Egyptian Journal of Medical Human Genetics

CASE REPORT Open Access

Challenges in blood transfusion caused by anti-Hr_0: A rare case of D-- Phenotype in Asia Abstract

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Abstract

Introduction D–Phenotype is linked to abnormal expression of *RHCE* gene. Consequently, individuals with this condition may develop antibodies against high-prevalence Rh antigens when exposed to a normal Rh phenotype, leading to challenges in blood matching.

Case presentation A 90-year-old male was admitted to the hospital due to prostatitis and acute retention of urinary. Surgery was planned after evaluation by urologist. A request was made for 2 units of red blood cells (RBC). The result for unexpected antibody screening was positive, and the antibody was identified as anti-Hr_0 alloantibody , a rare *RHCE * CE-D (5) – CE* was discovered by genetic testing. The patient has only one history of blood transfusion, which may result in the production of the anti-Hr_0 antibody. Compatible blood donors for this patient were not found, even after extensive screening.

Conclusion This report records a rare case of a D-- phenotype individual who produced anti-Hr_0 alloantibody. Subsequent analysis of *RH* gene sequencing revealed a genotype that has not been previously reported in Asia.

Keywords Rh deletion D--, Genotyping, Rh blood group, Anti-Hr_0 antibody

Background

The Rh blood group system which consists of two genes, *RHD* and *RHCE* is second only to the ABO blood group system in importance for clinical transfusions. To date, there are 56 antigens identified in this blood group system, with five antigens (D, C, E, c, and e) holding particular clinical significance [1]. Due to the high homology and close linkage of duplicated genes *RHD* and *RHCE*, they are prone to recombination, deletions, and mutations, leading to the emergence of variant Rh antigens and continuous discovery of new alleles [2]. One such rare variant is the Rh deficient type D--, which lacks the C, E, c, and e antigens but demonstrates enhanced expression of the D antigen on red blood cells. D-- phenotype is extremely rare. Despite an increase in case reports due to advancements in laboratory technology and genetic testing popularity, the mechanisms of genetic variation remain diverse [3]. The identification of a previously unreported genotype in Asia has been achieved through comprehensive serological analysis and genetic testing.

Case presentation

A 90-year-old male patient with one history of blood transfusion was admitted to the hospital for prostatitis and acute retention of urinary. After evaluation by the urologist, he underwent the proposed surgery. Routine blood analysis revealed a hemoglobin (Hb) level of 106g/L, and 2 units of red blood cells were applied for blood preparation before operation. The patient was group A, RhD positive, with positive result of unexpected antibody screening. Crossmatching tests were performed

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on four donors, revealing major matching incompatibilities, which needs further testing.

The patient's antibody screening showed a positive result with the microcolumn agglutination anti-human globulin(MA-AHG) test and a negative result with the saline medium, while direct antiglobulin test (DAT) was negative. Alloantibody was suspected in this patient . Antibody identification was conducted using a sixteen-panel cells, resulting in positive findings for MA-AHG with agglutination observed at 2+. Since the patient's DAT was negative, autoantibodies were ruled out. Additionally, the absence of anti-Kp^b, anti-Js^b and anti-Lu^b antibodies were confirmed through antigen typing in this patient. Crossmatching tests were conducted with 40 donors, revealing 2+ to 3+ agglutination on the major, while all minor results were negative. Therefore, it is suspected that the patient produces combined antibodies or high-prevalence antigen antibodies.

Rh typing for patients' red blood cells demonstrated agglutination with IgM monoclonal anti-D, while no agglutination was observed with IgM monoclonal anti-C, anti-c, anti-E, and anti-e, indicating that the Rh blood group as D-- phenotype. There was no difference in agglutination intensity IgM monoclonal anti-D by direct agglutination between D antigen in this patient and normal individual, so titer detection was performed. The titer of human anti-D (The reagent was prepared in our laboratory from an individuals who was RhD negative and produced anti-D) detected by red blood cells from DCCee blood donors with the same ABO type as the patient was 4, whereas the titer of human anti-D detected by this patient's red blood cells was 16. To evaluate the expression of D antigen, we designed absorption and elution tests, these tests were conducted using two types of red blood cells and human anti-D serum. O+ red blood cells were utilized for anti-D titers in the elution solution. The anti-D titer of elution solution from the red blood cell classified as DCCee was 2, while the titer of elution solution from this patient's red blood cell was 4. This indirectly indicates a higher expression of the D antigen in this patients' red blood cells compared to normal D+ red blood cells. The red blood cells with three different Rh phenotypes (DCCee, DccEE, dccee) were washed to absorb this patient serum, and acid release experiments were conducted. The pattern of absorbtion and elution solution of the three types was similar, albeit with varying intensities, raising suspicion of a combined composite antibody or anti-Hr_0 of the Rh blood group system. Refer to Fig. 1 for the detection process.

By analyzing the exon 1–7 sequence of the *RHCE* gene, homozygous mutations were identified in exons 1, 2, and 5. The specific mutation sites can be seen in Fig. 2. Referring to the *RH* (ISBT004) Blood Group Alleles: *RHCE (004RHCE Alleles v4.0-20180208)* data from the ISBT website, using *RHCE*01 (RHCE*ce, NM_020485* (mRNA)) as the reference sequence, and considering the sequencing results, it was observed that the 5th exon of the *RHCE* gene in this patient was substituted with the 5th exon of the *RHD* gene. The remaining sequence aligned with the characteristics of the *RHCE*C* allele, leading to the gene being named *RHCE*CE-D(5)-CE*.

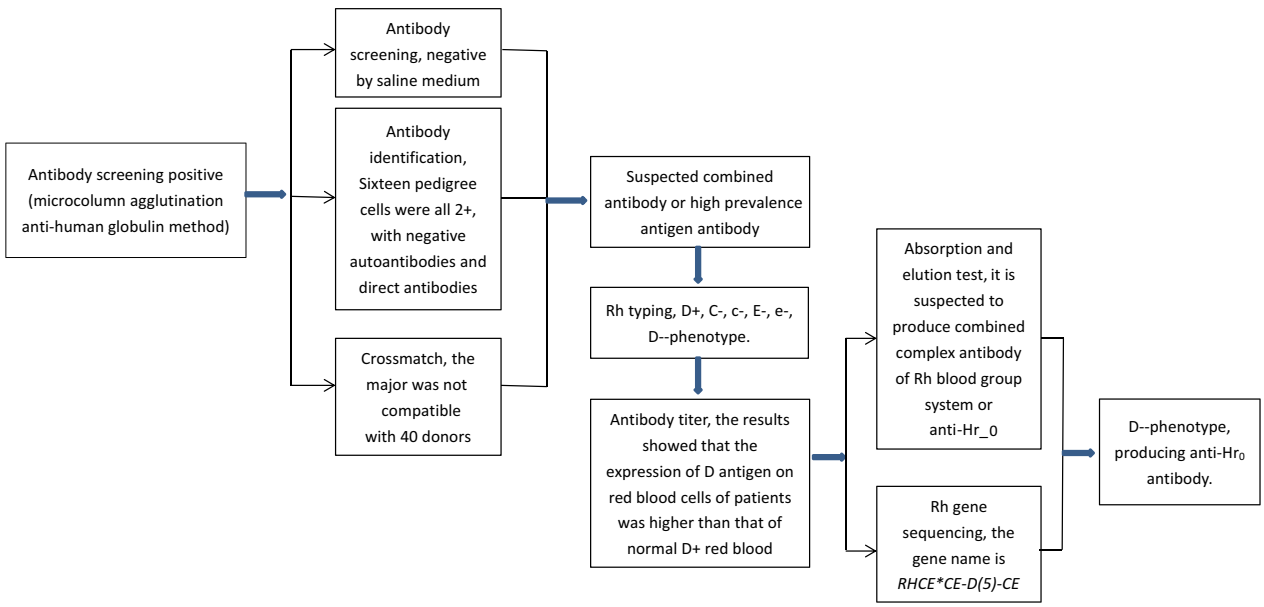

Fig. 1 Flow chart of antibody identification

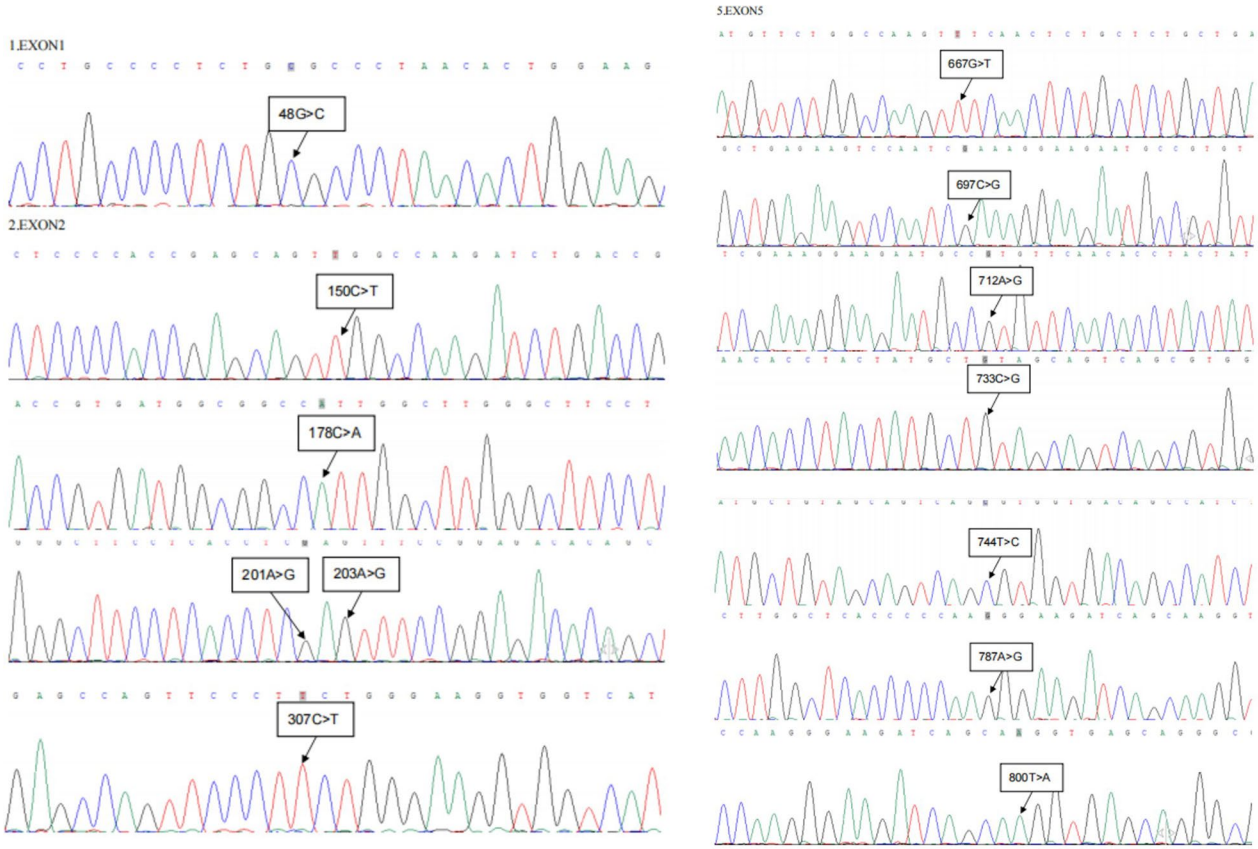

Fig. 2 Results from Rh gene sequencing (EXON1, EXON2, and EXON5). The black arrow indicates the base mutation site. No mutations were detected in EXON3-4 and EXON6-10

Discussion

The Rh blood group system is one of the most polymorphic and immunogenic systems known in humans . The Rh blood group gene consists of closely linked *RHD* and *RHCE* genes arranged in series. The two genes exhibit a high degree of homology, with the polymorphic *RHCE* gene encoding four antigens. The C and c antigens have four different amino acids, while E and e antigens have only one amino acid variant [4]. The D-- phenotype represents is a rare Rh variant, characterized by a lack of RhCcEe antigen expression on the surface of RBCs while exhibiting significantly elevated levels of the D antigen expression[5]. The RhD-- phenotype is predominantly observed in offspring of consanguineous unions, but researches have shown that heterozygosity in the parental generation with a normal phenotype can result in its complete absence in future generations [6, 7]. The rare Rh-deficience phenotypes are characterized by the absence of one or more Rh antigens (known as Rhnull, D--, Dc-, etc.), of which D-- phenotypic individuals can produce a rare alloantibody called anti-Rh17 (Hr0) due to blood transfusion or pregnancy, which may lead to severe hemolytic transfusion reaction (HTR) and haemolytic disease of the fetus and newborn (HDFN) [8].

The anti-Hr_0 antibody produced by D-- phenotype targets the common product of the other four antigenic gene loci in addition to the *D* gene. It exhibits agglutination with common Rh red blood cells, making it difficult to find compatible blood. The result of this patient's antibody screening was positive, with 2+ agglutination observed in the cells of antibody identification panel. Initially autoantibody was suspected due to lack of specificity, however, the negative DAT results ruled out the presence of autoantibodies. Through Rh typing, there was suspicion of antibodies to high-prevalence antigens or a combination of antibodies. Rh typing results revealed RhCcEe deficiency in this patient's RBC, which raised suspicion of anti-Hr_0 antibodies. The expression of the D antigen on the patient's RBC was higher than on the normal D + red cells, consistent with the D-- phenotype characteristics. Genetic testing showed that the patient had the *RHCE*CE-D(5)-CE* variant, reported in Caucasians but not in Asians and Blacks. While there

have been reports of D--phenotype in China in recent years, the mutation site does not align with this patient.

Rh antibodies are produced as a result of blood transfusions and pregnancy due to the immune responses of RBC. While some antibodies occur naturally, their exact production mechanism remains unclear. The blood group system exhibits a high frequency of antibody production, often resulting in the formation of combined antibodies. Prior to visiting our hospital, this patient had undergone a blood transfusion within three months. Consequently, due to antigen stimulation, the patient developed anti-Hr_0 antibody that target a prevalent antigen in the Rh blood group system. This antibody agglutinates all red blood cells except RhD--, Rhnull and Rhmod RBC, making it challenging to match compatible blood [9]. More unfortunately, due to the patient's advanced age, sole filial status, and failure to meet the criteria for autologous blood collection, compatible blood could not be found, leading to the cancellation of the surgery. This case highlights the importance of conducting antibody screening and identification prior to blood transfusions. When rare blood types are identified in advance, it is important to thoroughly assess their condition, minimize antigen stimulation, avoid blood transfusions whenever possible, and consider autologous blood donation for eligible surgical patients to preserve the opportunity for emergency blood transfusions. Furthermore, a rare blood group bank should be established to freeze and preserve the blood of D--phenotype donors, so as to meet the needs of patients with rare blood groups and ensure the safety of clinical transfusion.

Acknowledgements
Thank you to colleagues in the department for verifying serological experiments.

Author contributions
CYM and LHF were involved in the serological diagnosis of the rare phenotype and the patient blood management; YYL and YY drafted and critically evaluated the manuscript.

Funding
The authors did not receive any funding.

Availability of data and materials
All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Declarations

Ethics approval and consent to participate
This study protocol was reviewed and approved by Medical Ethics Committee of the General Hospital of the People's Liberation Army of China, approval number S2019-188-01. Written informed consent was obtained from legal guardians for publication of the details of their medical case and any accompanying images. The patient's identity is not disclosed in the paper.

Consent for publication
The patient's family members are allowed to disclose the details of the case.

Competing interests
The authors declare that they have no conflict of interest.

Received: 3 February 2024 Accepted: 26 March 2024
Published online: 22 May 2024

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