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TRANSFUSION

ONE-PAGE REPORT OF NEW ALLELES OR ANTIGENS

BLOOD GROUP GENOMICS

Identification of a novel *RHAG* allele with a c.500A>G variation associated with Rhmod phenotype

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The Rh blood group system is highly polymorphic and Rhmod is a rare deficiency phenotype, mainly due to *RHAG* gene variants that cause a significant reduction in RhD and RhCE antigens expression.[1] Several RHAG allele variants have already been reported to be associated with the Rhmod phenotype.[2] Here we report a novel *RHAG* allele associated with rare Rhmod phenotype in a Chinese individual.

1 | BRIEF METHODS

The proband was a patient with anemia, manifesting only as a reduction in red blood cell hemoglobin. RhD and RhCE antigens on the surface of red blood cells were detected by manual tube testing with IgM antibodies (anti-D, Clone: RUM-1; anti-C, Clone: MS-24; anti-c, Clone: MS-33; anti-E Clone: MS-80, MS-258; anti-e, Clone: MS-16, MS-21, MS-63; Shanghai Blood Biotechnology, Shanghai, China). The RhD antigen was further examined by indirect antiglobulin test (IAT) with another three anti-D reagents (1-IgM/IgG blend, IgM Clone D175-2, and IgG Clone D415 1E4, Dominion Biological limited, Canada; 2-IgM/IgG blend, IgM Clone GAMA401 and IgG Clone F8D8, IMMUCOR Ltd., USA; 3-IgG blend, Clone MS-26, Shanghai Blood Biotechnology, China) via tube and microcolumn gel test.[3] Irregular antibody screening was performed using a panel of test red cells from Sanquin (Sanquin, Amsterdam, Netherlands) by microcolumn gel test.

Genomic DNA was extracted from blood cells using a commercial DNA isolation kit (RBC Bioscience Corporation, Taiwan, China) according to the manufacturer's instructions. The 10 exons of *RHD* and *RHCE* gene were amplified using polymerase chain reaction sequence-based typing (PCR-SBT) according to the previous method established in our laboratory.[3] The RHD, RHCE, and RHAG alleles full-length sequences were analyzed using long-read sequencing based on PacBio technology platform, which was provided by Xi'an Haorui Genomics Technology Co., LTD. The clean data were compared and annotated with the reference sequences of *RHD* (NG_007494.1) and *RHCE* (NG_009201.3) and *RHAG* (NG_011704.1), and the subsequent analysis was carried out using Snapgene software (V6.2.2) (Table 1).

2 | RESULTS AND DISCUSSION

The proband's RhD, C, c, E, and e antigens were all negative based on the tube tests. Therefore, Rhnull phenotype

Abbreviations: Rh, Rhesus; RHAG, Rhesus-associated glycoprotein; RhD, Rhesus D; RhCE, Rhesus CE.

TABLE 1 Characteristic of phenotype and genotype of the proband.

Phenotyping								Genotyping			Novel allele		
Anti-D (IgM)	Anti-C (IgM)	Anti-c (IgM)	Anti-E (IgM)	Anti-e (IgM)	Anti-D1 (IgG, tube/gel)	Anti-D2 (IgG, tube/gel)	Anti-D3 (IgG, tube/gel)	Phenotype	*RHD*	*RHCE*	*RHAG*	Nucleotide change	Amino acid change	Accession no.
0	0	0	0	0	±/+	±/+	±/+	Rhmod	*RHD*01/RHD*01*	*RHCE*Ce/RHCE*Ce*	*RHAG*01 (c.500A>G)/RHAG*01 (c.500A>G)*	c.500A>G	p.Asp167Gly	PP590632

Note: 0 = no agglutination.

(Rh antigens not expressed) or Rhmod phenotype (weakly expressed) was speculated and further analyzed. The IAT results using tube test showed very weak agglutination (±) with three different clone anti-D reagents, while the microcolumn gel showed slightly stronger agglutination (+). Thus, the proband was inferred to be Rhmod phenotype. Serum antibodies screening was negative. Unfortunately, the indirect antiglobulin and adsorption-elution tests could not be performed due to the absence of IgG-type antibodies for RhCE antigens.

The 10 exons of the RHD and RHCE genes were present and no variants were identified by PCR-SBT. The genotypes were *RHD*01/RHD*01* and *RHCE*02/RHCE*02*, respectively. The full-length sequences of *RHD, RHCE*, and *RHAG* genes were obtained using the PcaBio long-read sequencing. The sequence results for *RHD* and *RHCE* were consistent with those of the PCR-SBT. Further, the full-length sequencing of the *RHAG* gene revealed that the proband had a homozygous c.500A > G missense variant in exon 4 of the *RHAG* gene.

The novel variant resulted in an amino acid change from aspartic acid to glycine at residue 167 (p.Asp167Gly) for the RhAG protein. Previous studies have shown that this Asp residue is located in the third extracellular ring of RhAG protein, which is closely related to ammonium salt transport.[4] It is speculated that the variant can lead to the change of its epitope, or affect the binding with *RHD* and *RHCE* proteins, thus forming the Rhmod phenotype. The novel allele sequence has been deposited into the National Center for Biotechnology Information (NCBI): the accession number is assigned as PP590632. The c.500A>G variant of the *RHAG* gene was not found in the NCBI dbSNP database. The novel Rhmod allele was provisionally designated as *RHAG*01M.18* by Red Cell Immunogenetics and Blood Group Terminology (RCIBGT) Working Party of the International Society of Blood transfusion (ISBT).

In conclusion, a novel *RHAG* allele with a c.500A>G variation was identified, which was responsible for the rare Rhmod phenotype.

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CONFLICT OF INTEREST STATEMENT

The authors have disclosed no conflicts of interest.

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