## A new *h* allele detected in Europe has a missense mutation in $\alpha(1,2)$ -fucosyltransferase motif II

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**BACKGROUND:** The *FUT1* gene encodes an  $\alpha(1,2)$ fucosyltransferase (H transferase), which determines the blood group H. Nonfunctional alleles of this gene, called h alleles and carrying loss-of-function mutations, are observed in the exceedingly rare Bombay phenotype. Twenty-three distinct h alleles have been characterized at the molecular level in various populations. The FUT2 (SE) gene is highly homologous to FUT1 (H). STUDY DESIGN AND METHODS: The FUT1 gene of an Austrian proband with the Bombay phenotype was characterized by nucleotide sequencing of the full-length coding sequence. A PCR method using sequence-specific primers for FUT2 genotyping in whites was developed. The plasma  $\alpha(1,2)$ -fucosyltransferase activity was determined. The distribution of the mutations underlying 24 h alleles and 7 se alleles was analyzed. **RESULTS:** The proband carried a new *h* allele. Two nucleotide changes, G785A and C786A, in codon 262 of the FUT1 gene resulted in the replacement of serine by lysine. No  $\alpha(1,2)$ -fucosyltransferase activity was detected in the proband's plasma. The proband was homozygous for the seG428A allele. Six of 17 missense mutations in nonfunctional h and se alleles occurred in highly conserved fucosyltransferase motifs. No loss-offunction mutation was observed in the aminoterminal section encompassing the transmembraneous helix. CONCLUSION: The missense mutation S262K in the FUT1 gene caused the loss of H transferase activity. The analysis of the distribution of mutations in nonfunctional FUT1 and FUT2 genes can point to functionally important domains in the H transferase.

he human blood group systems ABO (ISBT number 001), Lewis (ISBT number 007), and H (ISBT number 018) are determined by oligosaccharides. Their biosynthesis depends on distinct but structurally similar glycosyltransferases, which add monosaccharides to precursor molecules in a sequential manner.

The *FUT1* (*H*) and *FUT2* (*SE*) genes are highly homologous and located closely adjacent at the chromosomal position 19q13.3.<sup>1</sup> Both genes encode  $\alpha(1,2)$ -fucosyltransferases. The H transferase produces type 2H antigen that is required for the expression of H antigens on RBCs. The Se transferase can produce type 1H and 2H antigens but can do so only in the secretions, because of its tissue distribution. The type 1H antigen formed by the Se transferase can be modified by the LE transferase to form the Le<sup>b</sup> antigen. The main antigens of the ABO blood group, type 2A and 2B, derive from the type 2H antigen and hence depend on a functional H transferase.

About 20 percent of whites are homozygous for an enzyme-inactivating nonsense mutation (Trp143→ter) in the

**ABBREVIATIONS:** HGH = human growth hormone; SNP = single-nucleotide polymorphism; SSP = sequence-specific primers.

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*FUT2* gene, in correspondence to the frequency of the nonsecretor phenotype in many populations.<sup>2</sup> Thus, this nonfunctional *FUT2* allele (*seG428A* allele) represents about 45 percent of all *SE* alleles in whites, whereas, among Japanese, Indonesian, Filipino, Chinese, Thai, and South African Xhosa populations, other nonsense mutations, missense mutations, deletions, and gene rearrangements coding for inactive SE transferases are known.<sup>3-11</sup> No rapid *SE* genotyping method using PCR with sequence-specific primers (SSP) to detect the G428A single-nucleotide polymorphism (SNP) prevalent in whites is available.

Unlike in *FUT2*, there is no prevalent nonfunctional *FUT1* allele (*h* allele).<sup>12</sup> To date, 23 distinct sporadic *h* alleles have been found,<sup>12-18</sup> with a cumulative frequency of about 1 in 350 alleles of whites.<sup>12</sup>

The Bombay phenotype (O<sub>h</sub>; H–; H-deficient RBCs, nonsecretor<sup>19</sup>) is expressed when an individual is homozygous for h alleles and lacks a functional Se allele. In a person of the "true" Indian Bombay phenotype, this is due to a deletion of the Se gene and a missense mutation in the H gene.<sup>18</sup> In most other Bombay persons, the nonsecretor phenotype is due to the homozygous presence of the seG428A allele. The Bombay phenotype lacks antigens H, A, and B on RBCs (H-deficient RBCs) and in secretions (nonsecretor). Strong and clinically relevant anti-H, anti-A, and anti-B occur in the serum. Carriers are exceedingly rare, 1 in about 300,000 whites,<sup>20</sup> but they are invariably detected by their pathognomonic anti-H. The slightly more frequent para-Bombay phenotype (H-deficient RBCs, salivary ABH secretor<sup>19</sup>) occurs when a person is homozygous for h alleles but has at least one functional Se allele. Antigens H, A, and B are not properly expressed on RBCs (H-deficient RBCs) but may occur in secretions (secretor) and plasma. Type 1H antigens formed by the SE transferase and the derived type 1A and 1B antigens are present on glycolipids in the plasma and adsorbed onto RBCs much as Lewis antigens are. As carriers cannot produce anti-H, they are missed by standard blood grouping. Hence, no direct estimate of their population frequency has been established.

We describe two adjacent SNPs causing an enzymeinactivating missense mutation in the *FUT1* gene in an Austrian proband with the Bombay phenotype. A method for *SE* genotyping the European population by PCR-SSP is presented. The accumulated data are analyzed in the context of potentially functional important segments in the *FUT1* transferase.

## MATERIALS AND METHODS

#### Serologic tests

All serologic testing and titrations were done with a gel matrix test (DiaMed-ID Micro Typing System, DiaMed, Cressier sur Morat, Switzerland). H antigen was tested with commercial lectins (Biotest AG, Dreieich, Germany; DiaMed; Gamma Biologicals, Houston, TX; Molter, Neckargemünd, Germany; Serac, Bad Homburg, Germany). The adsorption-elution study was performed as described.<sup>21</sup> In brief, 1 mL of packed RBCs was incubated with 2 mL of anti-A (Bioclone murine monoclonal IgM clones MH04 and 3D3, Ortho, Raritan, NJ) for 30 minutes at room temperature. Elution was accomplished with an acid-elution kit (Red Cell Elution System, Immucor, Rödermark, Germany). As controls, group A cells were used.

#### Nucleotide sequencing of the FUT1 gene

The full-length coding sequence of the *FUT1* gene was amplified as described previously.<sup>12</sup> PCR amplicons were sequenced in the sense direction with primers h21, h13, h22, and h15 and in the anti-sense direction with primers h8, h4, and h12,<sup>12</sup> by the use of a DNA-sequencing unit (Prism Big Dye, ABI 377, Applied Biosystems, Weiterstadt, Germany).

#### **Review of additional inactivating mutations**

Including this work, 24 h alleles carrying mutations associated with a Bombay or para-Bombay phenotype have been described.<sup>12-15,17,18</sup> Seven alleles contained frameshift or nonsense mutations, and 17 alleles carried missense mutations. Two missense mutations (A12V and E348K) were exclusively observed in alleles carrying additional highly disruptive missense mutations and were not considered to be inactivating. For the E348K substitution, 10-percent residual activity has been reported.<sup>16</sup> Because FUT1 and FUT2 are highly homologous, mutations in FUT2 were also reviewed and the homologous positions in FUT1 determined. Ten se alleles have been described.<sup>3,4,7-11</sup> Five of these alleles contained nonsense or frameshift mutations; one represented a fusion gene with Sec1. Two alleles contained in-frame deletions resulting in the loss of the amino acid homologous to Val<sup>260</sup> in FUT1. Of two missense mutations, seC202T affecting the amino acid homologous to Pro129 and A385T affecting the amino acid homologous to Leu<sup>157</sup>, the latter was associated with a weak Se phenotype. Hence, a total of 17 missense mutations and two in-frame deletions affecting 17 amino acid positions and 12 premature ends of the coding sequence were available for analysis.

#### Searches for homology

Initially, sequences homologous to human *FUT1* were searched for by use of an iterative protein database program (Psi-Blast, NCBI: can be found at www.ncbi.nlm.nih.gov/BLAST).<sup>22</sup> For *Caenorhabditis elegans*, a search using the proteins with accession numbers CAB16868 and AAB70395 was done to align the potential *C. elegans* fucosyltransferase genes and identify stretches homologous to human *FUT1*. Those stretches of *FUT1* that aligned to nonhomologous parts of the two different *C. elegans* genes were considered to give no consistent alignment. For the other sequences,

the sequences homologous to the relevant parts of CAB16868 were used for sequence compilation.

A pattern search was done with another database (PROSITE, ExPASy, Swiss Institute of Bioinformatics, Geneva, Switzerland).<sup>23</sup> Accession numbers were human *FUT1*, NP\_000139 (gorilla, AAF14067; gibbon, AAF14062; chimpanzee, AAF14065; orangutan, AAF42964; rhesus ape, AAF14069; Macaca fascicularis, AAF42967; lemur, AAF14063; green monkey, BAA24047; Saimiri, AAF25584; Callithrix jacchus, AAF42965; rat, Q10980; mouse, NP\_032077; rabbit, Q10979; cow, AAF07933; pig, Q29043); human FUT2, Q10981 (gorilla, BAA31128; gibbon, AAF25585; chimpanzee, BAA31127; orangutan, BAA31129; M. fascicularis, AAF25581; lemur, AAF25583; green monkey, BAA31692; C. jacchus, AAF25582; rat, BAA21742; mouse, P97353; rabbit, CAA62669; cow, Q28113; pig, Q10982); orangutan Sec1, BAA21880 (gibbon, BAA21879; rhesus monkey, AAF14070; M. fascicularis, AAF42968; rat, AAD24470; mouse, AAC16887; cow, AAF03411); C. elegans fucosyltransferase F, CAB07352 (G, AAC25844; H, AAC25845; I, AAB70365; J, AAB53053; K, P34302; L, AAB66030; M, CAB01447; N, AAB70387; O, AAB70395; further homologues AAB94261; CAB04377; CAB02972; AAC17760; CAB07284; CAB16868; CAB04857; CAB76717; CAB03432; CAB03434; CAB03440; Q10017; AAD12816; AAF59635); Leishmania fucosyltransferase homologue, AAC24622 (Haemophilus pylori, AAC99764; Vibrio cholerae, BAA33632; Lactococcus lactis, AAC45235; Bacillus fragilis, AAD40713; Yersinia enterocolitica, AAC60771).

#### Fucosyltransferase assay

The determination of fucosyltransferase activity was done with plasma samples as described previously.24 The reaction mixture contained, in a total volume of 20 µL, 0.5 mM or 1.0 mM acceptor (p-nitrophenyl-β-D-galactosid, Nacetyllac-tosamine, or lacto-N-biose), 0.25 mMGDP-[U<sup>14</sup>]fucose (5000 cpm nmol<sup>-1</sup>, specific activity 200-370 mCi mmol<sup>-1</sup>; Amersham, Vienna, Austria), 0.1 M2-(N-morpholino)ethanesulfonic acid/HCl at pH 6.5, 10 mM MnCl<sub>2</sub>, 0.1 percent Triton X-100, 5 mM AMP, and 5 µL of plasma. After a 3-hour incubation at 37°C, the reaction was terminated by adding 0.5 mL of ice-cold stop solution (10 mM sodium borate, 2 mM EDTA). Then, the samples were added to Pasteur pipettes filled with 0.5 mL of ion exchange resin (Clform, 100-200 mesh; Dowex AG 1×8, BioRad, Vienna, Austria) and eluted with water. The eluate was mixed with 2 mL of scintillation cocktail (Pico Aqua, Canberra Packard, Vienna, Austria) to determine the radioactivity. Product formation was calculated by subtracting the activity detected in controls without acceptor. All tests were done at least in duplicate. Protein concentrations were checked by the bicinchoninic acid method (Pierce, Vienna, Austria).

## Se genotyping

The prevalent nonfunctional se allele in whites carries a Trp143ter nonsense mutation caused by the SNP G428A, for which we developed two PCR-SSP detecting the G ("Se," active gene product) or the A ("seG428A," inactive product). The positive control was a 434-bp PCR fragment of the human growth hormone (HGH).<sup>25,26</sup> Concentrations of the specific primers (Fig. 2A) were 0.25 µM and those of the control primers were 0.075 µM. Amplifications were carried out in a final volume of 10 µL containing 50 mM KCl, 10 mM Tris/HCl (pH 8.3), 0.01-percent gelatin, 5.0-percent glycerol, 100  $\mu g$  per mL of cresol red, 200  $\mu M$  of each dNTP, 100 ng of genomic DNA, and 0.4 units of Taq polymerase (Perkin-Elmer Cetus, Norwalk, CT). MgCl<sub>2</sub> at a concentration of 1.5 mM was present in all reactions. Both PCR reactions were designed to work under the same thermocycling conditions as used previously (PCR System 9600, Perkin-Elmer Cetus).<sup>25</sup> PCR fragments were size-separated in a 2percent agarose gel containing 0.5 µg per mL of ethidium bromide.

#### ABO genotyping

We applied a previously described *ABO* genotyping method,<sup>27</sup> which consisted of eight PCR-SSP and was designed to detect nucleotide sequence differences specific for the  $O^1$ ,  $O^2$ ,  $A^1$ ,  $A^2$ , and *B* alleles of the *ABO* gene.

#### **Statistics**

The deviation from the uniform distribution was tested according to Kolmogoroff-Smirnov.<sup>28</sup>

#### RESULTS

#### An Austrian proband with Bombay phenotype

**Blood group serologic tests.** The RBCs of the proband typed  $O_h$  ccddee Le(a+b-). The RBCs were not agglutinated by five anti-H reagents. A strong example of anti-H (titer 256), anti-B (titer 4), and anti-A<sub>1</sub> (titer 1) isoagglutinins were detected in her serum. Crossmatch was negative with RBCs of known Bombay phenotype. We tried to adsorb anti-A and anti-B. However, the eluate of the proband's RBCs was negative; the titers of the anti-A before and after adsorption did not differ.

An *h* allele of the *FUT1* gene. We sequenced the *FUT1* gene of the proband. A new *h* allele was found and dubbed *hG785A/C786A*. The proband was apparently homozygous for her *h* allele, as shown by the electrophoretograms of our sequencing approach from genomic DNA (Fig. 1), although the possibility of large deletions at the *FUT1* gene locus in *trans* position cannot formally be excluded. Two nucleotide changes (G $\rightarrow$ A at 785 and C $\rightarrow$ A at 786) resulted in the replacement of serine by lysine at codon 262. The nucleic acid

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Fig. 1. Electrophoretograms, nucleotide (nt) sequences, and amino acid (aa) sequences are shown for the proband with the Bombay phenotype (A) and a control with the prevalent *FUT1* allele (B). There are two adjacent nt polymorphisms (indicated by arrows and asterisks), located at nt positions 785 and 786, that distinguish both alleles. Both polymorphisms that are diagnostic for the hG785A/C786A allele cause a single missense mutation at codon 262. There are no double bands visible at either nt position of the electrophoretograms, which renders the presence of a second h allele unlikely. All nucleotides are in capitals, which indicates an exon sequence.

sequence was deposited in the EMBL nucleotide sequence database under the accession number AJ276886.

Lack of  $\alpha(1,2)$ -fucosyltransferase activity. The activity of the  $\alpha(1,2)$ -fucosyltransferase in the plasma of the

proband was checked (Table 1). No enzyme activity resulting in  $\alpha(1,2)$ -fucosylation was detected, whereas the activities producing  $\alpha(1,3)$ -fucosylation and  $\alpha(1,4)$ -fucosylation were normal.

*FUT2 (SE)* genotyping method by PCR-SSP. We developed a primer set for detecting the *Se* and *seG428A* alleles prevalent in whites (Fig. 2A). Representative results of this PCR-SSP method are also shown (Fig. 2B). *FUT2* genotyping was evaluated in 23 random Austrian blood donors (Fig. 2C) and found to be concordant with the serologic Lewis blood group results. The observed frequency of about 22 percent for *seG428A* homozygotes correlated well with the expected frequency of the nonsecretor phenotype in whites.<sup>2,29</sup> It should be noted that this method should be used only in European populations without ethnic admixture, because other Se-inactivating mutations are known to occur in Asian populations.

SE and ABO genotyping. In a person of the "true", Indian Bombay phenotype with an Se deletion, both Se and se reactions are expected to be negative. However, in a person of the Bombay phenotype who has a European ethnic background, nonsecretor status consistent with the Le(a+b-) phenotype and the *FUT2* genotype seG428A/seG428A is expected. In *FUT2* genotyping by the PCR-SSP method, the proband lacked the active Se allele and possessed an SE allele with a G428A mutation (Fig. 2B). This most probably represented an seG428A/seG428A genotype, although the possibility that an SE deletion occurred in *trans* to an seG428A allele (seG248A/-) could not be formally excluded. In ABO genotyping of the proband, the PCR-SSP reaction pattern indicated an  $A^1O^1$  genotype (not shown).

# Gene structure and positions of nonfunctional alleles

Enzyme-inactivating mutations may point to functionally important and highly conserved residues. Thus, we updated a current analysis<sup>30</sup> with seven additional missense mutations, including the one observed in this study.

Distribution of nonsense and frameshift mutations. Nonsense mutations, deletions, and insertions cause premature stop codons and can occur throughout the *FUT1* or *FUT2* coding sequence. It was unexpected to observe the 12 aberrations almost exclusively in the second half of the *FUT1* coding sequence and the homologous regions of *FUT2* (Fig. 3). This observed distribution deviated significantly from the uniform distribution (p<0.05, Kolmogoroff-Smirnov test).<sup>28</sup>

Distribution of missense mutations and conservation of the sequence. Seventeen missense mutations and two in-frame deletions affected 17 different amino acid positions. Excluding A12V that was not considered causative, no loss-of-function mutation was observed in a large stretch of the aminoterminal section encompassing the transmembraneous helix. We compiled the human amino acid

| TABLE 1. Activity of $\alpha(1,2)$ -fucosyltransferase in plasma |  |                    |                                   |  |  |  |  |  |  |
|--|--|--------------------|-----------------------------------|--|--|--|--|--|--|
|  | Rate of fucose incorporation per mg of protein (pmol/3 hours)* |                    |                                   |  |  |  |  |  |  |
| Acceptor   | Proband<br>(n = 3)   | Controls $(n = 4)$ | Type of<br>linkage tested         |  |  |  |  |  |  |
| p-nitrophenyl- $\beta$ -D-galactoside                            | —†   | 12.1 ± 1.8         | α(1,2) only                       |  |  |  |  |  |  |
| lacto-N-biose (Gal-β(1,3)GlcNAc)                                 | 41.7 ± 0.1   | 39.7 ± 2.4         | $\alpha(1,2)$ and $\alpha(1,4)$   |  |  |  |  |  |  |
| <i>N</i> -acetyl-lactosamine (Gal- $\beta$ (1,4)GlcNAc           | c) 41.3 ± 0.1  | 39.1 ± 1.8         | $\alpha$ (1,2) and $\alpha$ (1,3) |  |  |  |  |  |  |

The proband's plasma was tested three times. Four plasma samples, one each of blood group AB, A, B, and O, were used as controls; mean ± SD are shown. Not detectable

#### Δ Primers used

| Name of primers | DNA sequence of primers  | Specificity           | Size of<br>PCR product |
|-----------------|--------------------------|-----------------------|------------------------|
| FUT2-Se-128-s   | 5-CCGGCTACCCCTGCTCGTG-3  | Se (forward)          | 132 bp                 |
| FUT2-se1-428-s  | 5-ACCGGCTACCCCTGCTCGTA-3 | se (forward)          | 131 bp                 |
| FUT2-all-523-as | 5-CCGGCTCCCGTTCACCTG-3   | Nonspecific (reverse) |                        |

#### Representative results в

|                  | Controis<br>Se se | Se sø | Se se | Proband<br>Se se |
|------------------|-------------------|-------|-------|------------------|
| 434 bp<br>131 hp |                   |       |       |                  |
|                  | - +               | + +   | + -   | - +              |

#### С Correlation of Lewis phenotype with FUT2 genotype

| FUT:      | 2                 | Lewis phenotype |            |       |   |  |  |  |
|-----------|-------------------|-----------------|------------|-------|---|--|--|--|
| geno      | genotype Le(a–≿+) |                 | ) Le(a-b-) | Total |   |  |  |  |
| Se/S      | e 6               | 0               | 1          | 7     |   |  |  |  |
| Se/se     | e 11              | 0               | 0          | 11    |   |  |  |  |
| sc/sc     | . 0               | 5               | 0          | 5     |   |  |  |  |
| <br>Total | 17                | 5               | 1          | 23    | _ |  |  |  |
| Expe      | cted 29 72%       | 22%             | 6%         | 100%  |   |  |  |  |

Fig. 2. FUT2 (SE) genotyping by PCR-SSP. The nucleotide sequences of the primers used for SE PCR-SSP are shown (A). The Se- and seG428A-specific forward primers were used with a nonspecific reverse primer for both the Se and the se alleles. Two PCR amplifications were done per sample. The first reaction detects a G at nucleotide position 428 of the FUT2 gene, which is "diagnostic" of the Se allele; the second detects an A at the same position, which indicates the presence of an seG428A allele. Representative results for se/se, Se/se, and Se/Se samples are shown (B). The proband of this study was se/se homozygous. Control PCR amplicons of 434-bp size occurred in all reactions as described previously.<sup>25</sup> A study in 23 Austrian blood donors was done to correlate their Lewis blood group phenotypes with FUT2 genotyping results (C).

sequences in the range of the 17 known missense mutations and compared it to the sequences in other mammalian, invertebrate, and bacterial  $\alpha(1,2)$ -fucosyltransfer-ase genes

(Fig. 4). All mutations were in peptide segments of high homology throughout the mammalian genes. Six of the mutations were located in the previously described fucosyltransferase motifs I, II, and III.<sup>30-32</sup> This included the S262K mutation observed in the Austrian proband, which was located in motif II. Three other mutations (positions 241, 242, and 349) were located in two additional highly conserved regions that were conserved even in the bacterial genes. The

W at position 349 was present in all species with only four exceptions in C. elegans. Six mutations occurred in regions that were not overtly conserved in the invertebrate or bacterial genes. The N327T substitution<sup>15</sup> obliterated one of the three N-glycosylation sites occurring in the H transferase.

#### DISCUSSION

Since the profound polymorphism of nonfunctional FUT1 alleles was recognized,<sup>12</sup> substantial numbers of distinct h alleles have been found. The present observation of the hG785A/C786A allele adds to these numbers. We identified two adjacent SNPs in the hG785A/C786A allele causing an enzyme-inactivating missense mutation in the H transferase protein of an Austrian proband with a Bombay phenotype. The observed homozygosity of the proband for the hG785A/C786A allele is explained by the small but relevant inbreeding in human populations and is in congruence with previous findings.<sup>12</sup>

The present allele was considered an *h* allele on the basis of the Bombay phenotype of the proband detected by standard serologic procedures and the lack of plasma  $\alpha(1,2)$ -fucosyltransferase activity. Because we did no expression studies, it is possible that trace amounts of residual fucosyltransferase activity could be demonstrated in such studies. However, even such alleles are generally considered h alleles.<sup>17</sup>

We updated current analyses of functionally important segments,<sup>30-32</sup> including 7 additional missense mutations and 12 new invertebrate and bacterial genes with homology to  $\alpha(1,2)$ -fucosyltransferase (Figs. 3 and 4). All inactivating missense mutations occurred in highly conserved protein segments. In addition to the previously described fucosyltransferase motifs I to III, we identified a tryptophane residue conserved throughout all species.

The analysis of missense mutations in nonfunctional alleles and the observation of homologies between species complement each other in the identification of functionally important residues. Further possible approaches are the observation of mutations occurring in functional alleles<sup>7</sup> and studies of expression after site-directed mutation.<sup>16</sup> The observation of missense mutations in the population occurs only by chance and is unlikely to identify all muta-



Fig. 3. Schematic representation of the distribution of frameshift and nonsense mutations underlying nonfunctional *h* and *se* alleles. The bar symbolizes the 365-amino acid protein sequence of the H transferase. The positions of the nonsense mutations (Stop) and frameshift mutations (FS) observed in 7 *h* alleles and 5 *se* alleles are indicated above and below the bar, respectively. For the *FUT2* mutations (*SE* gene), both the homologous position in the H protein (upper number) and the position in the Se protein (lower number) are indicated.

tions abrogating functionality. Homology searches directly disclosed regions that were highly conserved between species and led to the identification of three highly conserved  $\alpha(1,2)$ -fucosyltransferase motifs.<sup>30,32</sup> Limitations derive from the restricted ability of computer programs to identify homologous sequence stretches and from the lack of information about the functional activity of many genes considered fucosyltransferase homologues. Most *C. elegans* genes were predicted from genomic sequences and might also represent pseudogenes or have acquired different functions. Thus, it was reassuring that 6 of 17 missense mutations occurred in one of the  $\alpha(1,2)$ -fucosyltrans-ferase motifs that represented only a small part of the whole protein.

An interesting finding was the scarcity of nonsense and frameshift mutations in the first half of the protein. Among possible explanations, a functional active gene product starting at a secondary start codon might be considered. Overall, the 5' sequence is much less conserved among the species than the 3' terminal parts. In bacterial fucosyltransferases, there are no sequences corresponding to the 80 aminoterminal amino acids in the human fucosyltransferase.

Because the amino acid substitutions found in any of the 17 *h* alleles do not occur in the *FUT2* gene, the mutations may not have arisen by gene conversion events between the closely adjacent and highly homologous *FUT1* and *FUT2* genes. Thus, the mechanism of the gene polymorphism is different from that often occurring at the *RHD/ RHCE* (RH blood group<sup>33</sup>) and *GYPA/GYPB* (MNS blood group<sup>34</sup>) gene loci. Another difference from these loci was that no phenotypically distinct mutation occurred only in the transmembraneous helix of the single-pass FUT1 protein or within 80 amino acids of that helix. It may be possible that no membrane integration is required for H transferase function or a massive reduction in function is not responsible for the Bombay phenotype. Individuals lacking any membrane-integrated H transferase in their Golgi apparatus might still produce H antigen in some tissues and express a "para-Bombay" phenotype, even if they are *se/se* homozygous.

The *FUT1* and *FUT2* genes are a very useful model for analyzing the general occurrence of sporadic mutations in genes.<sup>12,33,35</sup> Although many h alleles have been characterized to date, we think further analysis would be useful. As exemplified by the present case, newly identified persons with the Bombay phenotype very often carry novel h alleles. The characterization of functionally disruptive mutations may be relevant for other structurally similar glycosyltrans-ferases, such as ABO. This

approach expediently utilizes the large population databases available to transfusion medicine and allows the rapid delineation of molecular genetics data relevant for many genes.

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#### A NEW h ALLELE IN EUROPE

|                                     |                                  |                  |                             |                   |                       |                        | Notif I                  |                      | Hotif []               |                |               | Motif []   |                    |                          |
|-------------------------------------|----------------------------------|------------------|-----------------------------|-------------------|-----------------------|------------------------|--------------------------|----------------------|------------------------|----------------|---------------|--|--------------------|--------------------------|
|                                     | 1                                | 1                | 1                           | 1 1               | -                     | 1                      | ż                        | 22                   | 22 2                   | 2              | 2             | 3  | 3                  | 33                       |
|                                     | 1                                | 2                | 4                           | 55                | 6                     | ?                      | 2                        | 64                   | 56 6                   | 6              | 7             | 1  | 2                  | 10                       |
|                                     | r                                | 3                | в                           | ਸ਼ਾ               | - 5                   | 1                      | v                        | 12                   | 24 K                   | ć              | -0<br>N       | ů<br>v   | -                  | 69<br>KC                 |
| бело                                | ¥                                | г                | Ŷ                           | CE                | н                     | с                      | c                        |                      |                        | •              |               |  |                    |                          |
| FUEL Homan                          | FRUPATHAALAPVE                   | 17LPVL           | WRELQLHDWRS:                | EEYADL            | FLKLSG-F              | PCSWTFF                | GVHVRRGDY                | DSAYLROA             | PVEVVTSNG              | RMCK           | QGCV7P        | IGTEGEWAAYLA   | YLANFTL            | 7 LP EWVGTN              |
| FILL Gibboo                         | FLEPAMHAALAPVE<br>FTLEAMHAALAPVE | ITLEVE<br>ITLEVE | WRELQLHDWHS.                | EETADL<br>EEVADL  | FLELSG-FE             | PCSWTFF                | GVNVRRGDY<br>OME DRCDV   | DSAYLROA             | PVPVVTSNC9             | IEWCK          | QGEVIE        | ISTEGEWAAYLA   | YLANFTL<br>VLANDTL | ELPENVICEN<br>TERMUNICEN |
| FUT1 Chimpanzed                     | FLUCAMHAALAPVE                   | ITIPVL           | WRELOLHIMHS.                | EEYADL            | YLKLSS-FR             | PCSWTFF                | GVHVRRGDY                | DSAYLROA             | PVPVVTSNG              | SEWCK          | DGEVTF        | IGIEGEWAAYLA   | YLANFTL            | ILPENVGIN                |
| 7011 Orangotar                      | FULPAPHAALAPVE                   | ITLPVL           | WEELQLHCORS.                | EFYADL            | PLKLSG-F              | CSWTFF                 | GVHVRRGDY                | DSAYLSQA             | PVFVVTSNG              | EACK           | OGEVTE        | IGTEGEWAAYLA   | YLANFTL            | 7 LF EWVGIN              |
| 2011 Rhesus                         | FTLPAKHAALAPVF                   | ITEPVI.          | WRELQLHDWKS.                | EFYADL            | FLKLSG-FR             | PCSWIFT                | GVNVRRGDY                | DSAYL2QA             | PVPVVTSNG              | EWCK           | QGEVTF        | IGTEGEWAAYLA   | YLANFTL            | 7 LPEWVGIN               |
| FUEL K fascieviaris                 | FTGPAKHAAGAPVF                   | ITLPVI,          | WRELOLHT & S                | ECYALIC.          | FLELSS-FE             | PCSWIFF                | GVHVRRGDY                | DSAYLRCA             | PVFVVTSNG              | (EWCK          | QGEVTF        | IGTEGEWAAYLA   | YLANFTL            | FIPENVGIN.               |
| 70Ti Grean soskev                   | FTLPAKHAILAPVE                   | TTIPVI.          | WAELGLILLAMS                | ESYVDI.           | FINLSG-FR             | PCSWIFF                | GANAKERDI                | DEATLODA             | PVFVVTSNG<br>PVFVVTSNG | ABWCK<br>ABWCK | DEEVIE        | LES FGEMAATLA<br>LES FGEMAATLA   | YLANFTL<br>YLANFTL | TUPENVGIN                |
| 7071 Saimiri                        | FILPAMHAALOPVE                   | ITLPVL           | WOELRLHTARIS.               | SEYADL            | FLELSG-FR             | PCSWTPT                | GVIVERGDY                | XSAYLREA             | PVFVVTSNG              | MEWCR.         | KGEVNE        | ISTEGEWAAYLA   | YLANFTL            | E                        |
| 7071 Callichtix                     | FULPAMHAALAPVE                   | 172FVI.          | WARLOUGHDAMS.               | SEYADL            | PLKLSJ-FR             | PCSWIFF                | GVHVRRGBY                | DSAYLEQA             | PVFVVTSNG              | IEWCK          | <b>OGEVTF</b> | IGIFGFWAAYLA   | YLANFTL            | E                        |
| 7UT1 Rat                            | FIOPERSHTTLAPVE                  | ISLPVL           | WORLVLHDAMS:                | EEYSHL            | PLKL35-FI             | CSWTFF                 | GVHVRRGDY                | DRAYLQKA             | FIFVVTSNG              | RWCL           | HGEVVF        | IGIFGFWAAYLA   | YLANFTL            | FLPEVVGIN                |
| ZUTE PADELA                         | FTOPERHAALAPV2<br>FTOPERHAALAPV2 | ISLEVL<br>TTLEVL | WORLVERDWAS.                | SEYSHL<br>CEVENT  | FLKLS3-FF             | PCSWTPF                | GVHVRRGDY                | DRAYLOOA             | PIFVVTSNG              | IKIYCE.        | DETAND        | IGTEGEWAAYLA   | YLANFTL<br>VLANFTL | FLPEWVGIN<br>6LPEWVGIN   |
| FUTI Cow                            | FILTANEAALAPVE                   | TIPVL            | WRELOLHDWIS:                | EEYADL            | FLXLSG-F              | PCSWYFT                | GVHVRRGDY                | DSAYLROA             | PVFVVTSNO              | (EVCK          | OGEVIP        | ISTEGEWAAYLA   | YLANFTL            | FLPEWVGIN                |
| FUT1 Plg                            | FIQPAMHAVLAPV7                   | ITLPVL           | WRELELKOWNS                 | EDYAHL            | WEKLIG-FR             | PC5WTFT                | GVHVARGOY                | DGAYLQQA             | TVEWVTSNOL             | EVCR           | PGEVIF        | ISTEGEWAAYLA   | YLANFTL            | FLEEWVOIN                |
| FUT2 Human                          | FIFAQMESTLAPIF                   | ITLYVL           | WONTHLNDAME                 | <b>BAUNI</b>      | YVRF7G-Y              | POSINTEY               | GVHVRRGDY                | DARYLOOA             | LIFVVISIO              | INCR           | HULWYF        | LOTEG IWAAYL?  | VLANYTI            | PLOTMARY STR             |
| FUT2 GOF1115                        | FIFAQMENTLAPIF                   | JTLPVL<br>LTLPVL | WENYHLNEWME                 | SEYRHI            | YVEFR-YE<br>VICTOR-YE | PESNTEY                | GVHVRRGDY<br>GSRC70 DGDY | DARYLOGA             | PIFVVTSNO              | ANCR           | HGEVVP        | LUTEGIWAAYLT   | VLANYTL            | 7 DEENTGIA               |
| FUT2 Chimpanzee                     | FIFAOMHSTLAPIE                   | 1TLPVL           | WORYNLNDARE:                | SEYRNI            | YVEFTG-YE             | PCSWTFY                | GYHVRRGDY                | DRRYLOGA             | PIFWTSNO               | AWCR           | HGEVVE        | ISTEGIWAAYLT   | YLANYTI.           | FLPENHCIA                |
| FUT2 Orangutan                      | FIZAQMENTLAPIE                   | 1TLPVL           | WORYHLNDWME:                | EKYRHI            | YVELTG-YE             | PESWIFY                | GYHVRRGDY                | DARYLOOA             | PIFVVTSNO              | AWCO           | HSLWVF        | 1:STEG:WAAYLA  | YLANYTL            | FLPENTGIA                |
| FUT2 P Fanctoria-14                 | FIFAQMRSTLAP15                   | 1TLFVL           | W2NYHLHDMME                 | EKYRHI            | AAETLO-AE             | PCSWTTY                | GVHVRRGDY                | DARYLOOA             | PIFVVTSNO              | AWCR           | HGEVVF        | ISTFOIWAAYLA   | YLANYTL            | FLPEWIGIA                |
| FUT2 Lence felves                   | FIFTRMESTLAPIF                   | TELPVL           | WONYS ROWNE                 | ERYRHI            | YVELTG-YE             | PCSWIFY                | GVHVRRGDY                | DARYLEOA             | PVPVVTSNC              | ALCR.          | HGEVVE        | ISTEGINARYLA   | YLANYTL            | FLPEWNGIP                |
| Inic Ciney mounds                   | FIFAUMEST LAFIE                  | TIDEVD<br>THERE  | WONTHERDAME                 | CKYKHI<br>CXYRHT  | SAFTLE-11             | POSWIFY                | GVNVRHGEIT               | DERVIQUA             | PIPVVISNE              | CANCE.         | NGCYVY        | IGTEGIWAAYLA   | YLANYTL            | FLPEWIGIA                |
| FUS2 FAt                            | FIFASMENALAPIE                   | SLEVI            | WONYRENDAMES                | EKYRH1            | EVENCG-SI             | PESWIFY                | GVHVBBGEY                | DRIVLEKA             | 7VFVVTSNE              | GWCR           | ROFTINE       | LOTEGINAAY? A  | YLANYTL            | FLPENARIS                |
| FUTZ Mouse                          | FIFASMESTLAPIF                   | ISLPVL           | WOWYHLEDAME:                | ERYRHI            | YVAFTG-Y              | CSWIFY                 | GVHVRRGDY                | DRGYLEXA             | 2VFVVTSDD              | AWCR           | RGEVAF        | LGTEGIMAAYLT   | YLANFT2            | YLPEWVGIA                |
| FU72 Fabbit                         | YIFAQMHSTUAPIF                   | <b>TSLPVL</b>    | MONARTHOWNE:                | SRYRHI            | YVELTG-Y              | PCSWIFY                | GVHVBLRGDY               | DRGYLEQA             | PVFVVTSNG              | CANCE.         | RGEVVF        | ISTEGIWAAYLI   | YLANYTL            | FLPEWVGINC               |
| FUTZ COM                            | FIRPOMESTLAPIE                   | TTLEVI           | WORTHLADAME.                | 1HHYOS            | YVKLTG-YI             | PCSW7FY                | GVHV9RGDY                | DRRYLEOA             | PLFVVSSNG              | EAWCR.         | RGEVVF        | ISTEGIWAAYLA   | YLANYTL            | FLEXWIGIP                |
| FUT2 Pig<br>Seci Otena              | FIRRENTST AFTE                   | TTLEVL           | WONTHER MOMME:              | SRYRHI<br>Zrydut  | YVELTG-YP             | PCSWIFY                | GVHVRRGDY                | DRRYLEGA             | PVFVVSSNG              | GWCR           | RGEVVF        | ISTEGINAAYLA   | YLANYTL<br>VLANYTL | FLPEWIGIE                |
| Seci Gibbon                         | FIFAGMESTLAPIF                   | TTLPVL           | WONAHTHERWICE               | REVENT            | YVCLMG-Y              | PCTWTFY                | SVIVILOUS                | DROTING              | PUPArtstor             | LANCE.         | LCEVER        | VETFERMAAYLA   | YLANETI.           | FLERENVOLA               |
| Seci Fhesus                         | FITVOMESTLAPEF                   | 1TLPVL           | WONYTELWINME                | EKYRHI            | YVALTG-Y              | PCSYTEY                | GYHVRRGDY                | DRGYLORA             | PVTVVTSDD              | ANCR           | TOEVVP        | VETPOWAAYLA  | VLANETL            | TLPENVGIA                |
| Sect K. Lescirolaris                | FIFVOMHSTLAPIF                   | ITLEVL           | WONTHLENNINE                | EKYRHI            | YVRLIG-YI             | POSTTY                 | OVHVRRGDY                | DRGYLORA             | PVFVVTSDD              | ANCR.          | VGEVVE        | VETEGWAAYLA  | YLAKFTL            | FLPENVGIA                |
| Soci Rat                            | FIRASMESTLAPIE                   | 1SLPVL           | W_KYHLNDWME                 | ERYRHI            | YVRETG-YI             | PCSYTPY                | GYHVRRGDY                | DAGYLEKA             | PVFVVASDD              | IST'CR         | RGEVAF        | LSTEGIWANYL7   | YLANPTO            | YLPERVGIA                |
| Seci Mouse                          | FISESMENALAPIP                   | 155775           | WONTELNDWME<br>SYNDYLTMINME | KKYKAI<br>COVENIT | YVIC IG-Y             | NCSETEY<br>PROSPERV    | GVHVXKGEY                | DRGYLEKA             | JUPOVISIAS             | CANCE.         | LECAAL        | 131FSIWAAYLA   | YLANYTL            | FILPERARGIE              |
| C. elegane F                        | aca                              | nce              | DCS.                        | 001 MII           | YLHLTGTH              | COSWEY 7               | CVHVRRGDF                | DENFILSS             | tatVFFGDD)             | EFHD           | LC.           | HSTEGNINGYES   | YYTDIOF            | YLPHWIPL                 |
| C. elegens G                        | DCB                              | pca.             | DC.                         |                   | FLVIDGOY              | 25FKYF                 | CVHIRRGOT                | SSYFTIRA             | glvylisod              | kwr            | nce           | 5 STEGAWLGYM5  | YYRDIQE            | FFFTWKKL                 |
| C. elegans X                        | NCA                              | hca              | nca                         |                   | FUIDEQV               | -051 KY -              | CVHI RRGDZ               | DAVETIRA             | glirtespo              | EWVR           | nca           | SSIFCAWIGYMS   | YYRDIQE            | FERIMKET                 |
| C. clegans I                        | nca                              | DCB.             | nce                         |                   | YLHLDGRF              | (QAVKYF                | CVHIRRODZ                | DEWTIKKA             | VHVVLFGDDI             | PERK           | nca           | REFERENCES   | YYMDIRE            | ATSHALL                  |
| C. elegans J                        | nce                              | DCA              | n;a<br>nca                  |                   | VINITCHEN             | CORNER CORNER          | CVHIREOD                 | ASSETTA              | MUNUTIANDE             | CHIVE.         | nca .         | SSTFGAW ATLS   | NVODTAS            | TUPCETCT                 |
| C. elegans L                        | nca                              | nca              | nca                         |                   | YLHLTGHE              | OSWKYP                 | CVHARRSD7                | DEDETENS             | rkWIFGDD               | EFMR           | nca           | KSTERMIGTES  | FYRDIAY            | 5CB                      |
| C, elegans M                        | nce                              | hda              | nca                         |                   | YLHLAGYYY             | CONKYF                 | CAHTRRODZ                | DPTFVERA             | SRIVLFGDDE             | KFMX           | nça           | HSTFOLMSYFS  | FYNDIRE            | FMPSWTPL                 |
| C. elogons N                        | nca                              | ņ.св.            | NC4                         |                   |                       | SSVKYF                 | CVH5 ARGDE               | DPKFNKHA             | CKIVLECODE             | KIMR           | nca           | RSTFG W JYFS   | YYLDIXH            | FLPHNTPL                 |
| C. slegans O                        | nca                              | hta<br>naa       | nua                         |                   | YLHLSGIP              | roewaxya<br>Loweana    | C HTRRGU/                | dskflkns<br>Portsmol | TRIVIEDD               | KFLK.          | nca           | HSTFOWLGYFS  | YYMPLAY<br>MAMETER | YPTHWTPL<br>WHDUNING     |
| C. elegans :<br>C. placans Q        | CCH CCH                          | nca<br>hca       | nca<br>nca                  |                   | FLVIEGHYS             | COSYKY7                | CVHVERCOP                | DSNYTISL             | THE INTERCOOL          | IN LUIN        | nça<br>nca    | SSIFGWIGTES  | VYRDIRE            | YPPTWNK_                 |
| C. eispane R                        | nca                              | LCB              | hte                         |                   | FLVIDGHE              | PORYKY7                | CVHVRRGDT                | DETFTIKA             | of IVMISEDS            | DWUC:          | nco           | SSTEGWIGTES  | YYRDI RB           | PESTWIC:                 |
| <ol> <li>eleçans (new)</li> </ol>   | ticь                             | a.c.e            | n:te                        |                   | YT.HITGEFT            | LOEWKY7                | CVHVRKCDE                | DIMTVESA             | KGKESINFTI             | FFSS           | nca           | HSTEGMUCYES  | YYMDIRE            | FLPHWTP1.                |
| <ol><li>a) agains (new)</li></ol>   | trea.                            | siça             | tr=¢                        |                   | TIAPLOORA             | COSTRY7                | CVHIRRSDE                | EK218EV1             | VS FVI FGDD:           | FKR            | nca           | ESTEGWAMGY15   | YYADIRG            | YLPHWIPV                 |
| <ol> <li>elegans (New)</li> </ol>   | nes                              | nca              | N74                         |                   | NLILDOHY              | EDREKAĘ                | CTHVRRGDE                | DATFILAA             | VMVVVLGND:             | EFAY           | nca           | 5STEGINLS:LA   | YYRDILL            | YPPEWIKL                 |
| o, etegana (ne%)<br>≅ e/enaba (ne%) | hear                             | nce<br>nce       | n_a<br>n_a                  |                   | NULINGVY:             | r yar kir<br>Pospryf   | CPHINKLD?<br>CVHTKOTOL   | DEFETRS4             | VTVAT LGND             | DATE:          | nca           | TSSEGANLATIA   | YYRDØXE            | TETEWINE<br>YSNSWSKI     |
| <ol> <li>elegana (new)</li> </ol>   | nca                              | nca              | hôạ                         |                   | YLILEGHY              | FOSYKTE                | C'fHIHRG5?               | DPRFTRVA             | isvvvlgddi             | P IVD          | nça           | TSTEGAN AYLA   | YYRDIED            | YPKEWRTL                 |
| С. вједера (лем)                    | nca                              | nca              | nca                         |                   | HLFLNGHYI             | POSFXYP                | CTHINRSOF                | DENETRAA             | TTVVPGHD               | NESK           | nce           | SSTFGWLSYLA  | nsa                | hća                      |
| C, elegens (new)                    | ncə                              | nca              | nca                         |                   |                       | 52KYZ                  | CTHIRRGOF                | D25FTRAA             | TWWFGHOV               | NEAK           | nca           | SSTEGWALSYLA   | YYRDILE            | YPPEWIKL .               |
| C. elegans (new)                    | пса<br>                          | r.ca             | nca                         |                   | SLMVKGNG3             | FK3EKY?                | CVHAT                    | NKNCSVKA             | WHILIASPES             | EF             | 008           | ASTHGWANGYLA   | YFEPDMF            | EDDKWKKT                 |
| ( elegens took)<br>( elegens (pek)  |                                  | nce<br>nce       | nça<br>DCA                  |                   | YLHLSGKY)             | CONTRACTS<br>CONTRACTS | CVHIREDOF                | DALLETWAY            | AS AT ATPRIATE         | AFT.F          | nca           | nca  | nca                | пса                      |
| h. major                            | nca                              | VIVPET           | nca                         |                   | VFHNIG-F              | OSDAF?                 | ALHVARGDY                | VVEYYDAV             | TEPTAN                 | FGR            | AVMVSL        | NSTESSMORATLY  | SCRRVIA            | YCP3W1L1                 |
| H. pylari                           | nca                              | nce              | nca                         |                   | LTYFFG-Y3             | ODPRY7                 | <b>TVHI RRGDY</b>        | GIDYCKEA             | HELEVPCEDI             | KPT2           | pce           | NSTYSWAAY1M  | nr e               | LCKEWVKI                 |
| V. cholerae<br>I terrin             | nce                              | nca              | nca                         |                   | nca                   |                        | SERVERGEY                | DEDYYKRA             | <b>PVFEVFSKD</b>       | ANCE           | pca           | NSSI SWAARLN   | hùs                | EGYKWVPD                 |
| H. iragilis                         | ncsi<br>nesi                     | r.ca<br>r.ca     | nça.                        |                   | nyYLSG-Fi             | NEELEY7                | OVSIREDY                 | DINYFIES             | KVICF-SHOV             | EW'K           | nca           | MSSISWWTEIL-   | nca                | S RENALY                 |
| Y. enteracolitica                   | nca                              | n.ce             | nca                         |                   | NYHY-GYPC             | DE PD==                | BTHT MARIN               | DERYTERA             | TREEVESIO              | DWCK           | nce           | GSTESWWAA 11   | DGA                | kiri manye               |
|                                     | -                                |                  |                             |                   |                       |                        |                          |                      |                        |                |               | and the second s |                    |                          |

Fig. 4. Comparison of the mammalian *FUT1 and FUT2* genes and the localizations of the 17 missense mutations and the in-frame deletion in all known *h* and se alleles. Mammalian *FUT1*, *FUT2*, and *Sec1* fucosyltransferases and their homologues in *C. elegans*, Leishmania, and bacteria are compiled. The  $\alpha(1,2)$ -fucosyltransferase motifs and stretches of amino acids in the range of the amino acid positions with known mutations are depicted. The induced amino acid substitutions are shown; at position 154, both histidin and cystein have been observed. The *FUT2* mutations at positions 101, 129, and 230 are indicated at the homologous positions 129, 157, and 260 of the *FUT1* gene. The valine at position 230 of *FUT2* homologous to 260 of *FUT1* affected by an in-frame deletion is indicated by an asterisk. The substitutions at positions 157 and 348 are associated with alleles that have relevant residual function. Lower-case sequences indicate amino acids that were aligned manually. The accession numbers of the sequences are listed in the Methods section. nca = no consistent alignment obtained by Psi-Blast. *M. fascicularis = Macaca fascicularis*.

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