A Missense Mutation in the FUT6 Gene Results in Total Absence of \( \alpha_3 \)-Fucosylation of Human \( \alpha_1 \)-Acid Glycoprotein*

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The major \( \alpha_3 \)-fucosyltransferase activity in human plasma is encoded by the gene for fucosyltransferase VI (FUT6). A missense mutation (Gly-739 \rightarrow Ala) in this gene is responsible for deficiency of enzyme activity in plasma. To examine whether this fucosyltransferase is the sole enzyme responsible for the \( \alpha_3 \)-fucosylation of serum glycoproteins in the liver, we studied the fucosylation of three glycoproteins in sera of individuals with or without inactivated FUT3 and/or FUT6 gene(s) but with a functional FUT5 gene. \( \alpha_3 \)-Acid glycoprotein was used as the principal reporter protein for liver \( \alpha_3 \)-fucosyltransferase activity, because of its high fucose content. In all individuals with the FUT6 missense mutation Gly-739 \rightarrow Ala in double dose, no fucosylation of \( \alpha_3 \)-acid glycoprotein was found. This \( \alpha_3 \)-acid glycoprotein was not intrinsically resistant to fucosylation, since it was susceptible to in vitro fucosylation using an \( \alpha_3 \)-fucosyltransferase isolated from human milk. The same result was found for \( \alpha_1 \)-antichymotrypsin and \( \alpha_1 \)-protease inhibitor. On the other hand in all individuals with \( \alpha_3 \)-fucosyltransferase activity in plasma, \( \alpha_3 \)-fucosylated glycoforms of the glycoproteins studied were found. The degree of fucosylation of \( \alpha_3 \)-acid glycoprotein was correlated with \( \alpha_3 \)-fucosyltransferase activity (Rs = 0.82). These data indicate that the product of FUT6, but not of FUT3 or of FUT5, is responsible for the \( \alpha_3 \)-fucosylation of glycoproteins produced in liver and suggest that this organ is a major source of \( \alpha_3 \)-fucosyltransferase activity in plasma.

The \( \alpha_3 \)-fucosyltransferases (\( \alpha_3 \)-FTs)\textsuperscript{1} show a high degree of sequence similarity (1–5). Five genes encoding Fuct-III (EC 2.4.1.65), -IV, -V, -VI (EC 2.4.1.152), and -VII have been cloned and named FUT3 to FUT7 in the Genome Data Base (1–5). These enzymes differ in capacity to transfer fucose to distinct oligosaccharide acceptors, sensitivity to N-ethylmaleimide inhibition, cation requirements, and tissue-specific expression patterns (1–13). The FUT4-encoded enzyme (myeloid type, FucT-IV) is highly expressed in all tissues of 5–10-week-old human embryos and is then replaced by other \( \alpha_3 \)-FT enzymes. In adults, the FUT4 gene remains only expressed in myeloid cells and brain (6). The FUT3-encoded enzyme (Lewis type, FucT-I) has been found in gallbladder, kidney, and milk (2, 6), whereas the tissue distribution for the FUT5-encoded enzyme (FucT-V) is unknown (3, 11). The FUT6-encoded enzyme (plasma type, FucT-VI) is present in plasma, renal proximal tubules, and hepatocytes (6, 7, 11, 13), whereas the FUT7 gene is expressed in leukocytes (4, 5). In these cells, the FUT4 gene product seems to be responsible for the synthesis of the Lewis\textsuperscript{a} (Le\textsuperscript{a}) antigen, whereas the FUT7-encoded enzyme (leuko type, FucT-VII) appears to be a major \( \alpha_3 \)-FT involved in the synthesis of the sialyl-Lewis\textsuperscript{a} (SLex\textsuperscript{a}) antigen. Because of high sequence similarity and overlapping acceptor specificities of the \( \alpha_3 \)-FTs, it has not been possible to establish unequivocally which of the \( \alpha_3 \)-FTs is responsible for the \( \alpha_3 \)-fucosylation in human hepatocytes (13).

The tissue origin of the \( \alpha_3 \)-FT activity in plasma is not known. In an \( \alpha_3 \)-FT-deficient individual with a congenital kidney anomaly only about 10% of \( \alpha_3 \)-FT activity remained in plasma, and this activity appeared to have a myeloid enzyme character (6, 14). As the FucT-IV activity in the leukocytes of this individual was normal, the remaining activity in plasma may originate from leukocytes. The majority of the \( \alpha_3 \)-FT activity in plasma must therefore have another tissue origin. In the above-mentioned patient, however, there was no expression of Le\textsuperscript{a} antigen in the kidney (14), suggesting that this organ may contribute to the \( \alpha_3 \)-FT activity in plasma (6, 14). In addition to the kidney the liver is a likely candidate because it expresses the plasma-type enzyme (FUT6 gene product) (6, 7, 13). This organ is also known to be the source of the majority of plasma proteins as well as of another plasma glycosyltransferase, the \( \alpha_6 \)-sialyltransferase (15, 16). Plasma \( \alpha_3 \)-FT activity is mainly controlled by the FUT6 gene, and the Gly-739 \rightarrow Ala inactivating missense mutation results in the deficiency of this \( \alpha_3 \)-FT activity (11). We have exploited this experiment of nature to examine in sera of individuals with or without inactivation of FUT3 and/or FUT6 genes, but with functional FUT5 gene (11, 17), whether the \( \alpha_3 \)-fucosylation of \( \alpha_3 \)-acid glycoprotein (AGP), \( \alpha_3 \)-antichymotrypsin (ACT), and \( \alpha_1 \)-protease inhibitor (PI), three serum glycoproteins secreted by the liver, was affected. This would identify the \( \alpha_3 \)-FT responsible for the \( \alpha_3 \)-fucosylation of glycoproteins in the liver. AGP was selected as the principal reporter protein in this study because of its high fucose content and because it carries fucose residues in \( \alpha_3 \)-linkage only (18–22). The extent of \( \alpha_3 \)-fucosylation of AGP, ACT, and PI was measured in the sera of individuals with and without inactivating point mutation(s) in the FUT3 and FUT6 genes.
Missense Mutation in FUT6 Results in No Fucosylation of AGP

Table I

| Individual | Lewis phenotypes | Mutation(s) in genotypes | Plasma 3-FT activity | Relative distribution of AGP glycoforms in serum |
|------------|------------------|--------------------------|----------------------|-----------------------------------------------|
|            |                  |                          | A0       | Aw     | As   |
| Code 1a    | Code 2b          | FUT3d                  | dpm  %   | %      | %    |
| 1          | J.P. E           | -e                     | 1067/1067 | 739/739 | 80   |
| 2          | M.L. E           | -e                     | 59/59; 1067/1067 | 739/739 | 90   |
| 3          | M.P. E           | -e                     | 1067/1067 | 739/739 | 100  |
| 4          | D.I              | -e                     | 1067/1067 | 739/739 | 109  |
| 5          | E.S             | -e                     | 1067/1067 | 739/739 | 122  |
| 6          | A.S             | -e                     | 1067/1067 | 739/739 | 132  |
| 7          | J.L             | +e                     | 1067/1067 | 739/739 | 69   |
| 8          | J. S            | +e                     | 1067/1067 | 739/739 | 52   |
| 9          | 167S           | e                      | 1067/1067 | 739/739 | 10342|
| 10         | 163S           | e                      | 1067/1067 | 739/739 | 11357|
| 11         | 160S           | e                      | 1067/1067 | 739/739 | 13761|
| 12         | S.T. E          | -e                     | 59/7477 | 39/31 | 30 |
| 13         | G.D. E          | -e                     | 1067/1067 | 739/739 | 11500|
| 14         | L.O. E         | -e                     | 1067/1067 | 739/739 | 23360|
| 15         | B.E             | -e                     | 1067/1067 | 739/739 | 15160|
| 16         | H.E             | -e                     | 1067/1067 | 739/739 | 14300|
| 17         | H.9            | -e                     | 1067/1067 | 739/739 | 7477  |
| 18         | C.B. E          | -e                     | 1067/1067 | 739/739 | 21351|
| 19         | G.L. E         | -e                     | 1067/1067 | 739/739 | 320  |
| 20         | A.E. E         | -e                     | 1067/1067 | 739/739 | 32360|
| 21         | 118S           | -e                     | 1067/1067 | 739/739 | 11500|
| 22         | 137S           | -e                     | 1067/1067 | 739/739 | 12000|
| 23         | A.2            | -e                     | 1067/1067 | 739/739 | 1600  |
| 24         | C.2            | -e                     | 1067/1067 | 739/739 | 4060  |
| 25         | G.1            | -e                     | 1067/1067 | 739/739 | 13260|
| 26         | A.5            | -e                     | 1067/1067 | 739/739 | 3230  |

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Missed antigen expression, FUT3 and FUT6 inactivating mutations, 3-FT activity in plasma, and distribution of AGP glycoforms

For 26 individuals Leα and Leβ antigen expression on erythrocytes and in saliva were determined (22). Immunoelectrophoresis in a second perpendicular dimension in the presence of a precipitating monospecific antiserum (Dakopatts, Glostrup, Denmark) in a 1% agarose (Bio-Rad) gel resulted in precipitation of the separated glycoforms, which were stained with Coomassie Brilliant Blue R250 (Sigma), and the areas under the curves, representing the relative amounts of glycoprotein, were determined with a Summagraph (ACEDAD D-9000) (25).

In vitro Fucosylation—Nonfucosylated glycoproteins were fucosylated in vitro using an α3-FT preparation isolated from human milk (26). The incubation mixtures (25 μl) contained 10–12 μl of serum of an individual with the FUT6 mutation (containing 10 μg of AGP, 4 μg of ACT, and 15 μg of PI); 4 mM ATP, 20 mM MnCl2, 0.2 mM GDP-fucose, 123 microunits of human milk fucosyltransferase, 50 mM sodium cacodylate (pH 7.2), 0.1 M NaCl, 50% glycerol, 0.05% sodium azide. The mixtures were incubated at 37 °C for 2 h.

Statistics—The correlation between 3-FT activity in plasma and reactivity of AGP from these sera with the fucose-specific lectin AAL was determined using the Spearman rank correlation coefficient.

RESULTS AND DISCUSSION

In 26 individuals of different FUT3 and FUT6 genotypes the α3-FT activity in plasma was compared with the extent of fucosylation of AGP. Table I shows Lewis antigen expression, mutations in FUT3 (17, 27–29), and FUT6 (11) genes, α3-FT activity, and relative distribution of the differently fucosylated glycoforms of AGP. No inactivating mutation in FUT5 has been found as yet (11).

The extent of α3-fucosylation of AGP in sera of these individuals was established by CAIE with the fucose-specific lectin AAL. By this method AGP is fractionated into a nonreactive form (A0), AGP fractions that are not reactive with AAL, Aw, AGP fractions that are weakly reactive with AAL; As, AGP fractions that are strongly reactive with AAL (cf. Fig. 1), and strongly reactive glycoforms of AGP, with fucose in in-
The same results were obtained with two other serum glycoproteins, ACT (Fig. 1, d and e) and PI (Fig. 1, g and h). In vitro fucosylation of PI in individuals with the Gly-739 → Ala mutation gives an almost similar fractionation pattern as found for PI of normal sera (cf. Fig. 1, h and i). The retarded glycoforms of PI present in sera of individuals with the Gly-739 → Ala mutation is due to fucosylation of a 6-fucosylation of PI, which is also retarded by AAL (22, 30).

Only the FUT6 Gly-739 → Ala mutation in double dose results in lack of α3-FT activity in plasma (11). FUT3 alleles containing Thr-1067 → Ala or Gly-508 → Ala mutations or the allele containing both Thr-202 → Cys and Cys-314 → Thr mutations encode for proteins with no Lewis enzyme activity (17, 27–29). Inactivating mutations are present in individuals 1–6 in both FUT3 and FUT6 genes. Therefore, from the results obtained with these individuals alone it cannot be concluded whether the product of FUT3 or FUT6 is responsible for the fucosylation of these glycoproteins in liver. However, the fact that AGP, ACT, and PI are not α3-fucosylated in individuals 7 and 8, who show the FUT6 mutation in double dose and the FUT3 mutations in single dose, proves that these proteins are only α3-fucosylated by the FUT6-encoded enzyme (FucT-VI) and not by the FUT3-encoded enzyme (FucT-III) in liver. This conclusion is further supported by the fact that individuals 9–26, all showing α3-FT activity in plasma, which has been suggested before (13). In contrast with the report of serum glycoproteins of individuals 1–8, all deficient in α3-FT activity in plasma, these proteins were α3-fucosylated (Fig. 1, c, f, and i) in individuals 9–26, all showing α3-FT activity. Furthermore, this α3-FT activity in plasma in the latter group of individuals strongly correlates to the extent of fucosylation of AGP (Rs = 0.82, Fig. 2). This correlation in apparently healthy individuals is in accordance with earlier findings in individuals with certain pathologies. Increased activity of human plasma α3-FT activity has been described in liver cirrhosis (32) and for various cancers (33–35). An increase in fucosylation of human AGP (36) and other serum glycoproteins has also been found in these conditions (37–39). During acute inflammation we found an increase in fucosylation of α3-FT activity in plasma (data not shown). So far, no particular pathology was found in individuals showing no α3-FT activity in plasma and having nonfucosylated AGP. Several immunomodulatory activities have been described for this glycoprotein that are dependent on its glycosylation (40). Therefore, the implications of the presence of nonfucosylated AGP should be further investigated.

In conclusion, this study shows that AGP, ACT, and PI are α3-fucosylated by the product of the FUT6 gene in liver and that no other fucosyltransferase genes, like FUT3 or FUT5,
Plasma fucosyltransferase activity (dpm)

FIG. 2. Fucosylation of AGP versus α3-FT activity in plasma. The fucosylation of AGP was correlated with the α3-FT activity in plasma in individuals with this plasma activity. The Spearman rank correlation coefficient was determined between these two parameters in individuals with fucosylation of AGP (open circles) and was 0.82 (p < 0.001) between these two parameters.

take over when inactivating mutations in the FUT6 gene are present.

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