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

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Summary

About 20%–25% of Caucasian individuals are nonsecretors who fail to express soluble A, B, H, and Lewis b histo-blood group antigens in secretory organs and secretory fluids because of the absence of the *Secretor* gene (*FUT2*)–encoded α(1,2)-fucosyltransferase (Se enzyme) activity. Recently, the *FUT2* and a pseudogene have been isolated, and an Se enzyme–deficient allele (*se*) caused by a nonsense mutation (G428A, *se1*) in Caucasians has also been reported. Although we were unable to find the *se1* allele, we have found a missense mutation (A385T, *se2*) and two nonsense mutations (C571T, *se3* and C628T, *se4*) in the Japanese Se enzyme–deficient alleles. In addition, we have found a fusion gene, which consisted of the 5'-region of the pseudogene and the 3'-region of the functional *FUT2*, as a Se enzyme–deficient allele (*se5*). The DNA sequence analysis of the fusion gene indicated that the crossover region corresponded to regions between bases 253 and 313 of the pseudogene and between bases 211 and 271 of the *FUT2*. This finding suggested that the fusion gene was generated by homologous but unequal crossover. A population study on 141 randomly selected Japanese has indicated that the *se2* is a common Se enzyme–deficient allele in the Japanese population. The results suggest that Se enzyme–deficient alleles are race specific.

Introduction

ABO(H) histo-blood group antigens are oligosaccharide antigens (Watkins 1995) constructed by sequential action of specific glycosyltransferases (Oriol et al. 1986; Clausen and Hakomori 1989). These antigens are expressed not only in erythrocyte membranes but also in

tissues such as salivary gland and digestive mucosa. Since H antigen is a precursor of A and B antigens, the α(1,2)-fucosyltransferase is one of the essential enzymes for the tissue expression of ABH antigens, which are composed of type 1, type 2, type 3, and type 4 chains. The type 2 chains are expressed mainly in erythrocyte membranes as insoluble glycolipids, while saliva and digestive mucosa mainly have the type 1 antigens as soluble glycoproteins (Oriol et al. 1986; Clausen and Hakomori 1989; Wang et al. 1994a).

Recent biochemical and genetic studies have indicated that, at least, two distinct α(1,2)-fucosyltransferases are present in human tissues (Oriol et al. 1981; Kumazaki and Yoshida 1984; Le Pendu et al. 1985; Sarnesto et al. 1990, 1992). One is the H gene (*FUT1*)–encoded α(1,2)-fucosyltransferase (H enzyme) that regulates expression of the ABH antigens in erythrocyte membranes, and the other is the *Secretor* gene (*FUT2*)–encoded a(1,2)fucosyltransferase (Se enzyme) that regulates expression of ABH antigens in secretory glands and secretory fluids (Oriol et al. 1986; Clausen and Hakomori 1989). Rare individuals (Bombay and para-Bombay phenotypes) fail to express the H antigen in erythrocyte membranes because of lack of the *FUT1*-encoded enzyme activity (Kelly et al. 1994), while ∼20% of Caucasian individuals (nonsecretors) fail to express the H antigen in secretory fluids because of lack of the *FUT2*-encoded enzyme activity. In contrast to Caucasians, 20%–25% of Orientals are thought to be red cell Lewis(a+b+) and weak-secretor phenotype instead of red cell Lewis(a+b−) and nonsecretor phenotype (Henry et al. 1995).

Recently, Lowe and his collaborators have isolated *FUT1*, *FUT2*, and a pseudogene of *FUT2* (Larsen et al. 1990; Kelly et al. 1995; Rouquier et al. 1995). These genes and the pseudogene shared a high degree of DNA sequence homology (∼70%) and were located within a 100-kb region in chromosome 19q (Larsen et al. 1990; Kelly et al. 1995; Rouquier et al. 1995), suggesting they have evolved by gene duplication and subsequent divergence. Kelly et al. (1995) have also analyzed a molecular defect in the *FUT2* and have found a nonsense mutation of codon 143 (Trp-ter) (*se1*) responsible for the nonsecretor phenotype in Caucasians (Kelly et al. 1995).

Table 1

Primer Sequence for PCR Amplification of the *FUT2* and the Fusion Gene

Primer	Primer Sequence	Position of Primer
5'-FUT2	CCTTTCTCCTTTCCCATGGCCCACTTCATC	(−15)–15 bp of *FUT2*
385A or T	GACTGGATGGAGGAGGAATACCGCCAC<u>A</u>(or T)	358–385 bp of *FUT2*
571C or T	GCACCTTTGTAGGGGTCCATGTTCGC<u>C</u>(or T)	545–571 bp of *FUT2*
5'-pseudo	TTTGGCAGGGGGTGGGTGAAGAGACTCCTG	(−54)–(−25) bp of pseudogene
3'-common	GGCACTCATCTTGAGGGAGGCAGAGAA	1030–1056 bp of *FUT2*

NOTE.—Nucleotide sequences numbered following the notation of Kelly et al. (1995).

In the present study, we investigated the *FUT2* deficiency in the Japanese population. Although we did not find the *se1* allele, we found three point mutations and a fusion gene that consisted of the *FUT2* and its pseudogene, which were independently responsible for the Se enzyme deficiency in Japanese individuals.

Material and Methods

Typing of Blood-Group Phenotypes

Saliva and blood samples were collected from 10 healthy Japanese individuals in our laboratory, whose ABO, Lewis, and secretor phenotypes were determined as described by Wang et al. (1994a, 1994b). A further 141 blood samples were supplied by the Japan Red Cross Blood Center in Fukuoka, and the Lewis phenotype of each sample was determined by hemagglutination. In this study, we did not detect Lewis(a+b+) individuals, who are common among Orientals. Since Lewis b detectability in Lewis (a+b+) phenotype depends on the potency of antiserum used (Henry et al. 1995), the anti-Le^b (Kokusai Reagents) used is expected to give false red cell Lewis(a+b−) phenotype on the Oriental Lewis(a+b+) phenotype. Therefore, we classified Lewis(a+b−) individuals in this study as Se enzyme−deficient phenotype that contains both weak-secretors and nonsecretors.

PCR Amplification and Subcloning of FUT2 and Fusion Genes

Since the open reading frame of *FUT2* is encoded in a single exon, genomic DNA isolated from peripheral leukocytes (Sambrook et al. 1989) was subjected to PCR amplification (Saiki et al. 1988). 5'-FUT2 primer or 5'-pseudo primer and the same primer for 3'-side (3'-common) were used for amplification of *FUT2* or the fusion gene (35 cycles, 94°C 0.5 min, 65°C 1 min, and 72°C 1 min). The primer sequences and their positions in the *FUT2* and its pseudogene are shown in table 1. The resulting PCR products were subcloned into plasmid pGEM by use of a pGEM-T vector system I (Promega), and then the DNA sequence was determined by the dideoxynucleotide chain−termination method using an

AutoRead DNA sequencing kit and an ALF DNA sequencer (Pharmacia).

To detect A385T and C571T mutations, sequence-specific PCR was performed. The same primer for 3'-side (3'-common) (table 1) was used in all sequence-specific PCR. The PCR was carried out as follows: 385A (wild-type) or 385T (mutated) primer—1.2 U *EXTaq* DNA polymerase (Takara), 200 μM dNTP mix, 0.5 μM each primer, 50 ng genomic DNA in 25 μl *EXTaq* buffer (Takara) (28 cycles, 94°C 1 min and 72°C 1.5 min); 571C (wild-type) or 571T (mutated) primer—the same reaction mixture as above except 0.25 μM each primer was used (25 cycles, 94°C 0.5 min and 72°C 0.5 min). Since a C628T mutation creates a new endonuclease *Age*I site, the PCR products with 5'-FUT2 and 3'-common primers were digested by *Age*I to detect this mutation. All PCR products were then analyzed using 1.4% agarose gel electrophoresis.

Southern Blot Analysis

Restriction enzyme−digested DNAs (5−7.5 μg) were separated by 0.8% agarose gel electrophoresis, and then Southern blot analysis was carried out. Preparation of a digoxigenin-labeled *FUT2* probe (nucleotide residues 369−564) and detection of hybridized bands were performed using a DIG DNA labeling and detection kit (Boeringer Manheim).

Transient Expression of FUT2 Alleles in COS Cells and Assay of α(1,2)-Fucosyltransferase Activity

The *FUT2* alleles or the fusion gene was subcloned into a mammalian expression vector pRc/CMV (Invitrogen). The plasmid with DNA inserted in sense direction of the *FUT2* alleles was transfected into COS cells by DEAE-dextran (Gonzalez and Joly 1995). After 48 h of culture, the expression of H antigen on cells was examined using anti-H 1E3 monoclonal antibody, which reacts with the H types 1−4 chains (by courtesy of Dr. K. Furukawa, Gunma University) and fluorescein-labeled anti-mouse IgM antibody (Koda et al. 1993; Nakajima et al. 1993; Wang et al. 1994a), and the enzyme activity was also determined in cell extracts (Kelly
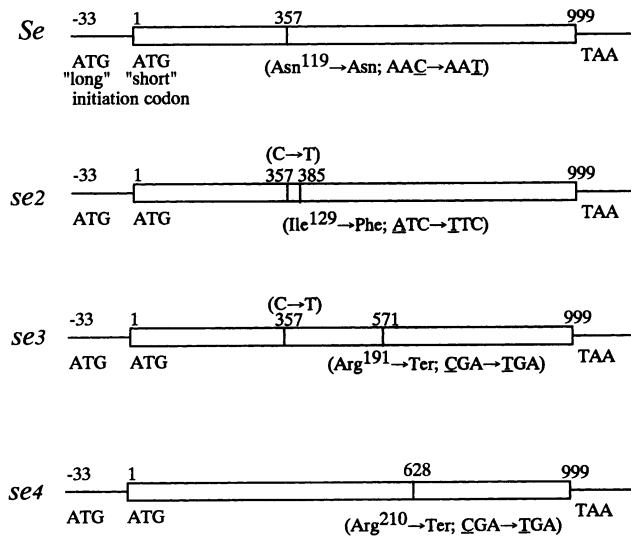
Figure 1   Location of nucleotide substitutions of the *FUT2* from Japanese secretors and nonsecretors. The coding region of the *FUT2* is represented by open rectangles. The relative positions of the mutations and amino acid substitutions are indicated below the coding region. *Se* represents *FUT2* isolated from secretors; *se2, se3,* and *se4* represent *FUT2* isolated from nonsecretors.

et al. 1995). The α(1,2)-fucosyltransferase activity was measured in 100 μl of 25 mM sodium phosphate buffer (pH 6.5) containing 100 μM GDP-L-[U-^{14}C]-fucose, 5 mM ATP, 10 mM MnCl$_2$, cell extract (50 μl containing ~50 μg protein), and 5 mM acceptor (lacto-N-biose I or N-acetyllactosamine) (Kelly et al. 1995; Masutani and Kimura 1995). After incubation at 37°C for 1 h, the products were separated from GDP-^{14}C-fucose by Dowex 1-X8 (chloride form) column (0.5 ml). No more than 15% of GDP-fucose was transferred prior to assay termination. A parallel control reaction without acceptor was performed for each reaction. To normalize the transfection efficiency, the pSV-β-galactosidase con-

trol vector (Promega) was cotransfected, and the activity of β-galactosidase was measured using the β-galactosidase enzyme assay system (Promega).

Results

To identify Se enzyme–deficient alleles (*se*), DNA sequence analysis of *FUT2* in five secretors and five apparent nonsecretors of our laboratory was performed. As shown in figure 1, we found an A-to-T missense mutation at position 385 (A385T, *se2*) that may result in an amino acid replacement of isoleucine by phenylalanine in codon 129, in *FUT2* from a Lewis(a+b−) individual. We also found a nonsense mutation (C571T, *se3*) in *FUT2* from another Lewis(a+b−) individual. Direct sequencing of the PCR product of *FUT2* indicated that four Lewis(a+b−) individuals were homozygous for *se2* and one Lewis(a+b−) individual was heterozygous for *se3/se5*, while three secretors were heterozygous for *se2* and two secretors had neither *se2* nor *se3* in *FUT2*. In addition, we found a neutral mutation (C357T) in all 20 *FUT2* alleles sequenced.

The A385T (*se2*) and C571T (*se3*) mutations were examined by sequence-specific PCR in 141 blindly selected Japanese. As shown in table 2, 21 of the 26 Lewis(a+b−) individuals were homozygous for *se2/se2*. However, we did not find the *se3* allele in any of the 141 Japanese individuals. In one Lewis(a+b−) individual (16) with one allele different from *se2* and *se3* by sequence-specific PCR, we analyzed the DNA sequence of this allele and found a new nonsense mutation (C628T, *se4*) but without the C357T neutral mutation (fig. 1). PCR-RFLP analysis indicated that only individual 16 had the *se4* allele.

No *se* allele was detected in one Lewis(a+b−) individual (96), analyzed by sequence-specific PCR for *se2* and *se3* and PCR-RFLP for *se4*. We obtained the PCR product of the *FUT2* of individual 96 when the sequence-

Table 2

Distribution of the *FUT2* Genotypes and Allele Frequencies in a Japanese Population

	Lewis Phenotypes				
Genotypes	Le(a−b+)	Le(a+b−)	Le(a−b−)	Total	Allele Frequencies
Se/Se	30	0	7	37	
Se/se2	52	0	5	57	
Se/se5	10	0	1	11	*Se* = .504
se2/se2	0	21	10	31	*se2* = .436
se2/se4	0	1	0	1	*se4* = .004
se2/se5	0	3	0	3	*se5* = .057
se5/se5	0	1	0	1	

Note.—The distribution of alleles in the Japanese population studied was in good agreement with that suggested by the Hardy-Weinberg equilibrium (χ^2 = 5.43; df = 6; .25 < P < .5).

specific primer (385A or 571C) and the 3'-common primer were used (not shown) but did not obtain the product when the 5'-FUT2 and the 3'-common primers (table 1) were used (see fig. 3, Se in lane 3), suggesting some deletion in the 5'-region of the *FUT2*. To investigate a possible deletion in the 5'-region of the *FUT2* in individual 96, Southern blot analysis was performed using the probe of nucleotide residues 369–564 of *FUT2*. As described by Kelly et al. (1995), two *Eco*RI fragments corresponding to the *FUT2* (18.5 kb) and the pseudogene (8.2 kb) were detected in a Lewis(a−b+) individual (fig. 2*A*, *Eco*RI, lane 1), while only a single 17.0-kb fragment was detected in individual 96 (fig. 2*A*, *Eco*RI, lane 3). In another Lewis(a+b−) individual (27) with one allele *se2* and the other different from *se2*, *se3*, and *se4*, three *Eco*RI bands were detected (fig. 2*A*, *Eco*RI, lane 2). A single *Nco*I fragment (2.7 kb)—different from both the *FUT2* band (1.7 kb) and the pseudogene band (3.6 kb)—was also detected in individual 96 (not shown). When genomic DNA from an Le(a−b+)
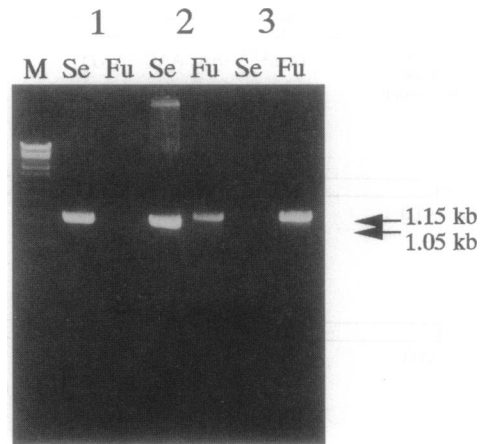

Figure 3   PCR amplifications for detecting the fusion gene. PCR was performed using 5'-FUT2 primer and 3'-common primer (Se) or 5'-pseudo primer and 3'-common primer (Fu) with DNA from a secretor (lane 1) and individuals 27 (lane 2) and 96 (lane 3). M represents *Eco*T14 I–digested λ-DNA. The resulting PCR products were analyzed using 1.4% agarose gel electrophoresis. The expected sizes of the fragments are indicated by arrows.
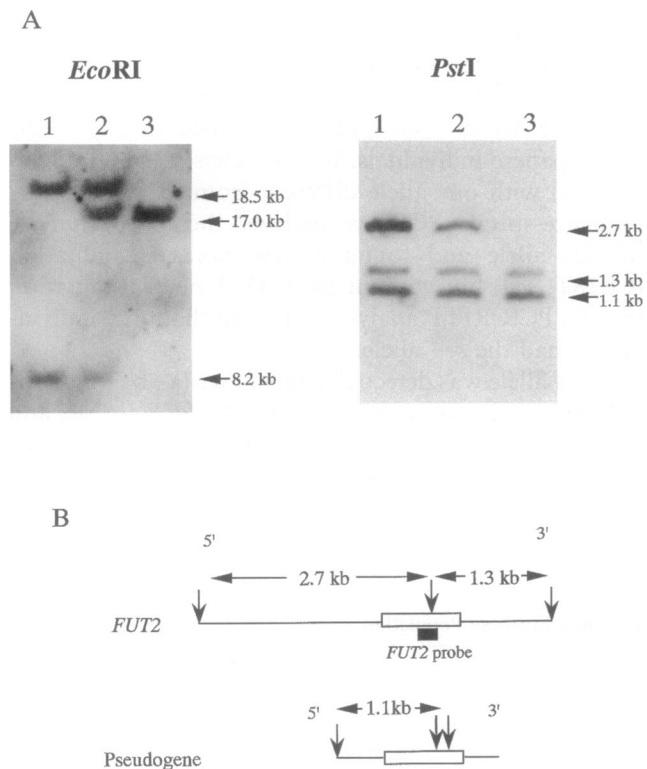

Figure 2   Southern blot analysis of genomic DNAs by use of the *FUT2* probe. In panel *A*, genomic DNAs (5–7.5 μg) from a secretor and individuals 27 and 96 (lanes 1, 2 and 3, respectively) were digested with *Eco*RI or *Pst*I, and then Southern blot analysis was performed. Sizes of hybridized bands are indicated by arrows. In panel *B*, the position of the *FUT2* (between bases 369 and 564) probe is indicated by the black box. The open reading frame of *FUT2* and the corresponding pseudogene sequence are indicated as open rectangles. *Pst*I sites are positioned by vertical arrows, following the notation of Kelly et al. (1995).

individual was digested by *Pst*I, three *Pst*I fragments (2.7 kb, 1.3 kb and, 1.1 kb) were detected by Southern blot analysis (fig. 2*A*, *Pst*I, lane 1). The 2.7-, 1.3-, and 1.1-kb bands corresponded to the 5'-region of *FUT2*, the 3'-region of *FUT2*, and the 5'-region of the pseudogene, respectively (Kelly et al. 1995) (fig. 2*B*). However, the 1.3-kb and 1.1-kb, but not the 2.7-kb, *Pst*I fragments were detected in individual 96 (fig. 2*A*, *Pst*I, lane 3). These PCR and Southern blot analyses suggested that a part in the 3'-region of the pseudogene and in the 5'-region of the *FUT2* were deleted. However, the 3'-region of *FUT2* and the 5'-region of pseudogene were both present in the genomic DNA of individual 96. The deletion in the 3'-region of the pseudogene was also suggested by the failure of the PCR amplification in the 3'-region of the pseudogene (between bases 570 and 1051) of individual 96 (not shown).

In closely linked gene clusters such as α-globin, β-globin, steroid 21–hydroxylase, glucocerebrosidase, and photopigment genes, a fusion gene presumably generated by homologous but unequal crossover has been reported (Neitz et al. 1989; Sinnott et al. 1990; Zimran et al. 1990; Metzenberg et al. 1991). To investigate the possibility of a fusion gene, PCR amplification was performed using a set of primers with the upper primer in the 5'-region of the pseudogene and the lower primer in the 3'-region of the *FUT2* (5'-pseudo and 3'-common primers, table 1). In individual 96, the expected size (1.15 kb) of the PCR product was amplified (fig. 3, Fu in lane 3), while the *FUT2* DNA fragment (1.05 kb) with the 5'-FUT2 and the 3'-common primers was not (Se in lane 3). Both DNA fragments of the fusion gene
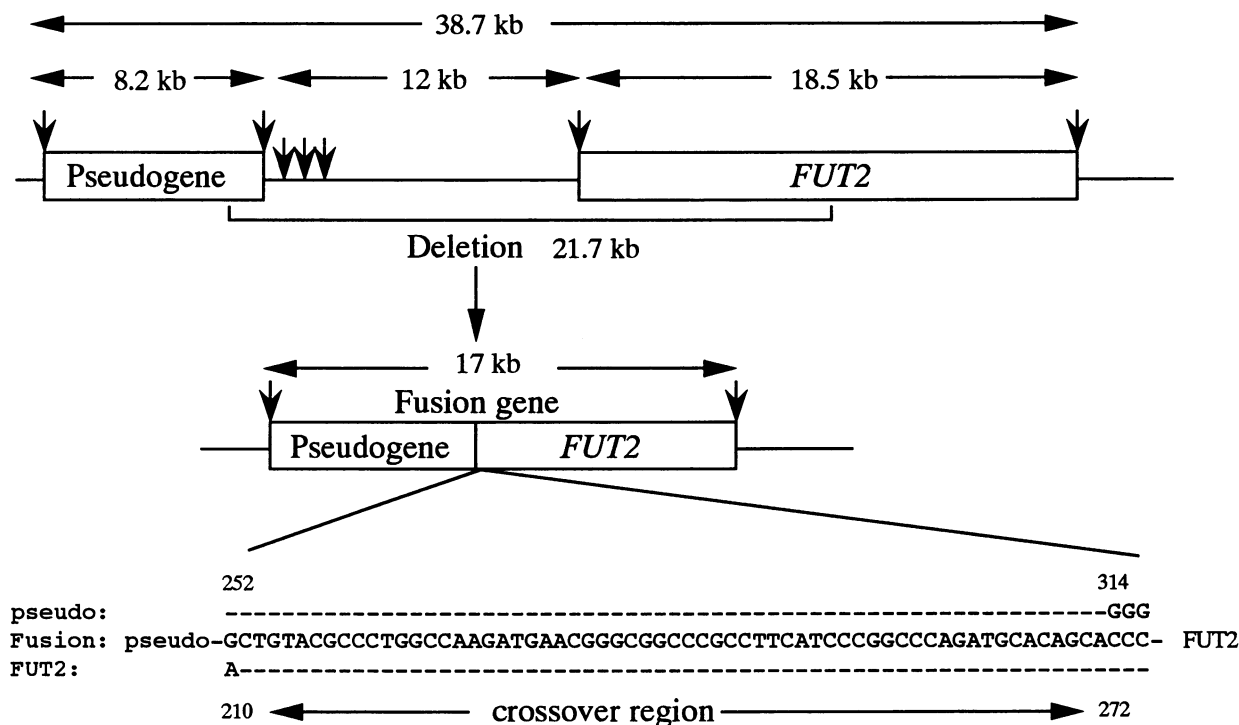
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←————————————————————— 38.7 kb —————————————————————→

←— 8.2 kb —→  ←———— 12 kb ————→  ←—————— 18.5 kb —————→

   ↓              ↓↓↓            ↓                    ↓
 ┌──────────┐   ┌─VVV─────────┐ ┌──────────────────────┐
─│ Pseudogene│   │             │ │        FUT2          │─
 └──────────┘   └─────────────┘ └──────────────────────┘
     └──────────────── Deletion   21.7 kb ──────────────┘

                              ↓

                   ←———— 17 kb ————→
                     Fusion gene
                  ↓                  ↓
                ┌─────────┬──────────┐
              ──│Pseudogene│  FUT2   │──
                └─────────┴──────────┘
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252   314
pseudo: --GGG
Fusion: pseudo-GCTGTACGCCCTGGCCAAGATGAACGGGCGGCCCGCCTTCATCCCGGCCCAGATGCACAGCACCC- FUT2
FUT2:   A--
         210 ←——————— crossover region ———————→ 272

Figure 4   *Eco*RI restriction map between the pseudogene and the *FUT2*, and the DNA sequence of the crossover region in the fusion gene. *Eco*RI fragments containing the *FUT2*, the pseudogene, or the fusion gene are indicated as open rectangles. *Eco*RI sites are shown by vertical arrows. Sizes of the *Eco*RI-DNA fragments and of the calculated DNA deletion are indicated. The DNA sequence of the crossover region of the fusion gene (fusion) was aligned with the corresponding region in both the pseudogene (pseudo) and the *FUT2* (FUT2). The nucleotides of the pseudogene and of the *FUT2* identical to that of the fusion gene are indicated by dashes.

and of the *FUT2* were amplified by PCR in 3 of the 26 Lewis(a+b−) individuals, 10 of the 92 Lewis(a−b+) individuals, and 1 of the 23 Lewis (a−b−) individuals (fig. 3, Se and Fu in lane 2). In other individuals, only the *FUT2* fragment was amplified (fig. 3, Se and Fu in lane 1). These results indicated that individual 96 was homozygous for the fusion gene (*se5*), and 14 of the 141 individuals were heterozygous for this fusion gene (table 2).

By DNA sequencing of individual 96, the fusion gene was demonstrated to consist of the 5′-region of pseudogene and the 3′-region of *FUT2* but without the C357T neutral mutation. The crossover region was within a 61-bp stretch corresponding to regions between bases 253 and 313 of the pseudogene and between bases 211 and 271 of the *FUT2* (fig. 4) in all fusion genes (six alleles) tested.

The effects of the point mutations and of the unequal crossover event reported here on the α(1,2)-fucosyltransferase activity were examined after transfection of pRc/CMV with or without *FUT2* alleles (pRc/CMV-*Se* and pRc/CMV-*se2*~*se5*). The enzyme activity in pRc/CMV-*Se*-transfected cell extracts was 14.7 nmol/mg/h with lacto-N-biose I (type 1 chain) as acceptor and 2.1 nmol/mg/h with N-acetyllactosamine (type 2 chain) as ac-

ceptor (table 3). A little activity (~2%) was detected in the pRc/CMV-*se2*-transfected cell extracts with lacto-N-biose I as acceptor. No α(1,2)-fucosyltransferase activity was detected in pRc/CMV-*se3*-, pRc/CMV-*se4*- or pRc/CMV-transfected cell extracts. The enzyme activity of the fusion gene (pRc/CMV-*se5*)–transfected cell ex-

Table 3

α(1,2)-Fucosyltransferase Activity in COS-7 Cells Transfected by Plasmid DNA

	ENZYME ACTIVITY (nmol/mg/h)	
TRANSFECTED VECTOR	Type 1	Type 2
pRc/CMV-*Se*	14.7	2.1
pRc/CMV-*se2*	<.3	nd
pRc/CMV-*se3*	nd	nd
pRc/CMV-*se4*	nd	nd
pRc/CMV-*se5*	2.8	.5
pRc/CMV	nd	nd

NOTE.—Values are the mean of two experiments. Enzyme activities were determined with lacto-N-biose I (type 1) or N-acetyllactosamine (type 2) as an acceptor. nd = not detected.

tracts was found to be ~20% of that of the pRc/CMV-*Se*-transfected cell extracts. However, while the transfection of the *Se* allele into COS cells expressed H antigen on cell membranes, detected by anti-H 1E3 (anti-H type 1-4 monoclonal antibody), the H antigen was not detected on cells transfected with the *se5* allele (not shown). These results indicated that three point mutations and the fusion gene may be responsible for nonsecretor or weak-secretor phenotypes.

Discussion

In the present study, we have identified four different Se enzyme–deficient alleles (*se2–se5*). In 141 individuals, 22% (31/141) of the individuals were homozygous for the *se2* allele, and the incidence of this mutation was 44% (123/282 alleles). These results indicate that the *se2* is a common Se enzyme–deficient allele, while the *se3* and *se4* are rare Se enzyme–deficient alleles in the Japanese population. Recently, Kelly et al. (1995) have reported that all the Caucasian nonsecretors they have tested (six individuals) were homozygous for Trp[143]-ter mutation (G428A, *se1*), and ~20% of 52 randomly selected American individuals were homozygous for this mutation. Their results suggested that the *se1* allele was a common nonsecretor mutation in Caucasians, whereas we were unable to find this mutation in a Japanese population. Although the incidence of the Se enzyme deficiency is ~20%–25% in both countries, the mutations responsible for the Se enzyme–deficient allele are different between these two countries. This was also the case for the *Lewis* gene (*FUT3*), in which point mutations in the null allele of the *FUT3* were different between Japanese, Indonesian, and Swedish populations, despite the incidence of Lewis-negative individuals being similar (~10%) in the three areas (Koda et al. 1993; Elmgren et al. 1993, 1996; Mollicone et al. 1994; Nishihara et al. 1994). Therefore, the point mutations of the *FUT2* and *FUT3* might have occurred after the races diverged, while the mutations in the *ABO* genes are conserved in nearly all races examined (Yamamoto et al. 1990; Franco et al. 1994). It is of interest and informative to examine the race-specific mutations of the null alleles of *FUT2* and *FUT3*. Moreover, we found a neutral mutation at position 357 (C357T) in all 20 *FUT2* alleles from five selected Lewis(a−b+) individuals and five Lewis(a+b−) individuals, all Japanese, suggesting that this neutral mutation is also race specific. Alternatively, the most common allele may have T, not C, at this position.

In addition to point mutations, we found a fusion gene that consisted of the 5'-region of the pseudogene and the 3'-region of the functional *FUT2*. According to the size of *Eco*RI-fragments containing the fusion gene (17 kb) (fig. 2*A*), the *FUT2* (18.5 kb) and the pseu-

dogene (8.2 kb) and the distance between the *Eco*RI fragments of the pseudogene and the *FUT2* (12 kb, reported by Kelly et al. [1995]), the DNA deletion generated by the homologous but unequal crossover was estimated to be ~22 kb (fig. 4). We tried to find the reciprocal product of the unequal crossover event (a chromosome with a new fusion gene consisting of the 5'-region of *FUT2* and the 3'-region of the pseudogene and the *FUT2* in line from upstream) by PCR analysis but have not yet found it.

The *FUT2* and its pseudogene share 78% homology in their DNA sequences, but the DNA sequences in the 5'-region and the derived N-terminal amino acid sequences of both genes (Kelly et al. 1995) are quite different. Recently, two rabbit α(1,2)-fucosyltransferase genes have been isolated (Hitoshi et al. 1995). One rabbit gene (RFT-II) and the pseudogene of *FUT2* have high DNA sequence homology (~80%), suggesting that the pseudogene is a human homologue of the rabbit RFT-II. The pseudogene has frameshift and nonsense mutations that disrupt the reading frame relative to the *FUT2* reading frame, thereby causing no α(1,2)-fucosyltransferase activity to be detected after transfection of the pseudogene in COS cells (Kelly et al. 1995). On the other hand, although we isolated the fusion gene as one of the null alleles of *FUT2*, the fusion gene appears to code a structurally functional protein because the 5'-region belongs to the pseudogene that is highly homologous to the functional RFT-II and the 3'-region belongs to the functional *FUT2* gene. Indeed, we detected some α(1,2)-fucosyltransferase activity in the fusion gene–transfected cell extracts (table 3). Kelly et al. (1995) have reported that the pseudogene mRNA has not been detected by Northern blot in normal human tissues. Since the promoter region of the fusion gene is expected to be identical to that of the pseudogene, the transcripts of the fusion gene as well as of the pseudogene may not be expressed in tissues. This could explain why we have found the *se5* as a null allele in spite of the 20% of activity found in its cognate enzyme product.

After submission of our manuscript, the C571T mutation (*se3*) has been reported among Polynesians as a relatively common nonsecretor allele (Henry et al. 1996). The results further suggest that Se enzyme–deficient alleles are race specific. In addition, the A385T mutaion (*se2*) was previously found as a weak secretor allele from individuals with Lewis(a+b+) red cell phenotype in Taiwan (Yu et al. 1995). In the present study, we found the *se2* (A385T mutaion) in double dose in individuals with Lewis(a+b−) red cell phenotype in a Japanese population. Since it is known that phenotyping of the Lewis system has some complexities and difficulties (Henry et al. 1995), this apparent discrepancy may be due to a difference on the reagents used and the apparent red cell Lewis(a+b−) phenotype in the present

study are probably red cell Lewis(a+b+) phenotype not detected by the Lewis antiserum used.

In the present population study, we collected blood samples from 141 Japanese individuals. Furthermore, we estimated the secretory status by the red cell Lewis phenotype, and therefore we were unable to determine the secretor phenotype of Lewis-negative individuals. Therefore, we cannot rule out the possibility of the presence of *se* allele(s) other than *se2–se5*. However, this is the first report to identify that the three point mutations and the fusion gene are independently responsible for the Se enzyme deficiency in Japanese individuals and that the *se2* allele is a common Se enzyme–deficient allele in the Japanese population studied.

Acknowledgments

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