Received: 20 May 2022 | Revised: 5 July 2022 | Accepted: 22 July 2022

DOI: 10.1111/trf.17082

REPORT OF NEW ALLELES OR ANTIGENS

TRANSFUSION

A novel *RHCE*Ce710A* allele (p.237Asp) in a Japanese patient with weak expression of C and no detectable e

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KEYWORDS: immunohematology (RBC serology, blood groups); molecular biology; RBC transfusion

1 | BACKGROUND

Rh is the most complex of the red blood cell (RBC) blood group systems, comprising 56 antigens encoded by two homologous, closely linked genes, *RHD* and *RHCE*. Nucleotide changes or gene rearrangements can lead to RhD and/or RhCE proteins with qualitatively or quantitatively altered expression.[1] ISBT lists more than 180 *RHCE* alleles. Here, we report the investigation of a patient sample with an ambiguous RhCE.

2 | BRIEF METHODS

Serologic testing was performed by automation by gel card testing (Grifols, Barcelona, Spain and Ortho Clinical Diagnostics, Raritan, NJ, USA) and in microplates with erythrocyte magnetization (Diagast, Loos, France). Genomic DNA was isolated from 400-µl whole blood samples using MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche, Meylan, France) according to the manufacturer instructions. *RH* genotyping was performed by Real-time PCR (ABI 7500 Fast Real-Time PCR System-Thermofisher, Les Ulis, France) and Sanger sequencing of *RHD* and *RHCE* exons 1–10 and flanking intron regions (3500 Dx genetic analyzer – Thermofisher, Les Ulis, France).

3 | RESULTS

The patient was a 41-year-old pregnant woman from Japan. The RBC typed D+, C + w, E+, c+, e− (RH: 1, w2, 3, 4, −5). The RBCs were non-reactive in microplate testing with P3X25513G8 + MS24 anti-C blend (IgM, Diagast) and weakly reactive by gel-based testing with MS24 anti-C (IgM, 0.5+ and 2+ with Ortho and Grifols, respectively). The RBCs were non-reactive in all methods with anti-e IgM blends tested (P3GD512 + MS63, Diagast and MS21 + MS63 + MS16, Grifols and Ortho). Adsorption-elution could not be performed for lack of a fresh sample. These serologic findings appeared consistent with a *DCE/DcE* haplotype combination. The *DCE* haplotype is often associated with a weak expression of C antigen in the absence of *RHCE* genetic alteration.[2] However, E/e Real-time PCR assay performed was discordant with the initial phenotype and revealed the presence of *RHCE*E* and *RHCE*e* alleles thus excluding the *DCE* haplotype. *RHCE* sequencing confirmed the heterozygous c.676 C/G (E/e) and identified an additional heterozygous C > A change at position c.710. This single nucleotide variation is predicted to encode an alanine to asparagine substitution at amino acid position p.237. Molecular analysis did not reveal any change in *RHD*.

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4 | BRIEF SUMMARY

We report a new allele *RHCE*Ce710A* (pAla237Asp) (Genbank accession no. ON542593) in a Japanese woman leading to weakened C and apparent loss of e reactivity. This change was found as rs540803248 in a single individual of African/African American ancestry in gnomAD v3.1.2. The change is located within the eight transmembrane domains, below the fourth extracellular loop bearing the p.Ala226 encoding the e antigen. This proximity may explain the absence of reactivity with routine anti-e reagents. Without adsorption-elution, very low level of e expression could not be ruled out. No transfusion history or immunization was reported for the proband and the partial nature of the new variant could only be estimated based on similar changes reported in *RHD* and *RHCE*. The c.710C > A substitution has been reported in the *RHD* gene with no phenotype data (GenBank accession no. GU998825). In *RHD*, an alanine-to-valine substitution induced by the c.710C > T change and associated with a weakened D phenotype has been reported twice, as *RHD*weak D type 155*, in a Chinese donor[3] and in a pregnant woman of Indian origin with anti-D.[4] In *RHCE*, the c.712A > G change involving p.238 on the RhCE protein is found in alleles responsible for the rare RH:-18 phenotype, including *RHCE*ceBI* and *RHCE*ceSM*, two alleles with no additional change in exon 5.[5,6] Based on these elements, we recommend caution for this newly discovered variant. Individuals carrying this allele should receive C− e− blood units for transfusion and, donors should be considered as C+ e+ to prevent alloimmunization of recipients. This study confirms that the phenotype does not always reflect the genotype and the importance of associating serologic and molecular methods for the detection of new RH alleles and ensuring patient safety.

ACKNOWLEDGMENT

We thank Laurent Devaux, Kévin Gaillard and Vintuya Muralitharan, from Etablissement français du sang, for valuable technical assistance. We also thank Drs Thomas Modot and Frédérique Sardo, from Etablissement français du sang, for contributing serological data.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to this manuscript submitted to TRANSFUSION.

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How to cite this article: Barrault A, Floch A, Pirenne F, Tournamille C. A novel *RHCE*Ce710A* allele (p.237Asp) in a Japanese patient with weak expression of C and no detectable e. Transfusion. 2022;62(10):E59–60. https://doi.org/10.1111/trf.17082