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Missense Mutation of *FUT1* and Deletion of *FUT2* Are Responsible for Indian Bombay Phenotype of ABO Blood Group System

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The Bombay phenotype fails to express the ABH antigens of ABO blood group system on red blood cells and in secretions because of a lack in activities of the *H* gene (*FUT1*)- and *Secretor* gene (*FUT2*)-encoded α(1,2)fucosyltransferases. In this study, we have examined the *FUT1* and the *FUT2* from three unrelated Indian individuals with the Bombay phenotype. These three individuals were found to be homozygous for a T725G mutation in the coding region of the *FUT1*, which inactivated the enzyme activity. In addition, we did not detect any hybridized band corresponding to the *FUT2* by Southern blot analysis using the catalytic domain of the *FUT2* as a probe, indicating that the three individuals were homozygous for a gene deletion in the *FUT2*. These results suggest that the T725G mutation of *FUT1* and the gene deletion of *FUT2* are responsible for the classical Indian Bombay phenotype.

The ABO histo-blood group antigens are oligosaccharide antigens (1) that are expressed not only on red cell membranes but also in secretions and tissues such as the salivary gland and the digestive mucosa (2, 3). The H antigen is a common precursor for both A and B antigens. Therefore, α(1,2)fucosyltransferase, which forms the H antigen by catalyzing the transfer of fucose in the α(1,2) linkage to the terminal galactose of a precursor molecule, is an essential enzyme for the expression of A and B antigens. Several lines of evidence have indicated that at least two distinct (H type and secretor type) α(1,2)fucosyltransferases are present in human tissues (4, 5). The *H* gene (*FUT1*)-encoded α(1,2)fucosyltransferase (H enzyme) is thought to regulate the expression of H antigen and thereafter A and/or B antigens in the erythroid lineage and in vascular endothelia, while the *Secretor* gene (*FUT2*)-encoded α(1,2)fucosyltransferase (Se enzyme) is thought to regulate the expression of these antigens in the secretory glands and the digestive mucosa.

Rare individuals of Bombay and para-Bombay phenotypes fail to express the H antigen on erythrocyte membranes, while about 20% of individuals (non-secretor) fail to express this antigen in saliva because of a lack of Se enzyme activity. The Bombay phenotype is characterized by the complete lack of ABH antigens both on red blood cells and in secretions as a result of the absence of both H and Se enzyme activities. The para-Bombay (H-deficient secretor) phenotype is characterized by a lack of, or a very weak expression of, the H antigen on red cells, and by the presence of ABH substances in secretions because of the absence of H enzyme activity and the presence of Se enzyme activity (1, 6).

Recently, *FUT1*, *FUT2* and the pseudogene (*Sec1*) have been isolated (7-9). These genes share a high degree (about 70%) of DNA sequence homology and are located within a 100 kb region in chromosome 19q13.3 (7-9), suggesting they have evolved by gene duplication and subsequent divergence.

Several groups, including us, have isolated and analyzed some *FUT1*- and/or *FUT2*-deficient alleles (10-17). In the present study, we have examined the *FUT1* and *FUT2* alleles from three unrelated Indian individuals with the Bombay phenotype, and we have found that a missense mutation of *FUT1* and a gene deletion of *FUT2* are responsible for H enzyme and Se enzyme inactivation, respectively.

MATERIALS AND METHODS

Indian Bombay individuals. The *FUT1* and *FUT2* from three unrelated Indian individuals with the Bombay phenotype in South Africa were analyzed in this study.

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PCR amplification and subcloning of FUT1 and FUT2. Genomic DNA was prepared from peripheral leukocytes by an organic solvent method (18). The complete open reading frames of *FUT1* and *FUT2* were amplified from genomic DNA by PCR as single products for cloning (12, 19). The coding region of *FUT1* was amplified as described previously (13). Resulting PCR products were subcloned into the pRc/CMV plasmid (Invitrogen, San Diego, CA) for DNA sequence and expression studies. DNA sequence was determined by the dideoxynucleotide chain termination method using an ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit and an ABI 373 sequencer (Perkin Elmer Japan ABI, Chiba, Japan). The coding region of *FUT2* was amplified as described previously (12). Recently, we have identified a non-coding exon 1 of *FUT2* approximately 7 kb upstream of exon 2 (20). Exon 1 and a part of the intron of *FUT2* were amplified from genomic DNA using the sense primer (5′-CAG AGG GAA CAC TGA GGT GCC TGC CCA ACC-3′) encompassing residues 39-68 of exon 1 of *FUT2* and the antisense primer (5′-GGC TTT GTC ACC AGC TGT TCC CGT G-3′) located at residues 790-814 in the intron of *FUT2* (35 cycles, 98°C-10 sec, 65°C-1 min and 72°C-1 min). PCR was carried out in 25 μl Ex Taq buffer (Takara, Tokyo, Japan) containing 1.2 U Ex Taq DNA polymerase (Takara), 200 μM dNTP mix, 0.4 μM each primer and 50 ng genomic DNA.

Detection of the T725G mutation by PCR-RFLP. PCR products of the *FUT1* alleles described above were subjected to digestion by restriction endonuclease *Nae*I and then analyzed by 1% agarose gel electrophoresis.

Transient expression of FUT1 alleles in COS cells and assay of α(1,2)fucosyltransferase activity. Mammalian expression vectors containing *FUT1* alleles in the sense orientation were transfected into COS-7 cells by the DEAE-dextran method (21). After 48 hours of culture, the expression of H antigen on cell surfaces was examined using anti-H 3A5 monoclonal antibody specific to the H type 2 chain (22) and fluorescein-labeled anti-mouse IgM antibody, as described previously (13, 23), and enzyme activities in cell extracts were determined (24). The α(1,2)fucosyltransferase activity was measured in 100 μl of 25 mM sodium phosphate buffer (pH 6.5) containing 30 μM GDP-L-[U-^{14}C]-fucose, 5 mM ATP, 10 mM $MnCl_2$, cell extract (50 μl containing about 50 μg protein), and 25 mM phenyl-β-galactoside as an acceptor. After incubation at 37°C for 20 min, the products were separated from GDP-^{14}C-fucose by a Sep Pak C18 cartridge (Waters, Milford, MA). A parallel control reaction without acceptor was performed for each reaction. To normalize the transfection efficiency, the pGL2 control vector (Promega, Madison, WI) was co-transfected, and the activity of luciferase was measured using a luciferase assay system (Promega).

Southern blot analysis. Restriction enzyme *Nco*I-digested DNAs (5 μg) were electrophoresed on a 0.8% agarose gel and transferred to a nylon membrane prior to Southern blot hybridization. A digoxigenin-labelled *FUT2* probe (nucleotide residues 358-1056 of exon 2) was prepared by PCR. The reaction mixture contained 1.2 U Ex Taq DNA polymerase, 200 μM DIG dNTP-labeling mix (Boehringer Mannheim, Mannheim, Germany), 0.4 μM each primer (385A primer, 5′-GAC TGG ATG GAG GAG GAA TAC CGC CAC A-3′, nucleotide residues 358-385, and 3′ common primer, 5′-GGC ACT CAT CTT GAG GGA GGC AGA GAA-3′, nucleotide residues 1030-1056) (12) and 100 pg pRc/CMV-Se in 25 μl Ex Taq buffer (35 cycles, 98°C-10 sec, 65°C-1 min and 72°C-1 min). To check the quality of genomic DNAs, the same membrane was rehybridized with the digoxigenin-labeled H type α(1,2)fucosyltransferase gene (*FUT1*) probe (25). Hybridized bands were detected using a DIG luminescent detection kit for nucleic acids (Boehringer Mannheim).

RESULTS

The FUT1 alleles in H-deficient individuals. We have sequenced the *FUT1* alleles of control (wild type) and H-deficient individuals to identify differences in the DNA sequences. As shown in Fig. 1A, we found a mutation T725G in the coding region of the H-deficient allele. The sequence difference is predicted to result in an amino acid change, L242R. The base substitution introduces a cleavage site for restriction endonuclease *Nae*I that was used to genotype individuals for this mutation in the *FUT1*. As represented in Fig. 1B, the PCR products of *FUT1* alleles of three Indian Bombay individuals were completely digested by restriction enzyme *Nae*I, indicating that these individuals were homozygous for the T725G mutation.

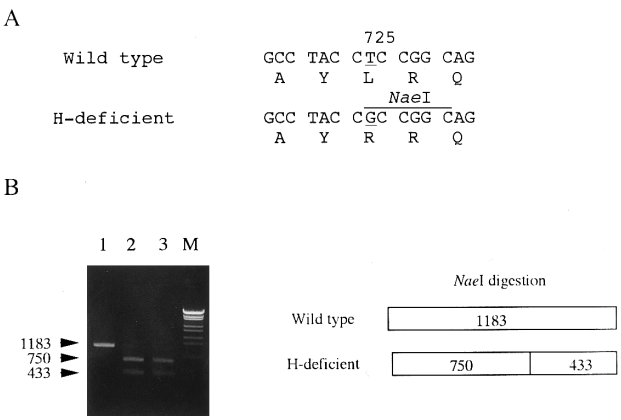

FIG. 1. An allele-specific restriction site polymorphism at position 725. (A) A different nucleotide in the H-deficient allele from the wild type allele is underlined. The *Nae*I site in the H-deficient allele is overlined. (B) PCR products of the *FUT1* were digested by restriction enzyme *Nae*I and were fractionated by 1% agarose gel electrophoresis. The *Nae*I digestion of PCR products from the H-deficient allele yielded two fragments of 750 and 433 bp, whereas one *Nae*I 1183-bp fragment was obtained from the wild type allele. Lane 1 represents a PCR product from the wild type individual, and lanes 2 and 3 represent PCR products from the Bombay individuals. The markers for molecular size (*Eco*T14-digested λ phage DNA) are in lane M.

Transient expression of FUT1 alleles in COS cells and assay of α(1,2)fucosyltransferase activity. To confirm whether the T725G mutation is responsible for the inactivation of the *FUT1* allele, we constructed plasmid DNA containing the T725G mutation (pRc/CMV-h) and transfected this into COS cells that have no endogeneous α(1,2)fucosyltransferase. Cells transfected by the wild type construct (pRc/CMV-H) showed a substantial amount of α(1,2)fucosyltransferase activity (4.3 $\pm$ 0.7 nmol/min/mg), while those transfected by the H-deficient allele construct (pRc/CMV-h) showed no detectable activity when phenyl-β-galactoside was used as an acceptor. We also tested for the expression of the H antigen on cell surfaces after transfection by *FUT1* alleles using anti-H 3A5 (anti-H type 2-specific monoclonal antibody) (22). The transfection by the *H* allele into COS-7 cells resulted in the expression of H antigen on the cell surface, whereas transfection with the *h*

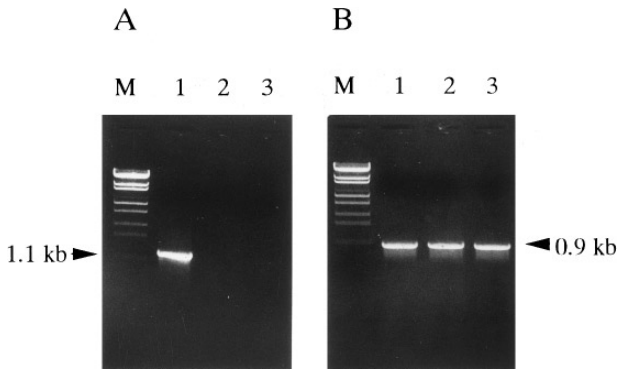

FIG. 2. PCR amplification for detecting the *FUT2*. PCR fragments for a total protein coding region of the *FUT2* (exon 2) (A) and for exon 1 and a part of intron of the *FUT2* (B) with DNAs from a secretor (lane 1) and Bombay individuals (lanes 2 and 3). Lane M, *Eco*T14 I-digested λ DNA. Resulting PCR products were analyzed using 1.0% agarose gel electrophoresis. Expected sizes of the fragments are indicated by arrowheads.

allele had no detectable H antigen (data not shown). These results suggest that the T725G mutation is responsible for the complete inactivation of the *FUT1*-encoded enzyme activity.

Identification of a gene deletion of the FUT2 from Bombay individuals. Since individuals lacked the H antigen not only on red blood cells but also in saliva, we next investigated the *FUT2* of Bombay individuals. However, we failed to amplify any DNA fragment corresponding to the protein coding region of *FUT2* by the previously described PCR reaction (12) (Fig. 2A, lanes 2 and 3). In the previous study, we found a fusion gene consisting of *Sec1* and *FUT2* as one of the Japanese non-secretor alleles (12). When the genomic DNA from an individual homozygous for the fusion gene allele was used as a template, the total protein coding region of *FUT2* was not amplified by PCR (12). To investigate the possibility of the presence of this fusion gene or another type of gene rearrangement, Southern blot analysis was carried out using the *FUT2* probe (nucleotide residues 358-1056 of exon 2). As described previously (12), two *Nco*I fragments corresponding to *FUT2* (1.7 kb) and *Sec1* (3.5 kb) were detected in a control individual (Fig. 3, lane N), while an additional 2.6 kb band corresponding to the fusion gene was detected in an individual heterozygous for the fusion gene (lane F). We failed to detect a 1.7 kb *FUT2* fragment, although we detected a single 3.5 kb *Sec1* fragment in three Indian Bombay individuals (lane B). To check the quality of the genomic DNAs, the same membrane was rehybridized with the probe for the *FUT1* and we found a 4.5 kb *FUT1* fragment in all samples (Fig. 3B). These results suggested that Indian Bombay individuals were homozygous for a gene deletion of the *FUT2* and that the gene deletion was responsible for the failure of the expression of the H antigen in secretions (Recently, the same mutation of the *FUT1* and a gene deletion of the *FUT2* among Indian immigrants in the Reunion island were found at Dr. Oriol's laboratory, personal communications, see a review article (17) also). Recently, we have isolated exon 1 of *FUT2* that is located 7 kb upstream of exon 2 (20). As shown in Fig. 2B, both exon 1 and a part of the intron were amplified from templates from a control donor and three Indian Bombay individuals. Therefore, the 5′ break point of the gene deletion is present within the intron (7 kb) of the *FUT2* (Fig. 4).

DISCUSSION

In the present study, we identified a single base substitution (T725G) in the Indian H-deficient allele. Recently, several groups, including us, have isolated and analyzed some *FUT1*-deficient alleles (10, 11, 13-17). These results indicated the heterogeneity for H-deficient alleles even in the same population. However, we found that three unrelated Indian individuals with the Bombay phenotype in South Africa were homozygous for the T725G mutation. The same mutation was found in Indian Bombay individuals in the Reunion island (17).

In addition to *FUT1,* we have also analyzed the *FUT2* and found that the Bombay individuals are homozygous for a gene deletion of *FUT2*. The deletion was identified both by PCR and Southern blot analyses. Recently, we have isolated the 5′ end of *FUT2* cDNA by rapid amplification of cDNA ends

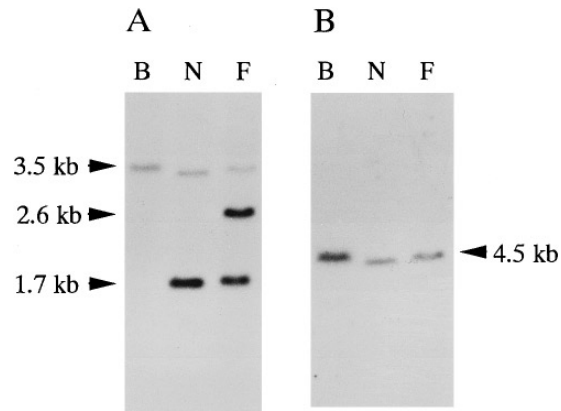

FIG. 3. Southern blot analysis of genomic DNAs using a protein coding region of the *FUT2* or the *FUT1* as a probe. Genomic DNAs (5 μg) from a control individual (N), a Bombay individual (B), and an individual who is heterozygous for the fusion gene consisted of the *Sec1* and the *FUT2* (F) were digested by *Nco*I. (A) Coding regions of the *FUT2* (1.7 kb) and the *Sec1* (3.5 kb) were visualized by a *FUT2* probe. (B) The *FUT1* was visualized by a *FUT1* probe.

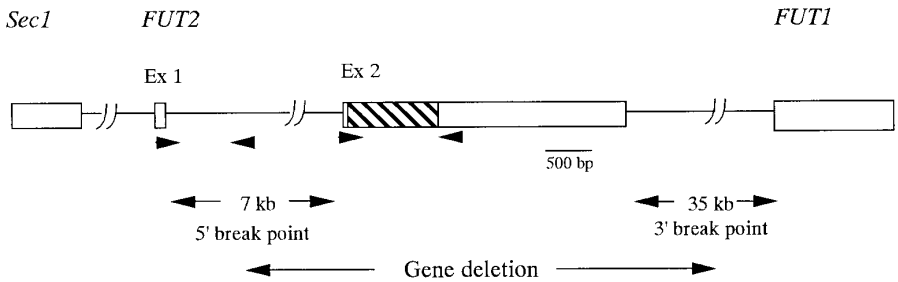

FIG. 4. A scheme of α(1,2)fucosyltransferase gene cluster. The *Sec1, FUT1,* and exons of the *FUT2* are indicated as rectangles. The protein coding region is indicated by a shaded line in exon 2 of the *FUT2.* Arrowheads indicate positions of PCR primers. The expected positions of the 5′ and 3′ ends of the gene deletion of the *FUT2* are indicated. The expected gene deletion of the *FUT2* is shown by a horizontal arrow. The length of 500 bp is given in the lower right corner of exon 2.

(RACE) analysis and found this gene consists of two exons (20). Exon 1 (0.1 kb) is located about 7 kb upstream of exon 2 which contains the total protein coding region. PCR analysis showed that exon 1 of *FUT2* was present in the three Bombay individuals (Fig. 2B). Rouquier et al. (8) indicated that *FUT1* is located about 40 kb downstream of *FUT2.* Since we have also isolated the 5′ end of cDNA and the 5′ flanking region of the *FUT1* (25), we analyzed the genomic DNA in these three Bombay individuals. Southern blot analysis (Fig. 3B) and PCR analysis (data not shown) suggested the presence of the complete *FUT1* (from promoter region to poly-adenylate signal) and of exon 1 of *FUT2* in these three Bombay individuals (Fig. 2B). Our results suggest that the 5′ break point of the gene deletion is within the intron (7 kb) of *FUT2* and the 3′ break point of the gene deletion is within the 35 kb sequence between *FUT2* and *FUT1* (Fig. 4). The results of analyses of *FUT1* and *FUT2* in this study suggest that the homozygosity for these two unusual and linked genetic mutations are common in individuals with the classical Indian Bombay phenotype.

Recently, we found a recessive allele in Japanese non-secretor donors that was due to a fusion of *Sec1* and *FUT2* rather than a simple point mutation (12). This allele was thought to be resulted from unequal crossing over followed by deletion of 22 kb between the two loci. However, the results of Southern blot analysis in this study indicated that the gene deletion observed in three Indian Bombay individuals was different from that observed in the fusion gene. Since closely related gene clusters such as the α- and β-globin genes and the haptoglobin gene are known to be hot spots for gene recombination (i.e. unequal crossing over, gene conversion, duplication and deletion) (26), our results suggest that the α(1,2)fucosyltransferase gene cluster is one of such hot spots for gene recombination.

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REFERENCES

1. Watkins, W. M. (1995) *in* Glycoproteins (Montreuil, H., Vliegenhart, J. F. G., and Schachter, H., Eds.), pp. 313–390, Elsevier, Amsterdam.
2. Oriol, R., Le Pendu, J., and Mollicone, R. (1986) *Vox Sang.* **51,** 161–171.
3. Clausen, H., and Hakomori, S. (1989) *Vox Sang.* **56,** 1–20.
4. Oriol, R., Danilovs, J., and Hawkins, B. R. (1981) *Am. J. Hum. Genet.* **33,** 421–431.
5. Le Pendu, J., Cartron, J. P., Lemieux, R. U., and Oriol, R. (1985) *Am. J. Hum. Genet.* **37,** 749–760.
6. Daniels, G. (1995) Human Blood Groups. Blackwell Science, Oxford.
7. Larsen, R. D., Ernst, L. K., Nair, R. P., and Lowe, J. B. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 6674–6678.
8. Rouquier, S., Lowe, J. B., Kelly, R. J., Fertitta, A. L., Lennon, G. G., and Giorgi, D. (1995) *J. Biol. Chem.* **270,** 4632–4639.
9. Kelly, R. J., Rouquier, S., Giorgi, D., Lennon, G. G., and Lowe, J. B. (1995) *J. Biol. Chem.* **270,** 4640–4649.
10. Kelly, R. J., Ernst, L. K., Larsen, R. D., Bryant, J. G., Robinson, J. S., and Lowe, J. B. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 5843–5847.
11. Johnson, P. H., Mak, M. K., Leong, S., Broadberry, R., Duraisamy, G., Gooch, A., Lin-Chu, M., Makar, Y., Okubo, Y., Smart, E., Koepsall, E., and Ewers, M. (1994) *Vox Sang.* **67**(Suppl. 2), 2 (abstract).
12. Koda, Y., Soejima, M., Liu, Y. H., and Kimura, H. (1996) *Am. J. Hum. Genet.* **58,** 343–350.
13. Wang, B. J., Koda, Y., Soejima, M., and Kimura, H. (1997) *Vox sang.* **72,** 31–35.
14. Yu, L. C., Yang, R. E., Broadberry, R. E., Chen, Y. H., and Lin, M. (1997) *Vox Sang.* **72,** 36–40.
15. Wanger, F. F., and Flegel, W. A. (1997) *Transfusion* **37,** 284–290.
16. Kaneko, M., Nishihara, S., Shinya, T., Kudo, T., Iwasaki, H., Seno, T., Okubo, Y., and Narimatsu, H. (1997) *Blood* **90,** 839–849.

17. Costache, M., Cailleau, A., Fernadez-Mateos, P., Oriol, R., and Mollicone, R. (1997) *Transfus. Clin. Biol.* **4,** 365–380.
18. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
19. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., and Erlich, H. A. (1988) *Science* **239,** 487–491.
20. Koda, Y., Soejima, M., Wang, B. J., and Kimura, H. (1997) *Eur. J. Biochem*. **246,** 750–755.
21. Gonzalez, A. L., and Joly, E. (1995) *Trends Genet.* **11,** 216–217.
22. Liu, Y. H., Akiyama, K., Jia, J. T., and Kimura, H. (1996) *J. Clin. Lab. Anal.* **10,** 144–148.
23. Koda, Y., Kimura, H., and Mekada, E. (1993) *Blood* **82,** 2915–2919.
24. Masutani, H., and Kimura, H. (1995) *J. Biochem*. **118,** 541–545.
25. Koda, Y., Soejima, M., and Kimura, H. (1997) *J. Biol. Chem*. **272,** 7501–7505.
26. Meetzenberg, A. B., Wurzer, G., Huisman, T. H. J., and Smithies, O. (1991) *Genetics* **128,** 143–161.