Submitted: 06 September, 2023 Accepted: 08 October, 2023 Published: 30 November, 2023 DOI:10.22514/jomh.2023.123

CASE REPORT

JOMH
Journal of Men's Health

ABO discrepancy due to the para-Bombay phenotype: a case report

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Abstract

In this study, we aimed to determine and analyze the genetic differences of the *FUT1* and *FUT2* genes within an individual exhibiting the Para-Bombay phenotype. Standard serological tests were conducted to analyze the ABO phenotype. The amplification of *FUT1* and *FUT2* genes was conducted by polymerase chain reaction and amplicons were sequenced. The determination of the *ABO* genotype was conducted by sequencing exons 1 to 7 of the *ABO* gene. The Lewis phenotype was defined as Le(a−b+); Despite this, red blood cells (RBCs) did not show signs of A, B and H antigens using the serological method utilized in this study. However, Analyses of sequencing and cloning demonstrated the existence of a *FUT1* mutation at position c.658C>T and an inactive allele *FUT1*01N.06* (c.551_552delAG). Genetic testing for ABO blood types was subsequently determined to be *ABO*B.01/ABO*B.01* (c.297A>G; c.526C>G; c.657C>T; c.703G>A; c.796C>A; c.803G>C; c.930G>A). Heterozygosity for indel and missense mutation within the coding region of *FUT1* are likely to be responsible for development of the Para-Bombay phenotype.

Keywords

ABO discrepancy; Para-Bombay; *FUT1*; *FUT2*

1. Introduction

The para-Bombay blood phenotype is an infrequent bloody type that has been rarely documented globally. Individuals exhibiting Para-Bombay phenotypes are identified by the lack of H antigens on the surfaces of their red blood cells (RBCs) [1]. In humans, regulation of the H antigen, which serves as a precursor for antigens A and B of the blood group ABO, involves the activity of two types of fucosyltransferase (*FUT*) enzymes [2]. The *FUT1* gene encodes for an enzyme known as H, which plays a crucial role in modulating the expression of H antigens on both erythrocytes and endothelial cells. *FUT2* encodes an additional H enzyme that is accountable for the biosynthesis of this antigen in bodily secretions [3]. *FUT1* gene mutations can result in the absence or severe reduction of H, thus causing the Para-Bombay phenotype. The Para-Bombay phenotype is characterized by the complete or partial loss of the H antigen on the surface of red blood cells, thus resulting in the presence of anti-H antibodies in the serum which leads to a discrepancy between the forward and reverse ABO blood groups [4]. It is extremely important to determine the blood type of Para-Bombay individuals who have anti-H antibodies since they suffer from severe hemolytic reactions when given RBCs containing the H antigen [5]. In this investigation, we present a unique and infrequent case of an individual exhibiting the Para-Bombay phenotype. We performed serological and genetic analysis to investigate the molecular mechanisms underlying the formation of the Para-Bombay phenotype.

2. Materials and methods

A male patient, aged 68 years, presented at our medical facility for further treatment after he was treated for diverticulitis of the ileocecal region and appendicitis three months previously. Otherwise, the patient provided no notable previous clinical history and had not undergone any previous blood transfusions. Upon admission, ABO blood group typing was determined by a YBXK-4A Automatic Blood Bank System (Wuxi Yuanbo Biotechnology Co. Ltd., Jiangyin, China); the ABO bloody type was further confirmed by manual tube tests. The Methods for tubing involved monoclonal anti-Le^a and anti-Le^b antibodies; Makropanel 16 (991026, 992025, 8000456588, Sanquin, Amsterdam, The Netherlands); anti-A, anti-B, anti-H antibodies and an ABO RBC Kit (20210608, 20201126, 20220407, Shanghai Hemo-Pharmaceutical & Biological Co. Ltd., Shanghai, China).

DNA was extracted from a sample of peripheral blood using a Universal Genomic DNA Kit (2272, Kangwei Century Biotechnology Co. Ltd., Jiangsu, China) in accordance with the manufacturer's instructions. A Human ABO, H Blood Group System Genotyping Test Kit (202112G, 202203D, Jiangsu Weihe Biotechnology Co., LTD, China) was used for polymerase chain reaction amplification and sequencing; amplicons were analyzed by a SeqStudio Genetic Analyzer

(Sequencing/Genetic Analysis, ThermoFisher Scientific, Waltham, MA, USA) [6, 7]. The sequencing profiles were read using Chromas Version 2.6.5 software (Applied Biosystems, Thermo Fisher Scientific) and resulting data were used to determine the *ABO, FUT1, FUT2* genotypes according to a database provided by the International Society of Blood Transfusion (ISBT) website.

3. Results

Upon admission, it was determined that the patient's blood type gel card exhibited a positive result for group O Rh D-positive during the forward grouping assessment. Nevertheless, upon conducting the reverse grouping, it was observed that the card showed a positive result for group B. The blood type was re-tested by the test tube method. The test tube results were consistent with those produced by the card method. However, further examination with O cells showed agglutination ($1+^{w}$). Consequently, the present case was subsequently referred to the Central Blood Station of Wenzhou for further investigation.

During forward grouping, the patient's RBCs did not possess any obvious ABO antigens (group O blood type). Further testing was performed; an agglutination test on the anti-H serum and the red blood cells did not show agglutination. A phenotype of Lewis is known as Le(a−b+) (Table 1). To evaluate for potential alloantibodies, samples of plasma were tested against 11 panels of the Makropanel 16 system with unique antigen profiles *via* standard tube methods at 4 °C, 37 °C and room temperature (Table 2). We were concerned that the alloantibodies (anti-H) were generally immunoglobulin (IgM).

Moreover, the *FUT1, FUT2* and *ABO* genes were genotyped separately by the Human H Blood Group and the Human ABO Blood Group Genotyping PCR Kit and sequenced directly. The patient's ABO gene genotype was determined to be *ABO*B.01/B.01* (c.297A>G; c.526C>G; c.657C>T; c.703G>A; c.796C>A; c.803G>C; c.930G>A), as determined by direct DNA sequencing. To investigate potential mutations in the *FUT1* and *FUT2* genes, we conducted amplification and sequencing of the complete coding regions of these two genes. Analysis of the *FUT1* gene sequence indicated that the patient possessed a compound heterozygous mutation, c.551_552delAG (p.Glu184Valfs*85) and c.658C>T, (p.Arg220Cys) (Fig. 1). In addition, the *FUT2* gene carried a homozygous single nucleotide variant, c.357C>T (p.Asn119=) (Fig. 2). According to these results, the patient had a Para-Bombay phenotype.

4. Discussion

The Para-Bombay phenotype is an uncommon phenotype and may potentially present challenges in the context of clinical blood transfusion and blood typing. Individuals who lack the H antigen are unable to generate the A antigen (referred to as substance A) or the B antigen (referred to as substance B) on their RBCs, irrespective of their alleles for blood groups A and B [8]. The serum of these individuals has the ability to generate an anti-H alloantibody which bears a similarity to the anti-A and -B iso hemagglutinins and is primarily composed of IgM. Furthermore, the serum exhibits activity at a temperature of 37 °C and can result in significant intravascular congestion [9]. Furthermore, it has been observed that these individuals exhibit a tendency to exhibit group O typing results when subjected to forward typing utilizing routine serological evaluation approaches. Nevertheless, it is important to note that due to their ability to express the H antigen in their secretions, their reverse typing results often reveal their genotype as ABO [10].

The Para-Bombay blood phenotypes are genetically heterogeneous and are distributed widely across the globe. The frequency of the Para-Bombay phenotype is 1:8000 to 1:10,000 in Taiwan, 1:15,620 in Hong Kong, and approximately 1:5000 to 1:16,200 in Southeast Asia [11]. To date, the prevailing genetic mutations of the *FUT1* gene associated with a phenotypic description of Para-Bombay are primarily deletions and point mutations of dinucleotides, specifically c.547delAG, c.880delTT and c.658C>T in Chinese individuals [12, 13]. The *FUT2* gene mutations associated with the Para-Bombay phenotype are point mutations (c.357C>T, c.716 G>A).

Our analysis indicated the absence of A, B and H antigens on the surfaces of RBCs, while reverse grouping identified the patient as group B according to serological results. These results suggested that the reason for the patient's ABO discrepancy was the Para-Bombay B phenotype with anti-H alloantibody. The patient's Lewis phenotype was determined to be Le(a−b+), while his genetic type of ABO was identified as *ABO*B.01/ABO*B.01*. These findings correspond with the secretor status of the patient and ABO reversing grouping, respectively. This Para-Bombay B condition features a classic mutation of *FUT1* (*FUT1*01N.06*) that impairs its functionality and stability; in addition, this mutation significantly downregulates the expression of the H antigen on RBCs.

This discovery demonstrates the significance of conducting serological examinations with O cell and anti-H tests in individuals with the Para-Bombay phenotype. Advanced molecular biology techniques can help to identify the Para-Bombay phenotype and can corroborate the results of complementary serological tests.

This study has a limitation that needs to be considered; the absorption dispersion test and the saliva test were not performed as these are time consuming. The systematic analysis of rare blood groups *via* serology and molecular biology can provide a theoretical basis for the correct identification of rare blood groups and the implementation of safe and effective transfusion therapy.

5. Conclusion

The presence of double deletions within the coding region of the *FUT1* gene is likely to be responsible for the Para-Bombay phenotype. In future, the molecular mechanisms responsible for the homozygous or heterozygous variations of the *FUT1* gene that are accountable for the Para-Bombay phenotype could be precisely defined by molecular diagnostic techniques.

TABLE 1. Serological analysis of ABH and Lewis antigens.

Forward grouping					Reverse grouping			Lewis	
anti-A	anti-B	anti-AB	anti-A1	Anti-H	Ac	Bc	Oc	anti-Le^a	anti-Le^b
-	-	-	-	-	4+	-	1+^{w}	-	+

Note: c, cells; -, negative; 1+–4+, positive according to agglutination strength; w, weak.

TABLE 2. Antibody screening.

	Antibody Identification
	(11 panels of Makropanel 16 (Sanquine))
IS	0
4 °C 10 min	1+ or 1+^{w}
37 °C 5 min	0

Note: IS, Immediately spin at room temperature; 11 panels; 1–11 of Makropanel 16 (Sanquine); 1+–4+, positive according to agglutination strength; w, weak.

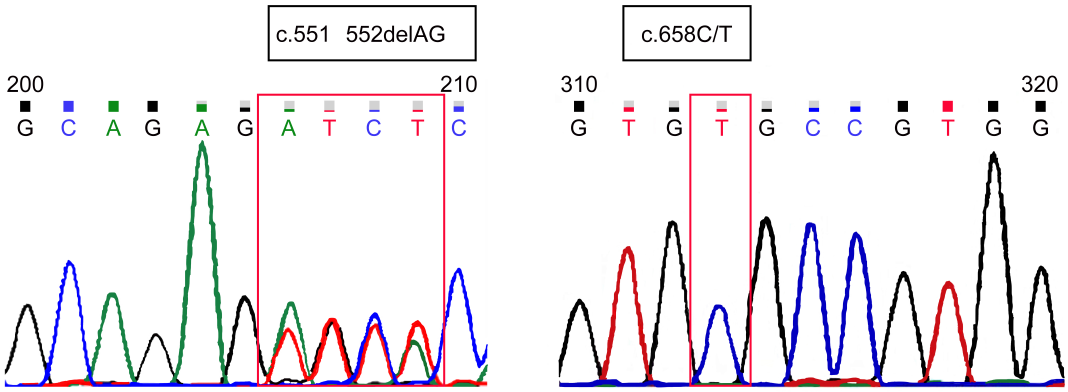

FIGURE 1. Direct sequencing revealed a compound heterozygous type in the *FUT 1* gene c.551_552delAG and c.658C>T.

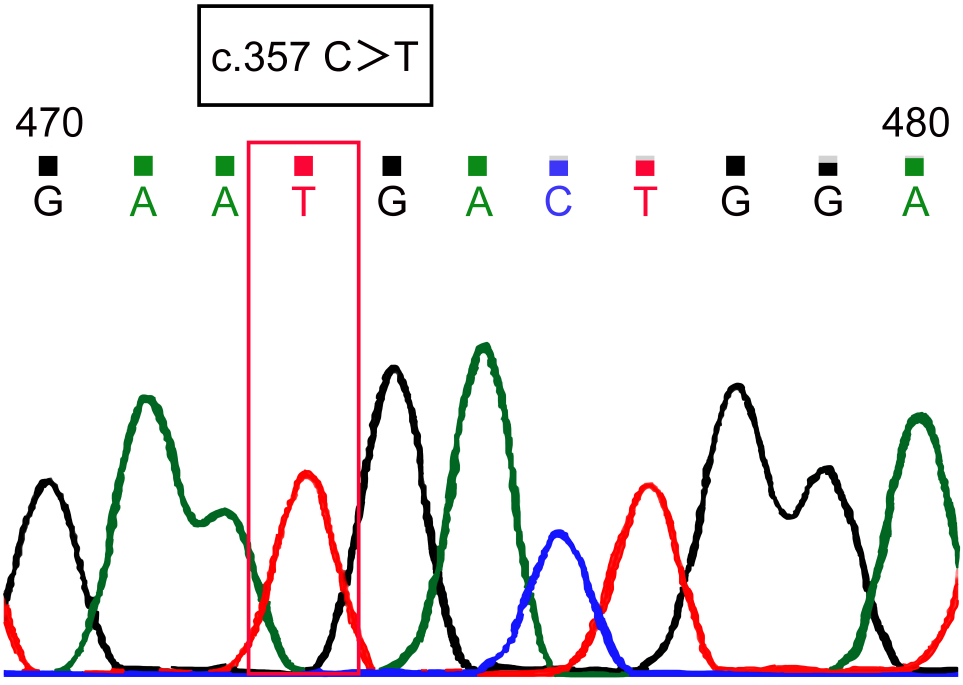

FIGURE 2. Direct sequencing revealed a homozygous variant in the *FUT 2* gene c.357C>T.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

YL, RCC and XZH—designed the study and carried them out, supervised the data collection, analyzed the data, interpreted the data, prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethics Committee of Wenzhou Central Hospital (L2023-03-062). Written informed consent was obtained from the patient for publication of this case report.

ACKNOWLEDGMENT

Not applicable.

FUNDING

This research received no external funding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Ying Lin, Rongcang Chen, Xingzhong Hu. ABO discrepancy due to the para-Bombay phenotype: a case report. Journal of Men's Health. 2023; 19(11): 116-119. doi: 10.22514/jomh.2023.123.