

A novel *RHCE*02* variant with a recombination of *RHD* exon 3 in a Chinese D+ voluntary blood donor with weak expression of e antigen

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Funding information

Changzhou Institute of Technology, Grant/Award Numbers: CJ20245046, CJ20241124

KEYWORDS: blood donor, *RHCE*02*, *RHCE*Ce-D(3)-Ce*, weak e expression

1 | BACKGROUND

The Rh blood group system comprises two closely linked homologous genes, *RHD* (MIM: 111680) and *RHCE* (MIM: 111700), which encode the common red blood cell-specific Rh antigens. The Rh system is one of the most diverse and currently comprises 56 antigens, with the main antigens being RH1 (D), RH2 (C), RH3 (E), RH4 (c), and RH5 (e).¹ To date, over 150 *RHCE* alleles have been identified through molecular analysis across various populations. Most rare *RHCE* phenotypes are often identified through the recognition of weak and partial expression of major RhCE antigens.²⁻⁴ For patients with Thalassemia, who frequently require blood transfusion, *RH* genetic polymorphisms that change the conformation of RH proteins in both patients and healthy blood donors contribute to compatibility issues. Aligning Sanger or NGS reads for RH, given *RHD-RHCE* homology, is challenging. Short reads hinder complete gene phasing and allele assignment, notably in samples with multiple polymorphisms.⁵ Sanger sequencing may not

address the problems caused by RH recombination. PacBio sequencing is a single-molecule sequencing technology that is capable of reading long DNA fragments and reduces ambiguity and overcomes the inherent limitations of Sanger sequencing by using additional assembly markers. We report a novel *RHCE* allele in a 28-year-old Chinese donor through long-read sequencing on the Pacific Biosciences (PacBio) platform with weak expression of e antigen.

2 | BRIEF METHODS

Fresh blood from the first-time blood donor was typed in the Rh blood group system by (1) an automated microplate testing and gel card typing, (2) the final result was confirmed by test tube method. A discrepancy prompted an investigation into the donor's *RHCE* gene. Serologic testing for C, E, c, and e antigens was conducted using standard hemagglutination techniques on the Microlab STARlet BG (Hamilton, Switzerland) for automated testing and the test-tube method for tube testing. Both methods utilized commercially available reagents from Shanghai Hematology Co., Ltd. (Shanghai, China). RH Gel card typing (Bioxun, Changchun, China) was

Abbreviation: CCS, Circular Consensus Sequence.

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conducted following the provided instructions. Genomic DNA was extracted using the MolPure[®] Blood DNA Kit (Yeason, Shanghai, China) from peripheral blood following the manufacturer's guidelines. Fifteen primer sets were developed to comprehensively amplify both *RHD* and *RHCE* genes. Sequencing was performed using Sanger sequence and long-read techniques on the Pacific Biosciences (PacBio) platform. Data analysis was conducted using SMRT Link v10.1.0 software, with subreads processed to generate Circular Consensus Sequence (CCS) reads. The CCS fragments relevant to *RHD* and *RHCE* were batched and realigned to the reference genomic sequence (*RHD* (NG_007494.1) and *RHCE* (NG_009208.3) genes) using pbmm2.

3 | RESULTS

The donor, a 28-year-old woman from China, had RBCs with the RH: 1, 2, 3, -4, 5w phenotype. In automated microplate testing and gel card methods, there was no agglutination observed with IgM monoclonal anti-e. However, the tube test revealed a C+, E+, c-, e+^w phenotype, as depicted in Figure S1. Sanger sequence results of *RHCE* showed eight nucleotide substitutions separately at *c.48G>C* and *c.105C/T* in exon 1, *c.150C>T*, *c.178C>A*, *c.201A>G*, *c.203A>G*, and *c.307C>T* in exon 2, and *c.676G/C* in exon 5, as shown in Figure S2. Considering that the *RHCE*cE* and *RHCE*ce* alleles differ only at *c.676*,⁶ and our sequencing results showed the *c.676* was heterozygous mutation, we conclude that the *RHCE* genotype yielded a *RHCE*cE* allele. The long-read

sequencing results of *RHCE* and the amino acid sequence are presented in Table 1 and Figure S3. In SNV2, a *RHCE-D* recombination was first detected in *RHCE*cE* allele backbone exons 3, similar to *RHCE*03.32*,⁷ resulting in a RH phenotype of cE^w. Since this sample's phenotype is C+E+c-e+^w, we speculate that the *RHCE*cE-D* (3)-*Ce* may also result in reduced expression of the e antigen, just as *RHCE*03.32* leads to reduced expression of the E antigen. *RHCE*03.32* was previously described only as *c.361A>T* *c.380C>T*, *c.383G>A*, *c.455C>A*. But with the full-length *RHCE* sequence from the PacBio platform, we've confirmed that our sample aligns with *RHCE*cE-D* (3)-*Ce*. Further, we can find the exact starting and ending regions of the reorganization, the starting region between *c.148-807* to *c.148-997* and the ending region between *c.486+6289* to *c.486+6696*.

4 | BRIEF CONCLUSION

We report a novel *RHCE02* with a replacement of exon 3 with *RHD*, which was identified for the first time in the Chinese people. The RH type of the donor was C+E+c-e+^w in serological methods. Long read sequencing revealed a *RHCE*cE-D*(3)-*Ce* recombination, which may be related to the e weak phenotype. Overall, we recommend caution for newly discovered variant. This study reminds us 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. On the other hand, Sanger sequencing's short reads cannot effectively resolve

TABLE 1 The nucleotide changes and resulting amino acid alterations were identified using PacBio long-read sequencing.

Allele name1	SNV1	Allele name2	SNV2	Predicted aminoacid change	Predicted phenotype
<i>RHCE*04</i>	<i>c.48G>C</i>	<i>new RHCE*02</i>	<i>c.48G>C</i>	p.Trp16Cys	C+E+c-e+w
	<i>c.105C/T^a</i>		<i>c.105C/T^a</i>	p.Asp35=	
	<i>c.150C>T</i>		<i>c.150C>T</i>	p.Val50=	
	<i>c.178C>A</i>		<i>c.178C>A</i>	p.Leu60lle	
	<i>c.201A>G</i>		<i>c.201A>G</i>	p.Ser67=	
	<i>c.203A>G</i>		<i>c.203A>G</i>	p.Asn68Ser	
	<i>c.307C>T</i>		<i>c.307C>T</i>	p.Pro103Ser	
			<i>c.361A>T</i>	p.Met121Leu	
			<i>c.380C>T</i>	p.Ala127Val	
			<i>c.383G>A</i>	p.Gly128Asp	
			<i>c.455C>A</i>	p.Thr152Asn	
	<i>c.676G/C</i>			p.Ala226Pro	
			<i>RHCE*cE-D</i> (3)- <i>Ce</i>		

^aHaplotype location of *c.105* is indeterminate.

issues arising from *RH* gene recombination, while long-read sequencing is demonstrating significant technical advantages in blood group genotype. The bioinformatics of these recombination alleles outstretched our knowledge of *RHCE* variants, which was pivotal for evaluating their impact to guide transfusion support and avoid immune-related blood transfusion reactions.

ACKNOWLEDGMENTS

The study was sponsored by Changzhou Institute of Technology (Grant Nos. CJ20245046 and CJ20241124).

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 on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

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

How to cite this article: Yang H-M, Ma S-F, Zou X, Xiong Y-Q, Yao Y, Fu W-Y, et al. A novel *RHCE**02 variant with a recombination of *RHD* exon 3 in a Chinese D+ voluntary blood donor with weak expression of e antigen. *Transfusion*. 2026. <https://doi.org/10.1111/trf.70091>