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ONE-PAGE REPORT OF NEW ALLELES OR ANTIGENS

TRANSFUSION

A novel *RHCE*03* with 255G > A, 538G > A, and Exon 9 of *RHD* in a Chinese individual encodes for altered c and E antigens

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Until 2022, there are currently 44 blood group systems recognized by ISBT. The RH system is one of the most polymorphic blood group systems due to the proximity and opposite orientation of *RHD* and *RHCE* genes. *RHD* encodes D and several partial D-associated low-prevalence antigens, whereas greater than 40 antigens, in addition to the five major Rh(D、C、c、E、e) antigens, are encoded by *RHCE* genes. Numerous alleles are described and can affect Rh protein expression, and this complexity is especially evident in some Chinese populations. Besides, the antigen epitope is a special site where the antibody binds to the corresponding antigen, and mutation(s) to the blood group gene may cause the change of the antigen epitope. *RHCE* genes variations, including gene rearrangements, can lead to altered Rh proteins with qualitatively or quantitatively altered expression of Rh antigens.[1,2]

1 | CASE STUDY AND AIMS

Rh phenotype on sample from a group A Rh D+ male patient with meningioma revealed the following results: C + c + ^{mf}E–e + (Figure 1A) showed the mixed field reactivity (mf) of c antigens. Before blood transfusion, a follow-up sample was requested, his RhCcEe phenotype was tested using another reagent, and RhCcEe phenotype showed negative c and E antigens (Figure 1B). The weak c antigen expression and discrepancy with second reagent were found and prompted further serologic and molecular investigation.

2 | MATERIALS AND METHODS

The patient's serum antibody was investigated by IAT assay. RhCcEe phenotypes were typed with micro-column gel card (Changchun bode biotechnology co., China; Sanquin reagents B.V, Cellbind Direct Type, Holland). Genomic DNA was extracted from the blood sample (Invitrogen, USA) and was subjected to the following molecular tests: sequence-specific primers polymerase chain reaction (PCR-SSP) and fluorescence quantitative PCR (FQ-PCR)[3,4] were performed for *RHCE* genes (ABI7500, Applied Biosystems 3500). *RHCE* Exons 1–10 were sequenced afterward by exon-specific amplification (the second-generation sequencing and the third-generation long-read technology)

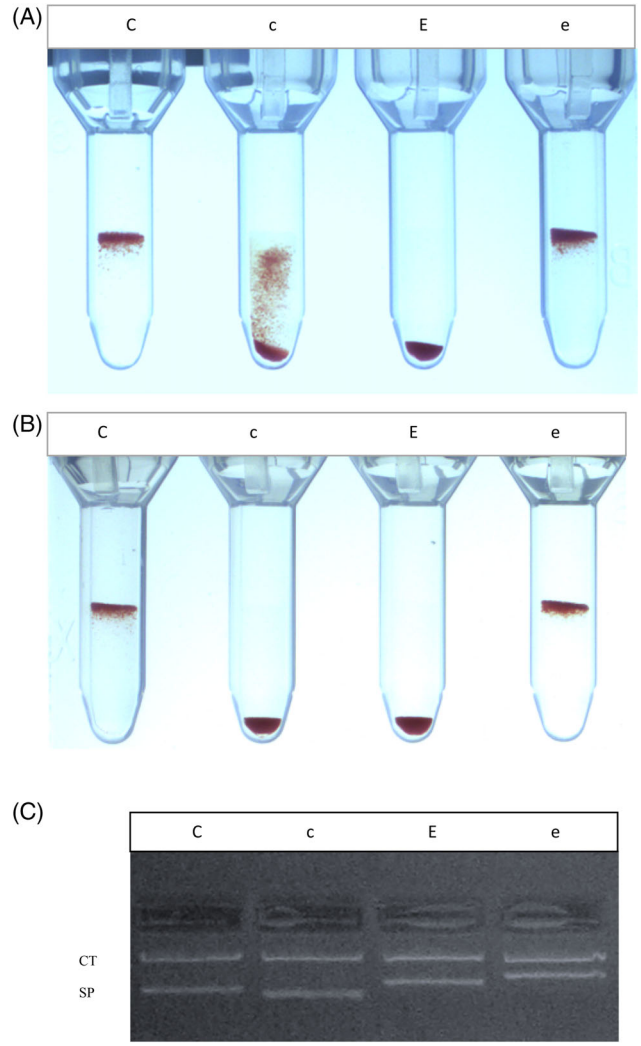

FIGURE 1 Results of RhCcEe phenotype and genotype. RhCcEe phenotype was unidentified accurately by micro-column gel card using different regent for the patient. As the RhCcEe phenotype showed C + c + ^{mf}E–e + (a mixed field reactivity of c antigen) by one regent (Figure 1A, Chuangchun, Bode Co.), but the RhCcEe phenotype showed C + c-E-e + by the other regent (Figure 1B, Sanquin reagents, Cellind direct Type, K7012). However, the *RhCcEe* genotype was determined to be *CcEe*, as shown in Figure 1C by PCR-SSP.

and analyzed using a program for sequence assembly and mutation detection (BioEidt sequence Alignment Editor and SMRT Link v10.1.0 software from Pacific Biosciences). RhCE protein model was established and analyzed according to the highest similarity of amino acid sequences on Swissmodel online.

3 | RESULTS

The IAT results showed no agglutination, and there were no unexpected antibodies in the patient serum. The results of micro-column gel card with anti-C, anti-c, anti-E, and anti-e revealed the decreased reactivity and mixed field (mf) reactivity of c antigen with one reagent, while tests with the same method revealed c antigen non-reactivity with another reagent (Figure 1A/B). PCR-SSP method revealed an apparent *RHCE*CcEe* genotype (Figure 1C), which is the same as the result of the fluorescence quantitative PCR (FQ-PCR) assay at the second time, but not consistent with the C + c + ^{mf}E–e + phenotype or C + c-E–e + phenotype. Sequence analysis revealed heterozygosity for the two novel mutations in one of the haploids of this sample, c.255G > A in Exon 2 and c.538G > A in Exon 4 were identified (Figure 2), which were not included in ISBT up to now. The c.255G > A was a synonymous mutation, causing no amino acid changes. However, the c.538G > A was a missense mutation, resulting in the change of p.Gly180Arg. Besides, a 5.5 k RHCE-D(9)-CE recombination fragment was found in the upstream and downstream intron regions in Exon 9, resulting in two new point mutations (c.1170C > T, c.1193 T > A) (Figure 3).

Further, we can find the exact starting and ending regions of the reorganization, showing the recombination initiation region for RHCE-D (9)-CE from g.59438 to g.60734 positions. The haploid 2 reverted to the RHCE homology from g.67557 to g.67883 positions (Figure 3). This amino acid change has been not reported in the dbSNP and ExAc databases (ExomeAggregationConsortium; http://exac.broadinstitute.org/, https://www.ncbi.nlm.nih.gov/snp/?term=rhce/).

4 | DISCUSSION AND CONCLUSIONS

We have described a novel *RHCE*cE* allele with point mutations c.255G > A and c.538G > A，which may give rise to an altered expression of both c and E antigens in Chinese individual. From ISBT, a point mutation of c.538G > C and c. 48G > C causes RH(c) weak (https://www.isbtweb.org/isbt-working-parties/rcibgt/blood-group-alleletables.html), whereas c.1170C > T, c.1193 T > A point mutation caused by RHCE-D (9)-CE recombination also may cause RH (E) and RH(c) weak. Besides, the presence of E antigen-specific expression of c.676G > C in one of the haploids may affect the expression of the E antigen. Therefore, we hypothesized that the predicted phenotype of this haploid was c/E weak. Combining the results of the other haploid, we finally predicted that the phenotype should exhibit weakened c/E antigens. Residue180 is associated with the start of the sixth

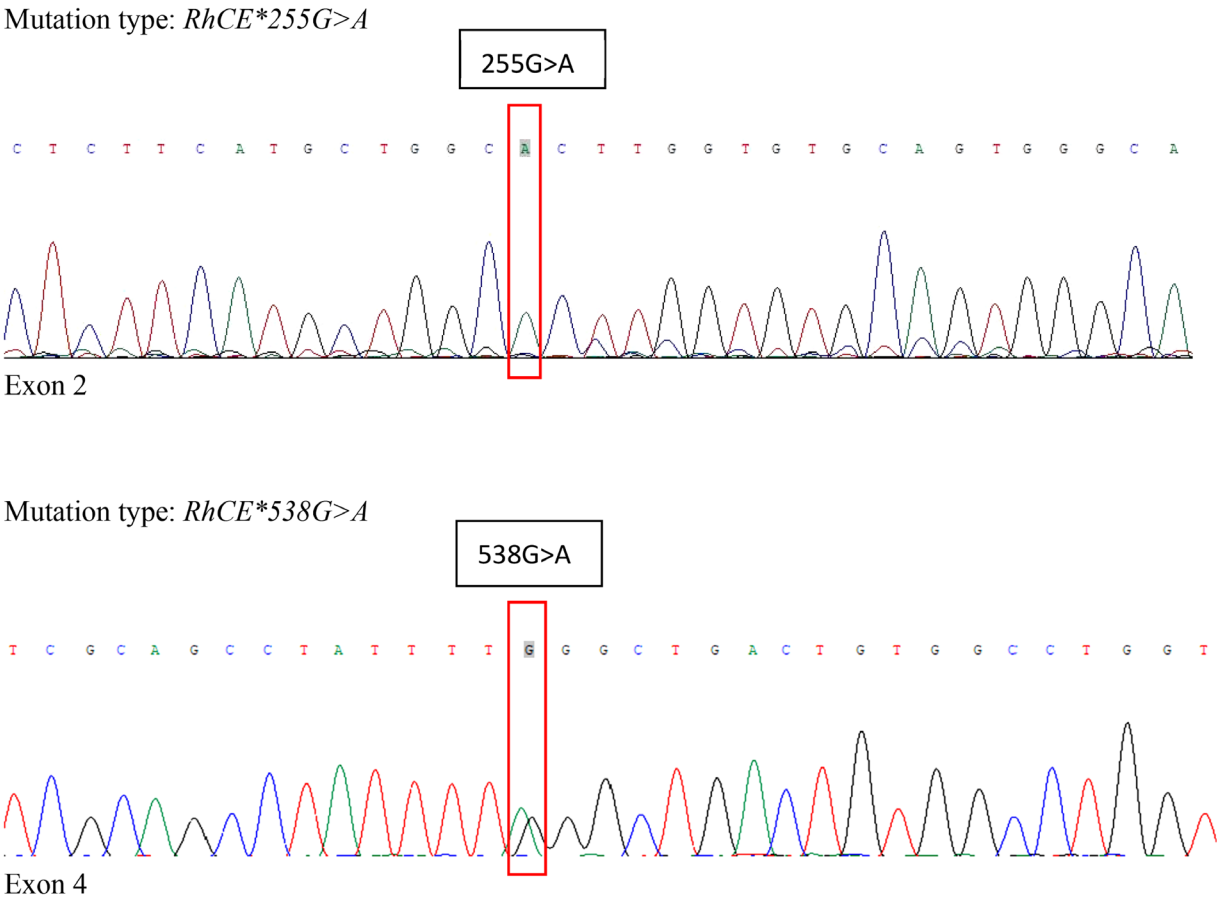

FIGURE 2 Alignment of the Exon 2, 4 sequence of the novel alleles. This novel alleles showed one nucleotide difference with *RHCE*cE* at position 255 G > A in Exon 2, 538G > A in Exon 4 respectively by sequencing.

transmembrane helix, and it is likely that a change from p.Gly to p.Arg could affect how the protein is inserted into the RBC membrane, c.255G > A was a synonymous mutation, causing no amino acid changes. However, this remains unproven in the absence of expression studies. The amino acid polymorphisms responsible for c and E antigens are encoded by Exon 4 and Exon 5, respectively. But due to point mutation in Exon 4 and the 180th amino acid substitution, RHCE molecular structure and the possible effects of the amino-acid residue variations were modeled and analyzed with Swissmodel software. The residue variations are very likely to change the peptide binding properties as well as antibody binding characteristics of the RHCE antigens. The failure of the RBCs to react with IgM-anti-c and IgM-anti-E together with no reactivity using one of the two monoclonal reagents including anti-c and anti-E suggests that the c and E antigens expressed by these alleles are partial antigens. This raises the question of whether this patient is at risk of producing anti-c or anti-E if challenged. DNA analysis of *RHCE* revealed heterozygosity for *RHCE*C/c* and *RHCE*E/e* as well as c.255G > A and c.538G > A. While further samples could not be obtained for additional serologic testing, *RHCE*cE*-specific sequence analysis confirmed that c.255G and c.538G were carried on these alleles.

We conclude that the *RHCE*cE* alleles containing the change c.255G > A, c.538G > A, and RHCE-D (9)-CE recombination give rise to c and E antigens that are both qualitatively and quantitatively altered and that individuals with this allele risk being not only mistyped for RhCcEe antigens but at risk of producing anti-c or anti-E. These data allow us to describe the characteristics of the RH system antigen and highlight a significant number of partial antigens with a risk of alloimmunization.

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CONFLICT OF INTEREST STATEMENT

The authors have disclosed no conflicts of interest.

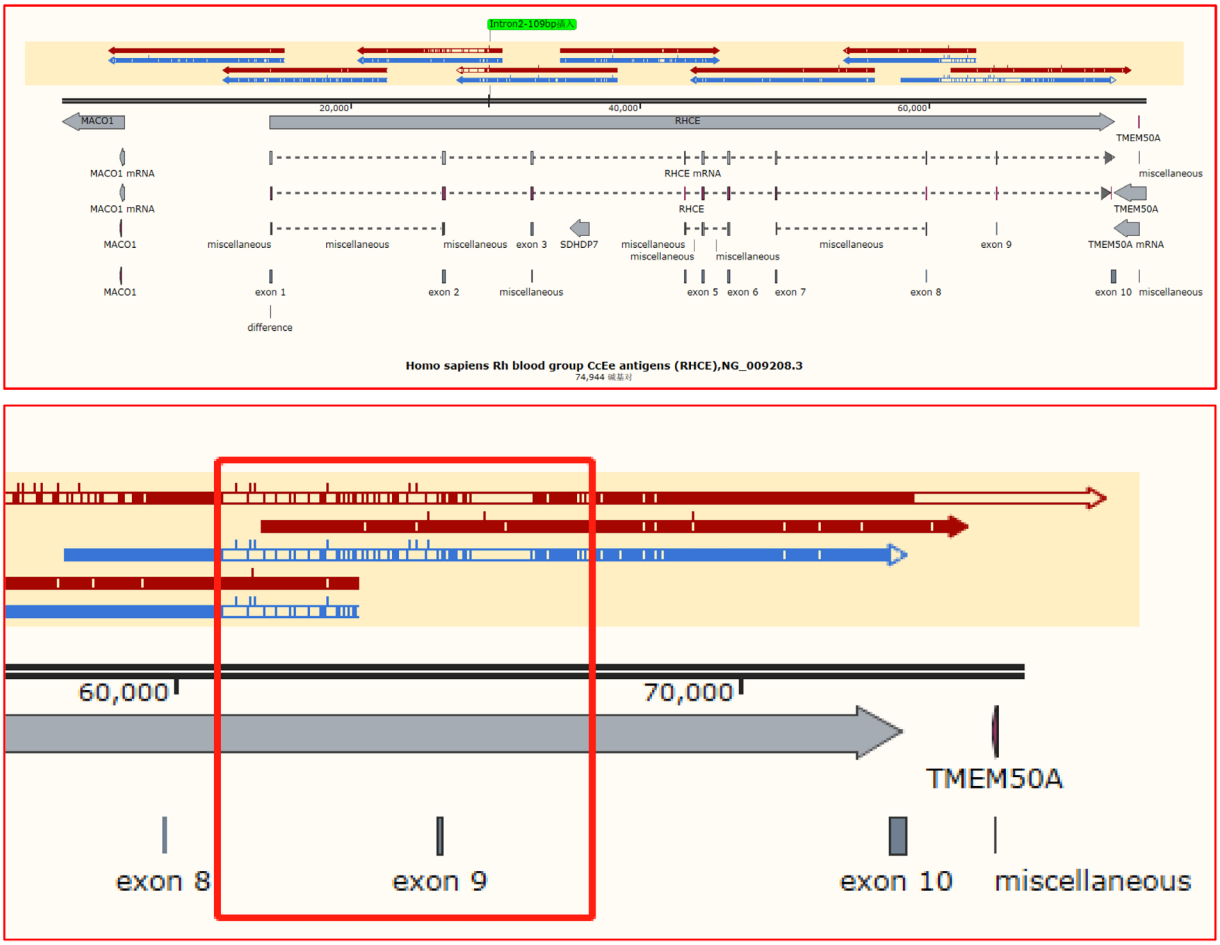

FIGURE 3 The characteristics of RHCE-D (9)-CE recombination. Two point mutations (c.1170C > T, c.1193 T > A) were found in Exon 9 and its upstream and downstream intron regions with a 5.5 k RHCE-D (9)-CE recombination fragment by the third sequencing; the start and stop regions of RHCE-D (9)-CE recombination showed that the recombination initiation region for RHCE-D (9)-CE from g.59438 to g.60734 positions. The haploid 2 reverted to the RHCE homology from g. 67,557 to g. 67,883 positions.

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