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ORIGINAL ARTICLE

Mutational Analysis of Bombay Phenotype in Iranian People: Identification of a Novel *FUT1* Allele

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Abstract Bombay phenotype is characterized by lack of ABH antigens on RBCs and in body secretions as a result of mutations in fucosyltransferase 1 (*FUT1*) and fucosyltransferase 2 (*FUT2*) genes. The aim of this study was a mutational analysis in Iranians with this phenotype. Serological analyses including ABH and adsorption-elution tests were performed in five unrelated Bombay individuals. ABO genotyping was determined by direct sequencing. The coding regions of *FUT1* and *FUT2* genes were amplified and sequenced directly or after cloning into suitable vector. A novel missense *FUT1* allele was detected (G1051T; G351C). Also four reported *FUT1* alleles were revealed. Molecular analysis of *FUT2* gene confirmed nonsecretor status in all individuals. This and our previous findings suggest the diversity and population specificity of *FUT1* alleles.

Keywords Bombay phenotype · *FUT1* · *FUT2* · ABH antigens

Introduction

The human ABH histo-blood group antigens are carbohydrate molecules which are made through the consecutive action of a series of glycosyltransferases [1–4]. The H substance, the precursor of these antigens, is an oligosaccharide molecule that is expressed on the surface of erythrocytes and a variety of epithelial cells. It is synthesized by alpha(1, 2) fucosyltransferase enzymes [3–6].

ABO antigens are synthesized by the action of glycosyltransferases on H substance and are expressed on the membrane of erythrocytes as well as in secretions and tissues like salivary glands. ABO gene which is located on long arm of chromosome 9q34 is essential for the synthesis of A and B antigens [6, 7].

There are two kinds of alpha(1, 2) fucosyltransferase in human tissues: Fut1 enzyme, encoded by *FUT1* gene, regulates the expression of ABH antigens on erythrocyte and endothelial cells. Fut2 enzyme is responsible for the expression of ABH antigens in gastrointestinal tract and secretions and is the product of *FUT2* gene [6–8]. *FUT1* and *FUT2* genes are located just close to each other on chromosome 19q 13.33 with 70% DNA sequence homology. The protein coding sequence of both genes has been confined to a single exon [7–12].

Bombay and *para*-Bombay phenotypes are rare genetic cases. These individuals are not able to express the ABH antigens on the membrane of erythrocytes and/or secretions, because of different silencing mutations in *FUT1* and/or *FUT2* [2–5]. The characteristic of Bombay phenotype is the lack of ABH antigens both on RBC's surface and in saliva while *para*-Bombay phenotype is the result of nonfunctional *FUT1* gene [4, 5, 13]. We previously reported two novel *FUT1* alleles in Iranian people with Bombay phenotype [8]. Here we describe a mutational analysis of five people with Bombay phenotype in Tehran, Iran.

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Materials and Methods

Samples and Serologic Typing

Samples were from five unrelated Iranian people who were referred to the Immunohematology laboratory of Iranian Blood Transfusion Organization due to discrepancy in ABO typing and incompatibilities in blood transfusion. After obtaining written consent from the individuals, 10 ml of peripheral blood with EDTA anticoagulant and saliva samples were collected.

The serological tests including the presence of ABH, and Lewis antigens were performed by standard tube agglutination. The secretor status was determined by testing saliva samples using standard serologic hemagglutination inhibition method as described previously [10].

Molecular Analysis

Genomic DNA was isolated from 0.2 ml of peripheral blood by a commercial assay (Yekta Tajhiz Azma; Tehran, Iran) according to manufacturer's instruction.

The full-length coding regions of *FUT1* and *FUT2* genes were divided into two overlapping fragments and amplified using primers Fut-1A, Fut-1B, and Fut-2A, Fut-2B respectively. The ABO genotype was determined by amplifying DNA fragments encompassing the exon 6 and 7 of ABO gene by polymerase chain reaction (PCR). The sequences of all primers are described elsewhere [8]. The amplification reactions were performed in a final volume of 50 µl, using 2 × PCR MasterMix (Yekta Tajhiz Azema; Tehran, Iran), 0.2 µM of each primer, and 50-100 ng of genomic DNA. Thermal cycling was carried out with an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 30 s at 95 °C, 45 s at 60 °C and 45 s at 72 °C, plus a final extension at 72 °C for 10 min. PCR products were separated by electrophoresis in 1.5% gel agarose containing 2 µl of Green Viewer (Pars Tous; Tehran, Iran) and visualized under UV light.

Sequencing reactions were performed using cycle sequencing kit (Big dyes Terminator Version 1.1; Applied Biosystems, Weiterstadt, Germany). When there was ambiguity in sequence analysis, PCR products were cloned in pDrive vector (QIAGEN PCR Cloning Kit; Hilden, Germany) and 4–6 colonies were sequenced.

Protein Structure Analysis

I-TASSER approach was used to predict protein structure of wild and mutant alleles of Fut1 enzyme. Molecular dynamic analysis was performed with Gromacs 4.6.5. RMSD greater than 2.0 was considered as a significant structural change.

Results

Table 1 represents serological and molecular analysis of ABH antigens. Lewis phenotype, the absence of H substance on RBCs and in saliva, and the presence of strong anti-H in sera confirmed the Bombay phenotype in all individuals.

Figure 1 represents gel electrophoresis of PCR products of *FUT1*. We found a novel *FUT1* allele in sample 94A (G1051C). This variation resulted in the substitution of glycine by cysteine at codon 351. Others four samples were also homozygous for reported point mutations as indicated in Table 2.

FUT2 mutational analysis revealed homozygosity of G428A in all individuals except sample 94E which generated no product with primers *FUT2*-A and *FUT2*-B. Genomic DNA of this sample was amplified with primers described by Koda et al. [14] which produced an approximately 1.8 kb fragment.

Protein prediction study revealed a structural change for Y224del allele when compared to wild-type allele (RMSD = 2.07). No significant change was observed for G1051C allele (RMSD = 1.2).

Discussion

Mutations of *FUT1* and *FUT2* genes result in Bombay phenotype, a rare genetic condition, which can be problematic when a person with this phenotype is in need of transfusion.

Five unrelated individuals with Bombay phenotypes were analyzed both serologically and with molecular assay. Sample 94A was homozygous for a novel mutation G1051T which caused the substitution of glycine by cysteine. Theoretically this amino acid change, the substitution of a hydrophobic amino acid by a polar one, renders the protein structure dramatically. However molecular dynamic analysis by Gromacs (RMSD = 1.2) did not show any significant change in the mutant protein. Further structural analysis especially contact-based measures described by Kufareva et al. [15] and also functional assay of the mutated enzyme can be conclusive as it is the next step of our research and the results will be published in our future paper. Sample 94B harbored a three nucleotides deletion 668-670del (Y224del). This variation was previously described [8] and it seems likely that is prevalent in our population. Molecular dynamic analysis predicted significant structural changes (RMSD = 2.07). Also

Table 1 Serological analysis of ABH and Lewis antigens

Sample	Hemagglutination			Antigen in saliva			Lewis phenotype	Anti-H (plasma)	ABO genotype
	A	B	H	A	B	H			
94A	–	–	–	–	–	–	Le(a + b −)	+	A_1A_1
94B	–	–	–	–	–	–	Le(a + b −)	+	O_1O_1
94C	–	–	–	–	–	–	Le(a + b −)	+	A_1B_1
94D	–	–	–	–	–	–	Le(a + b −)	+	A_1B_1
94E	–	–	–	–	–	–	Le(a-b −)	+	A_1O_1

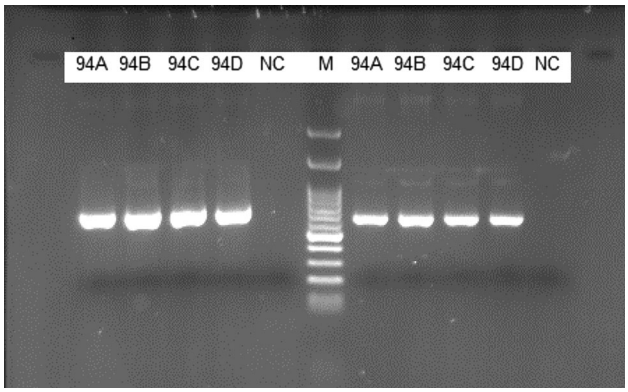

Fig. 1 Agarose gel electrophoresis of PCR reactions for FUT1 sequencing. The coding region of *FUT1* gene was divided into two overlapping amplicons, *FUT1*-A (left) and *FUT1*-B (right), generating two fragments of 670 and 660 bp length respectively. *NC* negative control, *M* molecular size marker 100 bp ladder

Table 2 Molecular findings of *FUT1* and *FUT2* mutational analysis

Sample	*FUT1*	*FUT2*
94A	G1051T **(G351C)**	G428A **(W143X)**
94B	668-670del **(Y224del)**	G428A **(W143X)**
94C	C826T **(Q276X)**	G428A **(W143X)**
94D	C826T **(Q276X)**	G428A **(W143X)**
94E	T725G **(L242R)**	Deleted

phylogenic comparison revealed this residue is conserved in different mammalians [8]. Samples 94C and 94D both were homozygous for a silencing mutation C826T (Q276X) which was first reported by Kelly et al. [16]. Sample 94E was homozygous for a missense mutation T725G (L242R), the classic Bombay mutation first described by Koda et al. in Indian people [17]. It is generally inherited as a haplotype with deleted *FUT2* gene and it is the case with this sample.

Investigation of *FUT2* mutations determined all samples to be homozygous for inactivating G428A allele except sample 94E. We found this allele to be the most frequent silencing mutation of *FUT2* in Iranian people (0.443, unpublished data). Sample 94E was homozygous for a partial deletion of *FUT2* including the coding sequence of exon 2. This is consistent with the findings of Koda et al. Non-coding sequence of this gene is rich in *Alu* repetitive DNA and as a result a hot spot for chromosomal recombination [14].

In conclusion, we found one novel mutation; three previously reported mutations of *FUT-1* gene and one silencing mutation also previously reported of *FUT-2* gene in five individuals with Bombay phenotype. This and our previous study indicate the diversity of mutations underlying this rare genetic condition.

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Author's Contribution FRM, MM and ZS performed the molecular and serological tests. MS designed the research and prepared the manuscript.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they do not have any competing interests.

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