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B para-Bombay phenotype in China caused by homozygous mutation for site 328 G>A of *FUT1* gene: a case report

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ABSTRACT

The aim of this paper is to accurately identify a case of B para-Bombay and to analyze the genetic mutation. ABO and Lewis blood groups were identified by standard serological methods, and trace antigens on RBCs were detected by adsorption-elution test, while blood group substances in the saliva were detected by agglutination inhibition test. The ABO gene exons 6–7, *FUT1* gene exon 4 and *FUT2* gene exon 2 were directly sequenced. Serological results showed that there were B antigens on RBCs without H antigens, anti-A and anti-HI antibodies in serum, and B and H blood group substances in the saliva. The Lewis phenotype was Le (a–b+). According to gene sequencing analysis, ABO, *FUT1* and *FUT2* genotypes were B101/O02, $h^{328G/A}h^{328G/A}$ and $Se^{357C/T}Se^{357C/T}$, respectively. This rare phenotype can be mislabeled as "O" if any of the detailed investigations are not performed. Therefore, in order to ensure the safety of blood transfusion, genetic and serological tests are necessary for the correct identification of difficult blood groups.

Keywords: para-Bombay, *FUT1*, homozygous mutation

INTRODUCTION

H antigen is synthesized by α-(1, 2)-fucosyltransferases that is encoded by *FUT1* and *FUT2* genes, which are located on 19q13.3. *FUT1* and *FUT2* genes are tightly linked[1]. *FUT1* gene, which has four exons, determines the expression of H antigen on red blood cells[2]. *FUT2* gene, which has two exons, regulates the synthesis of the H antigen in secretions[3]. H antigen is the precursor of A and B antigens. So, the Hh blood group system is closely related to the ABO blood group system. The para-Bombay phenotype is characterized by the absence of ABH antigens on red blood cells (RBCs) with the presence of ABH substances in body secretions or by the weak expression of ABH antigens on RBCs with the absence or presence of ABH substances in body secretions. Mutations of *FUT1* and *FUT2* form various para-Bombay phenotype alleles that have gradually been reported in many cases worldwide[4–5]. In this case, we reported an unusual para-Bombay phenotype in China caused by a homozygous mutation of 328G/A in the *FUT1* gene.

CASE REPORT

A 35-year-old female patient was admitted to the gynecology department of our hospital for upper abdominal pain and in appetence for 1 month, and was finally diagnosed with ovarian cancer. The patient was a member of the Lahu ethnic minority and had a history of childbearing, without blood transfusion. A specimen of the patient´s blood type was sent to our blood transfusion department for identification. The

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result of blood type detected by automatic matching and blood type analyzer (WADiana Compact, Diagnostic Grifols, S.A, Spain) was discordant with positive or negative type. Therefore, we used serological experiments and gene sequencing methods to further identify the discrepant blood group. The study was approved by the Second Affiliated Hospital of Chongqing Medical University´s ethics committee.

Blood grouping was performed using a standard serological technique with tube method. In positive type, the RBC agglutinated with anti-B in an intensity of 1+, and showed no reactivity against anti-A, anti-A_1 or anti-H (anti-A and anti-B provided by Shanghai Blood Bio-pharmaceutical Co., Ltd. Shanghai China; anti-A_1 and anti-H provided by Rapid Labs Ltd. UK). However, the plasma showed positive agglutina-tion with A, B and O cells at room temperature (ABO cells provided by Beijing Jinhao pharmaceutical Co., Ltd. Beijing, China). In addition, highly sensitive assays for the adsorption-elution test indicated the presence of weak B antigens on the patient´s RBCs. B and H blood-group substances were detected in the saliva (***Table 1***). Combined with the above results, the patient´s blood type was considered B para-Bombay phenotype. Moreover, anti-HI was found in the plasma. Because the plasma can agglutinate with adult group O cells but not with cord cells, the agglutination with group O cells of adults cannot be inhibited by group O secretor saliva[6]. The Lewis blood type was Le (a−b+) (anti-Lea and anti-Leb provided by Immucor, USA). The antibody screening test using the microcolumn gel card containing anti-human globulin was positive. Because we were unable to find blood donors with the same phenotype as the patient in Chongqing Blood Center, we randomly selected 6U type B^+ donors for microcolumn gel card cross matching. The results showed that 2U of erythrocytes matched with the patient. During this hospitalization, the patient was given a warm infusion of 2U type B^+ RBCs with matching blood. No adverse reactions were observed during the transfusion. Unfortunately, because the patient´s families were all in another province, we couldn´t make a genetic genealogy survey.

Table 1 **The results of serological experiments**

Reaction condition	RBCs with antiserum				Serum with reagents RBCs				Adsorption-elution experiment				Lewis		Blood type substance		
	Anti-A	Anti-B	Anti-A_1	Anti-H	A_c	B_c	O_c (adult)	O_c (cord)	Anti-A	Anti-B	Anti-A_1	Anti-H	Anti-Le^a	Anti-Le^b	A	B	H
RT	−	1+	−	−	4+	1+	2+	−	−	4+	−	−	−	2+	−	+	+
4℃	−	1+	−	−	4+	2+	3+	−	NT	NT	NT	NT	NT	NT	NT	NT	NT
37℃	−	1+	−	−	4+	−	1+	−	NT	NT	NT	NT	NT	NT	NT	NT	NT

NT: no experiment was done.

Direct sequencing of the 6th and 7th exons of the ABO gene had 261 delG, 297A>G, 526C>G, 646T>A, 657C>T, 681G>A, 703G>A, 771C>T, 796C>A, 803G>C, 829G>A, 930G>A mutations when compared with the alleles of the reference sequences (A1.01.1) (***Table 2***). These traits were consistent with B101/O02, which were in accordance with the patient´s serological characteristics (ABO 6−7 Exons Sequencing Kit of Jiangsu ZoJiwat Biomedical Co., Ltd, Jiangsu, China). In comparison with the reference sequence (NM-000148), the allele of the *FUT1* genotype showed *FUT1**01W.02/*FUT1**01W.02 (c.328G>A, p. Ala110Thr). The *FUT2* genotype was $Se^{357}Se^{357}$ allele, which is a synonymous mutation (c.357C>T, p. Asn119Asn), suggesting secretor status (***Fig. 1***).

Table 2 **ABO gene exons 6−7 sequencing results**

Sequences	Exon 6		Exon 7										
	261	297	467	526	646	657	681	703	771	796	803	829	930
Reference(A1.01.1)	G	A	C	C	T	C	G	G	C	C	G	G	G
Specimen	del/G	GG	CC	CG	AT	CT	AG	AG	CT	AC	CG	AG	AG
Specimen-B101	G	G	C	G	T	T	G	A	C	A	C	G	A
Specimen-O02	del	G	C	C	A	C	A	G	T	C	G	A	G

DISCUSSION

We experienced a rare case, in which a homozygous mutation of 328G/A in *FUT1* gene led to the formation of B para-Bombay phenotype. The sequencing results of *FUT1* were *FUT1**01W.02/*FUT1**01W.02, which is a very rare H^+ weak expression according to the International Society of Blood Transfusion. The mutation of G to A at site 328 resulted in the change of amino acid 110 from Ala to Thr, and finally reduced the activity of α-(1,2) fucosyltransferase, leading to the weak expression of H antigen on RBCs. So far as we know, this homozygous mutation of 328G>A in para-Bombay phenotype has only been reported in one Chinese individual[7], although the heterozygous mutation of 328G/A has been reported in the Chinese[8–9]. The latest research showed that a Korean study reported the same allele as this case in an Indonesian[10]. However,

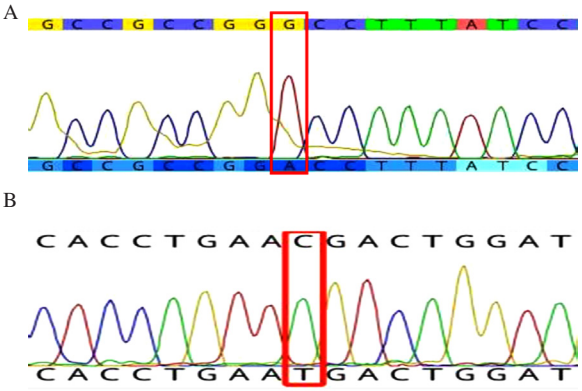

Fig. 1 **The results of *FUT1* and *FUT2* sequencing of the patient.** A: Homozygous mutation of the 328G/A in *FUT1*. B: Homozygous mutation of the 357C/T in *FUT2*.

this type of homozygous mutation of the 357C/T in *FUT2* is more common in the Chinese[11].

The alleles caused by *FUT1* and *FUT2* gene mutations have ethnic and geographic characteristics[12–13]. According to previous research in China, the incidences of the para-Bombay phenotype in Taiwan, Hong Kong and Fujian Province are 1/8 000, 1/15 620 and 1/8 500, respectively [14–16]. However, its incidence in the Lahu Nationality in Yunnan is much more common, which may be due to inbreeding[17]. This patient happened to be of the Lahu ethnic group. Luo G et al.[11] reported that the four most common alleles of *FUT1* in para-Bombay phenotypes in the Chinese population are h1(547delAG), h2(880delTT), h3 (658C>T), and h4 (35C>T).

Since anti-HI as the cause of hemolytic reaction had been reported previously[18], it is best to select a B para-Bombay phenotype donor for the patient. This may be obtained either from family donors or from a bank of rare blood types. Since the antibody of anti-HI had been detected in the patient´s plasma and we were unable to find a B para-Bombay phenotype donor, cross matching had to be done between a B^+ donor and the patient. Fortunately, 2U RBCs were found to be compatible. We conjectured that the cause might be the reduced activity of the anti-HI at 37°C and the relative low density of I antigen on the two donor´s RBCs.

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