SHORT COMMUNICATION

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The Fusion Gene at the ABO-Secretor Locus (*FUT2*): absence in Chinese populations

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Abstract The fusion gene (*se^{fus}*) is a null allele of the secretor type α (1, 2) fucosyltransferase gene (*FUT2*) and was first found in a Japanese population. It has not yet been reported in any other ethnic population. In the present study, we investigated the distribution of the fusion gene of the *FUT2* locus in five populations from three ethnic groups in East Asia. The fusion gene was found in two additional Japanese populations with a high frequency (0.0551 in Okinawa and 0.0792 in Akita) and, for the first time outside Japan, in a Korean population, at a very low frequency (0.0063 in Seoul). In contrast, we found no fusion gene in two Chinese populations. These findings showed that the *FUT2* fusion gene was ubiquitous in Japanese, but was rare in neighboring populations, suggesting that the *FUT2* fusion gene had emerged from within the Japanese. Additionally, a new null allele with a C-to-T substitution at nucleotide 658 was found in one individual native of southern China.

Key words ABO · Secretor · *FUT2* · Fusion gene · Japanese

Introduction

The expression of ABO antigens in secretions such as saliva is regulated by the secretor gene (*FUT2*)-encoded α (1, 2) fucosyltransferase (Se enzyme). The ABO secretor group

system has secretor and nonsecretor phenotypes. Secretors, who have ABO antigens in saliva, have at least one functional *Se* allele, and nonsecretors, who fail to express ABO antigens in saliva, are homozygous for the nonfunctional *se* allele. After the isolation of cDNA and genomic DNA of the *FUT2* gene (Kelly et al. 1995; Koda et al. 1997a), molecular analysis of *FUT2* has indicated that null alleles with a single base substitution in the protein coding region of *FUT2* (G428A, A385T, C571T, C628T and G849A) were responsible for the inactivation of *Se* enzyme (Kelly et al. 1995; Yu et al. 1996; Koda et al. 1996). The findings from these studies suggested that these null alleles were geographic-specific and perhaps therefore race-specific. The null allele with the G428A mutation (*se⁴²⁸*) was found to be common in Caucasians, while the null allele with the A385T (*se^{357, 385}*) was found only in Asian populations (a nucleotide change at position 357 (C to T) is a synonymous mutation) (Kelly et al. 1995; Koda et al. 1996). The presence of race-specific null alleles of *FUT2* is of interest because the frequency of the non-secretor phenotype is 20%–30%, and the total frequency of nonfunctional alleles (*se*) is 0.4–0.5 in all ethnic groups so far examined (Liu et al. 1998). In addition to the point mutations mentioned above, we have reported two unusual alleles of *FUT2*; one was the fusion gene (*se^{fus}*), consisting of the 5'-region of a pseudogene of α (1, 2) fucosyltransferase and the 3'-region of the functional *FUT2*, and believed to be generated by unequal crossover, as one of inactive alleles of *FUT2* in Japanese individuals (Koda et al. 1996), and the other was a complete deletion of the protein coding region of *FUT2* (*se^{del}*) in Indian individuals with the classical Bombay phenotype (Koda et al. 1997b). We have demonstrated the gene frequency of the *se^{fus}* in Fukuoka, a southwestern region of Japan close to Korea, to be 0.0567 (Koda et al. 1996). However, the fusion gene of the *FUT2* locus has never been found in any other ethnic group.

In this study, we investigated two Chinese populations, two additional Japanese populations, and a Korean population, to better understand the incidence of the *se^{fus}* allele in populations of East Asia, since a considerably high frequency of the *se^{fus}* has been found in Japanese populations (Koda et al. 1996; Narimatsu et al. 1998).

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Fig. 1. Gene frequencies of the fusion gene, *FUT2*, in various East Asian populations. Numbers in parentheses indicate numbers of the fusion gene/numbers of chromosomes tested. Data from Fukuoka and Tokyo are from Koda et al. (1996) and Narimatsu et al. (1998), respectively

Subjects and methods

Samples and Lewis phenotypes

Blood samples were collected from random donors in five populations drawn from three ethnic groups in East Asia, and included 138 Shenyang Chinese (from the northern region of China), 154 Guangzhou Chinese (from the southern region of China), 158 Seoul Koreans, 120 Akita Japanese (from the northern region of the Japanese mainland), and 136 Okinawa Japanese (from the southernmost island of the Japanese archipelago). The Lewis phenotype on red blood cells was determined by a hemagglutination method using mouse monoclonal anti-Lea and anti-Leb antibodies

(Kokusai Reagents, Kobe, Japan). Genomic DNAs were prepared from leukocytes by a proteinase K-sodium dodecylsulfate (SDS) method (Liu et al. 1999).

Screening mutations of *FUT2* by sequence-specific polymerase chain reaction (SSP-PCR) and PCR-restriction fragment length polymorphism (RFLP)

To detect the A385T and C571T mutations, sequence-specific PCR (SSP-PCR) was performed as described previously (Koda et al. 1996). The C357T and C628T substitutions were analyzed as described previously (Liu et al. 1998). To detect the G428A mutation, PCR products amplified by primers described by Kelly et al. (1995) were

Table 1 Comparison of the Allelic Frequencies of *FUT2* in East Asian populations

Alleles ^{a)}	Chinese		Korean	Japanese
	Guangzhou (n=154)	Shenyang (n=138)	Seoul (n=158)	Fukuoka ^{b)} (n=141)
<i>Se</i> (wild type)	0.1398	0.1595	0.1013	0.1028
<i>Se</i> ³⁵⁷	0.3506	0.3913	0.4051	0.4007
<i>se</i> ^{357,385}	0.4805	0.4312	0.4873	0.4362
<i>se</i> ^{357,571}	0.0097	0.0072	0	0
<i>se</i> ⁶²⁸	0	0	0	0.0036
<i>se</i> ^{fus}	0	0	0.0063	0.0567
<i>se</i> ⁸⁴⁹	0.0162	0.0036	0	0
<i>se</i> ⁴²⁸	0	0.0072	0	0
<i>se</i> ⁶⁵⁸	0.0032	0	0	0

^{a)} *Se* and *se* indicate a functional allele and a nonfunctional allele, respectively

^{b)} Data from Koda et al. (1996)

cleaved by *Mva*I and then analyzed using 8% polyacrylamide gel electrophoresis. The fusion gene was detected using SSP-PCR, Southern blot hybridization, and DNA sequencing methods as described previously (Koda et al. 1996). Since the G849A substitution created a new endonuclease *Dde*I site (Yu et al. 1996), PCR products amplified by primers 571C (nt 545-571) and 3'-common (nt 1030-1056) (Koda et al. 1996) were digested by *Dde*I and then analyzed using 2% agarose gel electrophoresis. During screening of the G849A mutation, we incidentally found different sizes of *Dde*I-fragments (113 bp and 399 bp) in an individual from a Guangzhou population, compared with sizes of the *Dde*I-fragments in the G849A mutation (302 bp and 210 bp). The direct DNA sequencing of the PCR product from this individual demonstrated a single base substitution C-to-T at nucleotide 658 in the coding region of the *FUT2*, which created a new endonuclease *Dde*I site (CTNAG).

Nucleotide sequencing

The fusion gene was amplified as described previously (Koda et al. 1996). The resulting PCR products were purified using Suprec-02 centrifugation tubes (Takara, Shiga, Japan), and then used for direct DNA sequencing with primers *Se*-seqU (5'-CCT GGC AGA ACT ACC ACC TG-3', nt 335-354) and *Se*-seqL (59-AGG GCC TGC TGT AGG TAT CG-3', nt 626-645) as described previously (Liu et al. 1998). Fifteen fusion genes and the *FUT2* of one individual with the C658T mutation were used for direct DNA sequencing.

Results and discussion

We investigated the distribution of the fusion gene in 120 individuals in Akita and in 136 individuals in Okinawa, and found that the frequencies of the fusion gene were

0.0792 in Akita and 0.0551 in Okinawa (Fig. 1). However, surprisingly, we found no fusion gene in either of the two Chinese populations (138 individuals in Shenyang and 154 individuals in Guangzhou). We also investigated *FUT2* in a Korean population (158 individuals in Seoul) and found two individuals having the *se*^{fus} in heterozygosity (frequency, 0.0063). The frequencies of other alleles of *FUT2* in the Chinese and Korean populations are given in Table 1. The heterozygosity rate for the Shenyang Chinese, Guangzhou Chinese, Korean, and Fukuoka Japanese populations was calculated to be 0.6286 ± 0.0152 , 0.6378 ± 0.0128 , 0.5882 ± 0.0131 , and 0.6376 ± 0.0149 , respectively. The *se*^{357, 385} was a common *Se* enzyme-deficient allele in the East Asian populations examined, and the *se*⁴²⁸, which is a common null allele in Africans and Caucasians (Liu et al. 1998), was quite rare in Asians (Table 1). Also, we found no fusion gene in 100 Caucasians and in 101 Africans (Xhosa) of South Africa (Liu et al. 1998). Accordingly, the fusion gene at the *FUT2* locus appeared to be ubiquitous in Japanese. To examine whether any other nucleotide substitution (s) were present in this unusual recombinant allele, we sequenced about 1 kb of 15 fusion genes from individuals in Fukuoka, Akita, Okinawa, and Korea, and found no accumulation of any other nucleotide substitution in the sequences of the 15 fusion genes, suggesting that the fusion gene had emerged considerably recently.

During this study, we found a new nonsense mutation (C658T), which resulted in a change in the codon for Arg220 to a stop codon that consequently truncated a large portion (112 amino acid residues) of the catalytic domain of the C-terminal of the enzyme. The frequency of an allele containing the C658T substitution was investigated in both Chinese populations and a Korean population, and we found only one individual having this mutation in heterozygosity in a Guangzhou population.

The fusion gene of the *FUT2* locus was found only in the Japanese and Korean populations and not in the two Chinese populations. The frequency of the fusion gene in the Japanese populations was 0.0475 - 0.0792, whereas in the Korean population it was 0.0063 (Table 1; Narimatsu et al. 1998). Other alleles of polymorphic DNAs found in Japanese and Koreans and not in Chinese were the Y2 allele of the DXYS5Y locus (Nakagome et al. 1992) and the *APRT**J allele of the adenine phosphoribosyltransferase gene (Kamatani et al. 1996), while the YAP⁺ allele of the DYS287 locus was present in the Japanese but not in the Koreans and not in the Chinese (Hammer and Horai 1995). However, the frequency of the YAP⁺ has been reported to be highest in sub-Saharan African populations, followed by North African and then European populations. Most Asian populations examined so far, except Japanese (42% frequency) and Tibetan (50% frequency) populations, have completely lacked the YAP⁺, demonstrating an irregular distribution of this polymorphism in Asia (Hammer and Horai 1995; Hammer et al. 1997). The ten fold higher frequency of the fusion gene in Japanese populations compared with a Korean population, and the finding of no fusion gene in Chinese popula-

tions, suggested that the *FUT2* fusion gene had emerged after the divergence of the Japanese-Korean group from other ethnic groups, and that the fusion gene had recently formed originally in the Japanese population and then very recently entered the Korean population. Further studies to determine the frequency of the *FUT2* fusion gene in other ethnic groups, especially in other Asian populations, are required, since the fusion gene could show an irregular distribution in Asia in a fashion similar to that of the *YAP*⁺ allele.

While there are many somewhat conflicting theories concerning the origin of the modern Japanese, Hanihara (1991) has proposed a dual structure theory of Japanese origins, based on the anthropometric characteristics of fossils, in which modern Japanese are the result of an intermixture between the upper Paleolithic native population of Japan and migrants from northeast Asia via the Korean peninsula during the Aeneolithic Yayoi period (300 BC-300 AD). An analysis of 25 classic genetic markers by Omoto and Saitou (1997) supported this dual structure theory. The analysis of the nucleotide sequences of the D-loop region of mitochondrial DNA from five East Asian populations by Horai et al. (1996) also supported this dual structure theory, and suggested that a great majority of the gene pool of the mainland Japanese was derived from the Asian continent during the Yayoi period. However, Nei (1995) argued against the dual structure theory by analyzing 18 protein polymorphic loci. He suggested that Japanese groups were derived from Northeast Asians and were formed 12 000 - 30 000 years ago, and that the contribution from migrants via the Korean peninsula to the Japanese gene pool was relatively insignificant (transformation theory). After the Japanese archipelago was formed by disconnection from the Asian continent about 12 000 years ago, the gene pool of populations in Japan was transformed independently of continental Asian groups to form the modern Japanese of the Japanese archipelago, consequently resulting in the ubiquitous presence of the *YAP*⁺ allele of the *DYS287*, the *Y2* allele of the *DXYS5Y*, the *APRT*J* allele, and the *FUT2* fusion gene in the Japan mainland.

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