Influence of Lewis α1–3/4-L-Fucosyltransferase (FUT3) Gene Mutations on Enzyme Activity, Erythrocyte Phenotyping, and Circulating Tumor Marker Sialyl-Lewis a Levels*

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Fucosylated glycoproteins carrying α1–4 fucose residues are of importance for cell adhesion and as tumor markers. The Lewis gene, FUT3, encodes the only known α1–4-fucosyltransferase (FucT), and individuals who are deficient in this enzyme type as Lewis-negative on erythrocytes. We examined the mutational spectrum of the Lewis gene in Denmark and found 6 different mutations. Five, T59G, T202C, C314T, G508A, and T1067A, were frequent, and one, C445A, was only detected in one out of 40 individuals. Allele-specific polymerase chain reaction-cleavage assays were established for the 202 and 314 mutations were co-located on the same allele. COS7 cells transfected with an allele having the 202/314 mutations lacked enzyme activity. Polymerase chain reaction-cleavage assays were established for the genotyping of healthy individuals as well as 20 genuine Lewis-negative cancer patients and 10 non-genuine. The latter have Lewis-negative erythrocytes but saliva α1–4FuCT activity. The genuine Lewis-negative individuals had mutations on both FUT3 alleles. In 66 healthy individuals, a gene dosage effect was detected as FUT3 heterozygous individuals had a lower α1–4FuCT activity in saliva than did homozygous wild-type individuals. The lower enzyme level in heterozygous individuals resulted in a significantly (p < 0.04) lower level of circulating sialyl-Lewis a structure in serum. This has the clinical impact that cut-off levels in tumor marker assays should be defined on the basis of genotyping. In the group of non-genuine Lewis-negative cancer patients, whose erythrocytes convert from Lewis-positive to Lewis-negative during the disease, FUT3 heterozygosity was significantly (p < 0.05) more common.

Fucosylated glycoprotein cell surface glycoconjugates belonging to the Lewis blood group system have recently been shown to be physiologically important. Not only do they present a challenge to transfection medicine, but they are 1) involved in cellular development, 2) serve as tumormarkers, and 3) are believed to be essential for the adhesion between leukocytes and vascular endothelium during the inflammatory reaction.

The fucosylated structures are formed by fucosyltransferases in the Golgi apparatus that act by transferring fucose from a nucleotide sugar donor to one or more acceptor substrates. The fucosyltransferases in mammals have been named according to the bond they create, α1–2 FuCT, α1–3 FuCT, and α1–4FuCT. These three distinct bonds can be catalyzed by a large repertoire of transferases, of which some are confined to certain tissues, and subjected to developmental regulation. Several genes encoding distinct fucosyltransferases have been cloned. Two of them encode α1–2-fucosyltransferases (4–7). FUT4, FUT5, FUT6, and FUT7 encode α1–3-fucosyltransferases (8–12), and FUT3 encodes an enzyme with both α1–3 and α1–4 fucosyltransferase activity (13). The predicted aminoo acid sequences of FUT3, -5, and -6 have a high degree of similarity, and may be the result of gene duplication (14, 15).

Mutations in these genes have been the subject of several recent studies. Thus, the lack of α1–3 FuCT activity in plasma in 9% of Indonesian individuals was shown to be caused by a missense mutation (G247K) in the catalytic domain of FUT6, which was inherited in the deficient families (16). Different inactivating mutations in the FUT3 gene have been reported from Japan and Indonesia. In Indonesia, the two mutations L20R and I356K have been seen to occur individually as well as together (17). The L20R mutation is located in the transmembrane segment and has been suggested to lead to improper membrane insertion without severely affecting the catalytic activity. The I356K mutation is located to the catalytic domain and is deleterious for the catalytic activity when it is alone, as well as when it is accompanied by L20R. In Japan, two types of coupled mutations are found, the L20R combined with either G170S or I356K (18), of which the G170S mutation is deleterious to enzyme function.

The amount of circulating sialyl-Lewis a structure is widely used as a tumor marker for colorectal, pancreatic, and gastric cancer (19) and is named the Ca19–9 cancer antigen (20, 21).

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1 The abbreviations used are: α1–2FuCT, GDP-fucose (β-D-N-acetylglucosaminidine 2-α-l-fucosyltransferase); α1–3FuCT, GDP-fucose (β-D-N-acetylglucosaminidine 3-α-l-fucosyltransferase); α1–4FuCT, GDP-fucose (β-D-N-acetylglucosaminidine 4-α-l-fucosyltransferase); Sialyl-Lewis a, NeuAcα2-3Galβ1-3(Fucα1–4GlcNAc) Lewis b, Fucα1–2Galβ1–3(Fucα1–4GlcNAc) PCR, polymerase chain reaction; AS-PCR, Allele-specific PCR; DIG, digoxigenin; ORF, open reading frame; bp, base pairs; MOPS, 4-morpholinopropanesulfonic acid.
Unfortunately, this tumor marker cannot be used in individuals who are Lewis-negative because of inactivating mutations in both FUT3 alleles. To use the tumor marker it is therefore important to establish which mutations inactivate the FUT3 alleles. In the present study of a Danish population, we identified two new FUT3 alleles with inactivating mutations, in addition to the four alleles already known. Furthermore, we have used FUT3 allelotyping to examine whether FUT3 heterozygous individuals have a lower amount of α1-4FucT activity in saliva and of circulating sialyl-Lewis in structure in serum than in individuals who are homozygous for the wild-type allele. This could be of clinical importance as the serum cut-off level between normal individuals and cancer patients would be lower in heterozygous individuals than in homozygous wild-type individuals. We found a significantly (p < 0.04) lower amount of serum sialyl-Lewis a in heterozygous individuals compared with homozygous wild-type individuals, and we suggest that allele-corrected cut-off levels should be used to improve the clinical performance of the Ca19–9 tumor marker in the future.

In transfection medicine, it has been reported that some individuals who type as Lewis-positive on erythrocytes can change their erythrocyte phenotype to Lewis-negative during pregnancy (22, 23). They have been named non-genuine Lewis-negative individuals as they have α1-4FucT activity in saliva (24). Due to this phenomenon, the Lewis-negative phenotype is more common among cancer patients (approximately 20%) than among healthy individuals (approximately 8%).

One aim of this study has been to investigate the mechanism that leads to the non-genuine phenotype by examining the FUT3 alleles in a number of non-genuine and genuine Lewis-negative cancer patients and Lewis-positive healthy individuals. In the majority of cases, we found the change from Lewis-positive to Lewis-negative to occur in FUT3 heterozygous individuals, and it is our hypothesis that FUT3 heterozygosity predisposes to the non-genuine Lewis-negative phenotype, due to the lower enzyme activity and lower production of Lewis structures.

**Experimental Procedures**

**Patients and Samples**—Delipitated blood clots, urine deposits, and paraffin-embedded tissue samples from colonic tumors, as well as normal colon far from the tumor, were obtained from a previously published patient material (25). Saliva, peripheral leukocytes for DNA extraction, and plasma for Ca19–9 measurements were obtained from paraffin-embedded tissue samples from colonic tumors, as well as normal colon tissue from patients who had never had colorectal cancer. DNA was extracted using a standard proteinase K-based method (26). DNA was extracted from paraffin-embedded tissue samples using a boiling method. 20–m sections were deparaffinized by boiling for 5 min, proteinase K was added, and the m sections were counted by liquid scintillation counting.

**Preparation of Genomic DNA**—DNA was extracted using a standard proteinase K-based method (28). DNA was extracted from paraffin-embedded tissue samples using a boiling method. 20–m sections were deparaffinized by boiling for 5 min, proteinase K was added, and the sections were incubated overnight at 50 °C. Nucleic acids were extracted once with Tris-buffered phenol and then five times with a chloroform:methanol:water, 10:5:1. The solvent was removed, and the glycolipid remaining at the origin was extracted with 2–5-ml chloroform:methanol:water, 60:40:9, containing 0.02% CaCl2 as the final concentration (1). Radioactive glycolipid bands were located by autoradiography, scraped from the plate, and counted by liquid scintillation counting.

**DNA Sequencing**—PCR was used to amplify the open reading frame (ORF) and the sequence flanking the start codon of FUT3. For amplifying ORF and the flanking region, the sequences of the sense and antisense oligonucleotide primers, the sizes of the product obtained, and the annealing temperature of the PCR reaction, respectively, were as follows: ORF, 5′-ATG GAT CCC CTG GGT GCA GCC ACC CAA CTC TCT CAA GAC-3′; flanking region, 5′-CA GGA CTG AGG GCC ACC CCC GCG GGG AAT GAG CAC-3′; flanking region 2, 5′-GAA AGA TGA GGT TCC CGG CAG CCC AAG GCA GAG CAG GCT GCC ACC CCC AGC-3′; flanking region 3, 5′-GGG AGT GGT GTC CTG TCG GGA GGA CCC ACT-3′; flanking region 4, 5′-GGG AGT GGT GTG TCG CTG TCG GGA GGA CCC ACT-3′, 264 bp, 72 °C. The PCR products were purified with Wizard PCR prep DNA purification system (Promega, Madison, WI) according to the manufacturer protocol. Nucleotide sequencing was performed on the ORF on 6 individuals, p115, p216, p215, p214, p138, and p246 (see Table II), by cycle sequencing using a direct blotting system (DBS system, Synthecore, Munich, Germany), TOA DNA polymerase, and several dioxigenin-labeled oligonucleotides corresponding to internal sequences of the wild-type FUT3 gene.

**Detection of Single Base Mutations of the FUT3 Gene Using PCR-cleavage Assay Methods**—The following primers were used: VE115ms, 5′-CATGGGCGGCGGCGCTGGGCTGGG-3′; VE32ms, 5′-GGGAGTGCTGCTGGTTCGGGAGGACCCAT-3′; VE5s, 5′-CTGCGGGTGCTGTCTTCTGGCAACGAC-3′; VE6s, 5′-GCTGCGGGTGCTGTCTTCTGGCAACGAC-3′. The T1067A mutation was detected by restriction fragment-length polymorphism to establish which mutations inactivate the FUT3 alleles. As an internal positive control, the amplification of a 248-bp fragment for the human
Functional Importance of Lewis Gene Mutations

Fig. 1. Thin layer chromatographic analysis of reaction products derived from \(^{14}C\)-fucose transfer to glycolipid acceptors catalyzed by saliva specimens from both Lewis-positive and genuine Lewis-negative donors. Lanes 1 and 2 show results from a Lewis-positive individual. Lanes 3 and 4 show results from a genuine Lewis-negative individual. Lanes 1 and 3 show transfer to LcOse,Cer and lanes 2 and 4 show transfer to LcOse,Cer. The reaction was conducted as described under “Experimental Procedures,” and the labeled glycolipids were separated on high performance thin layer chromatography plates (Baker) using a solvent system composed of CHCl\(_3\)/CH\(_2\)OH/H\(_2\)O, 60:40:9, prior to autoradiography with Kodak X-OMAT x-ray film. The identities of each band, which were confirmed in separate experiments by thin layer chromatography immunostaining with specific antibodies after transfer of unlabeled fucose to the indicated acceptors, is shown in the margins.

SCO gene was used. The PCR program included a 2 min, 94 °C initial denaturation step, followed by 30 cycles consisting of 30 s at 95 °C, 30 s at 68 °C, and 1 min at 72 °C. Subsequently, the allele-specific PCR products were digested with restriction enzyme NlaIII and analyzed in agarose gels.

Determination of Ca19–9—Serum and plasma concentrations of tumor-associated carbohydrate antigen Ca19–9 was performed by a solid-phase, two-site chemiluminescent enzyme immunometric assay for use with the Immulite automated analyzer according to the manufacturer instructions (Diagnostic Products Corp., Los Angeles, CA). The upper limit of normal serum level was defined as 37 units/ml.

Transfection of COS7 Cells—FUT3 ORF was amplified from the individuals p216, who was wild-type, and p214, who was homozygous mutated at 202/314, by the use of Expand high fidelity polymerase (Boehringer Mannheim). PCR was performed according to the manufacturer protocol with the same primers used for sequencing ORF and the annealing temperature at 68 °C. The PCR fragments were ligated into a PCR II vector by the use of the TA cloning system from Invitrogen (Carlsbad, CA) and sequenced to exclude PCR errors using the ABI 373A DNA sequencer (Perkin-Elmer) and the ABI PRISM dye terminator cycle sequencing ready reaction kit with AmpliTaq polymerase, FS (Perkin-Elmer).

The inserts were cut out with Nael and EcoRI and blunt end ligated (26) into pMPE, an AAV-based expression vector, containing a CMV promoter (a generous gift from Dr. Ramila Philip, Applied Immunoscience, Santa Clara, CA). The resulting plasmids were transfected into COS7 cells using calcium phosphate-DNA co-precipitation (29).

Flow Cytometry Analysis—Transfected COS7 cells were harvested 65 h after transfection and labeled with mouse monoclonal anti-Lea (BioClone, Ortho Diagnostic Systems, Dortmund, FRG) and mouse monoclonal anti-H (Dakopatts, Copenhagen, Denmark). After incubation with primary antibody, the cells were washed and subsequently stained with fluorescein isothiocyanate-conjugated rabbit anti-mouse IgG (DAKO). Flow cytometry analysis was performed with a Becton Dickinson FACSort.

Northern Blot Analysis—Total RNA was isolated from transfected COS7 cells using Parascript RNA isolation kit (Genta Systems Inc., Los Angeles, CA) according to the manufacturer protocol. Two to three μg were electrophoresed in a denaturing formaldehyde gel, 2.2 M in running buffer (0.04 M MOPS, 0.01 M sodium acetate, 0.001 M EDTA) according to standard procedures (26), and transferred to a nylon membrane (Zeta-Probe; Bio-Rad). An 1125-bp DIG-labeled probe was amplified with the same primers and annealing temperature as for sequencing ORF using the PCR DIG probe synthesis kit (Boehringer Mannheim) according to the manufacturer protocol. Hybridization was carried out at 42 °C for 16–20 h. Blots were subsequently rinsed in 0.2× SSC, 0.1% SDS at 62 °C. DIG-labeled probes were detected by an enzyme-linked immunosay using the DIG nucleic acid detection kit (Boehringer Mannheim) according to the manufacturer protocol.

RESULTS

Lewis Gene Alleles in the Danish Population—Sequencing of Lewis-positive and Lewis-negative individuals in the FUT3 ORF was carried out on two PCR templates, one of 1125 bp covering the coding sequence and 39 bp into the 3′-flanking region and one of 264 bp covering the 5′-untranslated region and the first 174 bp of the ORF. With this approach, we identified three mutations, T202C, C314T, C445A, in Danish Lewis-negative Caucasians that differ from those previously shown to be frequent in Lewis-negative Asian people (17, 18). Other mutations found were identical with those found in Asian people, T59G, G508A, and T1067A (Fig. 2, and Table II). A set of PCR-cleavage assays that detected all 6 mutations was established by the use of natural restriction enzyme cleaving sites or by introducing restriction sites with mismatch primers (Table I and Fig. 3). In the group of genuine Lewis-negative individuals, several had four different mutations, two on each allele, and it seemed that 202/314 mutations were almost always coupled, similar to 59/508 and 59/1067 in previous publications (17, 18).

To verify the localization of the 202/314 mutations, we made allele-specific PCRs with sense primers that either matched the T or the G at position 59 at the 3′-prime-end (Fig. 4). In order only to amplify the mutated or wild-type alleles at position 59, a mismatch was also introduced 3 bases from the 3′-end, as this led to a complete separation of T-59 and G-59. The PCR data showed that the position 59 mutation never occurred on the same allele as the 202 and 314 mutations (Fig. 4), and the 202 and 314 mutations always occurred together in 38 individuals, indicating that they exist on the same allele. Cloning of an allele with both the 202 and 314 mutations supported this result (see below). Patient 123 was an exception, as 59 and 1067 occurred on an allele that also had the 202 mutation (see le6 in Fig. 2).

The combination of 59/1067 or 59/508 mutations in one allele leads to the production of an enzymatically inactive protein (17, 18). The same seemed to be the case with the 202/314 mutations as 11 individuals, homozygous for both these mutations, were Lewis-negative on erythrocytes and had no saliva enzyme activity (Table II). To support this finding, an allele with the mutation couple of 202/314 as well as the wild-type allele were cloned and transfected into COS7 cells. The cloned genes were sequenced to verify the correct orientation and the fact that no PCR artifacts had been introduced.

COS7 cells transfected with the wild-type FUT3 gene (the enzymatically active allele Le in Fig. 2) expressed Lewis a structure, as evidenced by flow cytometry (Fig. 5), and showed α1–3-fucosyltransferase activity with a number of acceptors, as expected from the literature (Table III) (30). COS7 cells transfected with a FUT3 gene harboring the 202/314 mutation showed absence of both Lewis a structure and fucosyltransferase activity (Fig. 5 and Table III) although the transgene was actively transcribed, as evidenced by the presence of large amounts of mRNA in Northern hybridization with an FUT3 probe (Fig. 6).

An allele with the 59/202/1067 mutations was cloned from a Lewis-negative individual (patient 123) who was 202/314 on the other allele. Sequencing confirmed the existence of the mutations 59 and 202 on the same allele (data not shown). It is already known that the 1067 mutation is deleterious for the enzyme function (17). The C445A mutation was cloned from a non-genuine Lewis-negative cancer patient, and sequencing showed the existence of this mutation and the 59 mutation on the same allele (data not shown). The C445A mutation occurred in only one non-genuine Lewis-negative individual (patient 115) and was not found in cancer patients nor in the healthy controls examined. In one individual, patient 173, the 59 mutation was present on one allele and 202/314 on the other. This patient had saliva α1–4FucT activity, probably due
to the allele with the 59 mutation which is enzymatically active, and also demonstrated Lewis antigens on immunostaining of colorectal tissue (data not shown).

Based on our observations, we propose that there are at least seven different FUT3 alleles (see Le, le1, and le3–7 in Fig. 2) in Denmark, containing six single base mutations. These are located at positions 59, 202, 314, 445, 508, and 1067 (Fig. 2). The allele named le2 in Fig. 2 was first demonstrated in Indonesians (17) and has not been detected in Denmark.

**Heterozygosity of the Lewis Gene Determines Levels of Lewis Enzyme Activity in Saliva and Sialyl-Lewis a Structure in Plasma**—The saliva α1–4-fucosyltransferase activity was measured in 37 healthy Lewis-positive controls by the use of lactotetra- and lactoneotetraosylceramide glycolipid acceptors. The mobility on thin layer chromatography plates leads to immediate product identification (Fig. 1). Synthesis of α1–2, α1–3, and α1–4 products (H, Lex, Lea (28)) were all measured, as the ubiquitously present α1–3 activity served as control of enzyme preservation in the samples.

The activity of the α1–4-fucosyltransferase in saliva was correlated to the allelic status of the individuals (Fig. 7A), as the level in the heterozygous individuals was lower than that in the homozygous wild-type individuals. The level of sialyl-Lewis a structures circulating in plasma was measured by immuno-

**Fig. 2. FUT3 gene alleles.** Schematic presentation of the open reading frame of the Lewis (FUT3) gene (Le) and the mutated alleles (le), which are functionally inactive and lead to the Lewis-negative phenotype. The nucleotide sequence is the same as previously published (13), mutated codons are shown, and the mutated nucleotide is indicated by ×.

**Table I**

| Mutation | Primer pairs | Annealing temperature | Product size in base pairs | Restriction enzyme | Fragment sizes in base pairs |
|----------|--------------|-----------------------|---------------------------|--------------------|-----------------------------|
|          |              |                       |                           |                    | Wild-type | Mutated |
| T59G     | VE1mms<sup>a</sup> | 65 °C                 | 140                       | Msp I              | 140 | 24 + 116 |
|          | EL3as<sup>b</sup> |                       |                           |                    |               |
| T202C    | VE2mms       | 63 °C                 | 332                       | Rsa I              | 209 | 209 |
|          | VE3as        |                       |                           |                    | 123 | 23 + 100 |
|          | VE4as        |                       |                           |                    | 139 | 105 + 34 |
|          |               |                       |                           |                    | 65  | 65 |
| C445A    | VE5s         | 60 °C                 | 162                       | Nla III            | 126 | 101 + 25 |
| G508A    | VE4as        | 65 °C                 | 944                       | Pvu II             | 36  | 36 |
|          | EL3s         |                       |                           |                    | 851 | 509 + 342 |
| T1067A   | EL9as        | 60 °C                 | 109                       | Hind III           | 93  | 93 |
|          | sn6          |                       |                           |                    |               |
|          | sn7          |                       |                           |                    |               |

<sup>a</sup> Mismatch sense primer.  
<sup>b</sup> Anti-sense primer.  
<sup>c</sup> Sense primer.
Identification of Individuals Who Lose Lewis Antigen Reactivity in Erythrocyte Membranes during Disease and Become “Non-genuine” Lewis-negative Individuals—The frequency of the different Lewis genotypes was estimated by applying a set of PCR assays (Fig. 3). In Lewis-positive individuals with saliva α1-4-fucosyltransferase activity and Lewis-positive erythrocytes, the frequency of heterozygous individuals was 40%, and the rest were homozygous wild type. Among genuine Lewis-negative individuals, without saliva enzyme activity and with Lewis-negative erythrocytes, some were homozygous for the 202/314 or 59/1067 mutations, and some were compound heterozygous with a combination of the 59/1067 and 202/314 alleles. In 30 Lewis-negative individuals with cancer, the saliva enzyme activity, plasma sialyl-Lewis a structures, and FUT3 genotype were determined (Table II). 10 of the 30 were identified as non-genuine Lewis-negative individuals.

The allele distribution in the non-genuine Lewis-negative group was significantly different from that found in the Lewis-positive groups (Fig. 8). The number of heterozygous individuals with the mutations 59 and 202/314 was significantly increased (p < 0.02, Fisher exact test) in the group of non-genuine individuals, and also, the blood group A1 phenotype was significantly (p < 0.05, Fisher) more common in the non-genuine group. Two of the three non-genuine Lewis-negative individuals, who were not FUT3 heterozygous, were blood group A1.

Especially, the 59 mutation was over-represented in the non-genuine Lewis-negative cancer patients. Perhaps some dominant negative activity specific for alleles with this mutation is operating. Also, since Lea levels vary so widely within both the heterozygote and homozygote groups, it seems possible that more than one contributing factor is operating. Perhaps a threshold of FUT3 activity exists, below which individuals (heterozygous or not) tend to convert to Lewis-negative phenotype.

These data indicate that individuals who are FUT3 heterozygous, A1, or both are the ones most liable to develop the non-genuine phenotype in relation to a biological burden like cancer or pregnancy. Combined with the data above, this could very well be explained by the lower enzyme activity and the lower synthesis of Lewis structures in heterozygous individuals, which may, consequently, have a weaker phenotype with a lower density of Lewis epitopes in the erythrocyte membrane. The increased presence of A1 in the non-genuine group is not unexpected as it is well known that A1 individuals are more difficult to Lewis type than other ABO subgroups.

**DISCUSSION**

We have identified five common and one rare single base missense mutation within the coding region of the FUT3 gene in Denmark. Two of the common mutations, located at nucleotide position 59 and 1067, correspond to mutations frequent for Lewis-negative individuals in Indonesia. The 1067 mutation is in the catalytic domain where it inactivates the enzyme, whereas the 59 mutation leads to a reduced enzymatic activity with type 1 as well as type 2 chain acceptors in vitro. But it has the same affinity (K_m) as the wild-type enzyme (17).

However, COS cells transfected with the gene carrying the 59
mutation have no expression of Lewis structures. This might be explained by an inappropriate membrane insertion of the enzyme as the 59 mutation is located in the trans-membrane domain, and the transfected cells seem to secrete their enzyme, at least partly (17). The Japanese population has a 508 mutation in addition to the 59 and 1067 mutations (18, 31). This mutation completely abolishes enzyme function when expressed in COS cells (18). The mutations 202 and 314 have been associated with the Lewis-negative phenotype (32, 33) in Sweden, and this paper reports them to be frequent in Danes.

Northern blots of the transfected COS cells showed large amounts of mRNA to be present in the cells transcribing the FUT3 gene. The mutations 202 and 314 have been associated with the Lewis-negative phenotype (32, 33) in Sweden, and this paper reports them to be frequent in Danes.

On the basis of previously published data as well as data in

| Group          | Pat. | Path. | Eryt. | Plasma S-Lea | 1–3FT   | 1–2FT   | FUT3 Mutations |
|---------------|------|-------|-------|--------------|---------|---------|----------------|
| Non-genuine   | 115Sq Inv. O ND 240 610 ND | TG C A | bp59 bp202 bp314 bp445 bp508 bp1067 |
|               | 102 Inv. A1 12.8 1130 758 277 | TG | |
|               | 101 Inv. O 50.6 1290 581 ND | T C C T | |
|               | 145 Inv. A1 30.5 332 408 167 | T G T C C T | |
|               | 100 Inv. A1 ND 114 161 127 96 58 | T G T C C T | |
|               | 105 Inv. A1 ND 127 96 58 | T G T C C T | |
|               | 173 Inv. A1 ND 200 570 ND | T G T C C T | |
|               | 138Sq Inv. A1 ND 28 57 12 | T G | |
|               | 147 Inv. A1 188 351 202 123 | T G | |
|               | 174 Inv. A1 ND 440 550 ND | T G | |
|               | 137 Inv. A1 ND 0 14 0 | T G T C C T | |
|               | 139 Inv. A1 <2.5 0 182 115 T G T C C T | T A |
|               | 141 ND A1 <2.5 0 39 15 T G T C C T | T A |
|               | 142 ND A1 <2.5 0 30 8 T G T C C T | T A |
|               | 148 Inv. O <2.5 0 221 63 T G T C C T | T A |
|               | 143 Inv. A1 <2.5 0 ND ND T G T C C T | T A |
|               | 140 Nonin O <2.5 0 172 102 T G T C C T | |
|               | 146 Inv. A1 <2.5 0 187 121 C C T T | |
|               | 126 Nonin A1 <2.5 0 39 15 C C T T | |
|               | 144 Inv. O <2.5 0 36 15 G G A T A | |
|               | 136 Nonin O <2.5 0 46 18 C C T T | |
|               | 103 Nonin O <2.5 0 191 48 C C T T | |
|               | 104 Inv. O <2.5 0 115 58 C C T T | |
|               | 107 Inv. B <2.5 0 58 28 C C T T | |
|               | 106 Nonin O <2.5 0 139 42 C C T T | |
|               | 108 Inv. O <2.5 0 191 28 C C T T | |
|               | 109 Nonin O <2.5 0 123 33 C C T T | |
|               | 127 Nonin O <2.5 0 90 22 C C T T | |
|               | 146 Inv. O <2.5 0 187 121 C C T T | |
|               | 126 Nonin A1 <2.5 0 39 15 C C T T | |
|               | 144 Inv. O <2.5 0 36 15 G G A T A | |
|               | 128 Nonin A1 <2.5 0 129 36 G G A T A | |
|               | 123 Nonin O <2.5 0 157 78 T G C C C T | T A |
| Lewis-positive healthy controls | 215Sq A1 3.6 100 153 90 | T C C T | |
|               | 289 O <2.5 228 546 183 T C C T | |
|               | 118Tc A1 3.9 53 109 32 T C C T | |
|               | 149 A1 6 105 95 74 T G | T A |
|               | 249 A1 <2.5 32 55 ND T G | T A |
|               | 248Sq A1 <2.5 118 294 25 25 T C C T | |
|               | 262 A 13.6 343 444 200 | |
|               | 291 B 14.8 70 144 35 | |
|               | 216Sq A1 7.4 160 100 77 | |
|               | 298 B 23.3 60 105 ND | |
| Lewis-negative healthy controls | 214Sq O <2.5 0 ND ND | C C T T | |

* a1–2FT activity was measured with the Lea acceptor (see Fig. 1).

b 59 was present on one allele and 202/314 on the other, as there was no saliva enzyme activity and Lea and Leb antigens on tissue sections by immunostaining, the patient was included as non-genuine.

† 59 was present on one allele and 202/314 on the other, as there was saliva enzyme activity and Lea and Leb antigens on tissue sections by immunostaining, the patient was included as non-genuine.
this paper, it is possible to list those alleles known to be able to lead to alteration of *FUT3* activity and altered Lewis phenotype (Fig. 2). The mutations 59 and 1067 seem to be widespread, all over the world. They frequently occur as single mutations in Indonesia, whereas in Japan and Europe, they are almost always co-localized. A similar finding is the co-localization of 202/314 mutations. RNA molecular size standards in kilobases are indicated to the right.

**TABLE III**

| Acceptor | Wild-type | 202/314 Mutant | pcDNA1 |
|----------|-----------|----------------|--------|
| N-acetyllactosamine | 492       | 80             | 65     |
| Lacto-N-biose I | 1616      | 101            | 62     |
| Methylated LNBI | 2267      | 40             | 22     |
| Lacto-N-fucopentaose | 320       | 50             | 50     |

* A generous donation from Dr. K. L. Matta.
activity in saliva is lower in heterozygous individuals than it is in homozygous wild-type individuals. As the enzyme is the primary gene product, the explanation for this is unambiguous and is in accord with data published on Indonesians (17). A statistical recalculation of the plasma α1–3-fucosyltransferase activity published (17) gives a mean value in six homozygous wild-type individuals of 7573 dpm and in 17 heterozygous individuals (all having a 59/1067 allele) of 3549 dpm, which is significantly different (p = 0.04; Student’s t test).

It is surprising that the difference in α1–4-fucosyltransferase activity level leads to a different level of circulating sialyl-Lewis a structure in plasma. This indicates that the assembly line of fucosylated structures in the Golgi is not oversaturated with fucosyltransferase activity but rather that the amount of fucosylated structures produced by a given cell is sensitive to just a two-fold increase in fucosyltransferase activity. Furthermore, these data suggest that the transcription and translation of the FUT3 gene is relatively stable among different individuals, and if not so, the gene dosage effect would have been completely obscured by individual regulations of transcription and translation. The variation we found among wild-type individuals agreed well with previous publications (17), and it seemed to be specific for each individual as repeated testing of individuals with either high or low enzyme activity showed the level to be highly reproducible (data not shown).

With the knowledge of the gene dosage effect in mind, we examined if this effect could explain the long standing mystery of erythrocyte membranes losing their Lewis antigens during pregnancy and during diseases such as cancer. Under circumstances such as those, we, and others, have identified non-genuine Lewis-negative individuals who change from Lewis-positive to Lewis-negative during pregnancy. They are, however, also mutated in the secretor gene so no Lewis b is formed. Although they are homozygous wild-type in FUT3, the third group has a low amount of circulating sialyl-Lewis a. The amount of Lewis a produced is partly fucosylated to Lewis b as they are wild-type in the secretor gene. The fourth group, has a barely detectable amount of circulating Lewis a as it is heterozygous in FUT3, and the Lewis b formed is partly fucosylated to Lewis b.

In conclusion, we have identified four common and three rare FUT3 alleles in a Danish population. The relatively common allele with the 202/314 mutations was shown by expression studies to be enzymatically inactive. Furthermore, we have shown that the effect of heterozygosity for these FUT3 alleles is a relatively low α1–4-fucosyltransferase activity in saliva and a relatively low plasma level of the sialyl-Lewis a structure. The lower enzymatic activity in heterozygous individuals seemed to be causally involved, together with the A1 phenotype, in the conversion of genuine Lewis-positive individuals to non-genuine Lewis-negative individuals during disease periods or during pregnancy.

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Functional Importance of Lewis Gene Mutations
Influence of Lewis α1-3/4-L-Fucosyltransferase (FUT3) Gene Mutations on Enzyme Activity, Erythrocyte Phenotyping, and Circulating Tumor Marker Sialyl-Lewis a Levels

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