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Lewis (FUT3) genotypes in Taiwanese, Thai, and Filipino populations

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Abstract The Lewis (Le) blood type comprises two major antigens, Le^a and Le^b, which are encoded by α (1,2)-fucosyltransferase (FUT2) and α (1,3/1,4)-fucosyltransferase (FUT3). In this study, we analyzed the mutations of FUT3 in Taiwanese, Thai, and Filipino populations and correlated these with serologic phenotypes. One hundred and thirty-seven Taiwanese, 71 Thai, and 125 Filipino were studied unselectively. The frequency of the normal and four other mutant alleles for Taiwanese, Thai, and Filipino, respectively, were as follows: 187/274 (68.2%), 87/142 (61.3%), and 160/250 (64.0%) were wild type (Le); 14/274 (5.1%), 1/142 (0.7%), and 1/250 (0.4%) were a T202C/C314T mutation ($le^{202,314}$); 35/274 (12.8%), 15/142 (10.6%), and 22/250 (8.8%) had the G508A mutation (le^{508}); and 38/274 (13.9%), 39/142 (27.4%), and 67/250 (26.8%) carried the T1067A mutation (le^{1067}). The le^{445} and le^{1007} were not detected in this study. Our result provided the first genetic data of the FUT3 gene in these three populations, and the frequency distribution of mutant alleles among Taiwanese, Thai, and Filipinos demonstrates a significant difference ($P<0.001$). In our study, the $le^{202,314}$ mutation had considerable frequency in the Taiwanese, but the le^{1067} mutation had a higher frequency in Thai and Filipinos.

Key words Lewis blood group · α (1,3/1,4)-Fucosyltransferase (FUT3) · Taiwanese · Thai · Filipino

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Introduction

The Lewis (Le) blood group system, which is synthesized by the sequential action of fucosyltransferases, belongs to a family of structurally related oligosaccharides [8]. The molecules were first identified as red blood cell (RBC) antigens, but later they were also discovered in the exocrine secretions, including saliva [1, 8]. The expression of the two main Le antigens, Le^a and Le^b, is controlled by the interaction of the products of two genetically independent loci, the Secretor (Se) and the Le loci [5, 8, 11, 13, 20, 23]. These loci encode Se α (1,2)-fucosyltransferase (FUT2) and Le α (1,3/1,4)-fucosyltransferase (FUT3), respectively. The Se locus is also responsible for the formation of salivary ABH blood group substances, and thus correlation of the Le phenotype with the ABH Se status of an individual has long been observed.

The gene coding for FUT3 has been cloned [6]. The sequences of FUT3 alleles of Le negative individuals from several ethnic populations have revealed more than six point mutations that lead to a loss of enzyme activity [5, 12, 14, 15, 17, 18, 19, 21, 22]. These mutations were T202C, C314T (the mutations of T202C and C314T are linked closely), C445A, G508A, A1007C, and C1067A. Although several mutations of FUT3 were found in different ethnic populations, there were no reports based on the genetic analysis of the Le-negative FUT3 gene in Taiwanese, Thai, or Filipino populations. In this study, we developed a much reliable method – polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) – and investigated

all of the known mutations of FUT3 genes and their phenotypes in Taiwanese, Thai, and Filipino populations.

Materials and methods

Subjects

One hundred and thirty-seven Taiwanese, 71 Thai, and 125 Filipino were subjects of the study. There were 207 male and 126 female subjects with a mean age of 32 years (ranging from 19 years to 45 years), and no family members were included in this study. Blood samples were collected as described [3]. The RBC Le phenotype was determined using a manual polybrene method or a tube method using monoclonal antibodies (Gamma-CloneR, anti-Le^a, anti-Le^b Gamma Biologicals, Inc. Houston, Tex.).

DNA amplification and restriction enzyme analysis

Total genomic DNA was isolated from peripheral blood leukocytes as described previously [3]. The oligonucleotide primer and restriction enzyme analysis were the same as in our previous studies [3, 4]. The main purpose was to design an accurate and affordable method to create a restriction site either in mutations that do not have an authentic restriction site or in those situations where the restriction enzyme for the original natural restriction site is too expensive. The primers and restriction enzyme analysis for FUT3 mutations are shown in Table 1.

For the T202C mutation, the mutant creates a *SnaB* I restriction site. However, the enzyme is too expensive, so we introduced a mutagenic base, C, at nucleotide (nt) 202, which is located at the 3′ end of the upstream primer. This will create a *Msp* I site (CCGG) in a mutant allele after PCR amplification. The normal allele disrupts the restriction site. For the C314T mutation, the mutant creates a *Nla* III site, but there is another *Nla* III site near the nt 202 mutation which will interfere with the result. To resolve this problem, we created a new restriction site. The mutagenic base, T, was introduced as the second-to-last base of the 3′ end of the downstream primer, creating a TGATCA site after PCR. This site can be cleaved by *Bcl* I. The C445A mutation has an authentic *BstN* I site around nt 445 and nt 453. Therefore, we designed a mismatch primer at nt 453 to block this restriction site after PCR amplification. In addition, the C445A mutant will lose the authentic *BstN* I site. Regarding the G508A mutation, the mutant has a natural *Alu* I site, and another *Alu* I site is located near the downstream primer. In order to detect the A1007C mutation, a mutagenic base, A, the second base located at the 3′ end of the upstream primer, was introduced to create an *Alu* I site with base C of the mutant allele at nucleotide 1007. The normal allele will abolish this restriction site. For the T1067A mutation, two mutagenic bases, AT, the fourth- and fifth-to-last base of the 3′ end of the upstream primer were introduced to create a *Nsi* I restriction site with a normal T at nt 1067.

For the T202C mutation, a 158-bp fragment was amplified, and a new *Msp* I restriction site was created in the mutant allele. The normal allele was digested into 82 bp and 76 bp, which were unable to be separated under 3.5% agarose gel electrophoresis. The mutant allele was digested into 82 bp, 56 bp, and 20 bp (Fig. 1A). The C314T mutation, which created a *Bcl* I site, was cleaved to 133 bp and 25 bp fragments, but the normal allele had an undigested 158-bp band (Fig. 1B). For the C445A mutation, the amplified product was 284 bp. The 284-bp fragment remained undigested after *BstN* I digestion in the mutant allele, but it was digested to 254 bp and 30 bp for the normal allele (Fig. 1C). For the G508A mutation, the mutant allele was digested to 106-bp, 64-bp, and 25-bp fragments, and the normal allele was digested to 170-bp and 25-bp fragments (Fig. 1D). In order to detect the A1007C mutation, *Alu* I digestion of the PCR products from the normal allele yielded one fragment of 176 bp. However, *Nsi* I showed a 115-bp fragment in the mutants and two fragments (95 bp and 20 bp) in the normal allele (Fig. 1F).

Molecular cloning or direct sequencing of the Le allele

For uncertain cases, or those cases without any mutations mentioned above, direct sequencing or subcloning followed by sequencing of the PCR products was done as described [3]. The PCR products were either cloned into a T vector (pT7 Blue T-vector kit, Novagen, Inc. Madison, Wis.) or directly sequenced. DNA sequences were determined by the dideoxy chain termination method using an Amplicycle Sequencing Kit (Perkin Elmer, Foster City, Calif.).

The DNA amplification was performed as described [4] but with modification of the annealing temperature according to the melting temperature (Tm) of the primers. The amplified products were digested with the appropriate restriction enzymes followed by electrophoresis on 1.5–4% agarose gels. Positive and negative controls were confirmed by means of direct sequencing of the PCR products.

Statistical analysis

Description analysis was performed to present the frequencies and distributions of the demographic variables. A χ^2 test was used to examine the differences in distributions of measurement among the populations.

Results

The genotypes comprising the four common mutations (T202C, C314T, G508A, and T1067A) and the allele

Table 1 The primer sequences, locations,restriction enzyme, and restriction fragment sizes for analyzing the mutation in FUT3.*UP* upstream primer;*DP* downstream primer;*underlined* mutagenic base;*nt* nucleotide; * mutant

Mutation	Primer sequence	Location	Enzyme	Size of fragment (bp)
T202C and C314T	UP: CCACCCTCCTGATCCTGCTC	nt 182–201	*Msp* I (T202C)	82, 76 (82, 56, 20)*
	DP: GATATCCCAGTGGTGCACGATGATGATC	nt 315–339	*Bcl* I (C314T)	158 (133, 25)*
C445A	UP: identical to UP of T202 C and C314 T		*BstN* I	254, 30 (284)*
	DP: GAGATTGAAGTATCTGTCCAAGGC	nt 442–465		
G508A	UP: TCAACTTGGAGCCACACCCT	nt 404–423	*Alu* I	170, 25 (106, 64, 25)*
	DP: AGTTGGACACCGCCCAGGCCACCAG	nt 574–598		
A1007C	UP: GCTCCTTCCGCTGGGCACTAG	nt 986–1006	*Alu* I	176 (156, 20)*
	DP: TGGCCACAAAGGACTCCAGC	nt 1140–1161		
T1067A	UP: GTACCAGACGGTGCGATGCA	nt 1047–1066	*Nsi* I	95, 20 (115)*
	DP: identical to DP of A1007 C			

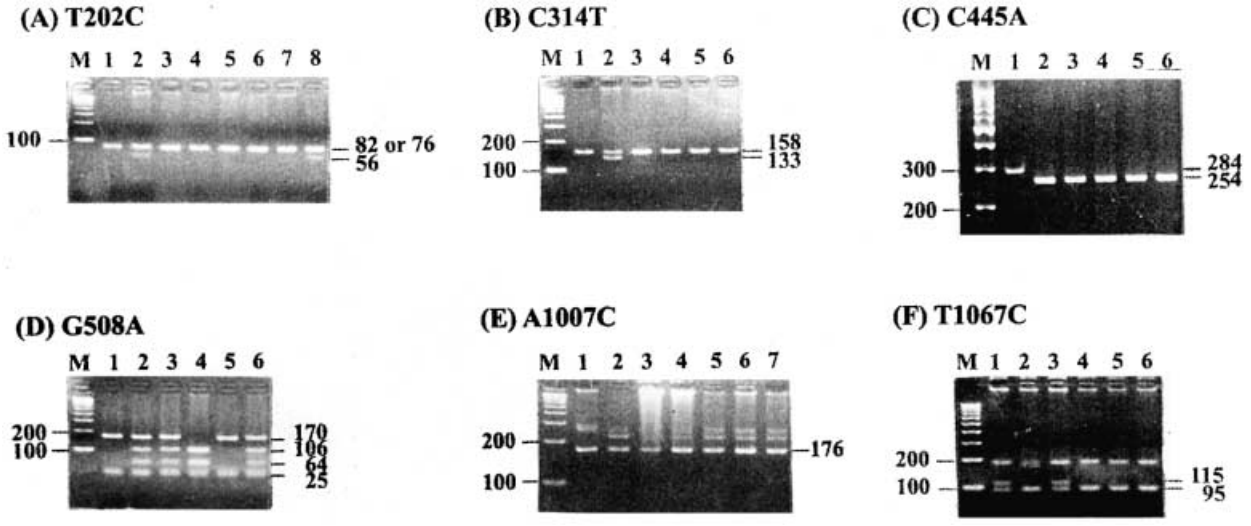

Fig. 1 The results of PCR products and restriction enzyme digestion. **A** For T202 C, lanes 1, 3–7 are wild-type alleles, and lanes 2, 8 are heterozygotes. **B** For C314 T, lanes 1, 3–6 are wild-type alleles, and lane 2 is a heterozygote **C** For C445 A, lane 1 is an uncut control, and lanes 2–6 are wild-type alleles. **D** For G508 A, lanes 1 and 5 are wild-type alleles, lanes 2, 3, 6 are heterozygotes of G508 A, and lane 4 is a homozygote of G508 A. **E** For 1007 C, lanes 1–7 are wild-type alleles. **F** For T1067G, lanes 2, 4–6 are wild-type alleles, lanes 1 and 3 are heterozygotes of T1067G, and the band near 200 bp is a non-FUT3 gene product

Table 2 FUT3 genotypes and Lewis blood phenotypes. Correlation in Taiwanese, Thai, and Filipino. *Ta* Taiwanese population; *Th* Thai population; *F* Filipino population

Genotypes	Lewis phenotype			
	Le (a–b+)	Le (a–b–)	Le (a+b–)	Le (a+b+)
	Ta/Th/F	Ta/Th/F	Ta/Th/F	Ta/Th/F
Le/Le	40/12/31	4/3/2	6/4/15	12/8/4
Le/le^{202,314}	6/1/0	1/0/0	0/0/1	3/0/0
Le/le^{508}	19/4/7	4/3/3	0/0/0	4/1/1
Le/le^{1067}	13/11/30	0/5/6	2/2/8	11/6/5
le^{202,314}/le^{508}	0/0/0	2/0/0	0/0/0	0/0/0
le^{202,314}/le^{1067}	0/0/0	2/0/1	0/0/0	0/0/0
le^{508}/le^{508}	0/0/0	1/2/2	0/0/0	0/0/0
le^{508}/le^{1067}	0/0/0	4/3/7	0/0/0	0/0/0
le^{1067}/le^{1067}	0/0/0	3/6/5	0/0/0	0/0/0
Total	174	69	35	35

frequencies in Taiwanese, Thai, and Filipinos are shown in Table 2 and Table 3. The four alleles, Le (wild-type allele), le^{202,314}, le^{508}, and le^{1067} were all detected in these three populations. Of the 137 Taiwanese, 71 Thai, and 125 Filipinos studied, 187/274 (68.2%), 87/142 (61.3%), and 160/250 (64.0%) were wild type (Le); 14/274 (5.1%), 1/142 (0.7%), and 1/250 (0.4%) had the T202C/C314T mutation; 35/274 (12.8%), 15/142 (10.6%), and 22/250 (8.8%) had the G508A mutation; and 38/274 (13.9%), 40/142 (28.2%), and 67/250 (26.8%) carried the T1067A mutation. However, we did not find the C445A and A1007C mutations in our study populations.

With regard to the molecular basis of phenotypes of Le (a–b+), 83 cases were wild type in both alleles, and 91 cases had a wild-type allele with a mutant allele of these four mutations. Concerning the phenotypes of Le (a+b–), 23 cases had Le/Le, and 12 cases were Le/1067. With regard to the phenotypes of Le (a+b+), 24 cases were Le/Le, and 31 cases had a wild-type allele and a mutant allele of le^{202,314}, le^{508}, and le^{1067}. Concerning the phenotypes of Le (a–b–), 9 cases had Le/Le, 22 cases had a Le and a mutant allele, and 38 cases were homozygous or double heterozygotes for the four common mutants.

Discussion

In this study, we found four le alleles in Taiwanese, Thai, and Filipino populations, which possess different mutations and encode non-functional Le enzymes. The non-functional enzymes are inactivated by these single missense mutations in the catalytic domain. The genetic detection of le alleles caused by mutation of the FUT3 gene is very difficult due to the highly homologous se-

Table 3 Distribution of the FUT3 allelic frequencies in Taiwanese, Thai, and Filipino. χ^2 test was used for statistical analysis. $\chi^2 = 30.50$; $P = 0.000$

Ethnicity (chromosome)	Mutations			
	Le	T202C/C314T	G508A	T1067A
Taiwanese (274)	187 (68.2%)	14 (5.1%)	35 (12.8%)	38 (13.9%)
Thai (142)	87 (61.3%)	1 (0.7%)	15 (10.6%)	39 (27.4%)
Filipino (250)	160 (64.0%)	1 (0.4%)	22 (8.8%)	67 (26.8%)

quences among the FUT3, FUT5, and FUT6 genes. In order to solve the problem, we selected the specific region of the FUT3 gene to design primers to amplify the FUT3 gene specifically. After sequencing part of the PCR products amplified by the specific primers, we found that our method was very specific for the FUT3 gene.

We have examined four common mutations in these three populations, and the frequency distribution between Taiwanese and Thai, and between Taiwanese and Filipinos, demonstrates significant differences ($P<0.001$; Table 3). In our study, the $le^{202,314}$ mutation had a considerable frequency in Taiwanese. There was a significant difference between Taiwanese and Thai ($P=0.04$) and between Taiwanese and Filipinos ($P=0.003$). However, there was no difference between Thai and Filipinos ($P=0.657$). The le^{1067} mutation had a higher frequency in Thai and Filipinos. There was also a significant difference between Taiwanese and Thai ($P=0.001$) and between Taiwanese and Filipinos ($P=0.002$). No difference was noted between Thai and Filipinos ($P=0.662$). We also compared the mutation frequencies of Taiwanese with two Chinese populations reported by Liu et al. [15]. There was no significance among these populations. Our result is consistent with the ethnic difference of the Le-negative alleles suggested by previous reports [5, 6, 12, 14, 22]. Our study shows that Thai and Filipinos are much more closely related than Taiwanese and Thai. The phenotype Le (a+b+) has been found in Taiwanese [2], Indonesians [9], Polynesians [7], Japanese [7, 24], and Australian aborigines [10, 25]. The Le (a+b+) phenotype is virtually absent in Caucasians but has a relatively high frequency (22–25%) in Taiwanese [2]. We compared the genotypes of FUT3 between Le (a+b+) and Le (a+b–) and found that there is no difference in genotypes between these two phenotypes. Most of the genotypes of Le (a+b+) were Le/Le or Le with $Le^{202,314}$, Le^{508}, and Le^{1067} of FUT3. In Le (a–b–) cases where both alleles or one allele is the wild-type FUT3 gene, the phenotype may be caused by the non-secretion of the FUT2 gene. However, most of these cases with double mutations in the FUT2 gene in our study had an A385T mutation [4]. Our results are different from previous studies [7, 16, 21]. The phenotype of Le (a–b–) individuals in these studies is caused by mutations of the Le gene only. Our results are consistent with the theory that the Le antigens are not synthesized by the erythropoietic progenitor cells but are taken up from plasma [16]. Since these cases were double mutations in the FUT2 gene, Le^a and Le^b will not be secreted into the blood and cannot be taken up by the erythrocytes.

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