Identification of a Missense Mutation (G329A; Arg\textsuperscript{110} → Gln) in the Human \textit{FUT7} Gene\textsuperscript{*}

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The human \textit{FUT7} gene codes for the α1,3-fucosyltransferase VII (Fuc-TVII), which is involved in the biosynthesis of the sialyl Lewis x (SLex\textsuperscript{a}) epitope on human leukocytes. The \textit{FUT7} gene has so far been considered to be monomorphic. Neutrophils isolated from patients with ulcerative colitis were examined for apparent alterations in protein glycosylation patterns by Western blot analysis using monoclonal antibodies directed against SLex\textsuperscript{a} and SLex\textsuperscript{a-related epitopes. One individual showed lower levels of SLex\textsuperscript{a} expression and an elevated expression of CD65s compared to controls. The coding regions of the \textit{FUT7} gene from this individual were cloned, and a G329A point mutation (Arg\textsuperscript{110} → Gln) was found in one allele, whereas the other \textit{FUT7} allele was wild type. No Fuc-TVII enzyme activity was detected in COS-7 cells transiently transfected with the mutated \textit{FUT7} construct. The \textit{FUT7} Arg\textsuperscript{110} is conserved in all previously cloned vertebrate α1,3-fucosyltransferases. Polymerase chain reaction followed by restriction enzyme cleavage was used to screen 364 unselected Caucasians for the G329A mutation, and a frequency of ≤1% for this mutation was found (3 heterozygotes). Genetic characterization of the family members of one of the additional heterozygotes identified one individual carrying the G329A mutation in both \textit{FUT7} alleles. Peripheral blood neutrophils of this homozygously mutated individual showed a lowered expression of SLex\textsuperscript{a} and an elevated expression of CD65s when analyzed by Western blot and flow cytometry. The homozygous individual was diagnosed with ulcer disease, non-insulin-dependent diabetes, osteoporosis, spondyloarthrosis, and Sjögren's syndrome but had no history of recurrent bacterial infections or leukocytosis.

Recruitment of leukocytes to sites of inflammation or infection is initiated by interaction of leukocytes with activated vessel wall endothelium leading to “rolling” of leukocytes along endothelial cell surfaces. This interaction subsequently leads to extravasation of leukocytes into the surrounding infected or inflamed tissue (1). E- and P-selectins, which are expressed on activated endothelial cells, are involved in this interaction (2–4). The third member of the selectin family, L-selectin, is involved when lymphocytes extravasate into secondary peripheral lymphoid organs, where it interacts with counter-receptors on the post-capillary high endothelial venules (HEV)\textsuperscript{3} (5).

All three selectins recognize glycoprotein counter-receptors that must be properly glycosylated for binding to occur. All glycans that have been described for efficient recognition by selectins are modified by α2,3-sialylation and α1,3-fucosylation, and the minimal common epitope for all selectins is the sialyl Lewis x (SLex\textsuperscript{a}, NeuAcα2–3Galβ1–4[Fuca1–3]GlcNAcβ1–3-) epitope (6).

The final step in the biosynthesis of the SLex\textsuperscript{a} antigen involves the action of an α1,3-fucosyltransferase (7). Of the six human α1,3-fucosyltransferases cloned so far, only three are expressed in leukocytes; Fuc-TIV (8–10), Fuc-TVII (11, 12), and the recently cloned Fuc-TIX (13). The expression levels of Fuc-TIX in human leukocytes is, however, significantly lower compared with Fuc-TIV and Fuc-TVII (13).

Fuc-TIV has a wide acceptor specificity for GlcNAc in polylactosamines and sialylated polylactosamines forming, for example, the Lewis x (Le\textsuperscript{a}, Galβ1–4[Fucα1–3]GlcNAcβ1–3) and CD65s (NeuAcα2–3Galβ1–4GlcNAcβ1–3Galβ1–4[Fucα1–3]GlcNAcβ1–3-) antigens. The Fuc-TVII acceptor specificity is restricted to the distal GlcNAc on α2,3-sialylated lactosamines forming the SLex\textsuperscript{a} antigen (14, 15). Although Fuc-TIV can synthesize SLex\textsuperscript{a} in vitro (14), Fuc-TVII has been proved to be crucial for the synthesis of SLex\textsuperscript{a} and selectin ligands on leukocytes (16, 17). In addition, Fuc-TVII expression in peripheral lymph HEV has been correlated with expression of L-selectin ligands (5, 18). Transfection of human lymphoid cell lines by antisense cDNA to selectively down-regulate Fuc-TIV suppressed SLex\textsuperscript{a} expression and E-selectin-mediated binding (19). Furthermore, mice made deficient in the Fuc-TVII enzyme showed blood leukocytosis, deficiency in expression of selectin ligand activity, impaired neutrophil trafficking in inflammation, and defects in lymphocyte recirculation, strongly establishing a role for Fuc-TVII in selectin ligand synthesis (20).

Several of the cloned α1,3-fucosyltransferases are highly polymorphic in humans. Point mutations inactivating or disrupting Fuc-TIII (α1,3/1,4-fucosyltransferase, Lewis enzyme) give rise to Lewis negative phenotypes (21–24). Inactivating mutations have also been found in the \textit{FUT6} gene coding for the plasma α1,3-fucosyltransferase, Fuc-TVI (25–28). However, there have been no reports on genetic polymorphism in

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\textsuperscript{3}The abbreviations used are: HEV, high endothelial venule; SLex\textsuperscript{a}, sialyl Lewis x; SLex\textsuperscript{a}, sialyl Lewis a; Le\textsuperscript{a}, Lewis x; Fuc-T, fucosyltransferase; PMN, polymorphonuclear leukocyte; LAD II, leukocyte adhesion deficiency type II; MOPS, 4-morpholinopropanesulfonic acid; FITC, fluorescein isothiocyanate; TBS, Tris-buffered saline; PCR, polymerase chain reaction; BSA, bovine serum albumin; PBS, phosphate-buffered saline; PBS-T, phosphate-buffered saline plus Tween 20; bp, base pair(s).}
was used to amplify the coding regions and immediately adjacent 5'- and 3'-flanking regions of FUT7 using 0.25 μg of cDNA as template. The PCR program used an initial temperature of 8 °C for 10 min, followed by 40 amplification cycles run for 15 s at 95 °C, 1 min at 59 °C, and 3 min at 68 °C. The last extension step was kept for 10 min at 68 °C. The PCR program used an initial temperature of 85 °C for 10 min, followed by 30 amplification cycles run for 15 s at 60 °C, 1 min at 59 °C, and 3 min at 68 °C. The last extension step was kept for 10 min at 68 °C. The 7-3s primer (25 pmol), VII-4as (5'-gtgattagttgctgtgcttgagagcttg-3'), is complementary to nucleotides 20 to 1 and contains additional nucleotides (lowercase) at its 5' end, including an EcoRI (underlined) restriction site. The antisense primer (25 pmol), VII-4as (5'-gtgattagttgctgtgcttgagagcttg-3'), was designed to cover the sequence over the splice site in the FUT7 gene from nucleotides 29 to 111; r2as, 5'-ccgtgccaag-3', corresponding to nucleotides 338 to 559, were used to amplify a 338-bp fragment of FUT7 cDNA. The G329A mutation abolishes the restriction site 3'-flanking regions of the FUT7 gene from DNA amplified from whole blood. The PCR program used an initial temperature of 85 °C for 10 min, followed by 30 amplification cycles run for 15 s at 60 °C, 1 min at 59 °C, and 3 min at 68 °C. The last extension step was kept for 10 min at 68 °C. The 7-3s primer (25 pmol), VII-4as (5'-gtgattagttgctgtgcttgagagcttg-3'), and the 7-4as antisense primer (30 pmol) were used. The 1404-bp PCR product was treated with NotI and analyzed by gel electrophoresis, the wild type allele was digested into two products of 247 and 91 bp, whereas the mutant allele remained intact. Complete sequencing of these ten clones was done on an Alf II-express the Cy5-dye terminator kit (Amersham Pharmacia Biotech). One wild type clone and one clone containing the G329A point mutation without PCR-induced errors were chosen for subcloning of the FUT7 insert into the pSI mammalian expression vector (Promega Corp.). The resulting plasmid containing the FUT7 wild type was called pSI-wt, and the plasmid containing the mutant construct was called pSI-329.

Cloning of the FUT7 Gene from Genomic DNA—PCR was used to amplify two coding regions and the 253-bp intron (33) as well as the immediately adjacent 5'- and 3'-flanking regions of the FUT7 gene from DNA amplified from whole blood. The PCR program used an initial temperature of 85 °C for 10 min, followed by 30 amplification cycles run for 15 s at 60 °C, 1 min at 59 °C, and 3 min at 68 °C. The last extension step was kept for 10 min at 68 °C. The 7-3s primer (25 pmol), VII-4as (5'-gtgattagttgctgtgcttgagagcttg-3'), and the 7-4as antisense primer (30 pmol) were used. The 1404-bp PCR product was digested into two products of 247 and 91 bp, whereas the mutant allele remained intact. Complete sequencing of these ten clones was done on an Alf II-express the Cy5-dye terminator kit (Amersham Pharmacia Biotech). One wild type clone and one clone containing the G329A point mutation without PCR-induced errors were chosen for subcloning of the FUT7 insert into the pSI mammalian expression vector (Promega Corp.). The resulting plasmid containing the FUT7 wild type was called pSI-wt, and the plasmid containing the mutant construct was called pSI-329.

Transfection—COS-7 cells (10^6 cells) cultured in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum were transfected with 10 μg of expression vector constructs using the DEAE-dextran method (34). The cells were transfected with pSI-wt and pSI-329 as well as with the AmpliTag DNA polymerase FS kit (PerkinElmer Life Sciences, Foster City, CA) on an Applied Biosystems 373A DNA sequencer (PerkinElmer Life Sciences).

Transfection efficiency was controlled using quantitative PCR analysis. Total RNA was isolated from transfected cells according to the manufacturer's instructions using a Total RNA kit (Promega Corp.) including treatment with DNase. A reverse transcription kit (Promega Corp.) was used according to the manufacturer's instructions to transcribe 1 μg of total RNA.

Quantitative PCR analysis was performed using the TaqMan PCR Core Reagent kit (PE Biosystems). Reactions for FUT7 quantification were performed in 30 μl with 0.2 μg of cDNA, 3 μl of 10× TaqMan Buffer A (500 mM KCl, 100 mM Tris-HCl, pH 8.3); 5 mM MgCl<sub>2</sub>; 200 μM each of dATP, dCTP, and dGTP; 400 μM dUTP; 0.3 units of uracil-N-glycosidase; 0.75 units of AmpliTaq Gold DNA polymerase; 50 nM FUT7 probe; and 100 nM FUT7 sense and antisense primers. The following FUT7 consensus primers and probe were used: r1s, 5'-CCCTGACTGACTCTGCC-3' (nucleotides −29 to −11); r2as, 5'-CTCCCCAGCTGCTG-3' (nucleotides 28 to 41); and FUT7 probe, 5'-CGTGGCTCGAGCTTCCA-3' (nucleotides −3 to 20). The PCR program used an initial temperature of 95 °C for 1 min and then 75 °C for 1 min, followed by 40 amplification cycles run for 15 s at 95 °C and 1 min at 60 °C. The...
amplifications were performed on an ABI Prism 7700 sequence detector equipped with a 96-well thermal cycler. Data were collected and analyzed with Sequence Detector version 1.6.3 software (PE Biosystems). Reactions for quantifying β-actin were performed exactly as described above except for using 3.5 mM MgCl₂, and 300 nM sense primer (5'-TCACCCACACTGGCCATCTAGCA-3') and 300 nM antisense primer (5'-CACGGCAACCCGCTAGGCTATTGCCAATGG-3'), and 200 nM β-actin probe (5'-ATGCCCCCCCATGCGATCTTGGT3'- (PE Biosystems). All analyses were performed in triplicate and with probes labeled with 6-carboxyfluorescin and 6-carboxytetramethylrhodamine.

**Immunofluorescence Analysis of Lewis Antigen Expression on the Surface of Transfected COS-7 Cells**—The transfected cells were trypsinized, washed, and incubated with primary antibodies against CD65s, SLε⁺, SLε⁻, and Le⁺. After 30 min of incubation with the primary antibody, the cells were washed with PBS without Ca²⁺ and Mg²⁺ and incubated another 30 min with fluorescein-conjugated rat anti-mouse IgG secondary antibody. After incubation with the secondary antibody, the cells were washed with PBS without Ca²⁺ and Mg²⁺. The cell pellets were then fixed with Mowiol (Hoechst, Frankfurt am Main, Germany) and paraformaldehyde (4%, pH 7.3) at a ratio of 1/3 (v/v) and mounted on glass. The cells were observed under a Leitz SM-LUX epifluorescence microscope. Immunofluorescence studies were also conducted on adherent cells in eight-well tissue culture slides (Nunc Inc., Naperville, IL) without using trypsin treatment.

**Flow Cytometry—Enzyme activity was analyzed by measuring the incorporation of GDP-[¹⁴C]fucose, 300 mCi/mmol (Amersham Pharmacia Biotech), to a sialylated type 2 acceptor substrate, NeuAc₂-β-Galβ1-4-GlcNAcβ1-s-biotin or a sialylated type 1 acceptor substrate, NeuAc₂-β-Galβ1-3-GlcNAcβ1-s-biotin (Syntosomes, Munich, Germany). COS-7 cells transfected with pSI, pSI-wt, or pSI-329 were lysed in 50 mM MOPS buffer (pH 7.5) containing 1% Triton X-100. Apparent Km for GDP-Fuc was determined using Lineweaver-Burk plots with GDP-Fuc concentrations between 2 and 10 μM and an acceptor concentration of 10 mM. Apparent Km for the sialylated type 2 acceptor was determined with acceptor concentrations between 0.25 and 10 mM and a GDP-Fuc concentration of 100 μM. The assay was initiated with the addition of cell lysate (45 μg of protein) to a reaction mixture containing GDP-Fuc, acceptor, 10 mM α-L-fucose, and 10 mM MnCl₂ in 50 mM MOPS buffer (pH 7.5). The mixture was incubated at 37 °C for 2 h. The product was purified by the Sep-Pak C₁₈ isolation procedure (35), and analyzed by liquid scintillation counting. Product formation was also measured at 0.5, 1, and 2 h and found to be linear in this time range.

**Mutation Screening by Restriction Endonuclease Analysis—Genomic DNA isolated from 5 ml of EDTA anticoagulated blood according to Ref. 36, was amplified by primers VII-15s and VII-5as. The 338-bp product was sequenced over the intron present in the FUT7 gene and GOI5 was found to be heterozygous in one allele. The G329A nucleotide change leads to an amino acid substitution between the two alleles from this individual. However, a G329A nucleotide change was present in both alleles from a control sample (lanes A and B). Molecular size standards are indicated to the left.

**RESULTS**

**Identification of a Patient with an Abnormal Expression of SLε⁺ and CD65s on her PMN—PMN lysates were analyzed by Western blot analysis to detect differences in expression of SLε⁺ and CD65s. All PMN samples analyzed from healthy volunteers expressed a similar set of SLε⁺-carrying glycoproteins with most intensely stained bands in the molecular mass region at ~90–115 kDa. A representative sample is shown in Fig. 1 (lane B). VIM-2 antibody directed against the CD65s epitope weakly stained glycoproteins with molecular masses of ~60–70 kDa (Fig. 1, lane D). Western blot analyses of PMN lysates from patients with ulcerative colitis showed staining patterns comparable to the healthy population. However, one of the patients (M. N.) exhibited a different staining pattern. The Western blot analysis of PMN lysate from this individual showed a significant reduction in the staining of SLε⁺-bearing glycoproteins. The staining intensity was about 60% compared with control samples for identical amounts of total protein (Table I and Fig. 1 (lane A)). In addition, staining of one band in the 100-kDa region was selectively lost. This pattern was seen using two different antibodies (KM-93, CSLEX-1) both known to react with SLε⁺, albeit with somewhat different binding properties (Ref. 37 and data not shown). Western blot analysis of PMN lysates from this patient (M. N.) using the VIM-2 antibody directed against the CD65s epitope, showed an increased staining (480%) compared with control samples (Fig. 1, Table I). This patient was analyzed both at the time of active disease and in clinical remission at several occasions during a 2-year period. The reduced expression of SLε⁺ and elevated expression of CD65s remained constant during this time.

**Lowered SLε⁺ Expression Correlates with a G329A Mutation in the Gene Coding for Fucosyltransferase VII—**The lowered SLε⁺ expression in PMN of patient M. N. indicated a potential defect in the Fuc-TVII enzyme. The gene coding for Fuc-TVII, **FUT7**, was amplified from genomic DNA by PCR and TA cloning. Plasmids were isolated from 13 bacterial clones and sequenced over the FUT7 insert. The two exons and the 253-bp intron present in the FUT7 gene were sequenced in both directions. There were no differences in the intron sequences between the two alleles from this individual. However, a G329A missense mutation was found in 7 out of the 15 bacterial clones, indicating that M. N. carried this mutation heterozygously in one allele. The G329A nucleotide change leads to an amino acid shift from arginine to glutamine at position 110. Sequence alignment (38) showed that FUT7-Arg¹¹⁰ is conserved in all 16 of the α1,3-fucosyltransferases cloned so far from vertebrate species (13, 39–41).

**Screening for G329A by Restriction Endonuclease Analysis—**A restriction fragment length polymorphism assay was
used to screen for the G329A mutation in DNA preparations from 106 plasma donors in Göteborg and 258 unselected adults in the Linköping area. In this population, three additional individuals carrying the G329A mutation heterozygously were identified. The overall frequency of the G329A mutation in the analyzed populations was 0.82%.

Identification of an Individual Homozygous for the G329A Mutation in FUT7—DNA from another of the identified heterozygotes (M. L.) was cloned and sequenced. This confirmed the presence of the G329A mutation in one allele and no other mutations or alterations in the coding sequences or in the intron sequence. NotI restriction endonuclease analysis of M. L. and 5 of her family members are summarized in Fig. 2. Apart from the heterozygous individual M. L., her brother (R. J.) and both of her daughters (A. L. and L. L.) also showed a cleavage pattern consistent with heterozygous expression of the G329A mutation. Her husband did not carry the G329A mutation. However, the PCR product obtained from the mother of M. L. (S. J.) was not digested at all, which indicated a homozygous expression of the G329A mutation (Fig. 2). The two FUT7 exons and the intron were completely sequenced in both directions from 14 clones obtained from this individual. All clones contained the G329A mutation. No other nucleotide changes were found. This individual thus carried the isolated mutation in both of her FUT7 alleles. When PMN lysates prepared from this individual were analyzed by Western blot using antibody KM93 directed against SLex, there was an almost complete lack of expression of SLex-binding glycoproteins compared with control samples (Table I and Fig. 3 (lanes A and B)). When the same samples were analyzed using the VIM-2 antibody directed against CD65s, a marked increase in the expression of this epitope was found for this individual (Fig. 3, lane C). The increased staining intensity was 980% compared with control samples (Table I and Fig. 3 (lane D)) and 205% compared with individual M. N.

Flow Cytometry Analysis of PMN from the Individual Homozygous for the G329A Mutation in FUT7—The expression of SLex on PMN from individuals with or without the G329A mutation was investigated using flow cytometry. Most of the PMN from the homozygous individual (S. J.) showed a KM93 staining just above background. However, a subpopulation of cells from this individual showed an intermediate staining with this antibody. Antibody KM93 reacted strongly with PMN from an individual lacking the G329A mutation (Fig. 4A). Staining of PMN with the anti-SLex antibody CSLEX-1 showed the same pattern with lower expression for the homozygous individual and a higher expression for an individual lacking the G329A mutation (Fig. 4B). In contrast to the results obtained by Western blot, there was no major differences between these individuals in staining of PMN with the anti-CD65s antibody VIM-2 (Fig. 4C). As expected, PMN from all analyzed individuals expressed a high level of Lea (Fig. 4D). Sialidase treatment of PMN prior to flow cytometry analysis reduced binding of KM93, CSLEX-1, and VIM-2 antibodies to background levels (data not shown).

Cell Surface Expression of SLex Is Not Detected on COS-7 Cells Transfected with FUT7 G329A cDNA—The Western blot and flow cytometry analyses of PMN from hetero- and homozygously mutated individuals indicated that the Arg$^{110}$ → Gln substitution affects Fuc-TVII activity. To confirm this, COS-7 cells were transiently transfected with plasmids containing either the mutated or the wild type FUT7 cDNA sequence (pSI-329 and pSI-wt, respectively). Mock transfectants using vector only (pSI) were used as negative controls. After transfection the expression of SLex, CD65s, Lea, and SLea was analyzed by immunofluorescence staining. Cells transfected with pSI-wt were clearly stained with anti-SLex antibody (Fig. 5A), whereas there was no staining of cells transfected with pSI-329 with this antibody (Fig. 5B). The same pattern was seen using both KM93 and CSLEX-1 antibodies (data not shown). This indicated that the G329A mutation significantly reduces the activity of Fuc-TVII in transfected COS-7 cells. Neither of the transfectants was stained with antibodies directed against

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**Table I**

| PMN lysates | SLex<sup>a</sup> (KM-93) | CD65s (VIM-2) |
|------------|-------------------------|---------------|
| M. N.      | 100*                    | 100*          |
| S. J.      | 0.2                     | 205.0         |
| Controls   | 169.3 ± 22.2            | 20.9 ± 17.5   |

<sup>a</sup> Value set to 100.
**FUT 7 Mutation**

**Fig. 4.** Flow cytometry analysis of isolated PMN from the FucT-VII Q/Q individual (S. J.) (gray histograms) and a FucT-VII R/R individual (white histograms). Negative control is shown as black histograms. A, KM93 antibody; B, CSLEX-1 antibody; C, VIM-2 antibody; D, anti-Lea antibody.

**Fig. 5.** Immunostaining of COS-7 cells transfected with pSI-wt (A) and pSI-329 (B). Transfected cells were incubated with primary antibody against SLex (KM-93) and fluorescein-conjugated rat antimouse IgG secondary antibody.

**TABLE II**

Analysis of fucosyltransferase VII activity

| Specific activity (pmol/(min mg protein)) | K_{m} acceptor | K_{m} GDP-Fuc (μM) |
|-----------------------------------------|----------------|-------------------|
| pSI-wt                                  | 10.4 ± 1.3     | 6.0 ± 0.8         |
| pSI-329                                 | 30.0 ± 0.6     | 5.0 ± 0.4         |
| pSI                                     | ND             | ND                |

* a NeuAcα2–3Galβ1–4GlcNAc-1-sp-biotin (10 mM).

**TABLE III**

Analysis of mRNA content; number of cycles before ∆Rn reaches the threshold

| FUT7          | Mean | S.D. | Mean | S.D. |
|---------------|------|------|------|------|
| pSI           | 40.00| 0.00 | 18.16| 0.54 |
| pSI-wt        | 30.37| 0.05 | 17.32| 0.09 |
| pSI-329       | 29.80| 0.50 | 17.62| 0.13 |
| pSI           | 40.00| 0.00 | 17.64| 0.04 |
| pSI-wt        | 31.22| 0.29 | 17.86| 0.11 |
| pSI-329       | 30.06| 0.70 | 18.23| 0.05 |

This mutation gives an amino acid shift from an arginine to a glutamine at position 110 in Fuc-TVII (Arg110 → Gln). When the G329A mutation was screened for in two small Swedish populations, 3 out of 364 individuals were found heterozygous for this mutation (Fuc-TVII R/Q). Although a larger population must be examined to ascertain the exact overall frequency of this mutation, this indicates that it might be carried by 1% of the population. FUT7 should thus be considered to be a polymorphic gene, especially since the G329A allele might be only one of several mutated alleles to be found in various populations around the world. Genetic analysis of the family members of one of the identified heterozygotes revealed an individual carrying the G329A mutation in both alleles (Fuc-TVII Q/Q). The two exons and the 253-bp intron of FUT7 (33) were fully sequenced in two of the identified heterozygotes (M. N. and M. L.) and in the homozygote. Apart from the G329A mutation, there was no other structural alteration compared with the wild type FUT7 sequence.

Western blot analysis of PMN lysates and flow cytometry of PMN showed that individuals carrying the G329A mutation had a lowered expression of SLex, which is consistent with the hypothesis that the G329A mutation affects Fuc-TVII activity.

**DISCUSSION**

When analyzing the expression of SLex and SLex-related antigens on PMN from patients with ulcerative colitis, one patient with decreased expression of SLex was identified. The FUT7 gene of this individual was cloned and sequenced, and a single point mutation, G329A, was found in one of the alleles.
CD65s. Flow cytometry analysis of PMN from the Fuc-TVII Q/Q individual also indicated an increased surface expression of CD65s compared to Fuc-TVII R/R individuals. However, the increase was not as pronounced as seen in the Western blot analysis. Since there is a possible competition among Fuc-TVII, Fuc-TIV, and Fuc-TIX for the same sialylated polylactosamine acceptor substrate, a lowered activity of Fuc-TVII would theoretically increase the substrate availability for Fuc-TIV and Fuc-TIX, which would explain the observed increase in CD65s expression (Fig. 7). Surprisingly, the major increase in CD65s antigens was detected on proteins in the 60–70-kDa region, whereas the expression of SLex was mainly detected on proteins migrating in the 90–115-kDa region. This would suggest that the observed phenotypic changes are not only explained by substrate availability. Previous studies have shown a reciprocal expression of Fuc-TVII and Fuc-TIV during differentiation of HL60 cells and in HL60 cells deficient in FUT7 expression (42, 43), indicating a linked transcriptional regulation of these enzymes. The possible effect of the FUT7 mutation on the transcriptional levels of fucosyltransferases in PMN must be studied further. Furthermore, there is always a possibility that the analyzed individuals in this study may have other differences in glycosyltransferase activity in addition to the lowered activity of Fuc-TVII, which would affect the glycoprotein profiles obtained in the Western blot analysis.

The phenotypic changes observed in individuals carrying the G329A mutation suggested a decreased activity of the Fuc-TVII enzyme. To study the effect of the G329A mutation in more detail, COS-7 cells were transfected with wild type and mutated FUT7 constructs. COS-7 cells transfected with the FUT7 gene containing the G329A mutation did not express SLex on the cell surface in contrast to cells transfected with the wild type FUT7 construct. In addition, there was no detectable α1,3-fucosyltransferase activity in whole cell lysate of COS-7 cells transfected with the mutated construct when an α2,3-sialylated lactosamine acceptor was used as substrate. The reported \( K_m \) values of Fuc-TVII are in the low millimolar range when Neu5Acα2-3Galβ1–4GlcNAc is used as acceptor (44, 45). The obtained \( K_m \) value for the sialylated type 2 acceptor used in the present study was 6 mM. \( K_m \) for GDP-Fuc was 5 μM. An acceptor concentration of 10 mM and a GDP-Fuc concentration of 100 μM would ensure an individual reaction rate at saturating acceptor concentrations, but still there was no detectable activity in the cells transfected with the pSI-329 construct. Even when incubation with the cell lysate was prolonged to 18 h, the activity in the COS-7 cell transfected with the mutant construct gave the same incorporation as the mock-transfected COS-7 cells, indicating that the Arg\(^{110} \rightarrow \) Gln substitution inactivates the Fuc-TVII enzyme. There was an overexpression of FUT7 transcripts in both cells transfected with the wild type and mutated constructs, and the levels of transcripts were similar for both constructs. This indicates that the decrease in enzymatic activity in the COS-7 cells was not an effect of reduced transcription efficiency for the mutated FUT7 construct. The lack of activity when an α2,3-sialylated type 1 chain was used as acceptor was to be expected, as only Fuc-TVII was overexpressed in the COS-7 cells and this enzyme specifically recognizes only the sialylated type 2 chain acceptor (11, 12, 15, 44).

Western blots using the polyclonal anti-Fuc-TVII antiserum positively identified the expected 39-kDa band in pSI-wt- and pSI-329-transfected cells in about equal quantities. Interestingly, the cells transfected with pSI-wt, but not those transfected with pSI-329, showed an additional specific band at 55 kDa. This heavier band might correlate to a heterodimer or a highly glycosylated form of the enzyme and imposes an interesting question on the structural and functional consequences of the G329A mutation. The molecular explanation for the lack of this band is now under focus and will be the subject of a separate publication.

Sequence alignment showed that Fuc-TVII-Arg\(^{110} \) is conserved in all 16 of the α1,3-fucosyltransferases cloned so far from vertebrate species (18, 39, 40). This amino acid is found just in between the hypervariable regions of α1,3-fucosyltransferases considered to be responsible for the acceptor binding domain and the peptide motifs presumed to be involved in the GDP-fucose binding (41). This arginine residue has not before been directly linked to enzymatic activity or specificity. It remains to be studied whether the Arg\(^{110} \rightarrow \) Gln substitution directly affects enzyme activity or if the substitution affects other functions of the enzyme such as ER or Golgi retention and degradation. One of the naturally occurring mutations found to inactivate the Lewis enzyme (Fuc-TIII) has been found to induce susceptibility to protease digestion rather than directly affecting enzymatic binding sites (46).

The role of Fuc-TVII in the synthesis of selectin ligands has been demonstrated in vivo using antisense oligonucleotides.

\[
\text{Neu5Acα2-3Galβ1-4GlcNAeβ1-3Galβ1-4GlcNAe-R}
\]

**FucT-VII**

**FucT-IV**

\[
\text{SLex} \quad \text{CD65s (VIM-2)}
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(19). The role of Fuc-TVII in vivo has also been clearly indicated by the generation of mice completely deficient in this enzyme (20). These mice showed blood leukocytosis, nonexistent binding of leukocytes to E- and P-selectin, impaired neutrophil trafficking in inflammation, and defects in lymphocyte recirculation. However, Fuc-TVII-deficient mice did not develop a phenotype as severe as mice deficient in E- and P-selectin. E/P-selectin-deficient mice exhibit extreme leukocytosis, systemic infections, and plasma cell proliferation (47), implying that lack of Fuc-TVII would not completely abolish all functional selectin ligands. The role of Fuc-TIV in generating selectin ligands has been debated. However, recent studies on mice deficient in Fuc-TIV and/or Fuc-TIV support a role for Fuc-TIV in selectin-dependent adhesion of leukocytes (48). Although Fuc-TVII seems to play the major role in generating selectin ligands, it is clear that inactivation of both Fuc-TIV and Fuc-TII is needed to completely inhibit leukocyte adhesion to activated endothelium. In addition, several studies have shown that specific cell lines can synthesize selectin ligands to activated endothelium. In addition, several studies have shown that specific cell lines can synthesize selectin ligands to activated endothelium. In addition, several studies have shown that specific cell lines can synthesize selectin ligands to activated endothelium. In addition, several studies have shown that specific cell lines can synthesize selectin ligands to activated endothelium. In addition, several studies have shown that specific cell lines can synthesize selectin ligands to activated endothelium. In addition, several studies have shown that specific cell lines can synthesize selectin ligands to activated endothelium. 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Identification of a Missense Mutation (G329A; Arg$^{110}$ → Gln) in the Human FUT7 Gene

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