Two Novel α 1,2-Fucosyltransferase Alleles in an H-Deficient Phenotype Individual

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Keywords

Blood group · H-deficient phenotype · *FUT1* gene

Summary

Background: Abnormal α 1,2-fucosyltransferase activity due to gene mutation results in decreased synthesis of H antigen and leads to an H-deficient phenotype. Here we studied the underlying molecular mechanisms in 7 Chinese blood donors with the H-deficient phenotype. **Methods:** Red blood cell typing was performed using standard serologic tests, and ABO genotype was analyzed using ABO polymerase chain reaction with sequence-specific primer tests. The coding sequence of the *FUT1* gene was amplified using the specific primers. The *FUT1* alleles were identified by a pCRII-TOPO carrier for TOPO TA cloning sequencing. **Results:** The H-deficient phenotype frequency was estimated to be approximately 1/30,000 (6/159,515) in the Chinese Han population. The *FUT1* gene mutation was demonstrated in 6 Chinese blood donors with the H-deficient phenotype. In only 1 case, no mutation was detected. Novel *FUT1* alleles were found in 1 donor. One of these novel *FUT1* alleles showed nucleotide 35C>T and 748C>T site mutations that resulted in amino acid substitution of Ala to Val and Trp to Arg at positions 11 and 250, respectively. Another novel *FUT1* allele had a nucleotide 655G>C site mutation, causing amino acid substitution of Leu to Val at position 219. **Conclusions:** Two novel *FUT1* alleles, 35T+748T and 655C, were identified that may greatly diminish the activity of α 1,2-fucosyltransferase and result in the H-deficient phenotype.

Introduction

Two types of α 1,2-fucosyltransferase encoded by the *FUT1* and *FUT2* genes have been found in human tissues. The *FUT1* gene encodes the α 1,2-fucosyltransferase which transfers fucose to the C-2 position of galactose on type II precursor substrate Gal β 1–4GlcNAc β 1-R. The *FUT1*-encoded enzyme is crucial for the generation of a precursor oligosaccharide H antigen that is processed to form both A and B antigens on red blood cells (RBCs). The *FUT2* gene is the human secretor gene which determines the formation of ABH antigens in secretions such as saliva.

The ABH antigen on RBCs of H-deficient secretors is absent or weakly expressed while normal amount of ABH antigen can be detected in secretion, Anti-HI/H was usually present in serum. Point mutation within the glycosyltransferase genes has been found to affect the enzyme substrate specificity and activity. The *FUT1* gene mutation may result in no or aberrant activity of α 1,2-fucosyltransferase, which subsequently leads to the formation of the H-deficient phenotype. A number of *FUT1* gene mutations causing impaired α 1,2-fucosyltransferase function have been reported [1–3], including missense, nonsense, and short deletions. More than 43 silencing or weakening *FUT1* mutations have been cited in the dbRBC database (www.ncbi.nlm.nih.gov/projects/gv/rbc/xs-lcgi.fcgi?cmd=bgmutsystems_info&system=hh).

The H-deficient phenotype results either from an inactive *FUT1* gene together with a normal or inactive *FUT2* gene. Therefore, the H-deficient phenotype can be divided into H-deficient non-secretor phenotype (Bombay phenotype), H-partially deficient non-secretor phenotype (classical para-Bombay phenotype), and H-deficient secretor phenotype (general para-Bombay phenotype). The molecular basis of the Bombay and para-Bombay phenotypes was reported in 1994 by Kelly et al. [4]. The Bombay phenotype was extremely rare in the Chinese Han population with an estimated

Fig. 1. The results of polymerase chain reaction – sequence-specific primer for 7 cases of para-Bombay type.

frequency of 1/300,000. However, in Bombay, India, its frequency is approximately 1/10,000. There seems to be a geographic variation in the frequency of the H-deficient phenotype within the Chinese Han population. The H-deficient phenotype frequency is approximately 1/8,000–10,000 in Fujian Province [5] and approximately 1/15,620 in Hong Kong [2]. In this study, we estimated the H-deficient phenotype frequency and reported 7 patients with the H-deficient phenotype, among whom two novel *FUT1* mutations were identified.

Material and Methods

Blood Samples

A total of 212,687 blood samples were selected at Dongguan Blood Center in 2009–2011. Upon exclusion of the repeat blood donors, the total number of donors was 159,515. Among them, 7 H-deficient phenotype cases were discovered. A total of 6 H-deficient phenotype cases came from the Dongguan Blood Center, while the remaining 1 came from Shaoguan Blood Center (also located in Guangdong Province). All 7 blood donors (4 men, 3 women) were ethnically Chinese Han and were not related. A routine health check was undertaken according to the health examination criteria of blood donors (GB18467–2011). This is the national standard of the People's Republic of China. Venous blood was collected in specimen tubes containing 3.2% trisodium citrate.

Blood Group Serology Methods

ABO serology was performed with standard serological tests. An adsorption-elution test was used to detect trace amounts of ABH antigens on the RBCs. The presence or absence of ABH antigens in the saliva was determined by the hemagglutination inhibition test. The Lewis antigen was tested to determine the secretion status if saliva was not available. Screening and panel cells were used to detect irregular antibody screening and identification.

H-Deficient Phenotype Screening Tests

The blood samples were tested for ABO and RhD typing. ABO blood grouping was performed using standard forward and reverse typing. If agglutination or hemolysis was detected with group O cells in the reverse

typing process, H antigen typing of the donor RBCs would be performed with 10% anti-H (extracted from *Ulex europaeus* lectin provided by Lorne Laboratories Limited, Lower Earley, Reading, UK). If no agglutination reaction was noted, the following tests would be carried out.

ABH Antigens on RBCs

Two serological methods were employed to further define the ABH antigens that were present on the RBCs. The routine method involved a hemagglutination test of donor RBCs with monoclonal anti-H (provided by Shanghai Hemo-pharmaceutical & Biological Co., Ltd., Shanghai, China). The more sensitive adsorption-elution test was employed for the detection of trace amounts of ABH antigens. For the detection of H antigens, 100 µl of washed and packed RBCs were incubated with monoclonal anti-H at 4 °C for 1 h. After centrifugation, the supernatant was discarded and the RBCs were washed three times with 5% saline. The antibody that bound to the RBCs was eluted by 10-min incubation at 56 °C. The antibody in the eluate was detected by hemagglutination with group O RBCs. A similar adsorption-elution test was used to detect trace amounts of A and B antigens on RBCs with polyclonal anti-A or anti-B instead of monoclonal anti-H.

Molecular Analysis

Sample genomic DNA extraction was performed using a QIAamp DNA Blood Kit reagent (Qiagen, Hilden, Germany).

ABO Genotype

ABO genotypes were tested using an ABO single specific primer-polymerase chain reaction (PCR) kit (G&T Biotech, Inc, Rockville, MD, USA). PCR amplification was carried out in a reaction volume of 9 µl containing 7 µl of PCR primer mixture, 1 µl of sample DNA, and 1 µl of Taq polymerase (0.33–0.5 U). The reaction was performed in a thermal cycler system using the following cycling parameters: 1 cycle of 95 °C for 5 min; 30 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 90 s, followed by a final extension at 72 °C for 5 min. The PCR products underwent 40 g/l agarose gel electrophoresis at 10 V/cm for 20 min, and the results were visualized with ultraviolet transillumination.

FUT1 Gene Amplification and Direct Sequencing of the PCR Products

To identify the mutations in the *FUT1*, a pair of primers (FUT1-F: 5'-GCATTTGCTAATTCGCC-3'; FUT1-R: 5'-GGCCTCTGAAGC-CACGTACT-3') was employed to amplify the *FUT1* gene. The PCR product encompassed the full coding region of 1,198 bp [6]. The 25-µl

Fig. 2. Results of partial cloning sequencing of *FUT1* gene. A: nt35C>T; B: nt748C>T; C: nt655G>C.

PCR master mix included 2.5 μ l of 10 $\times$ PCR buffer, 2 μ l of DNA (50–100 ng), 1 U of LATAq DNA polymerase (TaKaRa, Dalian, China), 0.2 mmol/l dNTP, and 1.7 mmol/l MgCl₂. The final primer concentration was 0.24 μ mol/l. The PCR cycles consisted of denaturation at 94 °C for 5 min, 94 °C for 30 s, 59 °C for 30 s, 72 °C for 90 s, 32 cycles, and 72 °C for 10 min.

The specificity of the PCR product was checked by electrophoresis with 2.0% agarose gel (10 V/cm) for 20 min. The PCR product was then purified with shrimp alkaline phosphatase enzymes (SAP 1 U/ μ l; Promega, Madison, WI, USA) and exonuclease enzyme I (Exo-I, 0.5 U/ μ l; TaKaRa). The 5- μ l PCR product was mixed with 0.75 μ l of SAP and 0.75 μ l of Exo-I and incubated at 37 °C for 30 min for the enzyme reaction, followed by 80 °C for 15 min for the enzyme inactivation. A big dye terminator v3.1 sequencing kit (Applied Biosystems, Foster City, CA, USA) was employed for bidirectional sequencing reaction. Sequencing primers FUT1-F and FUT1-R were used, and the sequencing product was cleaned and purified with ethanol/acetic acid. The product sequence was read with an ABI 3130 gene sequencing machine (Applied Biosystems).

Cloning and Sequencing

The gel-purified PCR product was cloned into the pCRII-TOPO vector (Tiagen Biotech Co., Ltd. Peking, China) using a TOPO TA cloning kit (Invitrogen, Groningen, the Netherlands) and then transfected into TOP-10 *Escherichia coli*. Recombinant colonies on LB plates were selected at random for each sample. Plasmid DNA was extracted from each colony and used as a template for the sequencing reaction.

Results

H-Deficient Frequency Estimate

H-deficient phenotype frequency in the Chinese Han population was estimated to be approximately 1/30,000 (6/159,515). However, this incidence could be underestimated

Table 1. Para-Bombay type of serology and genotype results

Case	Serotyping	ABO-PCR-SSP	Anti-HI/-H	Lewis group	<i>FUT1</i> gene mutation	Amino acid change
1	Am ^h	A/O	anti-HI	Le(a-b+)	880delTT/658C>T	294G/Arg220 Cys
2	Bm ^h	B/O	anti-HI	Le(a-b+)	328G>A/ 880delTT	Ala110 Val/294G
3	ABm ^h	A/B	anti-H	Le(a-b+)	no mutation	–
4	ABm ^h	A/B	anti-HI	Le(a-b+)	547delAG/658C>T	182fs)/Arg220 Cys
5	Am ^h	A/O	anti-HI	Le(a-b+)	658C>T/658C>T	Arg220 Cys/Arg220 Cys
6	Om ^h	O/O	anti-HI	Le(a-b+)	547delAG/880delTT	182G/294fs
7	Bm ^h	B/O	anti-HI	Le(a-b+)	35C>T,748C>T/655G>C	Ala11 Val,Trp250 Arg/Leu219 Val

because some H-deficient individuals might not produce strong anti-H or anti-HI.

Serologic Reactions of H-Deficient Phenotype

The samples of the 7 blood donors tested negative with monoclonal anti-H, but weak H antigen could be detected by the absorption-elution test. If an irregular screening test was positive, antibody identification and O cord cells were obtained to determine antibody specificity. Anti-H or anti-HI was found in all cases. The secretor status was identified with the Le (a-b+) phenotype or saliva study. The serologic tests were in line with the characteristics of the H-deficient secretor phenotype.

*Sequencing of the *FUT1* Gene*

Although the *FUT1* mutation was not detected in 1 case, the remaining 6 cases were all confirmed to be H-deficient with *FUT1* mutations (table 1). In this study, 3 *FUT1* alleles were found that resembled the 547delAG, 880delTT, and 658C>T mutations previously described by Yu et al. [7]. Case 2 also showed a nucleotide 328G>A mutation that resulted in amino acid substitution of Ala to Val as reported by Su et al. [8]. Case 7 displayed 2 new *FUT1* alleles that were first identified in the Chinese population. One novel *FUT1* allele carried two 35C>T and 748C>T nucleotide mutations that corresponded to the amino acid substitution of Ala to Val and Trp to Arg.

Although the 35C>T mutation was reported to be a missense mutation commonly found in the Chinese population [7, 9], there have also been reports that the 35C>T mutation only slightly diminishes fucosyltransferase activity [10]. Another novel *FUT1* allele was due to the 655G>C mutation, which resulted in the amino acid substitution of Leu for Val. The two novel alleles were submitted to the GenBank (under JX 078970 or JX 317627). No *FUT1* gene mutations were found in case 3; as a result, the serological tests were repeated, and the results showed an agglutination score with anti-A and anti-B at less than '1+' and no agglutination with anti-H. Weak A, B, and H antigens could be demonstrated by the absorption-elution test. A family study was not performed since the patient's relatives were living in other provinces. Therefore, it was not possible to confirm whether the H-deficient phenotype in this case was due to the *FUT1* mutation. Muta-

tions in other regulator genes causing transcription or translation problems might be at play.

Discussion

The independent H blood group system is notated ISBT018-Hh and has only a single type of H antigen. The H antigen is produced indirectly by the *FUT1* gene, which is located on 19q13.3. Its direct product is α -fucosyltransferase, which contains 365 amino acids and catalyzes the fucose sugar chain connected to the precursor to form the H antigen on the RBC surface. The expression of ABO antigens on the RBC membrane is in turn controlled by the H antigen. The *FUT1* and *FUT2* genes share high homology. The *FUT2* gene encodes α -fucose glycosyltransferase as well as the secretion of α -fucose radical enzyme activity and H antigens in the body fluid [11]. The incidence of the H-deficient phenotype has been reported to be 1 in 8,000 in the Taiwanese and Fujian populations [5, 12] and approximately 1–2 in 300,000 in the Japanese population [13]. In the current study, the incidence of the H-deficient phenotype in the Chinese Han population was found to be 1 in 30,000, which is close to the Taiwanese but far more than the Japanese. In 1994, Kelly et al. [4] first reported the molecular mechanisms of the para-Bombay and Bombay types, showing that the para-Bombay type was caused by a *FUT1* gene mutation. More than 43 missense, deletion, and insertion *FUT1* gene mutations have been reported to cause H antigen deficiency in RBCs [13]. It has been proposed that the different alleles be classified as 547delAG, 880delTT, 658C>T [7], 35C>T, 980A>C, 460T>C, and 522C>A mutations [12]. Both 547delAG and 880delTT are two-base deletions that result in frame shifts. One of the three AG repeats at nucleotides 547–552 was deleted and two of the three T bases at nucleotides 880–882 were deleted. It has a nucleotide 658C>T mutation that corresponds to an amino acid substitution of Arg to Cys.

Most H deficiencies are caused by homozygous mutation of the *FUT1* gene, while a few others are due to heterozygous mutations. In the current study, there was one case of 880delTT/658C>T, 547delAG/880delTT, 547delAG/658C>T and 880delTT and two cases of 658C>T. The 35C>T, 980A>C, 460T>C, and 522C>A mutations were not observed in the

current study. Thus, it appears that the latter mutations are not common in the Han population. Two novel alleles were detected as being compound heterozygote in one sample. The two novel alleles, 35C>T and 748C>T nucleotide mutations that corresponded to amino acid substitution of Ala to Val and Trp to Arg, and a 655G>C mutation that resulted in amino acid substitution of Leu to Val involve point mutations that may result in partial activity of α 1,2-fucosyltransferase and result in a weak expression of the H antigen on the RBCs. Weak A or B antigens based on type II chain on RBCs were made from the weak H antigens. Therefore, the weak activity of the α 1,2-fucosyltransferase encoded by 35C>T and/or 748C>T nucleotide mutations as well as the 655G>C mutation could explain these phenomena.

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Disclosure Statement

We certify that this study has not affiliation with or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in this manuscript. The manuscript has been seen and approved by all of the authors, is not under active consideration for publication, has not been accepted for publication, and has not been published elsewhere.

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