Yu-Hua Liu,[1,2,3] *M.D., Ph.D.; Yoshiro Koda,*[1,2] *M.D., Ph.D.; Mikiko Soejima,*[1,2] *B.S.; Hao Pang,*[1,2,3] *M.D.; Baojie Wang,*[1,3] *M.D., Ph.D.; and Hiroshi Kimura,*[1,2] *M.D., Ph.D.*

Lewis (*FUT3*) Genotypes in Two Different Chinese Populations

REFERENCE: Liu Y-H, Koda Y, Soejima M, Pang H, Wang B, Kimura H. Lewis (*FUT3*) genotypes in two different Chinese populations. J Forensic Sci 1999;44(1):82–86.

ABSTRACT: The allelic frequencies of the α(1,3/4)fucosyltransferase gene (*FUT3*) in two different Chinese populations (138 individuals in Shenyang and 154 in Guangzhou) were investigated using PCR-RFLP and nucleotide sequencing methods. The common alleles in the Oriental population, *Le* (wild type allele), $le^{59,508}$ (with the mutations at nucleotide (nt) 59T → G and nt 508G → A) and $le^{59,1067}$ (with the mutations at nt 59T → G and nt 1067T → A) were encountered, and also the rare alleles, le^{1067} (with the mutation at nt 1067T → A) and Le^{59} (with the mutation at 59T → G), were observed in these Chinese populations. In addition, the common allele in Caucasians, $le^{202,314}$ (with the mutations at nt 202T → C and nt 314C → T), was found in the Oriental population for the first time. The allelic frequencies of the *Le*, Le^{59}, $le^{59,508}$, $le^{59,1067}$, $le^{202,314}$, and le^{1067}, were 0.750, 0.011, 0.145, 0.054, 0.036, and 0.004 in the Shenyang population and 0.675, 0.026, 0.14, 0.123, 0.026, and 0.010 in the Guangzhou population, respectively. The presence of the alleles containing either the 59 mutation (Le^{59}) or the 1067 mutation (le^{1067}) suggested that the allele $le^{59,1067}$ may have originated by recombination between them.

KEYWORDS: Lewis gene, α(1,3/4)fucosyltransferase, Chinese population

The Le^a and Le^b antigens of human erythrocytes are structurally defined as neutral glycosphingolipids, are not synthesized by the erythropoietic progenitor cells, and are taken up from plasma (1,2). The production of these antigens is controlled by fucosyltransferases encoded by the secretor (*FUT2*) and Lewis (*FUT3*) genes (3–7). There are four phenotypes of the Lewis system: Le(a − b +), Le(a + b −), Le(a + b +) and Le(a − b −). Since the Lewis antigens of red blood cells are passively acquired from plasma, some complexities and difficulties are present in phenotyping of the Lewis system using the hemagglutination test. The occurrences of Lewis antigens and Lewis enzymes have been frequently observed in the saliva and in tissues of individuals with Le(a − b −) erythrocyte phenotype (3,8). The cDNA encoding the Lewis α(1,3/4)fucosyltransferase has been cloned (9). Molecular analysis of the *FUT3* in Japanese (6,10,11), Indonesian (3), Swedish (12,13) and Danish populations (14) has revealed six point mutations in the coding region of the *FUT3*—nt 59T → G (Leu20Arg), nt 202T → C (Trp68Arg), nt 314C → T (Thr105Met), 445C → A (Leu146Met), nt 508G → A (Gly170Ser) and nt 1067T → A (Ile356Lys)—responsible for inactivation of the enzyme activity. Since the mutations in null alleles of the *FUT3* appeared to be race-specific (11,13), analysis of these mutations would be of anthropological interest to compare the incidences of the different Lewis genotypes among ethnic groups. However, studies on these mutations of the Lewis gene among different populations have been few. In particular, the genotype distribution has not yet been reported in any Chinese population. In this study, we have investigated the distribution of the mutations on the Lewis (*FUT3*) locus in a northeastern Chinese (Shenyang) population and in a southern Chinese (Guangzhou) population using PCR-RFLP and nucleotide sequencing methods.

Materials and Methods

Lewis Phenotype of Erythrocytes and DNA Preparation

Blood samples were collected from 138 unrelated blood donors in a northeastern region (Shenyang) and from 154 in a southern region (Guangzhou) of China. The Lewis phenotypes of red blood cells were determined with monoclonal anti-Le^a and -Le^b antibodies (Ortho Clinical Diagnostics, Tokyo, Japan) as described previously (15). Genomic DNA was extracted from peripheral leukocytes using the proteinase K-SDS method as described previously (6).

PCR-RFLP and Nucleotide Sequencing Methods

PCR amplification was performed in 20 μL of LA Taq buffer containing 2.5 mM $MgCl_2$, 200 μM dNTPs, 25 pmol of each of a pair of primers, 1.0 unit of LA Taq DNA polymerase (Takara, Shiga, Japan), and 0.1 ~ 0.5 μg of genomic DNA as the template, as described previously (11,16,17). The oligodeoxynucleotide primers, 5′-CTC AAG CTT AAG CAG GAG ATT GTC ATC ACT GAC C-3′ (No. 9; −102 to −69 sense nucleotides) and 5′-CTC TCT AGA CGT GCC GTG ATG TCT CTC TGC AC-3′ (No. 10; 1528 to 1559 antisense nucleotides), were used to amplify the full length open reading frame of the Lewis gene. The restriction enzyme sites for *Hin*dIII and *Xba*I were introduced into each of the primers, No. 9 and No. 10, respectively, and indicated by underlining. PCR amplification involved initial denaturing at 94°C for 1 min followed by 30 cycles of denaturing at 94°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 2 min.

The resultant products were subsequently used as templates for nested PCR to detect the missense mutations at the positions of

[1] Visiting associate professor, associate professor, lecturer, visiting assistant professor, professor and professor, respectively.
[2] Division of Human Genetics, Department of Forensic Medicine, Kurume University School of Medicine, Kurume, Fukuoka 830–0011, Japan.
[3] Division of Serology, Faculty of Forensic Medicine, China Medical University, Shenyang, 110001, China.

Received 30 Dec. 1997; and in revised form 5 May 1998; accepted 7 May 1998.

59, 314, 508, and 1067 (Fig. 1). Amplification of all nested PCR was performed in 20 μL of Taq buffer containing 1.5 mM $MgCl_2$, 200 μM dNTPs, 25 pmol of each of the pair of primers, 1.0 unit of rTaq DNA polymerase (Toyobo, Osaka, Japan), and 1 μL of 1000-fold diluted first PCR product as the template. The nucleotide sequences of primers for nested PCR were as follows—the primers 5′-ATG GCG CCG CTG TCT GGC CGC CC-3′ (No. 1; 36 to 58 sense nucleotides) and 5′-GAG GAC CCA CTG GGA GCC CT-3′ (No. 2; 136 to 155 antisense nucleotides) for position 59, the primers 5′-GCA AGG TGT ACC CAC AGG CAG CCA-3′ (No. 5; 290 to 313 sense nucleotides) and 5′-TTC CAG GTG CTG GCA GTT AGG-3′ (No. 6; 421 to 441 antisense nucleotides) for position 314, the primers 5′-ATG TCC AAC CCT AAG TCA CG-3′ (No. 3; 340 to 359 sense nucleotides) and 5′-TGA GTC CGG CTT CCA GTT GGA-3′ (No. 4; 592 to 612 antisense nucleotides) for position 508, and the primers 5′-CGC TCC TTC AGC TGG GCA CTG GA-3′ (No. 7; 985 to 1007 sense nucleotides) and 5′-CGG CCT CTC AGG TGA ACC AAG AAG CT-3′ (No. 8; 1068 to 1093 antisense nucleotides) for position 1067. All nested PCR involved initial denaturing at 94°C for 1 min followed by 30 cycles of denaturing at 94°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 1 min. The nested PCR products were cleaved by *Msp*I, *Nco*I, *Pvu*II or *Hin*dIII, respectively, and then underwent electrophoresis on an 8% polyacrylamide gel for the mutations at 59, 314 and 1067 or on a 1.4% agarose gel for the mutation at 508 (Fig. 1) (11). The mutation at nt 202T → C was examined as follows: the first PCR product of individuals associated with the mutation at nt 314C → T was digested by *Hin*dIII and *Xba*I and then subcloned into plasmid pRc/CMV (Invitogen, San Diego, CA). The DNA sequence was then analyzed using an ABI PRISM 310 genetic analyzer (Perkin Elmer Japan ABI, Chiba, Japan).

Results and Discussion

It has been demonstrated that null alleles of the *FUT3* usually contained the two mutation sites in the coding region, such as $le^{59,508}$, $le^{59,1067}$ or $le^{202,314}$ (6,10–13). Generally, Lewis-negative individuals in Oriental populations have possessed a homozygous mutation at nucleotide 59 (10,11). However, we have found that three individuals with the Le(a − b +) phenotype showed a double mutation at nucleotide 59 and a single mutation either at nucleotide 508 or 1067, suggesting a new allele containing only the 59 mutation. Therefore, we subcloned the *FUT3* from them to identify their genotypes through DNA sequencing. The results revealed that the genotypes of the three individuals were $Le^{59}/le^{59,508}$ (two individuals) and $Le^{59}/le^{59,1067}$ (the other individual). We (6) and Nishihara et al. (10) have demonstrated that extracts of COS cells transfected with the plasmid containing only the mutation at nt 59T → G showed enzyme activity comparable to extracts transfected with the plasmid containing the wild type *FUT3*. Mollicone et al. (3) found the allele containing only the mutation at nt 59T → G in several Indonesian pedigrees, and their transfection study also demonstrated the activity of the Lewis enzyme, but a lower activity than that in extracts of cells transfected with the wild type allele. Consequently, we termed this allele containing only the mutation at nt 59T → G as Le^{59}, being a Lewis-positive allele. The presence of the alleles containing either the 59 mutation (Le^{59}) or the 1067 mutation (le^{1067}) suggested that the allele $le^{59,1067}$ may have originated by recombination between them. The $le^{59,508}$ allele also may have originated by a similar mechanism. Recently, an allele containing only the 508 mutation in a Japanese was found (H. Nishimukai, personal communication).

In the present study, we encountered four kinds of negative allele, that were marked as $le^{59,508}$, $le^{59,1067}$, $le^{202,314}$ and le^{1067}, and two kinds of positive allele, one of which was the wild type gene marked as *Le*, and the other of which was marked as Le^{59} (Table 1). The $le^{202,314}$ allele which is the common allele in Caucasians was found in Orientals for the first time. Lewis-negative individuals comprised 6.52% of those in Shenyang, and 9.74% of those in Guangzhou. Their genotypes were homozygous or heterozygous for Lewis-negative alleles. On the other hand, Lewis-positive individuals with the Le(a − b +), Le(a + b −) or Le(a + b +) phenotype comprised 93.48% of those in Shenyang and 90.26% in Guangzhou, and were found to possess at least one of *Le* and Le^{59}. The genotypes and allele frequencies of the *FUT3* in these two Chinese populations are shown in Table 1 and Table 2, respectively. The Lewis allele frequencies of a Japanese population (11) and African (Xhosa) and Caucasian populations in South Africa (18) are also shown in Table 2 for comparison. The distribution of the alleles of the Lewis locus in the two Chinese populations was in good agreement with the Hardy-Weinberg equilibrium. The power of discrimination (PD), and the excluding probability of paternity (EPP), was calculated to be 0.613 and 0.223 in the Shenyang population, and 0.717 and 0.301 in the Guangzhou population, respectively. The PD and EPP calculated from the Lewis genotypes were remarkably higher than those obtained from the Lewis phenotypes (PD = 0.122, EPP = 0.149 in Shenyang, and PD = 0.176, EPP = 0.166 in Guangzhou).

It seemed obvious that there would be a large genetic heterogeneity among Lewis-negative individuals in different populations (10–14,18). Apart from the wild type (*Le*), two dominating alleles were encountered among the two Chinese populations, one allele was with the mutations at nucleotides 59 and 508 ($le^{59,508}$), and the other was with the mutations at nucleotides 59 and 1067 ($le^{59,1067}$). A difference between the Chinese and the Japanese populations was found in the $le^{59,508}$, which was higher in the Japanese population (0.275) than in the Chinese populations (0.145 and 0.140) (Table 2). In contrast to the $le^{59,508}$ and $le^{59,1067}$, the $le^{202,314}$ allele, which is the common allele of the *FUT3* among Lewis-negative Caucasian individuals (0.140) (18), was not found in two Japanese populations studied independently (10,11) and was found in the two Chinese populations for the first time, but with a low frequency (0.036 and 0.026). The genotype distribution between the two Chinese populations showed no significant deviation ($\chi^2 = 9.504$, df = 7, $0.1 < p < 0.25$), but a significant difference in the genotype distribution was observed between Chinese (Shenyang and Guangzhou) and Japanese populations ($\chi^2 = 44.88$, df = 7, $p < 0.005$), between Chinese and Xhosa populations ($\chi^2 = 107.09$, df = 8, $p < 0.005$) and between Chinese and Caucasian populations ($\chi^2 = 92.27$, df = 12, $p < 0.005$) by the 2×1 contingency table test. Therefore, the heterogeneity among *FUT3* alleles would be a useful tool for personal identification and anthropological interest as a genetic marker.

Acknowledgments

We thank Mr. S. Kamimura for his technical assistance. This work was supported in part by Grant-in-Aids for Scientific Research and International Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

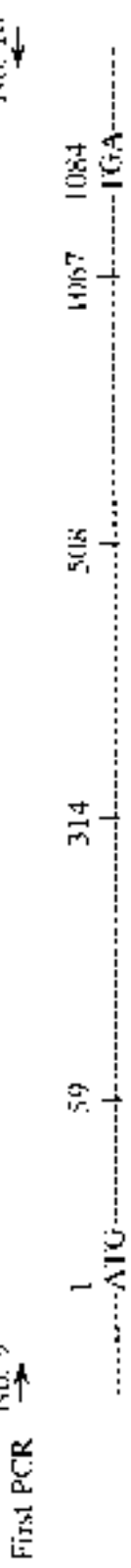

FIG. 1—*Schematic diagrams for* FUT3 *genotyping by PCR-RFLP. The positions of five primer sets for PCR are indicated by arrows. A set of primers, No. 9 and No. 10, was employed to amplify the full-length open reading frame of the* FUT3. *The primer sets, No. 1 and No. 2, No. 5 and No. 6, No. 3 and No. 4, and No. 7 and No. 8, were employed for detecting T59G, C314T, G508A and T1067A, respectively. The nested PCR product at a different position with the mutation (▲) was cleaved into two fragments by a corresponding endonuclease.*

TABLE 1—*Distribution of the Lewis genotypes in northeastern (Shenyang) and southern (Guangzhou) Chinese populations.*

Genotypes	Shenyang (n = 138)			Guangzhou (n = 154)		
	Le(a+b−)	Le(a−b+)	Le(a−b−)	Le(a+b−)	Le(a−b+)	Le(a−b−)
Le/*Le*	15	64	0	20	52	0
Le/Le^{59}	1	1	0	2	3	0
Le/$le^{59,508}$	9	21	0	10	22	0
Le/$le^{59,1067}$	3	5	0	6	15	0
Le/$le^{202,314}$	2	6	0	2	4	0
Le/le^{1067}	0	1	0	0	0	0
Le^{59}/$le^{59,508}$	0	0	0	1	1	0
Le^{59}/$le^{59,1067}$	0	0	0	0	1	0
Le^{59}/$le^{202,314}$	0	1	0	0	0	0
$le^{59,508}$/$le^{59,508}$	0	0	2	0	0	1
$le^{59,508}$/$le^{59,1067}$	0	0	5	0	0	6
$le^{59,508}$/$le^{202,314}$	0	0	1	0	0	1
$le^{59,1067}$/$le^{59,1067}$	0	0	1	0	0	4
$le^{59,1067}$/le^{1067}	0	0	0	0	0	1
$le^{59,1067}$/$le^{202,314}$	0	0	0	0	0	1
le^{1067}/le^{1067}	0	0	0	0	0	1

TABLE 2—*Comparing allelic frequencies of the* FUT3 *among various populations.*

Alleles of *FUT3*	Populations				
	Chinese (Shenyang)* (n = 138)	Chinese (Guangzhou)* (n = 154)	Japanese† (n = 148)	African (Xhosa)‡ (n = 100)	Caucasian§ (n = 100)
Le	0.750	0.675	0.607	0.500	0.675
Le^{59}	0.011	0.026	0	0	0.020
$le^{59,508}$	0.145	0.140	0.275	0.310	0.010
$le^{59,1067}$	0.054	0.123	0.114	0.025	0.130
le^{1067}	0.004	0.010	0.003	0	0.005
$le^{202,314}$	0.036	0.026	0	0.080	0.140
Others	. . .	. . .	. . .	0.085	0.030

* This study.
† From Liu et al. (11).
‡ From Pang et al. (18).
. . . = Not determined.

References

1. Sneath JS, Sneath PHA. Transformation of the Lewis group of human red cells. Nature 1955;176:172.
2. Marcus DM, Cass L. Glycosphingolipids with Lewis blood group activity: uptake by human erythrocytes. Science 1969;164: 553–5.
3. Mollicone R, Reguigne I, Kelly RJ, Fletcher A, Watt J, Chatfield S, et al. Molecular basis for Lewis α(1,3/1,4)fucosyltransferase gene deficiency (*FUT3*) found in Lewis-negative Indonesian pedigrees. J Biol Chem 1994;269:20987–94.
4. Henry SM, Oriol R, Samuelsson BE. Lewis histo-blood group system and associated secretory phenotypes. Vox Sang 1995;69: 166–82.
5. Kelly RJ, Rouquier S, Giorgi D, Lennon GG, Lowe JB. Sequence and expression of a candidate for the human secretor blood group α(1,2)fucosyltransferase gene (*FUT2*): homozygosity for an enzyme-inactivating nonsense mutation commonly correlates with the non-secretor phenotype. J Biol Chem 1995;270:4640–9.
6. Koda Y, Kimura H, Mekada E. Analysis of Lewis fucosyltransferase genes from the human gastric mucosa of Lewis-positive and -negative individuals. Blood 1993;82:2915–9.
7. Koda Y, Soejima M, Liu YH, Kimura H. Molecular basis for secretor type α(1,2)fucosyltransferase gene deficiency in a Japanese population: a fusion gene generated by unequal crossover responsible for the enzyme deficiency. Am J Hum Genet 1996;59: 343–50.
8. Ørntoft TF, Holmes EH, Johnson P, Hakomori S, Clausen H. Differential tissue expression of the Lewis blood group antigens: enzymatic, immunohistologic, and immunochemical evidence for Lewis a and b antigen expression in Le(a−b−) individuals. Blood 1991; 77:1389–96.
9. Kukowska-Latallo JF, Larsen RD, Nair RP, Lowe JB. A cloned human cDNA determines expression of a mouse stage-specific embryonic antigen and the Lewis blood group α(1,3/1,4)fucosyltransferase. Genes Dev 1990;4:1288–303.
10. Nishihara S, Narimatsu H, Iwasaki H, Yazawa S, Akamatsu S, Ando T, et al. Molecular genetic analysis of the human Lewis histo-blood group system. J Biol Chem 1994;269:29271–8.
11. Liu YH, Koda Y, Soejima M, Uchida N, Kimura H. PCR analysis of Lewis-negative gene mutations and the distribution of Lewis allele in a Japanese population. J Forensic Sci 1996;41:1018–21.
12. Elmgren A, Rydberg L, Larson G. Genotype heterogeneity among Lewis negative individuals. Biochem Biophys Res Commun 1993; 196:515–20.
13. Elmgren A, Bȍrjeson C, Svensson L, Rydberg L, Larson G. DNA sequencing and screening for point mutations in the human Lewis (*FUT3*) gene enables molecular genotyping of the human Lewis blood group system. Vox Sang 1996;70:97–103.
14. Ørntoft TF, Vestergaard EM, Holmes EH, Jakobsen JS, Grunnet

N, Mortensen M, et al. Influence of Lewis α1–3/4-L-fucosyltransferase (*FUT3*) gene mutations on enzyme activity, erythrocyte phenotyping, and circulating tumor marker sialyl-Lewis a levels. J Biol Chem 1996;271:32260–8.
15. Wang BJ, Akiyama K, Kimura H. Quantitative analysis of Le^a and Le^b antigens in human saliva. Vox Sang 1994;66:280–6.
16. Koda Y, Soejima M, Kimura H. Detection of G to A missense mutation of Lewis-negative gene by PCR on genomic DNA. Vox Sang 1994;67:327–8.
17. Koda Y, Soejima M, Kimura H. Detection of T to G mutation at position 59 in the Lewis gene by mismatch polymerase chain reaction. Int J Leg Med 1995;107:280–2.
18. Pang H, Liu YH, Koda Y, Soejima M, Jia JT, Schlaphoff T, et al. Five novel mutations of Lewis gene (*FUT3*) in African (Xhosa) and Caucasian populations in South Africa. Hum Genet 1998;102: 675–80.

Additional information and reprint requests:
Hiroshi Kimura, M.D., Ph.D.
Department of Forensic Medicine
Kurume University School of Medicine
Kurume, Fukuoka 830-0011, Japan
Fax: + 81-942-31-7700
E-mail: hkimura@med.kurume-u.ac.jp