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ONE-PAGE REPORT OF NEW ALLELES OR ANTIGENS

TRANSFUSION

Novel *RHCE*cE(341A)* allele

Rébecca Voreux[1] | **Sunitha Vege**[2] | **Odile Crépin**[3] | **Raynald Flahaut**[4] | **Céline Ricard**[4] | **Gorka Ochoa-Garay**[2] | **Chloe Homich**[5] | **Bruce S. Sachais**[2,5] | **Lynsi Rahorst**[6] | **Randall W. Velliquette**[2] | **Aurélie Barrault**[7] | **Christophe Tournamille**[7,8] | **Aline Floch**[7,8]

[1]Établissement français du sang Hauts-de-France-Normandie, Service d'immunohématologie de Lens, France

[2]Immunohematology and Genomics Laboratory, New York Blood Center Enterprises, Rye, New York, USA

[3]Laboratoire Central de Biologie, CH Béthune-Beuvry, France

[4]Établissement français du sang Hauts-de-France-Normandie, Service QBD secteur IHD, Loos, France

[5]Immunohematology Reference Laboratory, Blood Bank of Delmarva (a Division of New York Blood Center Enterprises), Newark, Delaware, USA

[6]Community Blood Center of Kansas City (a Division of New York Blood Center Enterprises), Kansas City, Missouri, USA

[7]Laboratoire de Biologie Moléculaire de Référence en Immunohématologie Moléculaire, Etablissement français du sang Ile-de-France, Créteil, France

[8]Univ Paris Est Créteil, INSERM U955 Equipe Pirenne «Transfusion et maladies du globule rouge», IMRB, Créteil, France

Correspondence
Aline Floch, Laboratoire de Référence en Immuno-Hématologie Moléculaire, Etablissement français du sang, 5 rue Gustave Eiffel 94017 Créteil cedex, Île-de-France, France.
Email: aline.floch@efs.sante.fr

1 | BACKGROUND

The Rh blood group system is highly polymorphic: 56 antigens described to-date.[1] Antigen immunogenicity and prevalence, contribute to clinical relevance and especially in pregnancy.

Here, we report a novel *RHCE* allele found in two unrelated probands: a 23-year-old pregnant woman of European descent in France (S1-A) whose red blood cells (RBCs) typed D + C-E + ^{w}c + e + serologically and a White donor in the USA (S2) whose RBCs typed C-E- serologically but predicted E-positive by genotyping. A sample from the father of S1-A, a healthy blood donor (S1-B), was also tested.

2 | BRIEF METHODS

For samples S1-A and S1-B, serologic typing was performed by automated column agglutination technology (CAT) with IH-1000 (Bio-Rad, Hercules, CA), Vision Max (Quidel Ortho, San Diego, CA) and Qwalys 3 Evo (Diagast, Loos, France) instruments and manual CAT with DG Gel Rh Pheno (Grifols, Barcelona, Spain). S2 was tested on automated PK7300 (Beckman Coulter, Brea, CA) with Diagast reagents and in tube with Bio-Rad reagents.

Genomic DNA was extracted from whole blood. S1-A was tested by: in-house Real-Time PCR assays, RHCE BeadChip (BioArray Solutions/Werfen, Warren, NJ) and Sanger sequencing of *RHCE* exons 1–10 and *RHD* exon

3 on a 3500 Dx genetic analyzer, (Thermo Fisher Scientific, Waltham, MA). *RHCE* exon 3 of S1-B was Sanger sequenced. S2 was tested by: PreciseType HEA and RHCE BeadChip, Sanger sequencing of *RHCE* exons 1 to 10, targeted *RH* next-generation sequencing (NGS) (MiSeq Illumina, San Diego, CA), and *RHCE*E*-specific Sanger sequencing for phasing.

3 | RESULTS

By automation S1-A RBCs typed E + ^{W} with Anti-E on IH-1000, Vision Max, and Qwalys Evo, and E negative by manual testing. See Table 1 for anti-E testing on all probands. S1-A RBCs also typed D+, C–, c+ and e+ with no ambiguity.

By real-time PCR, E (c.676C) and e (c.676G) were present (*E/e*). *RHCE*cEIV*,[2] frequent weak E allele in individuals of African and European descent, was excluded. RHCE Beadchip results were consistent with these findings. By Sanger sequencing, *c/c*, and c.676C/G (*E/e*) were detected. A heterozygous c.341G>A change (rs1238030431) was found in *RHCE* exon 3 and no other changes in sequenced regions. S1-A *RHD* exon 3 was also sequenced and no changes from conventional were found. RBCs from S1-B typed E + e + with multiple anti-E; and sequencing of *RHCE* exon 3 found no changes from wild type.

S2 RBCs typed E- negative by automation and by tube testing, but was predicted E+ by PreciseType HEA. RHCE BeadChip analysis showed *cE/ce*, with Sanger and NGS identifying c.341G>A in heterozygosity. c.341A was found in cis with c.676C (*E*) by E-specific sequencing. S2 RH NGS showed *RHD*01* and *RHD*01 N.01*.

4 | BRIEF SUMMARY

The c.341G>A change is predicted to encode p.-Arg114Gln, located in the 4th transmembrane domain of the RhCE protein, in close proximity with p.226Pro (Figure S1).[3] The same variant is responsible for *RHD*weak D type 25* and very different phenotypes for changes at amino acid 114 have been reported, suggesting conformational sensitivity in this region.[4]

*RHCE*ce(341A)* was described in 2009 by Hustinx et al.[5] in a Caucasian patient with an e+^{W}, JAL+ phenotype. The clinical significance of the variant was uncertain, in the absence of known immunization. *RHCE*cE (341A)* has been previously described, but with no associated serology.[6] RBCs from S1-A and S2 were not available for JAL testing. In S1-A, the change was inferred to be on a *RHCE*cE* background based on the patient's E + ^{w}, e+ phenotype and also on the absence of the variant allele or a E + ^{W} RBC type in S1-B. In S2, *RHCE*E*-specific Sanger sequencing confirmed *RHCE*cE(341A)*, explaining the discrepancy between phenotype and genotype. These cases highlight the value of having multiple samples to confirm serologic phenotype of novel variants.

TABLE 1 Serological typing results with monoclonal anti-E.

Manual testing		Sample
Grifols MS-260 (IgM)	0 (DT)	S1-A, France
Bio-Rad S12/MS260 (IgM)	0 (IS)	S2, USA
Automation		
IH-1000/MS-260 (IgM)	1+ (DT)	S1-A, France
Vision Max/C2 (IgM)	1+^{w} (DT)	S1-A, France
Qwalys Evo/906 (IgM)	1+^{w} (DT)	S1-A, France
PK7300/906 (IgM)	0 (IS)	S2, USA

Note: Results for S1-B, who did not have the novel allele, are not shown.
Abbreviations: DT, direct testing; IS, immediate spin.

The sequence was deposited in Genbank (Accession number PX392379), and given the ISBT designation *RHCE*03.37*. The effect of c.341A on the expression of c could not be assessed, due to *RHCE*ce in trans*.

Although no anti-D or anti-e have been reported associated with *RHD*weak D type 25* or *RHCE*ce341A*, respectively, the change is predicted to be located in the transmembrane region of RhCE, in close proximity with p.226. For transfusion, E-negative RBC units should be considered if transfusion is necessary for female patients of childbearing age with *RHCE*cE(341A)* in the absence of a conventional *RHCE*E in-trans*.

5 | WEB RESOURCES

New York Blood Center RHCE website https://www.nybce.org/national-center-for-blood-group-genomics/rhce-table/ [accessed January 11, 2026].

RHeference https://www.rheference.org/ [accessed January 11, 2026].

ISBT Blood Group Database https://blooddatabase.isbtweb.org/ [accessed January 11, 2026].

AUTHOR CONTRIBUTIONS

AF, RV, LR, CH, BSS, RWV and SV collected and analyzed the data, AF and RV prepared the original draft, OC, RF, CR and performed serologic testing, GOG, AB and CT performed molecular testing; and all authors reviewed and edited the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have disclosed no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Rébecca Voreux https://orcid.org/0009-0002-1087-082X
Sunitha Vege https://orcid.org/0000-0003-0243-266X
Gorka Ochoa-Garay https://orcid.org/0000-0003-3817-9416
Randall W. Velliquette https://orcid.org/0009-0003-3981-1791
Christophe Tournamille https://orcid.org/0000-0001-7889-5404
Aline Floch https://orcid.org/0000-0003-3238-4566

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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