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Serological and gene sequencing analysis of a case of para-Bombay phenotype Am^h

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ABSTRACT

The paper aims to study the serological and genetic characteristics of a case of para-Bombay Am^h. The serological method was applied to identify the proband´s ABO phenotype and PCR-SSP assay was used to analyze the genotype of the para-Bombay blood. DNA sequencing of the PCR products of the first exon of *FUT1* gene was used to analyze the genotype and nucleic acid sequence mutation. The serological results showed that the ABO phenotype of the proband was O-type. However, while after absorption-elution test, the ABO phenotype showed weak A-type. The serological test also showed that the irregular antibody anti-H was positive. PCR-SSP assay showed that the proband was *h4* para-Bombay type and sequence analysis showed a point mutation c.35C>T of *FUT1* gene. The study suggests that genetic analysis is necessary for blood typing in those who have elusive immunological typing results.

Keywords: blood group, para-Bombay phenotype, *FUT1* gene

INTRODUCTION

Para-Bombay phenotype is a rare blood group, often showing an inconsistent or unexpected ABO phenotype by using conventional serological techniques[1]. The underlying mechanism is as follows: H antigen is the precursor of A and B antigen on the surface of red blood cells (RBCs), and its synthesis depends on the activity of a-(1,2)-fucosyltransferase (FUT1). *FUT1* mutation can cause the failure of H antigen synthesis, which can result in the defective A and B antigens[2]. Para-Bombay individuals often have anti-H antibody and show the unconformity of forward and reverse typing. If para-Bombay patients are given RBCs containing H antigen, they will suffer severe hemolytic reactions[3]. Here, we reported a Chinese case of para-Bombay Am^h phenotype with unconformity of forward and reverse typing, caused by a disruptive mutation in *FUT1* of a pregnant woman. This case highlights the importance of determining blood type accurately before clinical blood transfusion for pregnant women.

SUBJECTS AND METHODS

Subjects

The proband was a 27-year-old Han female with 39 weeks gestation from Shandong Province. She had a history of one successful delivery without transfusion. The serological results showed unconformity of forward and reverse typing. Meanwhile, the irregular antibody screening results were positive and Rh is also positive. This study was approved by Ethics Committee in the hospital and the informed consent was ob–

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tained from the participant.

Reagents and instruments

Anti-A and anti-B serum (20171224, Shanghai Blood Biomedical Co., China; 8000176551, Sanquin, Amsterdam, Netherland); Anti-Le^a and anti-Le^b antibody (8000227742, Sanquin, Amsterdam, Netherland); Antibody screening cells (8000237560, Sanquin, Amsterdam, Netherland); Antibody identification spectrum cells (8000207012, Sanquin, Amsterdam, Netherland); Human erythroid Bombay blood group genotyping kit (PCR-SSP, Tianjin Super Biotechnology Development Co., Ltd.); Taq polymerase (Promega Company, USA); KA-2200 centrifuge (Kubota Company, Japan).

Detection of ABH antigens on red blood cells

ABO forward and reverse group typing of proband were determined by using the routine serological method. Further absorption and elution test was conducted to detect the trace amount of ABH antigens on the surface of RBCs. The secretion of ABH antigens in saliva was detected by the standard serologic hemagglutination inhibition method. Lewis blood group was also tested for identification of the secretory type.

PCR-SSP genotyping

The para-Bombay gene was detected by human erythroid Bombay blood group genotyping kit (PCR-SSP). The internal control was a conserved fragment (983 bp) of *hGH* gene. The primers´ sequences were seen in ***Table 1***. An ultraviolet gel imaging system was used to observe the results and record the image. PCR cycle parameters were: 96℃ , 2 min; 96℃ , 2 min, 1 cycle; 96℃ , 20 sec, 68℃ , 60 sec, 5 cycles; 96℃ , 20 sec, 65℃ , 45 sec, 72℃ , 30 sec, 10 cycles; 96℃ , 20 sec, 62℃ , 45 sec, 72℃ , 30 sec, 15 cycles; 72℃ , 2 min, 1 cycle; 4℃ preserved.

FUT1 and ABO gene sequencing

The *FUT1* and ABO gene *EXON1-7* were directly sequenced. The *FUT1* gene primer sequences: forward, 5´-CTGGGACTAAGGAGTCTGC-3´; reverse, 5´-CTGCCTGCCAGGCATC-3´. PCR products were sequenced using a cycle sequencing kit on ABI 3730 sequencer. The mutation site was determined by BLAST with existing exon1 sequence in GenBank.

Table 1 **The primers sequences of the eight holes and the names of genes**

Position	Direction	Primers	Sequences (5´–3´)
1	forward	h1	TCGTGCAGGGTGAAGTCTGC
	reverse	Nh4	ATCGTCAGCTCTGCGTGGC
2	forward	Nh1	GTCGTGCAGGGTGAAGTCTCT
	reverse	Nh4	ATCGTCAGCTCTGCGTGGC
3	forward	h2	GCACTGTGTGAGCAGGGGAG
	reverse	682A	CGTGGGGACTATCTGCAGGTTA
4	forward	Nh2	GCACTGTGTGAGCAGGGGAA
	reverse	682A	CGTGGGGACTATCTGCAGGGTA
5	forward	h3	TGTCGGCGTCCACGGGT
	reverse	Nh2	GCACTGTGTGAGCAGGGGAA
6	forward	Nh2	GCACTGTGTGAGCAGGGGAA
	reverse	Nh3	TGTCGGCGTCCACGGGC
7	forward	682G	GTGGGGACTATCTGCAGGGTG
	reverse	Nh2	GCACTGTGTGAGCAGGGGAA
8	forward	Nh1	GTCGTGCAGGGTGAACTGTCT
	reverse	h4	ATCGTCAGCTCTGCGTGGT

RESULTS

Serological identification

The testing of the patient´s RBCs with monoclonal ABO typing reagents indicated that the blood type of the proband was weak A type, but easily confused with O phenotype. During reverse blood group typing at room temperature, the serum was strongly reactive with Bc RBCs with 4+ intensity of agglutination, and weakly reactive with A1 and O cells. However, reverse typing showed the presence of anti-A1 (2+) and O cells at 4℃ , and the presence of anti-A1 (1+) and O cells at 37℃ (***Table 2***).

Table 2 **Serological results of blood group typing**

Reaction temperature	Antigen on RBCs					Antibody in serum			
	anti-A	anti-B	anti-H	anti-Le^a	anti-Le^b	A1c	Bc	Oc	Control
4℃	0	0	0	0	2+	2+	4+	2+	0
Room temperature	0	0	0	0	1+	1+	4+	1+	0
37℃	0	0	0	0	1+	1+	4+	1+	0

Note: ABO forward and reverse typing and Lewis type. Antigen typing using monoclonal reagents showed generally negative results (0), as opposed to positive reactions (slightly positive 1+~2+, and strongest positive 3+~4+, respectively).

Absorption-elution test, saliva-test and antibody identification

The absorption-elution test of proband showed weak reaction with Ac (2+). The presence of ABH substances in saliva was consistent with their Le (a–b+) phenotypes (***Table 3***). Anti-H was found in the serum of individual with titer of 1:32.

Table 3 **Absorption-elution test and saliva test results**

Contents	Absorption-elution test			Saliva test		
Reagents	Ac	Bc	Control	Anti-A	Anti-B	Anti-H
Reaction	2+	0	0	0	2+	0
Conclusion	Weak A antigen			A and H substance positive		

PCR-SSP genotyping

According to the base number of the target gene involved in the study, there are 241 bp, 264 bp, and 541 bp bands respectively at the 4, 6, and 8 in the classification table of reference gene results, which is confirmed as *h4* homozygotes, as shown in ***Fig. 1***.

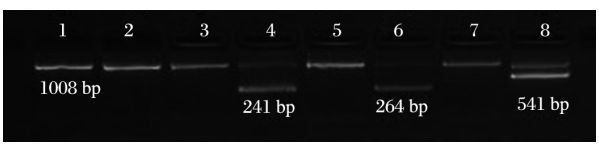

Fig. 1 **PCR-SSP genotyping results**

Gene sequencing

The direct sequencing analysis of *FUT1* PCR products showed that the point mutation at the 35th base was c.35C>T (***Fig. 2***). The sequencing results were consistent with the results of genotyping method. And the ABO sequencing was ABO*A1.02/ABO*O.02.01.

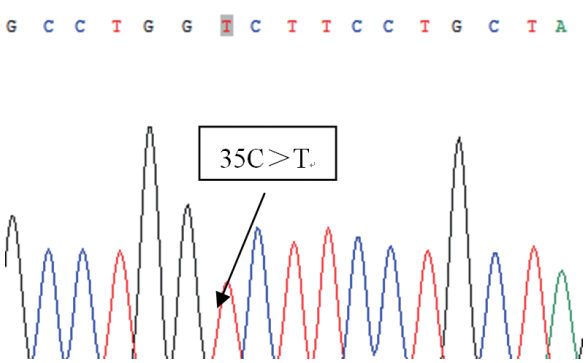

Fig. 2 **Sequence analysis results of *FUT1* gene**

DISCUSSION

The para-Bombay phenotype is characterized by the absence or weak expression of ABH antigens on RBCs, with the presence of ABH substances in body secretions[1,4]. In this study, one individual with the para-Bombay Am^h phenotype was detected. Unfortunately, her previous blood transfusion history was not known, and any family related data were not available. Anti-H antibody was observed in the patient´s serum and still active at 37℃ . The presence of anti-H antibody has clinical significance, since it can activate complement and cause hemolysis[5–8]. Therefore, when an individual with para-Bombay requires transfusion, it is necessary to use RBCs without H antigen. The para-Bombay Am^h phenotype is easily confused with O phenotype. So absorption-elution test combined with genotyping is necessary for this kind of blood group typing.

At present, there are more than 50 reported mutations in *FUT1* gene, including missense, deletion, and insertion mutations. The most common mutations are the following: *h1* (547delAG), *h2* (880delTT) and *h3* (658C>T)[9]. The genes of both Bombay type and para-Bombay type are polymorphic[10]. Most of the mutations are point mutations, or gene deletions at different position[11]. In our study, the para-Bombay PCR-SSP kit was selected to confirm the para-Bombay genotype. So far, nearly 50 different *FUT1* mutations capable of causing H antigen defects in RBCs have been reported[12–14]. The proband we found was *h4/h4* genotype. The molecular basis of mutation in *FUT1* was c.35C>T. This mutation brings to the amino acid change of α-(1,2)-fucosyltransferase from alanine (ALA) to valine (VAL), which would result in the termination of protein synthesis. The mutation caused the loss or decline of the enzyme activity and the para–Bombay serological panel emerged.

In this paper, we only observed the mutations of *FUT1* and the ABO genes, but failed to analyze *FUT2* gene. And it is necessary to study the family investigation in the future.

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References

[1] Lin JJ, Chen RC, Zhu SY, et al. Sequence analysis of α-(1, 2)-fucosyltransferase gene in nine Chinese individuals with para-Bombay phenotype[J]. *Gene,* 2019, 685: 21–3.

[2] Yang XJ, Xie HH, Peng YX. Identification and transfusion strategy of Bombay like type[J]. *Chinese Journal of Blood Transfusion (in Chinese)*, 2018, 31(5): 486–9.

[3] Luo GP, Wei L, Wang Z, et al. The summary of FUT1 and FUT2 genotyping analysis in Chinese para-Bombay

individuals including additional nine probands from Guangzhou in China[J]. *Transfusion,* 2013, 53: 3224–9.

[4] Zhao Q, Wang ZL, Su M, et al. Identification and molecular mechanism of para-Bombay phenotype[J]. *J Clin Hematol (in Chinese)*, 2019, 32(1): 159–61.

[5] Zhang JP, Zheng Y, Sun DN, et al. Formation of para-Bombay phenotype caused by heterozygous or homozygous mutations of FUT1 gene[J]. *Chinese Journal of Experimental Hematology (in Chinese),* 2014, 22(1): 195–8.

[6] Liao XC, Jiao W, Li HL, et al. Molecular mechanism of para-Bombay: a report of 2 families[J]. *Chinese Journal of Blood Transfusion (in Chinese)*, 2015, 28(12): 1475–8.

[7] Wang F, Mao W, Zhang T, et al. Study on the molecular mechanism of Bh para-Bombay[J]. *Chinese Experimental Diagnostics (in Chinese)*, 2017, 21(7): 1249–52.

[8] Chang HJ, Zhang NH. Serological characteristics and pedigree analysis of a para-Bombay[J]. *Journal of Clinical Transfusion and Laboratory Medicine (in Chinese)*, 2018, 20(5): 556–7.

[9] Guo ZH, Xiang D, ZhuZY, et al. Analysis on FUT1 and FUT2 gene of 10 para-Bombay individuals in China[J]. *Chin J Med Genet (in Chinese)*, 2004, 21(5): 417–21.

[10] Wang Ni, Song WQ, Xia YX, et al. Gene analysis of a Bmhpara-Bombay[J]. *Chinese Journal of Blood Transfusion (in Chinese)*, 2019, 32(8): 828–30.

[11] Matzhold EM, Wagner T, Drexler C, et al. FUT1 aberrant ABO B phenotype with irregular anti-B caused by a para-Bombay mutation[J]. *Transfus Med Hemother,* 2020, 47: 94–7.

[12] Yang XY, Wang GY. Advances in ABO gene expression regulation[J]. *Asia-Pacific Journal of Blood Types and Genes*, 2019, 3(2): 101–7.

[13] Er TK, Yang TC, Liu YH. Heterozygous FUT1 mutations causing a para-Bombay phenotype[J]. *Clin Lab*, 2018, 64: 1787–9.

[14] Liang W, Cai F, Yang L, et al. FUT1 four non-functional alleles were identified in seven Chinese individuals with para-Bombay phenotypes[J]. *Iran J Public Health*, 2018, 47: 1128–36.

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