inheritance study of RHD*DIVa/RHCE*ceTI(D2) haplotype in two families

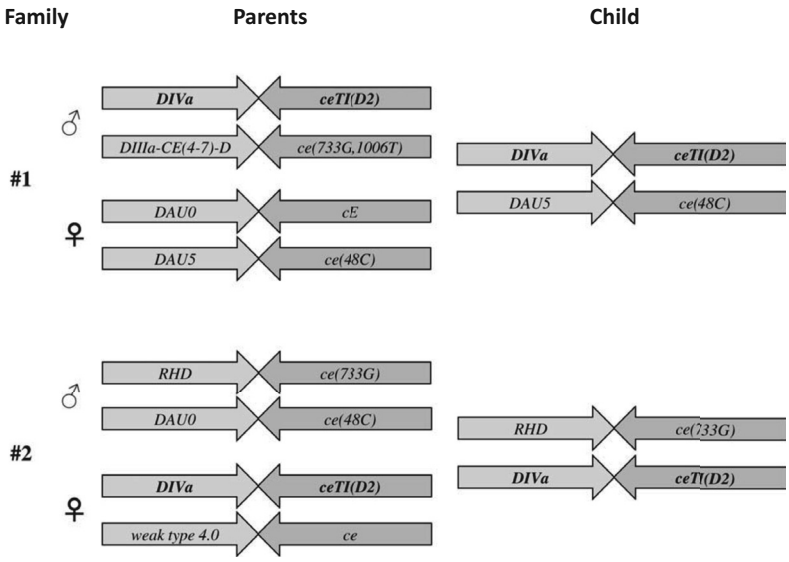


Table 4: DAT characterization of *DIVa/ceTI(D2)* and comparison with *DIVa/ceTI*

Technique	Anti-C clone	Reactivity	
		RHD*DIVa/ RHCE*ceTI(D2)	RHD*DIVa/ RHCE*ceTI
Tube	MS273	-	-
Plate	P3X2551368 + MS24	-	-
Gel matrix	MS24	-	-
	BS58*	+	+

* Reactivities using anti-Ce MoAb BS58 are shown in Fig. S1 (available as supporting information in the online version of this paper).

Results of RH genotyping were roughly similar to those of previous data.[12,16,17,20] The most common RHD alleles were belonging to the usual three African D clusters, that is, DAU, DIVa, and weak D Type 4.[21] As expected, alleles from the DAU cluster were the most frequent[12,22] and three out of five partial predicted D phenotypes were related to the *RHD*DAU3* allele. This supports the suggestion of Wagner and colleagues[3] that DAU cluster is a major source of D variability and anti-D immunization in patients of African ancestry. The frequency of the *RHD*DAU0.1* allele was 0.029 to 0.039 in Dogon compared to 0.017 in blood donors from Mali.[13] The frequency of *RHCE* alleles was broadly similar to previous reports with a high frequency of *RHCE*ce(48C)*.[12,20,23] However, frequencies of *RHCE*ceMO* and *RHCE*ceTI* were lower than that reported by Granier and coworkers[12] who investigated 220 African samples from six ethnic groups. The most likely explanation for this discrepancy is cohort size. Our data showed that the frequency of RHCE*ce(254G) was around 10% in both ethnic groups and that it accounted for all the partial predicted e phenotypes (3/3); lower frequencies (around 5%) were noted in SCD patients.[16,17]

Neither *RHD*DIIIa* allele nor *RHD*DAR/RHCE*ceAR/*ceEK* haplotype was detected in this study. This finding was surprising since the frequency of these alleles and haplotypes ranged from 3% to 5% in other studies[12,15] and indicated that African populations may be more heterogeneous than previously suggested.[12] The frequency of the *RHD*DIVa* allele (today considered to be the same allele as *RHD*DIVa.2*[24]) in Dogon was 3%. This is interesting since this allele was absent in samples from Congo-Brazzaville and Kenya whereas it showed a frequency of approximately 10% in two West African ethnic groups (Mandenkas and Yorubas).[12] This observation supports the notion that the *RHD*DIVa* allele is specific to or at least more frequent in West Africa. The geographic origin of investigated samples could at least partially explain the wide range of *RHD*DIVa* frequencies previously reported which were 0.018 and 0.019 in French blood donors from African origin and SCD from France, respectively compared to 0.009 in both sub-Saharan Africa and SCD from the United States.[12,16,17,20]

Several differences were observed between Dogon and Fulani. The frequency of *RHD*DAU0* was 0.217 to 0.247 in Dogon compared to 0.076 in Fulani. The *RHCE*Ce-D(4)-ce* allele was found exclusively in Fulani with a high frequency (0.195). Moreover, two of 46

samples were homozygous for this allele leading to the predicted RH:246 phenotype.[10] Surprisingly, two samples with *RHCE*Ce-D(4)-ce* in trans to *RHCE*ce* and *RHCE*-ce(254G)*, respectively, were typed C- with MS24 anti-C which reacts usually 31 in Ortho column agglutination technique. Unfortunately, no blood sample compatible with immunohematologic tests was available to confirm the typing after molecular investigations. The finding that 53.6% of C1 Fulani had a partial predicted C phenotype supports systematic search for *RHCE*Ce-D(4)-ce* allele in this population especially in hosting countries. This is also supported by the finding that 7.3% of C1 SCD patients from unknown ethnic group harbored *RHCE*Ce-D(4)-ce*.[5]

Another approach would be to consider all C1 patients from African ancestry as potentially being partial C and straightaway transfuse them with C-RBC units. However, such an approach would require the use of already scarce resources since most donors are from Caucasian ethnicity and only 32% are C-.

This study also identified the new *RHCE*ceTI(D2)* allele characterized by seven SNPs in Dogon. An inheritance study showed that transmission of this new allele was part of a haplotype with *RHD*DIVa*. Two of the polymorphisms, that is, 48G>C and 1025C>T, were in common with the *RHCE*ceTI* allele that encodes a partial e phenotype.[4] Since these alleles shared three intronic SNPs, that is, IVS2-91a>g, IVS2-32C>T, and IVS6 1 52C>T, it seems reasonable to think that they have the same origin. It is likely that the new allele arose from a rearrangement between *RHCE*ceTI* and *RHD*. Based on the nucleotide sequence observed in coding regions as well as on previously published data showing that the expression of C antigen is related to the exofacial serine 103 resulting from the 307C>T transition,[25,26] it can be thought that samples bearing *RHCE*ceTI(D2)* express all or part of C-antigen. Surprisingly, five of the seven samples bearing *RHCE*ceTI(D2)*, that is, five from the Dogon population in this study plus the two from children included in the inheritance study, typed C- with MoAb MS24 while the two remaining carried *(C)ceS Type 1* in trans to *RHCE*ceTI(D2)*. In the light of findings showing that *RHD*DIVa* expresses weak, variable, and unstable positive RBC reactions with some anti-C,[4] additional immunohematologic tests were carried out on samples with either *RHD*DIVa/RHCE*ceTI(D2)* or *RHD*DIVa/ RHCE*ceTI* haplotypes. Since anti-C yielded similar results on both samples, it was not possible to demonstrate specific C reactivity encoded by *RHCE*ceTI(D2)* allele. Use of a larger anti-C panel will be needed to clearly determine the C profile associated with this allele. Since *RHCE*ceTI* was shown to encode partial phenotypes for both antigens,[4] study of partial c and e antigen expression encoded by *RHCE*ceTI(D2)* would also have been useful but c and e investigation was rendered impossible because *RHCE*ceTI(D2)* was in trans to the *RHCE*ce* allele in all samples.

Taken together, our results revealed an uneven distribution of some RH variant alleles in Mali Africa, suggesting the need for further study in well-documented cohorts. A wider study in donors of African descent will also be required to determine the frequency of the new haplotype RHD*DIVa/RHCE*ceTI(D2) associating an allele encoding partial D, variant ce antigens, and aberrant reactivity with anti-C and to evaluate its potential impact on transfusion strategy.

REFERENCES

1. Flegel WA. Molecular genetics and clinical applications for RH. Transfus Apher Sci 2011;44:81-91.
2. Reid ME, Lomas-Francis C, Olsson ML. The blood group antigens FactsBook. 3rd ed. San Diego: Academic Press; 2012.
3. Wagner FF, Ladewig B, Angert KS, *et al*. The DAU allele cluster of the RHD gene. Blood 2002;100:306-11.
4. Westhoff CM, Vege S, Halter Hipsky C, *et al*. RHCE*ceTI encodes partial c and partial e and is often in cis to RHD*DIVa. Transfusion 2013;53:741-6.
5. Tournamille C, Meunier-Costes N, Costes B, et al. Partial C antigen in sickle cell disease patients: clinical relevance and prevention of alloimmunization. Transfusion 2010;50:13-19.
6. Roussel M, Poupel S, Nataf J, *et al.* RHD*DOL1 and RHD*DOL2 encode a partial D antigen and are in cis with the rare RHCE*ceBI allele in people of African descent. Transfusion 2013;53:363-72.
7. Noizat-Pirenne F, Lee K, Le Pennec PY, et al. Rare RHCE phenotypes in black individuals of Afro-Caribbean origin: identification and transfusion safety. Blood 2002;100:4223-31.
8. Le Pennec PY, Rouger P, Klein MT, *et al*. A serologic study of red cells and sera from 18 Rh:32,-46 (RN/RN) persons. Transfusion 1989;29:798-802.
9. Pham BN, Peyrard T, Juszczak G, *et al*. Heterogeneous molecular background of the weak C, VS1, hr(B)-, Hr(B)- phenotype in black persons. Transfusion 2009;49:495-504.
10. Nance ST, Lomas-Francis C. Where are we in efforts to unravel the complexity of Rh to guide transfusion decisions? Transfusion 2013;53 (11 Suppl 2):2840-3.
11. Sonneborn HH, Ernst M, Tills D, *et al*. Comparison of the reactions of the Rh-related murine monoclonal antibodies BS58 and R6A. Vox Sang 1990;58:219-23.
12. Granier T, Beley S, Chiaroni J, *et al*. A comprehensive survey of both RHD and RHCE allele frequencies in sub-Saharan Africa. Transfusion 2013;53(11 Suppl 2):3009-17.
13. Wagner FF, Moulds JM, Tounkara A, *et al*. RHD allele distribution in Africans of Mali. BMC Genet 2003;4:14.
14. Grootkerk-Tax MG, van Wintershoven JD, Ligthart PC, *et al*. RHD(T201R, F223V) cluster analysis in five different ethnic groups and serologic characterization of a new Ethiopian variant DARE, the DIII type 6, and the RHD(F223V). Transfusion 2006;46:606-15.
15. Hemker MB, Ligthart PC, Berger L, *et al*. DAR, a new RhD variant involving exons 4, 5, and 7, often in linkage with ceAR, a new Rhce variant frequently found in African blacks. Blood 1999;94:4337-42.
16. Chou ST, Jackson T, Vege S, *et al*. High prevalence of red blood cell alloimmunization in sickle cell disease despite transfusion from Rh-matched minority donors. Blood 2013; 122:1062-71.
17. ilvy M, Tournamille C, Babinet J, *et al*. Red blood cell immunization in sickle cell disease: evidence of a large responder group and a low rate of anti-Rh linked to partial Rh phenotype. Haematologica 2014;99:e115-7.
18 Jeremiah ZA, Buseri FI. Rh antigen and phenotype frequencies and probable genotypes for the four main ethnic groups in Port Harcourt, Nigeria. Immunohematology 2003;19:86-88.
19. Jeremiah ZA, Odumody C. Rh antigens and phenotype frequencies of the Ibibio, Efik, and Ibo ethnic nationalities in Calabar, Nigeria. Immunohematology 2005;21:21-4.

20. Kappler-Gratias S, Auxerre C, Dubeaux I, *et al*. Systematic TH genotyping and variant identification in French donors of African origin. Blood Transfus 2014;12 Suppl 1:s264-72.
21. Flegel WA, von Zabern I, Doescher A, *et al*. D variants at the RhD vestibule in the weak D type 4 and Eurasian D clusters. Transfusion 2009;49:1059-69.
22. Touinssi M, Chapel-Fernandes S, Granier T, *et al*. Molecular analysis of inactive and active RHD alleles in native Congolese cohorts. Transfusion 2009;49:1353-60.
23. Silvy M, Di Cristofaro J, Beley S, *et al*. Identification of RHCE and KEL alleles in large cohorts of Afro-Caribbean and Comorian donors by multiplex SNaPshot and fragment assays: a transfusion support for sickle cell disease patients. Br J Haematol 2011;154:260-70.
24. Reid ME, Ripaux M, Auxerre C, *et al*. DIVa and DIVa-2 are encoded by the same RHD allele [abstract]. Transfusion 2012;52:34A.
25. Noizat-Pirenne F, Mouro I, Pennec L, et al. Evidence that serine at position 103 is not sufficient for complete C antigen expression [abstract]. Transfusion 1999;39:103S.
26. Vege S, Johnson NC, Velliquette RW, *et al*. A novel allele with nucleotide 307C > T (Pro103Ser) on RHCE*ceAG (254C > G) encodes robust C antigen [abstract]. Transfusion 2013;53: 30A.