CORRESPONDENCE

Bombay Blood Group in a Turkish Family: Serological and Molecular Analysis

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Received: 10 February 2014 / Accepted: 24 July 2014 / Published online: 6 August 2014
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Dear Sir

The ABO blood group antigens are oligosaccharide antigens and expressed on erythrocyte membranes and in secretions. The H antigen is the precursor of the A and B antigens. A and B antigens are determined by the ABO locus, while the H antigen is determined by α - [1, 2]—fucosyltransferase genes FUT1 (H) and FUT2 (Se). FUT1 and FUT2 genes are responsible for H antigen expression on erythroid lineage and in secretions, respectively. [1, 2]. Individuals with the rare Bombay phenotype do not express H antigen because of the absence of both H and Se enzyme activities. As a result, they cannot make A antigen or B antigen on their red blood cells. Individuals with the para-Bombay phenotype do not express ABH antigens on red blood cells, but ABH substances are present in saliva [3, 4]. H deficiency is rare: it is found in 1 of 8,000 in Taiwan, 1 of 10,000 in India, and 1 per million in Europe [5]. Despite its rarity, its identification is critical in transfusion medicine, as patients with this phenotype can only be transfused with Bombay RBC units. Bombay blood group had been previously reported in Turkish individuals by using serological method [6]. Here, we report a Turkish donor with

Bombay phenotype, which was confirmed by serological tests, pedigree studies and sequence analysis (Table 1).

Blood group test was performed to a 38 year-old-Turkish man who came to the blood center in Istanbul for blood donation. Agglutination test of plasma with O red cells was four positive. As the blood center in Istanbul did not have the technical capability of performing an anti-H test, the donor was invited to our blood center in Ankara. Initially, the proband gave no prior history of blood transfusions nor did he have any underlying medical conditions. We studied serological tests to confirm the Bombay phenotype. The red cells were not agglutinated by anti-A, anti-B (Diamed AG, Switzerland) and anti-H from Ulex europeus for forward reaction. Interestingly, the proband has a different serologic profile from the known Bombay phenotype: Agglutinations test of plasma with A₁, A₂, B and O red cells (reverse reaction) resulted as mixed field, mixed field, four positive and four positive, respectively. Antibody screening and identification tests were performed with a 3-cell and 11-cell panels (Diamed AG, Switzerland), respectively. All of panel cells showed grade four agglutination with the patient's cells.

The proband's Lewis phenotype was Le (a-b-). The serological results indicated that the proband may be Bombay phenotype. Daughter of the proband was also identified with Bombay phenotype according to the results of serological tests. The pedigree of the proband's family is shown in Fig. 1.

FUT1 and *FUT2* gene mutation analysis was planned in order to determine the genotype of proband. Genomic DNA was isolated from anticoagulated peripheral blood samples. Primers were designed to amplify the coding sequence of *FUT1* and the related secretor gene, *FUT2*. For *FUT1* locus, an 1568-bp DNA fragment encompassing the full coding region was amplified with a forward primer (5'-

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Table 1 Primers were used in the study for sequencing

Gene	Exon	Direction	Sequence (5' → 3')	Amplicon length (base pair)
FUT1	4	Forward	CCGAAATTCCAAGGCTCTCATCC	1568
	4	Reverse	ATATGGGCTGGGAAGAGCAGGTG	
FUT2	2	Forward	TAAACACACTTGAGATACATGCCTGTGC	1361
	2	Reverse	GTTCACTGAGATTTCTGTTACTTGCAGC	

Fig. 1 Pedigree of the family members of the proband

CCG AAA TTC CAA GGC TCT CAT CC-3') and a reverse primer (5'-ATA TGG GCT GGG AAG AGC AGG TG-3'). For *FUT2* locus, an 1361-bp DNA fragment encompassing the full coding region was amplified with a forward primer (5'-TAA ACA CAC TTG AGA TAC ATG CCT GTG C-3') and a reverse primer (5'-GTT CAC TGA GAT TTC TGT TAC TTG CAG C-3') by polymerase chain reaction (PCR). For sequence analysis, the amplified fragments were purified using the NucleoFast® 96 PCR (MACHEREY–NAGEL GmbH), sequenced using the Nextera XT DNA Sample Prep Kit (Illumina, San Diego, CA) and then analyzed in an Miseq sequencer (Illumina, San Diego, CA). The sequences were analyzed with computer software (IGV 2.3, Broad Institute). The genetic analysis of the *FUT1* locus revealed a homozygous status for described mutation (422G > A) associated with Bombay phenotype. This mutation creates a stop codon. Mutations detected in the *FUT2* gene [461G > A (W154X) and 772G > A (G258S)] was evaluated as polymorphism due to high allele frequencies observed in the society. However, one of these mutations (W154X) causes “non-secretor” phenotype.

We report the first Turkish case of serologically-detected, sequencing-confirmed Bombay blood group phenotype. We also want to show genetic characterization of the mutated

alleles associated with this phenotype in Turkey. This proband and his family are the first step of this study. Further investigation will include all members of the family.

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