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Original Article

Allele frequency of A385T in *Fucosyltransferase 2* gene *(FUT2)* among Southern Thai population

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Abstract

Fucosyltransferase 2 gene *(FUT2)* gene plays an important role in control of enzymes that secrete blood type antigen substances. The mutation of *FUT2* at nucleotide position 385 from A to T (A385T) combined with secretor phenotype is associated with proneness to infections by some pathogens, on comparing with the non secretor phenotype. The objective set was to study the prevalence of A385T mutation with secretor phenotype. Among 132 Southern Thai individuals, 18(13.6%) had Le(a+b-) phenotype and 5(3.8%) had Le(a-b-) non secretor phenotype with genotypes AA 6(26.0%), AT 13(56.5%) and TT 4(17.3%); whereas 109(82.6%) having Le(a-b+) phenotype and 7(5.3%) having a Le(a-b-) secretor phenotype had genotypes AA 42(31.8%), AT 50(37.9) and TT 10(7.5%). The frequencies of genotypes AA, AT and TT among 264 alleles examined in a sample of 132 Southern Thai individuals were 48, 70, and 14%, respectively, indicating a rather wide distribution of A385T in *FUT2* gene in the Southern Thai population. The results show that the *FUT2* gene really encodes the secretor enzyme α(1,2)*fucosyltransferase* and indicate an ethnic group-specific nonsense or missense point mutation in the *FUT2* gene. This study provides substantial new information on the prevalence of the examined polymorphism.

Keywords: A385T, allele specific PCR, *FUT2* gene, Southern Thai

1. Introduction

The ABO blood group and secretor status are inherited independently by an individual. The ABH (*FUT 1*) gene codes for the ABO blood group. The secretor (*FUT 2*) gene interacts with *FUT 1* gene to determine the ability to secrete blood group antigens into body fluids and secretion. The secretor (*FUT 2*) gene encodes for an α (1, 2) fucosyltransferase that determines the blood group antigen secretor status and influences the Lewis phenotype of an individual. A person can either be a secretor (SeSe/Sese) or a non-secretor (sese) of ABH substances (Saboor, Ullah, Qamar, Mir, & Moinuddin, 2014). Secretors are persons who put their blood type antigens into their body fluid and secretions, while non-secretor do not.

The Lewis phenotype of a person is determined by the epistatic interaction of the α (1, 2) fucosyltransferase and α (1, 3/1,4) fucosyltransferase encoded by the secretor (*Se* or *FUT2*) and Lewis (*Le* or *FUT3*) loci, respectively. The Lewis antigens are adsorbed by the RBCs through circulating glycolipids, resulting in their Lewis phenotypes. They can also be detected in plasma, saliva and other secretions (Itoh, Taka hashi, Satoh, Nagao, & Fujime, 2004). The 4 major Lewis phenotypes are: Lewis negative secretor and non-secretor Le(a-b-), Lewis positive non-secretor Le (a+b-), Lewis positive secretor Le (a-b+), and Lewis partial secretor Le (a+b+), which is caused by an inefficient secretor transferase due to a missense in the Se allele, known as Se^{385}. While people with functioning *FUT2* are known as secretors, individuals (approximately 20% worldwide) who inherit null *FUT2* mutations are termed non-secretors (Gillver & Henry, 2003; Itoh *et al.*, 2004; Jaff, 2010.). Non-secretors are unable to synthesize and express ABO (H) or Le^b antigens on their mucosal glycans because of the *FUT2* mutation, but they can express Lewis a Le^a, Le (a+b−) phenotype, α1-3,4 fucosyltransferase antigens in their mucosa due to the action of *FUT3* (Previato *et al.*,2015). Both *FUT2* secretor and non-secretor status have been reported to be associated with either pro-

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tection against or susceptibility to different infections. Those who are secretors are less prone to infections by Helicobacter pylori, Campylobacter jejuni, HIV-1and Norovirus than those who are non secretors (Bernado *et al.*, 2016; Chen *et al.*, 2005; Hutson, Airaud, Le Pendu, Estes, & Atmar, 2005; Kurrse & Mirghani, 2016; Oba-Shinjo *et al.*, 2004; Orntoft *et al.*, 1996; Previato *et al.*, 2015; Salomaa *et al.*, 2000).

Le (a-b+) usually has the corresponding secretor status in saliva. In these individuals the *FUT2* enzyme is highly active and transforms most of the precursor substance into H type 1, which is in turn transformed into Le^b by the Lewis enzyme. In contrast, the Le (a+b-) phenotype is found in ABH-non secretors with at least one functional allele, but is inactive for both *FUT2* alleles.

The Le (a+b+) phenotype is almost absent in Caucasians while it occurs in 22-25% of the Asian population (Henry *et al.*, 1995). This phenotype is presumably due to a heterozygous variant in the *FUT2* gene (A385T).

Therefore the expression of H antigen is decreased; a proportion of precursor chains remains un-substituted and can be transformed into Le^a by the Lewis enzyme. So, both Le^a and Le^b are abundant in secretions and can be on RBCs (Clausen & Hakomori, 1989; Oriol, Le Pendu, & Mollicone, 1986). Individuals with the mutation on Lewis gene (*FUT3*) are labeled Le (a-b-), regardless of the ABH secretor type. The allele se^{385} is a common null allele in Asians (Svensson, Petersson, & Henry, 2000; Chang *et al.*, 1999). The aim of this study was to examine the genotype distribution of A385T within *FUT2* gene along with analysis of the secretor phenotype and the Lewis blood type among SouthernThai populations.

2. Materials and Methods

2.1 Study populations

The study was approved by the Ethics Committee of the Faculty of Medicine, Prince of Songkla University (project code 58-182-19-2). In a random sampling of healthy Southern Thai population, 132 who had no familial relationship in 2 generation were included. Blood samples were obtained with informed consent.

2.2 Blood sample collection and processing

Three ml of peripheral blood was collected in an ethylene diamine tetra acetic acid (EDTA) tube.

Lewis phenotypes were determined for fresh blood samples by standard hemagglutination methods. Typing was carried out with monoclonal Le^a and Le^b antibodies (Ortho-Clinical Diagnostics, Inc., Raritan, NJ, USA).

DNA was isolated from blood using Kit GF-1 Blood DNA extraction (Vivantis, Malaysia) The extracted DNA was stored at -20^0C until the genotype analysis.

2.3 Typing of Lewis blood group phenotypes of erythrocytes

Lewis blood group phenotypes of erythrocytes were determined as described previously (Previato *et al.*, 2015). In brief, anti-Le^a and anti-Le^b monoclonal antibodies were used for hemagglutination tests according to the manufacturer's instructions.

2.4 Saliva sample collection and processing of the secretor status

A total of 132 saliva samples were collected for detection of ABH antigen. Saliva (5-10 ml) was collected in a wide-mouthed test tube and centrifuged at 1,000 x g for 10 min. The supernatant was transferred to a clean test tube that was placed in a boiling water bath for 10 min to inactivate the salivary enzymes. After re-centrifugation at 100 x g for 10 min, the supernatant fluid was diluted with an equal volume of saline at -20^0C. The water-soluble secreted A, B and H antigens could be demonstrated in saliva via hemagglutination inhibition test with monoclonal A, B and H antibodies (Ortho-Clinical Diagnostics, Inc., Raritan, NJ, USA). Double dilutions of the appropriate blood grouping reagents were prepared beforehand for the selection of blood grouping reagent dilution. One drop of 3 % saline suspension of red cells was added to one drop of each reagent dilution. A_1, B or cells were used to determine the A, B, or H secretor status, respectively. Each tube was centrifuged and examined macroscopically for agglutination. The highest reagent dilution that gave 2+ agglutination was selected. For hemagglutination inhibition test, one drop of appropriately diluted blood grouping reagent was mixed with one drop of saliva, and the mixture was incubated for 10 min at room temperature. One drop of 3% saline suspension of washed indicator cells was added to each tube. Each tube was centrifuged and examined macroscopically for agglutination. Saline control tube was included in each test (Chang *et al.*, 1999; Svensson, Petersson, & Henry, 2000).

2.5 A385T allele detection in *FUT2* gene by Allele specific PCR

Two separate detections of 385A Allele and 385T allele were performed for each sample. 4 primers were designed for the *Homo sapiens* fucosyltransferase 2 (*FUT2*) gene (NC_000019.10:48695971-48705934), including F-primer (5' TGG-ACG_ATC-AAT-GCA-ATA-GG 3'), R-primer (5'TCT-CAA-AGA-ATG-GGC-CAG-CA 3'), Specific primer for normal 385A allele (5' GGA-GGA-GGA-ATA-CCG-C CA-CA3') and Specific primer for mutant 385T (5' GGA-GGA-GGA-ATA-CCG-CCA-CT3'). For the 385A allele detection, three primers were used to generate 2 specific fragments of 863 bp internal control band (F-primer and R-primer) and 654 bp 385A band (Specific primer for normal 385A allele and R-primer). For 385T allele detection, three primers were used to generate 2 specific fragments of 863 bp internal control band (F-primer and R-primer), 385T band (Specific primer mutant 385T and R-primer) and 385A band (Specific primer normal 385A and R-primer) as shown in Figure 1.

The PCR reaction mixture (50 uL) contained 50 ng genomic DNA, 1 uL of each primer (5 pmol), 200 uM dNTPs and 1X PCR Master Mix (One PCR plus, GeneDirex, Taiwan). The PCR cycling conditions in the T100 thermal cycler (Bio-Rad, Hercules, CA, USA) were as follows: (95°C 15 min), one cycle; followed by 30 cycles (94°C for 30 s, 58°C

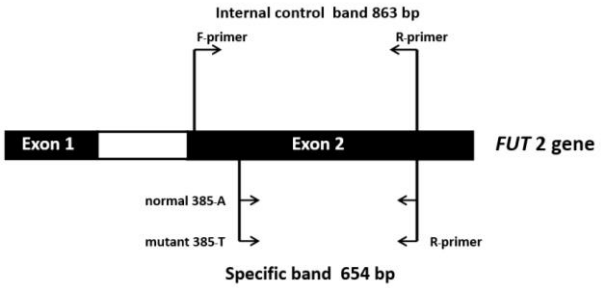

Figure 1. Identification of the A385T polymorphism on *FUT*2 gene by allele specific PCR methodology. The locations and orientations of F-primers, R-primer, normal 385A and normal 385T are indicated. The sizes of amplified DNA are depicted: the 863 bp fragment is internal control band also used for DNA sequencing, while the 654 bp fragment is specific for A or T of in 385 position.

for 30 s , 72◦C for 1 min); and final extension at 72°C for 5 min. Amplified DNA stained with SYBR® Safe DNA Gel Stain (Invitrogen, Ltd., Paisley, K) was directly subjected to 100 Volt electrophoresis on 1.5% agarose gel for 45 min.

2.6 Confirmation of A385T allele in *FUT2* gene by Direct DNA sequencing

The 132 purified amplification products were sequenced directly for *FUT2* genotyping. The complete segment of *FUT2* concluding at the 385 position was directly sequenced. The dideoxynucleotide termination sequencing reaction was performed using the ABI 3730 Genetic Analyzer platform and the results were analyzed using CHROMAS software.

3. Results

3.1 Distribution of 385 in *FUT2* genotyping among gender

Blood and saliva samples from 132 southern Thai subjects were tested. The 385 at *FUT2* genotyping using allele specific PCR and DNA sequencing showed that (Figures 2-5) 17 of them were males and 115 were females. The homozygous (AA), heterozygous (AT) and homozygous (TT) cases in males were detected for 8(47%), 5(29.4%) and 4(23.5%) cases, respectively, while the counts of females detected were similarly 40(34.8%), 65(56.5%) and 10(8.7%) (Table 1).

3.2 Distribution of 385 *FUT 2* A385Tand secretor status in saliva

We investigated secretor phenotypes in the saliva samples of 132 Southern Thais, distinguishing the presence or absence of A, B and H antigens in saliva. The counts of secretors and non secretors detected were 109(82.6%) and 23 (17.4%), respectively. The secretors and non secretor were homozygous in (AA) 42(38.5%) and 6(26%) cases, heterozy-

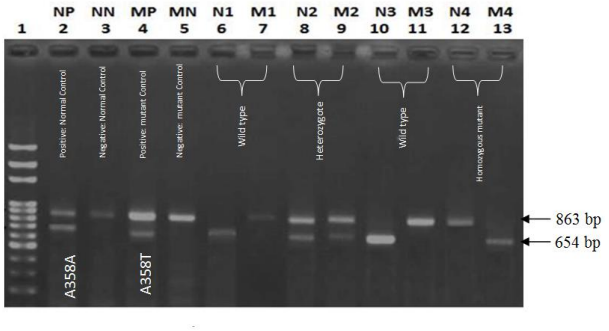

Figure 2. A representative agarose gel electrophoresis of two conditions for the detection of 385A (normal allele) and 385T (mutant allele) in *FUT2* gene, using allele specific PCR analysis. The combined result of those two conditions was used for genotyping identification. For example, the homozygous 385A /385A sample (wild type) is only positive for 385A (normal allele) condition, while homozygous 385T /385T sample is only positive for 385T (mutant allele). The heterozygous 385A /385T is positive for both 385A (normal allele) and 385T (mutant allele), shown in Lanes 2 and 4: Positive control for 385A (normal allele) and 385T (mutant allele); Lanes 3 and 5: Negative control for 385A (normal allele) and 385T (mutant allele); Lanes 6 and 7 as well as Lanes 10 and 11: a representative sample of homozygous 385A /385A ((wild type); Lanes 8 and 9: a representative sample of heterozygous 385A /385T; Lane 12 and 13: a representative sample of homozygous 385T /385T; and Lane 1: 100 bp ladder. (NP= positive control for 385A (normal allele), NN= negative control for 385A (normal allele), MP= positive control for 385T (mutant allele), NP= negative control for 385T (mutant allele), N(no.) = Number of samples detected with 385A (normal allele) condition and M (no.) = Number of samples detected with 385T (mutant allele) condition.

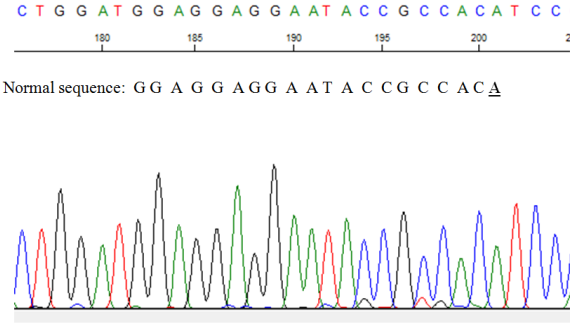

Figure 3. Representative DNA sequencing profile of *FUT2* gene demonstrating the homozygous allele A(wild type) at nucleotide 385 in Southern Thais.

Table 1. Distribution of 385 at *FUT2* genotyping by gender.

Gender	Homozygous AA (%)	Heterozygous AT (%)	Homozygous TT (%)	Total (%)
Male	8 (47.0)	5 (29.4)	4 (23.5)	17 (100)
Female	40 (34.8)	65 (56.5)	10 (8.7)	115 (100)
Total	48 (36.4)	70 (53)	14 (10.6)	132 (100)

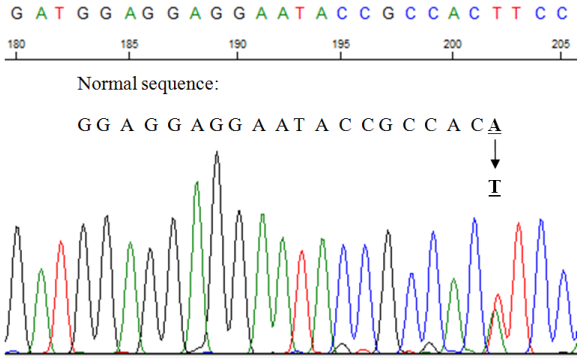

Figure 4. Representative DNA sequencing profile of *FUT2* gene demonstrating the heterozygous allele A→ T at nucleotide 385 in Southern Thais.

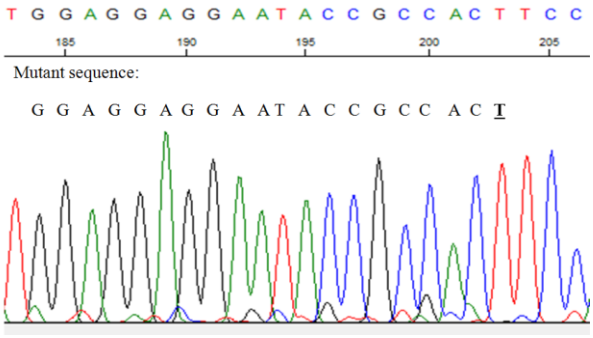

Figure 5. Representative DNA sequencing profile of *FUT2* gene demonstrating the homozygous allele T at nucleotide 385 in Southern Thais.

gous in (AT) 57(52.3%) and 13(56.5%) cases, and homozygous (TT) in 10(9.2%) and 4(17.3%) cases, respectively. The frequencies of A and T alleles were 166(76.1%) and 98 (44.9%), respectively (Table 2).

3.3 Association of genotyping with secretor, nonsecretor, and Le^a and le^b antigens

Among 109 subjects who had secretor phenotype, all those with AA and TT genotypes had Le(a-b+), while 7/50 subjects (14.0%) who had AT genotype had Le(a-b-). Among the 23 non secretors, all those with AA and TT genotypes had Le(a+b-) while 5/13 (38.5%) of subjects who had AT genotype had Le(a-b-) (Table 3).

4. Discussion and Conclusions

We investigated secretor phenotypes in the saliva samples of 132 Southern Thais focusing on the presence or absence of A, B and H antigens in saliva. The individuals were classified into secretor 109(82.6%) or non secretor 23 (17.4%) cases, as about 20% of the Asian population is non secretors. The non secretor individuals who lack Se enzyme activity would not synthesize the H type-1 structure that is also a precursor of A, B and Le^b antigens, resulting in lack of secretion of ABH antigens into saliva or no expression of Le^b antigens (Park, Song, Han, & Kim, 2005).

The Lewis phenotype in our Southern Thai population sample were found to be divided in three kinds: Le(a-b+), Le(a+b-), and Le(a-b-). Lewis antigens Le^a and Le^b are known to be distributed not only on erythrocytes but also in plasma, saliva and other secretions (Itoh, Takahashi, Satoh, Nagao, & Fujime, 2004).

The secretor enzyme (*FUT2*), an α-1, 2-fucosyltransferase, is responsible for the fucose transfer in an α-1,2 linkage to form the terminal H type 1 structure. This study examined the frequency of the *FUT2* (385 A-T) polymorphism by allele specific PCR with separate conditions for 385A and 385T alleles. In Table3 we summarize these results together with the finding that when tested in parallel, secretor, non secretor, Lewis phenotype, AA, AT and TT genotypes distinguished three Lewis phenotypes, Le(a-b+), Le(a+b-) and Le(a-b-) as well as secretors and non secretors. The previous study genotyping 209 Koreans showed AA 40(19.1%), AT

Table 2. Distribution of 385 at *FUT2* gene and frequency of secretor A385T polymorphisms by the secretor status in the saliva.

Genotype	Secretor (n=109, 82.6%)	Non secretor (n=23, 17.4%)	Total (n=132)
AA	42 (38.5)	6 (26.0)	48 (36.4)
AT	57 (52.3)	13 (56.5)	70 (53.0)
TT	10 (9.2)	4 (17.3)	14 (10.6)
Frequency	Secretor	Non secretor	Total (n=264)
A allele	141 (64.7)	25 (54.3)	166 (76.1)
T allele	77 (35.3)	21 (45.7)	98 (44.9)

Table 3. Association of genotyping with secretor, non-secretor, and Le^a and Le^b antigens.

Genotype	Secretor (n=109)			Non-secretor (n=23)			Total
	Lewis phenotype			Lewis phenotype			
	Le (a-b+)	Le (a+b-)	Le (a-b-)	Le (a-b+)	Le (a+b-)	Le (a-b-)	
AA	42 (31.8)	0	0	0	6 (4.5)	0	48 (36.4)
AT	50 (37.9)	0	7 (5.3)	0	8 (6.1)	5 (3.8)	70 (53)
TT	10 (7.5)	0	0	0	4 (3)	0	14 (10.6)
Total	102 (77.3)	0	7 (5.3)	0	18 (13.6)	5 (3.8)	132 (100)

111(53.1%), TT 58(27.8%), A allele 191(45.7%) and T allele 227(54.3%) (Park, Song, Han, & Kim, 2005), while in our study the counts were A allele 166(76.1%) and T allele 98 (44.9%). The reason is probably that Koreans have more than TT *genotype 14(10.6%)* than Southern Thais (Table2). It is reasonable that isolated populations have developed somewhat differing allele frequencies.

In summary, we established the genotypes and allele frequencies of A385T of the *FUT2* genes. The se^{385} was a common secretor enzyme deficient allele in Southern Thais. Our study provides substantial new information on the prevalence of the examined polymorphism.

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