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Molecular characterization of secretor type α(1,2)-fucosyltransferase gene deficiency in the Philippine population

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Abstract We analyzed the seven mutations which are responsible for the deficiency of the secretor type α(1,2)-fucosyltransferase gene product, Se enzyme, in the Philippine population. One hundred and one unrelated Filipinos in Taiwan were studied. A new mutation, a 3-base pair deletion from nt 688 through 690, was found in two (0.1%) of 202 chromosomes. The frequencies of six other mutated alleles were as follows: 71/202 (35.2%) were cDNA 385 A→T missensed mutation (se2), 28/202 (13.9%) were C571T nonsense mutation (se3), 16/202 (7.9%) were G849A nonsense mutation (se4), 4/202 (1.9%) were G428A nonsense mutation (se1), and 81/202 (40.1%) were wild-type allele (Se). No C628T nonsense mutations (se5) or fusion genes of pseudogene and FUT2 gene (se 6) were found in this population. For the molecular basis of phenotype Le(a+ b–): eight cases had se2/se2, six cases had se2/se3, two cases had se3/se4, one case was homozygous of se4, one case was se3/se1, and two cases were se2/se7. For the Le(a+ b+) phenotype: four cases had se2/se2, two cases had se2/se3, one case was se3/se3, and one case was se2/se4. For the Le(a– b+) phenotype: 16 cases were Se/Se, 21 cases were Se/se2, six cases were Se/se3, five cases were Se/se4, and two cases had Se/se1. Our results suggest that the genotypes of the α(1,2)-fucosyltransferase gene in phenotypes Le(a+ b+) and Le(a+ b–) are the same. Other factors that play important roles may cause the differences between these two phenotypes. Several hotspot mutations in the α(1,2)-fucosyltransferase gene are responsible for the nonsecretor phenotype.

Key words Filipino · α(1,2)-fucosyltransferase · Secretor phenotype · Nonsecretor · Mutation analysis

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Introduction

The human Lewis histo-blood group system belongs to a family of structurally related oligosaccharides that are synthesized by the sequential action of fucosyltransferase. The molecules were first identified as red cell (RBC) antigens, and later they were also discovered in tissues such as the salivary gland and digestive mucosa. In this polymorphic blood group system, the genes coded for the two fucosyltransferases responsible for the synthesis of the Le^a and Le^b glycoconjugates have been very well characterized [10, 15]. The Lewis gene (FUT3) codes for an α(1,3/1,4)-fucosyltransferase that can add fucose to either the type-1 precursor to form Le^a antigens or to the H-type 1 to form Le^b antigens [13]. The secretor gene (FUT2) codes for an α(1,2)-fucosyltransferase that adds fucose onto the type-1 precursor to form H-type1, the precursor of Le^b. Major RBC and salivary ABH secretor phenotypes result from the polymorphism of the Lewis and secretor systems, which are genetically independent, and the interaction of the transferase. The RBC phenotypes are (a) Lewis negative secretor and nonsecretor Le(a– b–), (b) Lewis positive nonsecretor Le(a+ b–), (c) Lewis positive secretor Le(a– b+), and (d) Lewis partial secretor

Le(a+ b+), which is caused by an inefficient secretor transferase.

Recently, six mutations were reported to be responsible for the Lewis phenotypes Le(a+ b–) or Le(a+ b+) [6, 7, 11, 18, 19]. A mutation caused by substitution of G→A at nucleotide 428 of cDNA was responsible for Le(a+ b–) in Caucasians [10]. Mutations C571T and G849A responsible for Le(a+ b–), and mutation A385T responsible for Le(a+ b+) were found in Taiwanese [18, 19]. Among the screened Japanese population, four mutations responsible for Le(a+ b–) were found. They include A385T, C571T, C628T, and a fusion gene consisting of the 5′ region of the pseudogene and the 3′ region of the functional FUT2 gene [11]. An A385T individual was also found in an Indonesian family [6]. To date, a similar study concerning FUT2 polymorphism in Filipinos has not been done. We developed a nonradioactive assay using normal or mismatched primers to explore the molecular basis of secretor FUT2 gene deficiency in the Filipino population.

Materials and methods

Subjects

DNA was extracted from blood samples of 101 unrelated Filipinos in Taiwan. The RBC Lewis phenotype was determined using a commercial antibody (Gamma-CloneR, anti-Le^a, anti-Le^b; Gamma Biologicals, Inc. Houston, Tex., USA) by tube test or by the manual polybrene method.

DNA amplification and restriction enzyme analysis

Total genomic DNA was isolated from peripheral blood leukocytes of the subjects as described previously [3]. We used the same strategy of oligonucleotide primer design and restriction enzyme analysis as in our previous studies [3, 4] (Table 1). The main purpose of our study was to design an accurate and affordable method for screening by creating a restriction site in mutations that do not have a natural restriction site. Three sets of amplification for five common mutations were used. The first set was used to detect A385T and G428A mutations, the second set to detect C628T mutation, and the third set to detect C571T and G849A mutations. For the A385T mutation, the mutant does not create any restriction sites (though the wild normal allele has a *Fok*I site). The mutagenic base T located at nt 383, introduced by an upstream primer with mutant T at nt 385, will create a CTCTTC site of *Ear*I. The normal allele CTCATC would disrupt the restriction site. For G428A, there is a *Bst N*I site (CCTGG) in a normal allele. Though the C628T mutation creates a new *Hph*I or *Age*I site, obtaining the enzymes is expensive. For the normal allele, we introduced an artificial base C at nt 618, with the base C at nt 628 to create *Bgl*I restriction site GCCN$_5$GGC. For C571T and G849A, both mutations have a natural *Dde*I restriction site (CTNAG). To detect the fusion gene, consisting of the 5′ region of the pseudogene and 3′ region of the functional FUT2 gene, we used the method of Koda et al. [11].

Molecular cloning or direct sequencing of se allele

For uncertain cases or those without any of the mutations mentioned above, a pair of primers specifically for the Sec 2 DNA segment encoding the secretor FUT2 gene was used to amplify the coding region. The upstream primer locates at nt –65 through –45 of the gene (5′-CTAAC GTGTCCCGTTTTCCTC-3′), 5′ to the first initiation codon. The downstream primer (5′-GTCCTGCTCATGGAACCATG-3′) is complementary to nt 1072 through 1091 within the 3′ untranslated region of FUT2 gene. The polymerase chain reaction (PCR) products were either cloned into T vector (pT7 Blue T-vector kit, Novagen, Inc., Madison, Wis., USA) or directly sequenced. DNA sequences were determined by means of the dideoxy chain termination method using an Amplicycle Sequencing Kit (Perkin Elmer Cetus, Foster City, Calif., USA).

The DNA amplification was performed as described previously [6], except that the annealing temperature was changed according to the melting temperature (Tm) of the primers. The amplified products were digested with appropriate restriction enzymes and then electrophoresed on 1.5–3.5% agarose gels. A few cases were confirmed by direct sequencing of the PCR product and were used as positive or negative controls.

Results

Results of the restriction map change of secretor type FUT2 gene deficiency after restriction enzyme digestion are shown in Fig. 1a–d. For A385T, a 243-base pair (bp) fragment was amplified by the appropriate primer pairs and a new *Ear*I (*Ksp*632 I or *Eam*1104 I) restriction site was created in the mutant allele. A 243-bp band identical to the undigested product was noted in the normal allele after *Ear*I digestion, whereas the mu-

Table 1 Primer sequences, restriction enzymes, and restriction fragment sizes of the mutation in FUT2 deficiency (*UP* upstream primer, *DP* downstream primer)

Mutation	Primer sequence	Enzyme	Size of fragment (bp)	
			N	M
A385T (se2)	UP: GATGGAGGAGGAATACCGCCT<u>T</u>C[a] DP: CCACTCTGGCAGGAAGGC-3′	*Ear*I	243	214, 29
G428A (se1)	Identical to A385T primer pairs	*Bst*NI	142, 64, 25, 12	142, 89, 12
C571T (se3)	UP:AGGAGATCCTCCAGGAGTTCA DP:AGAAGGAGAAAAGGTCTCAAAGG	*Dde*I	581	461, 120
G849A (se4)	Identical to C571T primer pairs	*Dde*I	581	394, 187
C628T (se5)	UP: AGTGTGGAAGGGGGTGGTG<u>C</u>CC DP: CCACTCTGGCAGGAAGGC	*Bgl*I	358	333, 25

[a] Underlined: mutagenic base

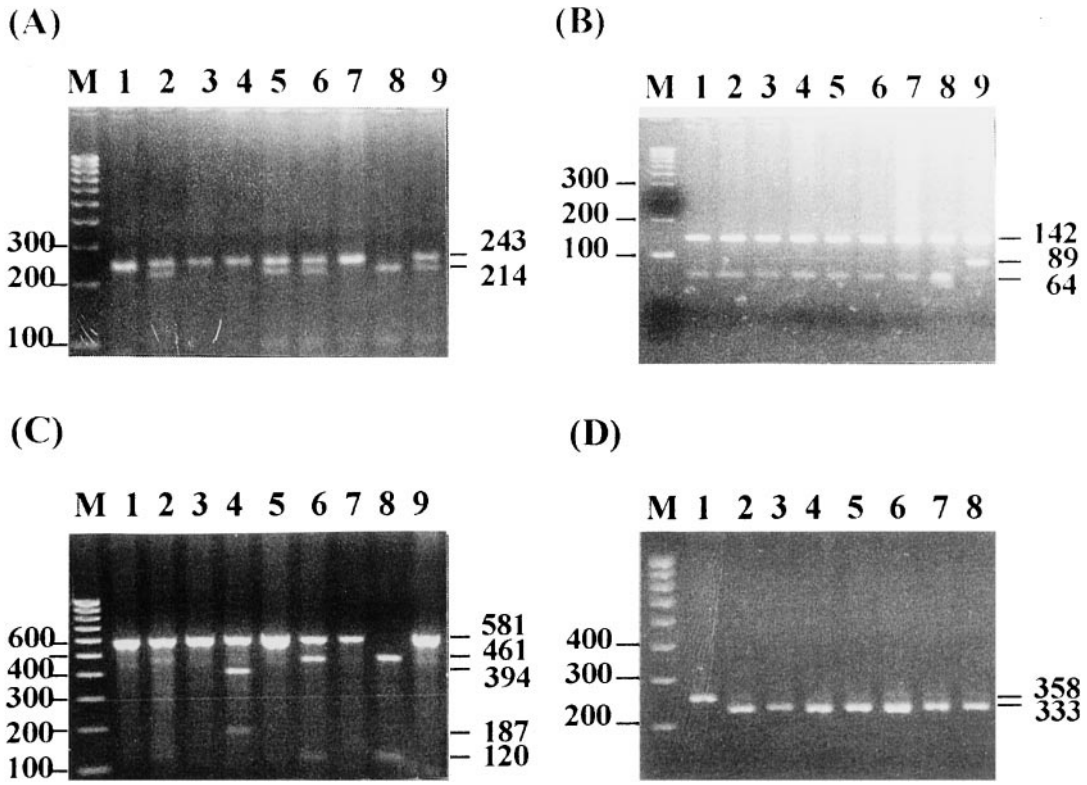

Fig. 1A–D (**A**) Detection of the A385T mutation: *lanes 2, 5, 6,* and *9* are heterozygous mutations, *lane 8* is a homozygous mutation, and *lanes 1, 3, 4,* and *7* have no mutation. (**B**) Detection of the G428A mutation: *lanes 1–8* have no mutation, and *lane 9* is a heterozygous mutation. (**C**) Detection of C571T and G849A mutations: *lane 6* is a heterozygous mutation of C571T, *lane 8* is a homozygous mutation of C571T, and *lane 4* is a heterozygous mutation of G849A. (**D**) The results of C628T mutation: *lanes 2–8* have no mutation, *lane 1* is an uncut control

tant product was cleaved into a 214- and a 29 bp fragment. A band of less than 35 bp was not well visualized in a 3.5% agarose gel (Fig. 1a). *Bst N*I cleavage of the normal allele PCR product yields four segments (142, 64, 25, and 12 bp), while three fragments (142-, 89-, and 12-bp bands) were generated for the G428A mutant case (Fig. 1b). For C571T and G849A mutations, a 581-bp fragment was PCR amplified, and this was followed by *Dde*I restriction enzyme digestion. As shown in Fig. 1c, the DNA size of the normal allele remained unchanged (581 bp); however, the products of C571T were cleaved into 120- and a 461-bp fragments and the products from G849A were cleaved into 394- and 187-bp DNA fragments. Digestion of the C628T mutation PCR products by *Bgl*I showed a 358-bp fragment and two fragments (333 and 25 bp) in a normal allele (Fig. 1d).

There were two Le(a+ b–) subjects found to have a heterozygous A385T mutation among the 101 cases (1.98%). In order to explore the relationship between phenotype and genotype, we amplified the whole FUT2 gene in these two cases and sequenced the PCR products directly. In addition to a heterozygous A385T mutation, after sequencing of the whole FUT2 gene these two cases also had a heterozygous three-base deletion (nt 688 through 690) (Fig. 2a) and the deletion abolished its authentic *BstE*II site (Fig. 2b). The PCR products used for detection of C571T and G849A mutations were also used to detect the deletion mutation. The 581-bp PCR products were digested using *BstE*II, and the wild-type allele was cut into 237- and 344-bp fragments. An undigested 578-bp fragment was noted in the mutant allele.

The serological data and results of mutation analysis of 101 unrelated Filipinos are summarized in Table 2. The results show that 35.2% (71/202 alleles) were A385T mutation (se2), 13.9% (28/202) were C571T mutation (se3), 7.9% (16/202) were G849A mutation (se4), 1.9% (4/202) were G428A mutation (se1), 0.1% (2/202) were nt 688 through 690 deletional mutation (se7), and 40.1% (81/202) were normal allele (Se). No C628T mutations (se5) or the fusion gene (se6) were found in this population.

The genetic basis for the Lewis phenotype in Filipinos differs from that in other populations. Among 23 individuals with Le(a+ b–), eight were homozygous for the se2 allele, six were found to be se2/se3, three were se2/se4, two were se3/se4, two were se2/se7, one was se3/se1, and one was homozygous for se4. Among eight Le(a+ b+) individuals, four were homozygous for se2, two were se2/se3, one was se2/se4, and one was homozygous for se3. Among 50 individuals with Le(a– b+), 16 were homozygous for Se allele, 21 were Se/se2, six were Se/se3, five were Se/se4, and two were Se/se1. Among 20 cases of Le(a– b–), five were Se/Se, three

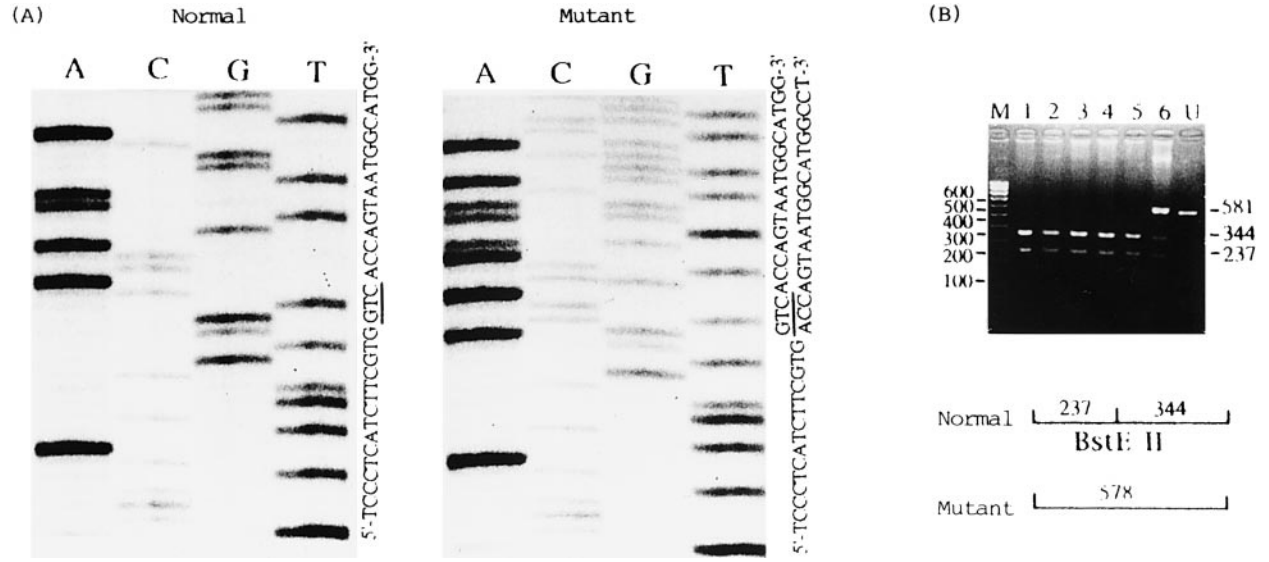

Fig. 2A,B (**A**) Direct sequencing data of a novel mutation (se7). The *left picture* is a normal control. The *right picture* shows the heterozygote of a three-base deletion (*GTC underlined*); the double bases in the same lane are due to a normal allele and a frameshift mutant allele. (**B**) Results of three-base deletion detected by *Bst E*II digestion. *Lanes 1–5* are normal controls, *lane 6* is a heterozygote of deletion from nt 688 to 690. (*M* 100-bp ladder marker, *U* uncut control)

were Se/se2, three were se2/se2, three were se2/se3, two were se3/se3, and there was one case each of the following: Se/se1, Se/se3, se3/se4, and se4/se4 (Table 2).

Discussion

In the genes of secretor type FUT2 gene deficiency, six mutations have been identified. All of them either create or abolish a normal enzyme restriction site. In this study, we devised a nonradioactive method for selective amplification of the part of the FUT2 gene or pseudogene with specific oligonucleotide primers, followed by digestion of particular restriction enzymes. The enzymes recognize the natural or artificial restriction sites. In a total of 101 individuals studied, 7.4% were homozygous for se2, and the incidence of this mutation was 35.2% (71/202 alleles). The results indicate that se2 is the most common Se enzyme-deficient allele found in this population. This is similar to the FUT2 polymorphism found in Japanese populations [11]. Two unusual polymorphic variants, the se5 allele and a relatively high (5.7%) fusion gene mutation, were commonly found in Japanese populations, but they were not found in this Filipino population. The rate of the most common nonsense mutation of G428A, responsible for the nonsecretor phenotype in Caucasians [10], was 1.9% in the current population. On tracing the family history of these subjects, we found that they are descendents of an inter-racial mixture of Filipino and Caucasian.

Table 2 Distribution of FUT2 genotypes and allele frequencies in the Filipino population

Genotype	Lewis phenotype				Total	Allele frequencies
	Le(a−b+)	Le(a+b−)	Le(a+b+)	Le(a−b−)		
Se/Se	16	0	0	5	21	
Se/se1	2	0	0	1	3	
Se/se2	21	0	0	3	24	se =.401
Se/se3	6	0	0	1	7	se1=.019
Se/se4	5	0	0	0	5	se2=.352
Se/se7	0	0	0	0	0	se3=.139
se2/se2	0	8	4	3	15	se4=.079
se2/se3	0	6	2	3	11	se7=.010
se2/se4	0	3	1	0	4	
se2/se7	0	2	0	0	2	
se3/se3	0	0	1	2	3	
se3/se4	0	2	0	1	3	
se3/se1	0	1	0	0	1	
se4/se4	0	1	0	1	2	

For the C571T (se3) mutation and the G849A (se4) mutation, which had been found in the Taiwanese indigenous Paiwan group, there is a high carrier rate in the Philippines. Data from previous studies of Japanese and Polynesians suggest that Se enzyme-deficient alleles are race specific [7, 11]. Our results suggest either that the Taiwanese Paiwan group may have migrated to or from the Philippines, or that these two populations have identical ancestors. Further investigations need to be done to support this hypothesis.

The three-base (nt 688 through 690) deletion mutation (se7) was first discovered in the present study. Among all the 202 alleles of the Filipino population, only two had the mutation, and both cases (se2/se7) had the Le(a+ b–) phenotype. These results indirectly demonstrat that se7 is a deficient mutant of FUT2 gene.

Results from this study are consistent with others reported in the literature [5, 6, 7, 10, 11, 18]. Several hotspot mutations in the FUT2 gene are responsible for the nonsecretor phenotype. Some mutations are race specific and are predominant, such as the G428A mutation found in Caucasians and the A385T mutation in Asians. We also analyzed the C357T polymorphism with two different alleles which have been reported in other studies [11, 12].We found similar results in the Filipino population.

The Lewis Le(a+ b+) phenotype has been found among Taiwanese [2], Indonesians [14], Polynesians [8], Japanese [9, 16], and Australian aborigines [1, 17]. The Le(a+ b+) phenotype is virtually absent in Caucasians but has a relatively high frequency (22–25%) in Taiwanese [2]. Upon comparison, no difference was found between the genotypes Le(a+ b+) and Le(a+ b–). This phenomenon suggests that some factors may modify the mutated se enzyme, which has the low activity of the FUT2 enzyme. If the activity of se increases after the reaction of these factors, the phenotype will be Le(a+ b+). When activity decreases, the phenotype will be Le(a+ b–). The current study provides a genetic basis that can be used to solve the discrepancy between the phenotypes Le(a+ b+) and Le(a+ b–).

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