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CASE REPORT

A Novel *RHD* Allele Similar to *RHD*DIV.4* with Additional c.1025T>C

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SUMMARY

Background: **Polymorphic RH is one of the most important blood group systems used for transfusion. Use of molecular technologies combined with serological testing help to identify the new *RHD* variants.**
Methods: **The traditional serological test of blood type was performed for a 28-year-old pregnant female. The result showed a weaker positive reaction result on two different microcolumn gel cards. A molecular genotyping assay was performed to get more information.**
Results: **The gene sequence study indicated 7 nucleotide changes in exon 7, compared with the reference allele. The third-generation sequencing using the long-read PacBio HiFi system showed these variants were all located in the same haplotype. The variant is replaced by the counterpart from *RHCE* gene in exon 7 with at least 37 bp. Most of its position was located in the sixth extracellular loop.**
Conclusions: **A novel *RHD* allele was identified with 7 missense mutations that is similar to *RHD*DIV.4* and likely causes a partial D phenotype.**
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KEYWORDS

RHD allele, serological test, molecular technology

INTRODUCTION

Polymorphic RH is one of the most important blood group systems used for transfusion. Serological testing of RhD sometimes shows weak reactions to *RHD* gene mutations. Using molecular technologies combined with serological testing, more than 460 *RHD* variants (classified as D-positive, D-negative, weak D, partial D, or Del) have been recorded by the International Society of Blood Transfusion.

CASE PRESENTATION

Herein, we report a case of a specimen from a female patient, her ethnicity is Han. The results showed a weaker positive reaction (3+) on two different microcolumn gel cards. A further molecular study indicated a novel variant consisting of 7 nucleotide changes (c. 1025T>C, c.1048G>C, c.1053C>T, c.1057G>T, c.1059

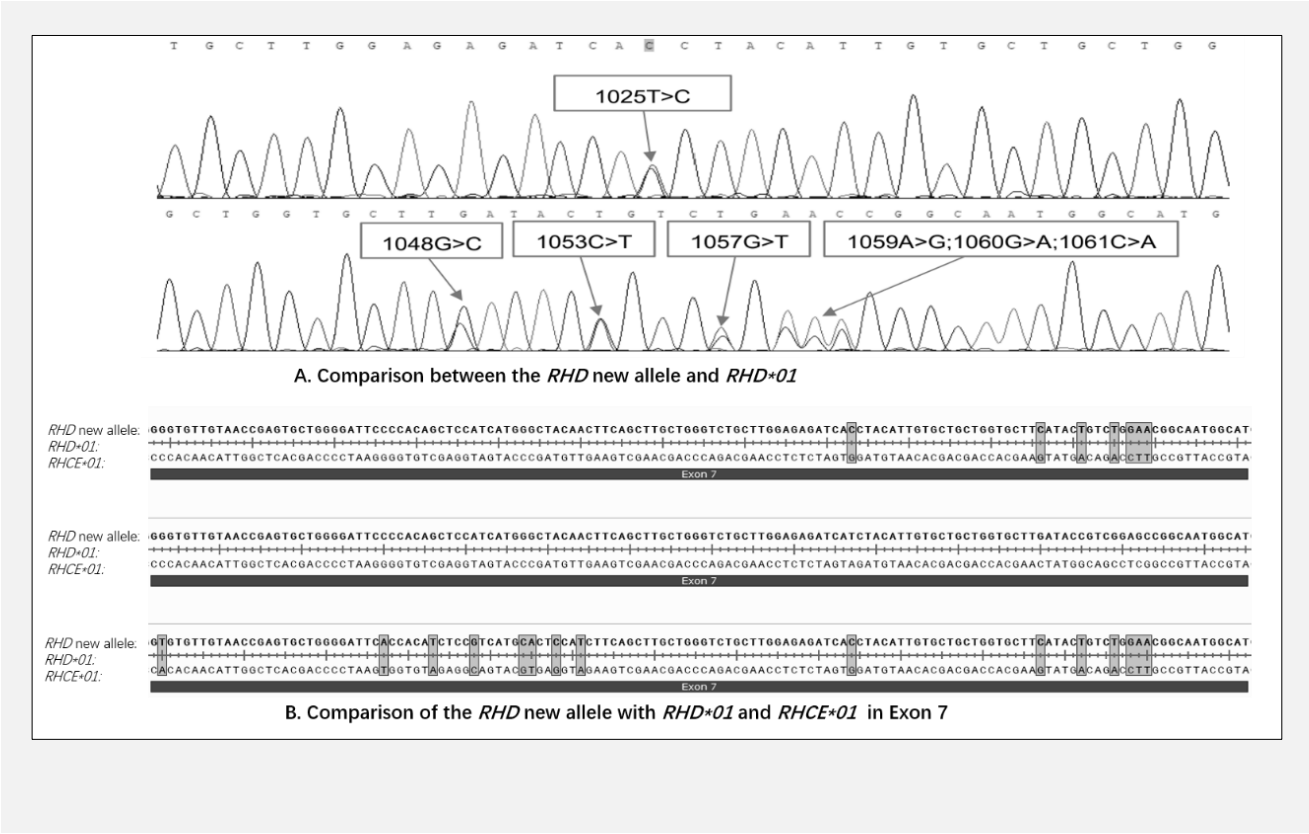

Figure 1. Sequence comparison of *RHD* new allele with *RHD*01* and *RHCE*01* in Exon 7.

A. Seven nucleotides of the *RHD*01* allele were replaced at 1025, 1048, 1053, 1057, 1059, 1060, and 1061 positions compared with the *RHD* new allele. B. Comparison of the Exon 7 (940 - 1,073) region of the RHD new allele, *RHD*01*, and *RHCE*01*.

A>G, c.1060G>A, c.1061C>A) in exon 7, leading to amino acid changes (p.Ile342Thr, p.Asp350His, p.Gly-353Trp, and p.Ala354Asn).

Serological typing was performed according to the manufacturer's instructions. Automatic blood group machines (AutoVue, Ortho; Erytra Grifols) were used to detect RhD blood groups. An indirect anti-globulin test was performed manually using a semi-automatic incubator and a centrifugal machine (DG, Grifols) with micro-column gel cards. The saline tube test was performed using a Kubota cell centrifuge (KUBOTA, Osaka, Japan). Three types of human standard monoclonal anti-D-IgM serum were used as follows: 1) human anti-D-IgM antibody (clone D7B8) for AutoVue Innova (Ortho, USA); 2) human anti-D-IgM antibody (clone P3 x 61) for Erytra (Grifols) and the semi-automatic instrument; and 3) another anti-D monoclonal antibody (IgM), anti-D monoclonal antibody (IgG) (Shanghai Hemo-Pharmaceutical & Biological Co. Ltd., Shanghai, China). The presence or absence of upstream and hybrid rhesus boxes was used to determine *RHD* zygosity. DNA sequencing of *RHD* exons 1 - 10 was performed using polymerase chain reaction-sequence-based typing [1]. The patient provided written informed consent before enrollment. This study was approved by the Ethics Committee of West China Second University Hospital, Sichuan University (2020051).

Weak D-antigen expression was detected using routine tests. It was first detected by a microgel card on the Grifols machine, and a 3+ positive reaction was obtained. A similar 3+ positive reaction was observed by another microgel card on the AutoVue machine. Then, 2+ positive results were observed under different conditions (at room temperature immediately spin; at 4℃ for 15 minutes and spin; at 37℃ for 15 minutes and spin) by saline tube tests. Additionally, a strong positive reaction (4+) was observed in the gel card test using anti-D-IgG reagents. However, a direct anti-human immunoglobulin test yielded negative results. A molecular genotyping assay was performed to get more information. The results of RHD gene typing showed that this variant was a heterozygous allele with 7 nucleotide changes in exon 7 of the RHD gene (Figure 1), compared with the reference allele, RHD*01 (NG_007494.1) [2]. To determine the RHD haplotype, we conducted third-generation sequencing using the long-read PacBio HiFi system [3] and found that these variants were all located in the same haplotype.

DISCUSSION

The variant is replaced by the counterpart from *RHCE* gene in exon 7 at least 37 bp, most of its position was located in the sixth extracellular loop, except for c.1025 T>C (p.Ile342Thr), located in the 11th transmembrane domain. We predicted that this variant belongs to the partial D phenotype and is prone to alloantibody production.

A similar *RHD* allele to our newly identified *RHD* allele was *RHD*DIV.4*. One variant (c.1025T>C, p.Ile342 Thr) was different from the *RHD*DIV.4* [2], and may influence RHD protein expression. The nucleotide sequence of this new *RHD* allele has been submitted to GenBank under accession number OR823847.

CONCLUSION

In conclusion, we identified a novel *RHD* allele with 7 missense mutations, that is similar to *RHD*DIV.4* with an additional c.1025T>C and likely causes a partial D phenotype. Using molecular technologies combined with serological testing helped to identify the blood type.

Declaration of Interest:

None of the authors have any conflict of interest in this study.

References:

1. Legler TJ, Maas JH, Köhler M, et al. RHD sequencing: a new tool for decision making on transfusion therapy and provision of Rh prophylaxis. Transfus Med 2001;11:383-8. (PMID: 11696232)

2. ISBT. (ISBT 004) RHD blood group alleles, International Society of Blood Transfusion (ISBT). https://www.isbtweb.org/resource/004rhd.html 2023

3. Gueuning M, Thun GA, Wittig M, et al. Haplotype sequence collection of ABO blood group alleles by long-read sequencing reveals putative A1-diagnostic variants. Blood Adv 2023;**7**:878-92. (PMID: 36129841)