A novel member of the human β-galactoside α2,6-sialyltransferase (ST6Gal) family, designated ST6Gal II, was identified by BLAST analysis of expressed sequence tags and genomic sequences. The sequence of ST6Gal II encoded a protein of 529 amino acids, and it showed 48.9% amino acid sequence identity with human ST6Gal I. Recombinant ST6Gal II exhibited α2,6-sialyltransferase activity toward oligosaccharides that have the Galβ1,4GlcNAc sequence at the nonreducing end of their carbohydrate groups, but it exhibited relatively low and no activities toward some glycoproteins and glycolipids, respectively. It is concluded that ST6Gal II is an oligosaccharide-specific enzyme compared with ST6Gal I, which exhibits broad substrate specificities, and is mainly involved in the synthesis of sialyloligosaccharides. The expression of the ST6Gal II gene was significantly detected by reverse transcription PCR in small intestine, colon, and fetal brain, whereas the ST6Gal I gene was ubiquitously expressed, and its expression levels were much higher than those of the ST6Gal II gene. The ST6Gal I gene was also expressed in all tumors examined, but no expression was observed for the ST6Gal II gene in these tumors. The ST6Gal II gene is located on chromosome 2 (2q11.2-q12.1), and it spans over 65 kb of human genomic DNA consisting of at least eight exons and shares a similar genomic structure with the ST6Gal I gene. In this paper, we have shown that ST6Gal II, which has been known as the sole member of the ST6Gal family, also has the counterpart enzyme (ST6Gal II) like other sialyltransferases.
glycosphingolipids, and sialyloligosaccharides. It has also been described previously (23, 24). ST6Gal II was amplified by PCR using primers 5'-TTGGAATCTCATCAGGTGACGTCATTTTGGTG-3' (nucleotides 1913–1931) and 5'-AGACGTCATTTTGGTGACACCTGAAG-3' (complementary to nucleotides 1491–1519 in Fig. 1A, the synthetic EcoRI site being underlined) and 5'-ACTTGGATCATACAGACATACGACACATGTTG-3' (complementary to nucleotides 1797–1819) and 5'-CGAGTTGTCGACCACTCCCGAG-3' (nucleotides 1514–1533) and 5'-CAAGAATTCTTCTCTAGGAAAGAGGATGCTG-3' (complementary to nucleotides 1475–1502, the synthetic EcoRI site being underlined), with the first strand cDNA of human colon was designed pcDNA-ST6Gal II/short.

The expression vector of the soluble ST6Gal II was constructed as follows. The DNA fragment encoding the whole coding region of human ST6Gal II was amplified by PCR using primers 5'-TTATGATTCACACCTGAAG-3' (nucleotides 309–331 of GenBank™ accession number NM_003032) and 5'-CCGGTGTTCTCCAGGATGTAAG-3' (complementary to nucleotides 1519–1539) with human liver cDNA as a template and cloned into plBamHI site of pcDNS-1 vector. Then the 1.1-kb EcoRI fragment encoding a truncated form of ST6Gal II (lacking the first 43 amino acids of the coding region) was prepared and subcloned into the EcoRI site of pcDNA, which was designated pcDNA-ST6Gal I.

We also constructed an expression vector containing the whole coding region of ST6Gal II. The 1.8-kb HindIII fragment containing the coding region of ST6Gal II was prepared from the cloned cDNA and subcloned into the HindIII site of the expression vector pRC/cmV, which was designated pRCcmv-ST6Gal II.

Preparation of Soluble Sialyltransferases—For production of soluble forms of sialyltransferases, COS-7 cells were transfected with the above pcDNA vectors using LipofectAMINE™ reagent (Invitrogen) and cultured as described previously (23). The amino-acid sequence for sialyltransferases expressed in the medium was assayed by IgG-Sepharose gel (Amersham Biosciences) and used as the enzyme source.

Sialyltransferase Assays and Product Characterization—Sialyltransferase assays were performed as described previously (25, 26). In brief, enzyme activity was measured in 50 mM MES buffer (pH 6.0), 1 mM MgCl₂, 0.5 mM CaCl₂, 10% glycerol, and 0.5 μM CMP-[14C]NeuAc and 1 μM acceptor substrate, and an enzyme preparation in a total volume of 10 μl. As acceptor substrates, 10 μg of glycoproteins, 5 μg of glycolipids, or 10 μg of oligosaccharides were used. The enzyme reaction was performed at 37 °C for 3–20 h. For glycoproteins, the reaction was terminated by the addition of SDS-PAGE loading buffer, and the reaction mixtures were subjected directly to SDS-PAGE. For glycolipids, the reaction mixtures were applied to a Sep-Pak Vac C₁₈ column (100 mg; Waters, Milford, MA), and purified glycolipids were subjected to high performance thin-layer chromatography (HPTLC) (Silica-Gel 60; Merck) with a solvent system of chloroform, methanol, and 0.02% CaCl₂ (55:45:10). For oligosaccharides, the reaction mixtures were directly subjected to HPTLC with a solvent system of 1-propanol, aqueous ammonia, and water (6:1:2.5). The radioactive materials were visualized and quantified with a Fuji BAS2000 radioimage analyzer. The intensity of the radioactivity was converted into moles using the radioactivities of various amounts of CMP-[14C]NeuAc (12.0 GBq/mmol, 925 kBq/ml) as standards. Quantification was performed within the linear range of the standard radioactivity.

For kinetic analysis, the reaction was performed as described above except using various concentrations of acceptor substrates. Under these conditions, the product formation from the individual acceptor substrates was linear up to 4 h. Kinetic parameters were determined by Lineweaver-Burk plots.

For linkage analysis of sialic acids, [14C]NeuAc-incorporated Galβ1,4GlcNAc with ST6Gal I or II was digested with β-galactosidase (from bovine testes; Sigma) or linkage-specific exosialidase. NANAase I (specific for α₂,3-linked sialic acids; Glyko, Inc.) or NANAse II (specific for α₂,3- and α₂,6-linked sialic acids; Glyko, Inc.). After the above treatment, reaction mixtures were subjected to HPTLC with a solvent system of 1-propanol, aqueous ammonia, and water (6:1:2.5). The radioactive materials were visualized with the BAS2000 radioimage analyzer.

Analysis of ST6Gal I and II Gene Expression in Various Human Tissues and Tumors—Relative expression levels of ST6Gal I and II mRNAs were estimated by RT-PCR using human multiple tissue cDNA panels. For the analyses of ST6Gal I gene expression, ST6Gal II-specific primers 5'-AGAGCTCAATTTTGTTGCTGCTTGGG-3' (nucleotides 1264–1286) and 5'-TTAACAGGTTGAATGACCGTGG-3' (complementary to nucleotides 1745–1765) were used. For the analysis of ST6Gal II gene expression, ST6Gal I-specific primers 5'-TTATGATTCACACCTGAAG-3' (nucleotides 309–331 of GenBank™ accession number NM_003032) and 5'-CAAGAATTCTTCTCTAGGAAAGAGGATGCTG-3' (complementary to nucleotides 658–680) were used. As a control, glyceraldehyde 3-phosphate dehydrogenase (G3PDH) gene expression was also measured using G3PDH-specific primers 5'-GGATCCACAGCTGTCGATACC-3' and 5'-AAGCTTACCACCCCTGGTCTGTA-3' (27). PCR mixture was performed in a total volume of 50 μl containing 10 mM Tris-HCl (pH 9.4) for 1 h at 75 °C and 72 °C for 90 s for ST6Gal I and II genes and 25 cycles for G3PDH and 72 °C for 10 min. The PCR products were electrophoresed on a 2% agarose gel, stained with ethidium bromide and visualized under UV light.
A Novel Human β-Galactoside α2,6-Sialyltransferase, ST6Gal II

RESULTS

Cloning and Nucleotide Sequencing of a New Sialyltransferase cDNA—Using the human expressed sequence tag and high throughput genomic sequence databases, we found some sequences (GenBank™ accession numbers BE612797, BE613250, and BF038052 (EST clones) and AC005040, AC016994, and AC108049 (genomic sequences)) with similarity to human ST6Gal I. These sequences were distinct from those of the sialyltransferases cloned previously, suggesting that these clones encode a novel member of the sialyltransferase family. The above EST clones were obtained from the Image Consortium, but these clones did not contain the expected entire coding sequence of the novel sialyltransferase. As these EST clones were considered to lack the DNA sequence encoding the COOH-terminal region of the new sialyltransferase, we performed RT-PCR to obtain it with the human colon cDNA as a template. However, we could not amplify the corresponding region as a single fragment by RT-PCR with another primer set (see Fig. 5), and this fragment encoded the same amino acid sequence with the above clone. From the plasmid containing the combined DNA fragment, the 0.5-kb AatII-XhoI fragment was prepared, and this was ligated into the AatII-XhoI sites of the plasmid containing the EST clone BE612797, and the DNA fragment encoding the new sialyltransferase having the expected amino acid sequence was obtained.

The nucleotide sequence of the putative new sialyltransferase cDNA and its deduced amino acid sequence are shown in Fig. 1A. The predicted protein consists of 529 amino acids with a calculated molecular mass of 60,157 Da and with five potential N-linked glycosylation sites. The position of the initiation codon was estimated according to the Kozak consensus sequence (28). Hydrophathy analysis (29) indicated one prominent hydrophobic sequence of 19 amino acids in length in the NH2-terminal region, predicting that the protein has type II transmembrane topology characteristic of many other glycosyltransferases cloned to date (Fig. 1B). Comparison of the deduced amino acid sequence with those of other human sialyltransferases showed significant sequence identity in two regions, sialyl motifs L (41.7–65.9%) and S (26.1–56.5%). The overall amino acid sequence of the predicted protein showed the highest sequence identity with ST6Gal I (48.9%) (Fig. 2). These results strongly suggest that the predicted protein belongs to the sialyltransferase family, especially the ST6Gal-family. Thus, we tentatively designated the new sialyltransferase as ST6Gal II. It is striking that the stem region of ST6Gal II, which is located between the transmembrane domain and the active domain, is very long like mouse, chicken, and human ST6GalNAc I (30–32).

We also found by extensive database searches that ST6Gal II is related closely to KIAA1877 proteins (GenBank™ accession numbers AB058780 and XM_038616), whose cDNA clones were isolated as one of unidentified human genes by a sequencing project (33). The exon 1 of the AB058780 clone is different from that of the ST6Gal II gene (Fig. 1A). The start codon of the AB058780 clone is not identified, but this clone has essentially the same amino acid sequence with ST6Gal II except it has five additional amino acid residues in its NH2-terminal region. The XM_038616 clone has been reported as a protein consisting of 463 amino acids that shares the COOH-terminal 234 amino acids with ST6Gal II at first. The differences in the NH2-terminal region were caused by frame shifts of the coding region. However, this sequence was updated recently, and it was shown that the XM_038616 clone has the same amino acid sequence with ST6Gal II. In addition, there is a splicing variant of ST6Gal II that has short different amino acid sequence in the COOH-terminal region and lacks most of the sialyl motif (GenBank™ accession numbers BC008680 and BE613250; see Fig. 1A, Short form).

Sialyltransferase Activity of the Newly Cloned Enzyme—To facilitate determination of the enzymatic activity of the new sialyltransferase, we constructed the expression plasmid pcDNA-ST6Gal II, which allows expression of ST6Gal II lacking the transmembrane domain as a secretable protein fused with the IgG-binding domain of S. aureus protein A. The plasmid was then transfected into COS-7 cells, and the protein A-fused ST6Gal II expressed in the medium was adsorbed to IgG-Sepharose resin, which was used as the enzyme source. For comparative analysis, the protein A-fused ST6Gal II short form and ST6Gal I were also prepared. As shown in Table I and Fig. 3, ST6Gal II exhibited activity toward oligosaccharides Galβ1,4GlcNAc and lacto-N-neotetraose, both of which have Galβ1,4GlcNAc structure at the nonreducing end of their car-
bohydride groups. The apparent $K_m$ values of ST6Gal II for Gal$_{1,4}$GlcNAc and lacto-N-neotetraose were estimated to be 0.71 and 0.48 mM, respectively, which were significantly lower than that of ST6Gal I for Gal$_{1,4}$GlcNAc (2–10 mM) (34). However, ST6Gal II did not exhibit activity toward oligosaccharides such as Gal$_{1,3}$GalNAc, Gal$_{1,3}$GlcNAc, lactose, and lacto-N-neotetraose, all of which do not contain Gal$_{1,4}$GlcNAc structure at the nonreducing end of their carbohydrate groups. ST6Gal II also exhibited relatively low activity toward some glycoproteins, which are considered to have Gal$_{1,4}$GlcNAc structure at the nonreducing end of their carbohydrate groups. However, ST6Gal II did not exhibit activity toward glycolipids examined in this study, including paragloboside, which has Gal$_{1,4}$GlcNAc structure at the nonreducing end of its carbohydrate group. We also examined the enzymatic activity of the short form of ST6Gal II lacking most of the sialyl motif S (Fig.

**Table I**

Acceptance substrate specificity of human ST6Gal I and II

Various acceptor substrates were incubated in the standard assay mixture using soluble sialyltransferase fused with protein A as an enzyme source. Each substrate was used at the concentration of 0.5 mg/ml for glycolipids and 1 mg/ml for glycoproteins and oligosaccharides. Relative rates are calculated as a percentage of the incorporation obtained with Gal$_{1,4}$GlcNAc. $R$ represents the remainder of the $N$-linked oligosaccharide chain. *, 1.03 pmol/h/ml medium; **, 8.14 pmol/h/ml medium.

| Acceptors | Representative structures of carbohydrates | ST6Gal II | ST6Gal I |
|-----------|-------------------------------------------|-----------|-----------|
| Oligosaccharides | | | |
| Type II | Gal$_{1,4}$GlcNAc | 100* | 100** |
| Type I | Gal$_{1,3}$GlcNAc | 0 | 4.2 |
| Type III | Gal$_{1,3}$GlcNAc | 0 | 8.7 |
| Lactose | Gal$_{1,4}$Glc | 0 | 31.1 |
| Lacto-N-tetraose | Gal$_{1,3}$GlcNAc,1,3Gal$_{1,4}$Glc | 0 | 101.6 |
| Lacto-N-neotetraose | Gal$_{1,4}$GlcNAc,1,3Gal$_{1,4}$Glc | 86.2 | 101.6 |
| Glycoproteins | | | |
| Fetuin | NeuAc$_{2,3}$Gal$_{1,3}$GlcNAc-O-Ser/Thr | 0 | 13.0 |
| NeuAc$_{2,3}$Gal$_{1,3}$GlcNAc-O-Ser/Thr | 3.9 | 95.0 |
| NeuAc$_{2,6}$Gal$_{1,4}$GlcNAc-R | 0 | 0 |
| Asialofetuin | NeuAc$_{2,6}$GalNAc-O-Ser/Thr | 3.9 | 95.0 |
| NeuAc$_{2,3}$Gal$_{1,4}$GlcNAc-R | 0 | 0 |
| BSM | NeuAc$_{2,6}$GalNAc-O-Ser/Thr | 0 | 0 |
| Asialo-BSM | NeuAc$_{2,3}$Gal$_{1,4}$GlcNAc-R | 0 | 0 |
| Ovomucoid | NeuAc$_{2,3}$Gal$_{1,4}$GlcNAc-R | 0 | 0 |
| Asialovomucoid | NeuAc$_{2,6}$GalNAc-O-Ser/Thr | 0 | 0.3 |
| Sialic acid glycoprotein | NeuAc$_{2,6}$GalNAc-O-Ser/Thr | 0 | 0.3 |
| Asialo-Sialic acid glycoprotein | NeuAc$_{2,3}$Gal$_{1,4}$GlcNAc-R | 0 | 0.3 |
| Glycolipids | | | |
| Lactosylceramide | Gal$_{1,4}$Glc$_{1,4}$Cer | 0 | 0 |
| GM1a | Gal$_{1,4}$GlcNAc,1,4Gal$_{1,3}$Glc$_{1,4}$Cer | 0 | 0 |
| GM1b | Gal$_{1,4}$GlcNAc,1,4Gal$_{1,3}$Glc$_{1,4}$Cer | 0 | 0 |
| GM3 | Gal$_{1,4}$GlcNAc,1,4Gal$_{1,3}$Glc$_{1,4}$Cer | 0 | 0 |
| Paragloboside | Gal$_{1,4}$GlcNAc,1,4Gal$_{1,3}$Glc$_{1,4}$Cer | 0 | 0 |

**Fig. 2. Sequence comparison of human ST6Gal I and II.** The conserved amino acid residues are boxed. Sialyl motifs L and S are double underlined and dashed underlined, respectively. The conserved His and Glu residues in sialyl motif VS are marked with asterisks.
RESULTS indicated that ST6Gal II transfers sialic acid on to galactose; thus we performed semiquantitative RT-PCR to examine the expression levels of the ST6Gal II gene in various tissues. The expression levels of the ST6Gal II gene in various tissues were too low to be detected by Northern blotting. The expression levels of the ST6Gal II gene in various tumors can be attributed to the ST6Gal I activity but not the ST6Gal II activity.

Genomic Organization of the ST6Gal II Gene—To know the genetic and evolutional relation of the ST6Gal II gene with other sialyltransferase genes, we analyzed the genomic organization of the ST6Gal II gene by database search. The genomic sequences containing the ST6Gal II gene (GenBank™ accession numbers AC005040, AC016994, and AC108049) were obtained and analyzed by the BLAST search of the human genome database using ST6Gal II-related cDNA sequences (GenBank™ accession numbers AB059555, BE613250, AB058780, and XM_038616) as queries. A schematic representation of the most probable genomic structure of the ST6Gal II gene is shown in Fig. 6. We found that the ST6Gal II gene is located on chromosome 2 (2q11.2-q12.1), and it spans over 85 kb of human genomic DNA consisting of at least eight exons (see Table II and Fig. 6A). The existence of exons 1a and 1b encoding different 5′-untranslated regions was suggested by sequence analysis of some ST6Gal II-related clones. The COOH-terminal region of the active form of ST6Gal II is encoded by exon 6b, whereas that of the short inactive form is encoded by exon 6a. The sequences of the splice junctions of the ST6Gal II gene obey the GT-AG rule (35) (Fig. 6B). Some amino acid residues in the exon/intron boundaries of the ST6Gal I and II genes are highly conserved (Fig. 6B). In our previous study, we found that codons for Arg in the sialyl motif L are highly conserved as a splice junction among many mouse sialyltransferase genes. Exceptionally, the codon for Asp in the sialyl motif L is a splice junction of the mouse ST6Gal I gene (36) (Fig.

![Fig. 3. Incorporation of sialic acids into various oligosaccharides by human ST6Gal I and II.](Image)

![Fig. 4. Linkage analysis of incorporated sialic acids by human ST6Gal I and II.](Image)
7). We also found that the codon for Asp in the sialyl motif L is a splice junction of the human ST6Gal II gene. In addition, the split patterns of the coding sequences for sialyl motif S of the ST6Gal I and II genes are different from the ST3Gal I and II genes and ST6GalNAc I and II genes (Fig. 7). Comparison of the exon/intron boundaries and exon sizes suggests that the ST6Gal I and II genes have a similar genomic structure (37) (see Fig. 6 and Fig. 8).

DISCUSSION

So far, ST6Gal I has been known as the sole member of the \( \beta \)-galactoside \( \alpha \),2,6-sialyltransferase family for more than ten years. However, the existence of other \( \beta \)-galactoside \( \alpha \),2,6-sialyltransferases that have different substrate specificities or preferences from ST6Gal I has been expected (34). With the progress of the human genome project, the extensive database search enabled us to detect the second type of \( \beta \)-galactoside \( \alpha \),2,6-sialyltransferase (ST6Gal II).

As shown in this study, ST6Gal II exhibited activity toward oligosaccharides containing Gal\( \beta \)1,4GlcNAc structure at the nonreducing end of their carbohydrate groups, but it exhibited weak or no activity toward glycoproteins and glycolipids, respectively. We also examined the \textit{in vivo} activity of ST6Gal II by transfecting the expression vector of full-length ST6Gal II cDNA (pRo/CMV-ST6Gal II) into several kinds of cultured cells. However, significant changes were not observed in the sialylation pattern of glycoproteins in these cells analyzed by S. nigra lectin blotting (data not shown). Together with the \textit{in vitro} substrate preference of ST6Gal II, it is most likely that in \textit{vivo} substrates of ST6Gal II are oligosaccharides containing Gal\( \beta \)1,4GlcNAc structure at the nonreducing end, although there remains a possibility that some glycoproteins and/or glycolipids may be sialylated specifically by ST6Gal II. The ST6Gal I knock-out mice exhibited great loss of cell surface Sia\( \beta \)2,6Gal\( \beta \)1,4GlcNAc structures and hallmarks of severe immunosuppression (22). These indicate that ST6Gal II are not involved in the production of cell surface Sia\( \beta \)2,6Gal\( \beta \)1,4GlcNAc structures and cannot compensate for the ST6Gal I activity in immune system (the existence of mouse ST6Gal II has been suggested by some EST clones; GenBank\textsuperscript{TM} accession numbers BB552328, BB633550, BB651169, and BB666153). Therefore, it can be said that ST6Gal II is an oligosaccharide-specific enzyme compared with ST6Gal I, which exhibits broad substrate specificities toward glycoproteins, glycolipids, and oligosaccharides. Although the main substrate of ST6Gal I \textit{in vivo} has been considered as glycoproteins, it is also likely that ST6Gal I is significantly involved in the synthesis of sialyloligosaccharides in some tissues. Our \textit{in vitro} analysis showed that ST6Gal I can sialylate not only Gal\( \beta \)1,4GlcNAc and lacto-\( N \)-neotetraose but also Gal\( \beta \)1,3GlcNAc, lactose and lacto-\( N \)-tetraose. On the other hand, ST6Gal II cannot sialylate Gal\( \beta \)1,3GlcNAc, lactose and lacto-\( N \)-tetraose (see Fig. 3 and Table I). This suggests that some
kinds of sialyloligosaccharides are produced by ST6Gal I only. The biological importance of sialyloligosaccharides produced by ST6Gal II is unclear at present, but expression of the ST6Gal II gene seems to be regulated developmentally or tissue-specifically (Fig. 5), suggesting that sialyloligosaccharides produced by ST6Gal II may play important roles in various biological phenomena. Sialyloligosaccharides are considered to play important roles in physiological functions in infancy, such as growth and development (38). Therefore, it may be possible that sialyloligosaccharides produced by ST6Gal II in the fetal brain are involved in brain development or function.

It has been reported that some sialyloligosaccharides in human milk have growth-promoting effects on bifidobacteria and lactobacilli present in the intestinal flora (38). The predomi-
nant bifidobacteria flora in the intestinal tract is considered to inhibit the growth of harmful bacteria, such as pathogenic strains of *Escherichia coli*, and protect infants against gastrointestinal diseases. Moreover, sialyloligosaccharides have been considered to have inhibitory activity against the binding of cholera toxin B subunit to its receptor, GM1 (38). Fluid accumulation of cholera toxin-induced diarrhea in rabbit intestine was also clearly reduced by the presence of sialyllactose (38). Many of the Sia-binding pathogens exhibit a preference for the /H231/H9252/2,3-sialyl linkage (39), but it is considered that compounds

![Fig. 7. Split patterns of sialyl motifs. Split positions of exons encoding sialyl motifs are indicated by vertical lines. The highly conserved amino acid residues are marked with asterisks.](#)

![Fig. 8. Comparison of the genomic structures of the human sialyltransferase genes. The genomic structures of 20 sialyltransferase genes are presented. The protein coding region and the untranslated region are shown by filled rectangles and open rectangles, respectively. Untranslated regions are not necessarily shown to scale. It should be noted that the genomic structure of the ST3Gal V gene would show more similarity to those of ST3Gal III, IV, and VI genes if the exons 6 and 9 of the ST3Gal V gene were split at appropriate positions. It should be also noted that the genomic structure of the ST8Sia III gene would show more similarity to those of the ST8Sia II and IV genes if the exon 3 of the ST8Sia III gene were split at an appropriate position. Sialyl motifs L (SM-L) are underlined in bold. Sialyl motifs S (SM-S) are underlined. Sialyl motifs (VS) are shown by asterisks. Sialyl motifs L and S of some genes are split by introns.](#)
containing an α2,6-sialyl linkage may act as decoys or smoke screens to foil potential pathogens (40). Therefore, it may be possible that sialyloligosaccharides produced by ST6Gal II in small intestine and colon contribute to the maintenance of the intestinal flora and protection against enteric infections.

So far, genomic structures and chromosomal localization of 18 human sialyltransferase genes have been analyzed (36, 41, 42). We have also performed an extensive database search to obtain more detailed information on genomic organization of 20 human sialyltransferase genes in this study. The results are summarized in Fig. 8 and Table II. Genomic structural analysis of the ST6Gal II gene revealed that this gene has a similar genomic structure with the ST6Gal I gene, suggesting that these genes share a common ancestral gene. The split pattern of the coding sequences for sialyl motif L and S of these genes are different from other sialyltransferase genes (Fig. 7), also suggesting that the ST6Gal I and II genes may have evolved independently or differently from the most ancestral sialyltransferase gene. Besides the ST6Gal I and II genes, there are several sets of sialyltransferase genes that encode similar enzymes and share similar genomic structures. Among them, ST6GalNAc I and II genes, ST6GalNAc III and V genes, and ST6GalNAc IV and VI genes are located close to each other on chromosomes 17, 1, and 9, respectively (Table II), suggesting that each gene pair is closely related from an evolutionary standpoint. Probably each gene pair has arisen from a common ancestral gene by tandem duplication. It should be noted that the genome sizes of each gene pair are also similar to each other (Fig. 8). On the other hand, other pairs of similar sialyltransferase genes, such as the ST6Gal I and II genes, are not located on the same chromosome. This suggests that these genes have arisen from a common ancestral gene by gene duplication and subsequently dispersed in the human genome by translocation. We found by database search that besides the functional sialyltransferase genes, there are significant amounts of sialyltransferase gene-related nonfunctional DNA sequences, such as pseudo genes and partial fragments of sialyltransferase genes, in the human genome. The existence of these remnants of sialyltransferase genes also suggests that dynamic events of the human genome have contributed to the evolution of sialyltransferase genes.

The human ST6Gal I gene is expressed ubiquitously, and its expression levels are much higher than those of the ST6Gal II gene (Fig. 5). It has been known that the expression of the ST6Gal I gene is regulated by physically distinct promoters in a tissue- and stage-specific manner (37, 43). In most cases, resultant ST6Gal I transcripts differ in the 5′-untranslated regions but encode the same protein. Multiple mRNA isoforms that differ only in the 5′-untranslated regions have been also identified in human ST3Gal IV-VI (44–46). These transcripts are produced from a single gene locus by a combination of alternative splicing and alternative promoter usage in a tissue- and stage-specific manner. We found by database search and genomic structural analysis of the ST6Gal II gene that there are some isoforms of the ST6Gal II mRNA that differ in the 5′-untranslated region and/or the regions encoding the COOH terminus of the protein and the 3′-untranslated region. These suggest that the ST6Gal II transcripts are also produced by a combination of alternative splicing and alternative promoter usage in a tissue- and stage-specific manner. Although the expression levels of the ST6Gal II gene are relatively low, above transcriptional regulation may contribute to the specific expression of the ST6Gal II gene. It should be noted that besides the transcripts encoding the active form of ST6Gal II, there are other transcripts encoding the short inactive form of ST6Gal II lacking most of the sialyl motif S. At present, we do not know the biological importance and function of the short form of ST6Gal II. However, it may be possible that the short form of ST6Gal II acts like a lectin and is involved in some interaction events, because it should be able to bind sialic acids through the sialyl motif L. The detailed analysis of transcriptional regulation of the ST6Gal II gene will help elucidate biological significance of each transcript.

The mammalian sialyltransferase family is supposed to consist of more than 20 sialyltransferases. It is interesting that all the members of so-far cloned sialyltransferases have the counterpart with similar enzymatic properties and genomic structure. The biological significance of these multiple genes is unclear at present. One interpretation is that they may be important for fine control of the expression of sialylglycoconjugates, resulting in a variety of developmental stage-specific and tissue-specific glycosylation patterns. All the members of the sialyltransferase family will be identified by the genome project in the near future. Characterization of each sialyltransferase and analysis of the transcriptional regulation of each gene will help elucidate the biological significance of each sialyltransferase and the sialylglycoconjugates they produce.

REFERENCES

1. Paulson, J. C. (1989) Trends. Biochem. Sci. 14, 272–276
2. Drickamer, K. (1993) Glycobiology 3, 2–3
3. Livingston, B. D., and Paulson, J. C. (1993) J. Biol. Chem. 268, 11504–11507
4. Glimcher, R. A., Harduin-Lepers, A., and Delannoy, P. (1997) Glycobiology 7, (2), v-vii
5. Datta, A. K., and Paulson, J. C. (1995) J. Biol. Chem. 270, 1497–1500
6. Datta, A. K., Sinha, A., and Paulson, J. C. (1998) J. Biol. Chem. 273, 9608–9614
7. Kitazume-Kawaguchi, S., Kabata, S., and Arita, M. (2001) J. Biol. Chem. 276, 15866–15873
8. Tsuji, S. (1996) J. Biochem. 120, 1–13
9. Tsuji, S. (1999) in Sialobiology and Other Novel Forms of Glycosylation (Inoue, Y., Lee, Y. C., and Troy, F. A., eds) pp. 145–154, Gakushuin Publishing Co., Osaka, Japan
10. Okajima, T., Fukushima, S., Miyazaki, H., Ishida, H., Kiso, M., Furukawa, K., Urano, T., and Furukawa, K. (1999) J. Biol. Chem. 274, 11479–11486
11. Okajima, T., Fukushima, S., Ito, H., Kiso, M., Hirahayashi, Y., Urano, T., Furukawa, K., and Furukawa, K. (1999) J. Biol. Chem. 274, 30557–30562
12. Ikehara, Y., Shimizu, N., Keno, M., Nishihara, S., Nakanihshi, H., Kitamura, T., Narimatsu, H., Tsuji, S., and Tatematsu, M. (1999) FEBS Lett. 463, 92–96
13. Okajima, T., Chen, H.-H., Ito, H., Kiso, M., Tai, T., Furukawa, K., Urano, T., and Furukawa, K. (2000) J. Biol. Chem. 275, 6717–6723
14. Takashima, S., Ishida, H., Ozan, T., Ando, T., Ishida, H., Kiso, M., Tsuji, S., and Tsujimoto, M. (2002) J. Biol. Chem. 277, 24030–24038
15. Tsuji, S., Datta, A. K., and Paulson, J. C. (1996) Glycobiology 6, (2), v-vii
16. Taka, S., Ito, H., Kiso, M., Urano, T., and Furukawa, K. (1999) FEBS Lett. 440, 297–301
17. Powell, L. D., Sigro, D., Sigro, R. V., Stamenkovic, I., and Varki, A. (1993) J. Biol. Chem. 268, 7019–7027
18. Sigro, D., Varki, A., Brassch-Ander, S., and Stamenkovic, I. (1993) J. Biol. Chem. 268, 7011–7018
19. Powell, L. D., and Varki, A. (1994) J. Biol. Chem. 269, 10628–10636
20. Hetherington, A., Sirard, J., Kappel, J., and Varki, A. (1995) J. Biol. Chem. 270, 14623–14629
21. Kono, M., Yoshida, Y., and Tsuji, S. (2000) FEBS Lett. 460, 1–4
22. Ikehara, Y., Kojima, N., Kurosawa, N., Hamamoto, T., and Tsuji, S. (1995) J. Biol. Chem. 270, 14623–14629
23. Kono, M., Yoshida, Y., Kojima, N., and Tsuji, S. (1996) J. Biol. Chem. 271, 29366–29371
24. Lee, Y.-C., Kaufmann, M., Kitazume-Kawaguchi, S., Kono, M., Takashima, S., Kurosawa, N., Liu, H., Pircher, H., and Tsuji, S. (1999) J. Biol. Chem. 274, 11958–11967
25. Takashima, S., Tachida, Y., Nakagawa, T., Hamamoto, T., and Tsuji, S. (1999) Biochem. Biophys. Res. Commun. 260, 23–27
26. Kozak, M. (1986) Cell 44, 283–292
27. Kono, M., Takashima, S., Tachida, Y., Inoue, M., Kurosawa, N., Liu, H., Pircher, H., and Tsuji, S. (2000) J. Biol. Chem. 275, 845–854
28. Kono, M., Takashima, S., Tachida, Y., Inoue, M., Tachida, Y., Narimatsu, H., and Tsuji, S. (2000) J. Biol. Chem. 275, 845–854
29. Ikehara, Y., Kojima, N., Kurosawa, N., Kudo, T., Kono, M., Nishihara, S., Isaki, S., Morozumi, K., Isakowitz, S., Tsuda, T., Nishimura, S., Tsuji, S., and Narimatsu, H. (1999) Glycobiology 9, 1213–1225
30. Nagase, T., Nakayama, M., Nakajima, D., Kikuno, R., and Ohara, O. (2001) DNA Res. 8, 85–95
31. Hamamoto, T., and Tsuji, S. (2001) in Handbook of Glycosyltransferases and
A Novel Human β-Galactoside α2,6-Sialyltransferase, ST6Gal II

Related Genes (Taniguchi, N., Honke, K., and Fukuda, M., eds) pp. 295–300, Springer-Verlag, Tokyo, Japan

35. Shapiro, M. B., and Senapathy, P. (1987) Nucleic Acids Res. 15, 7155–7174
36. Takashima, S., Kono, M., Kurosawa, N., Yoshida, Y., Tachida, Y., Izoue, M., Kanematsu, T., and Yagi, S. (2000) J. Biochem. 128, 1033–1043
37. Wang, X. C, Vertino, A., Eddy, R. L., Byers, M. G., Jani-Sait, S. N., Shows, T. B., and Lau, J. T. Y. (1993) J. Biol. Chem. 268, 4355–4361
38. Nakano, T., Sugawara, M., and Kawakami, H. (2001) Acta Paediatr. 42, 11–17
39. Karlsson, K. A. (1995) Curr. Opin. Struct. Biol. 5, 622–635
40. Gagneux, P., and Varki, A. (1999) Glyobiology 9, 747–755
41. Harduin-Lepers, A., Krzewinski-Recchi, M.-A., Hebbar, M., Samyn-Petit, B., Vallejo-Ruiz, V., Julien, S., Perrat, J. P., and Delannoy, P. (2001) Recent Results Dev. Cancer 3, 111–126
42. Harduin-Lepers, A., Vallejo-Ruiz, V., Krzewinski-Recchi, M.-A., Samyn-Petit, B., Julien, S., and Delannoy, P. (2001) Biochimie (Paris) 83, 727–737
43. Dall'Olio, F. (2000) Glycoconj. J. 17, 669–676
44. Kitagawa, H., Mattei, M. G., and Paulson, J. C. (1996) J. Biol. Chem. 271, 931–938
45. Kim, K.-W., Kim, S.-W., Min, K.-S., Kim, C.-H., and Lee, Y.-C. (2001) Gene 273, 163–171
46. Taniguchi, A., Kaneta, R., Morishita, K., and Matsumoto, K. (2001) Biochem. Biophys. Res. Commun. 287, 1148–1156
47. Svennerholm, L. (1964) J. Lipid Res. 5, 145–155
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