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Molecular Cloning and Characterization of a Novel Isoform of the Human UDP-Galactose Transporter, and of Related Complementary DNAs Belonging to the Nucleotide-Sugar Transporter Gene Family[1]

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We described recently the molecular cloning of human UDP-galactose transporter 1 (hUGT1) [Miura, N. *et al.* (1996) *J. Biochem.* 120, 236-241]. Now we have characterized its isoform, hUGT2, that is most likely generated through the alternative splicing of a transcript derived from the UGT genomic gene, that also codes for hUGT1. Introduction of the open reading frame sequence of hUGT2 into a mouse cell line, Had-1, that lacks the UDP-galactose transporter, complemented the genetic defect of the mutant, as judged from the lectin-sensitivity spectra of the transformants and the nucleotide-sugar transporting activity of microsomal vesicles isolated from them. UGT-related genes were found through a BLAST search of dbEST based on their significant similarity with hUGT genes. We report here cDNA clones belonging to two subfamilies of the nucleotide-sugar transporter gene family. One is the human CMP-sialic acid transporter gene, and the other is a group of homologous genes with an undefined function that are distributed in man, mouse, and rat, and show significant similarity to the yeast UDP-*N*-acetylglucosamine transporter.

Key words: CMP-sialic acid transporter, nucleotide-sugar transporter, UDP-galactose transporter, UDP-*N*-acetylglucosamine transporter.

The study of the nucleotide-sugar transporter has made definite progress quite recently, with almost simultaneous success in the molecular cloning of three transporters, namely the UDP-GlcNAc transporter from yeast (*1*), the CMP-sialic acid transporter from mouse (*2*), and the human UDP-Gal transporter (*3*). The similarity among these nucleotide-sugar transporters indicates that they constitute a family of related genes. Elucidation of other family members as well as their possible isoforms would facilitate further study toward the full understanding of their structure-function relationship, and their physiological and developmental regulation. As an extension of our previous work, we have now isolated and characterized a cDNA clone encoding a novel isoform of the human UDP-Gal transporter and several other related cDNA clones belonging to the mammalian nucleotide-sugar transporter gene family.

In our previous study, that involved the molecular cloning of the human UDP-galactose transporter, we utilized the 3′-RACE procedure to extend a truncated cDNA clone toward the 3′-end (*3*). When total RNA from TIG-1 cells was used as a template, we obtained a single 3′-RACE product derived from hUGT mRNA, whose sequence was determined and reported, and which we now rename human UDP-Gal transporter 1 (hUGT1). Interestingly, however, we obtained two distinct 3′-RACE products with different chain lengths with poly(A)$^+$ RNA from TIG-1 cells as a template instead (Fig. 1a). The longer PCR fragment represented the 3′-end of hUGT1 cDNA. Nucleotide sequence analysis of the shorter one indicated that the 5′ portion of the product was identical with hUGT1, while its 3′ portion was unique (Fig. 1b). A poly(A) addition signal was found in the unique portion of the product and was followed by a poly(A) tail. We therefore assumed that the 3′-RACE product was amplified from a mRNA transcribed and alternatively spliced from the genomic UGT gene (*4*), that also codes for hUGT1 mRNA. To test this assumption, we prepared PCR primers, as indicated in Fig. 1b. One of the primers, NI251 (5′-gaattcGTGGGGCAGGGGTGGTGGGGACAG-3′), was designed to hybridize to a sequence in the region unique to the shorter 3′-RACE product, and the other, NI243 (5′-gaattcCAACATGGCAGCGGTTGGGGCTGGTG-3′), was designed so that it represents the 5′-end of the ORF of hUGT1 with an artificially introduced *Eco*RI site, shown in lower case, at the 5′-terminus. The underlined part of the second primer is the codon for the initiating methionine. An RT-PCR product of 1.3 kbp with an ORF of 396 amino acid residues was uniquely amplified

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Abbreviations: GS-II, *Griffonia simplicifolia* II; UDP-Gal, UDP-galactose; UDP-GlcNAc, UDP-*N*-acetylglucosamine; WGA, wheat germ agglutinin.

from a human liver cDNA pool (Clontech QUICK-Clone Human Liver cDNA; Clontech, Palo Alto, CA), and its sequence was coincident with that of the shorter 3′-RACE product along the entire overlapping portion (Fig. 1, refer to the DDBJ/GenBank™/EMBL DataBank, accession number D88146, for the exact sequence). We therefore designated this cDNA as hUGT2. The deduced amino acid sequence is shown in Fig. 4 together with those of other related gene products described below. The amino acid sequence was identical with that of hUGT1 except for eight amino acids at the C-terminus. The structure of hUGT2 cDNA is consistent with the idea of that hUGT2 mRNA is alternatively spliced from the same gene with hUGT1 mRNA. It is also consistent with our preliminary results obtained on analysis of the structure of the human UGT genomic gene (to be published elsewhere).

To determine whether or not hUGT2 exhibits the ability to transport UDP-Gal, as hUGT1 does, an expression vector, pMKIT-neo-hUGT2, was constructed as described in one of our previous publications (*3*). The recombinant plasmid, pMKIT-neo-hUGT2, was transfected into Had-1 mutant cells by the procedure described previously (*5*), and then transformants were selected in ES medium (Nissui Pharmaceutical, Tokyo) supplemented with 2% FCS containing 1 mg/ml G418. The galactosylation of the cell

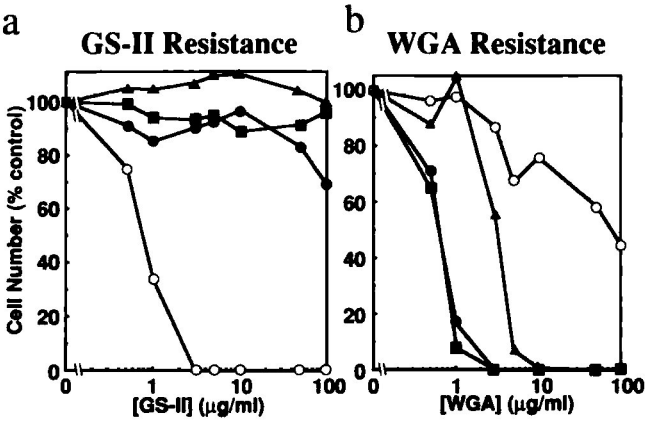

Fig. 2. **Reversal of the lectin-resistance spectrum of Had-1 cells to that of FM3A cells on the introduction of cDNAs for human UGT isozymes.** The same number of cells of each clone (10^4 cells in 1 ml of culture medium) was initially inoculated into ES medium supplemented with 2% FCS containing various concentrations of either GS-II (a) or WGA (EY Laboratories) (b), as indicated, and then grown for 3 days in a 24-well culture dish. The number of cells was then determined and presented as a percentage of that of a control culture grown in the absence of the lectin. ■, Had-1-hUGT2-GS-II-G5; ●, Had-1-hUGT1-GS-II-3; ○, Had-1-neo-2; ▲, FM3A-neo-2.

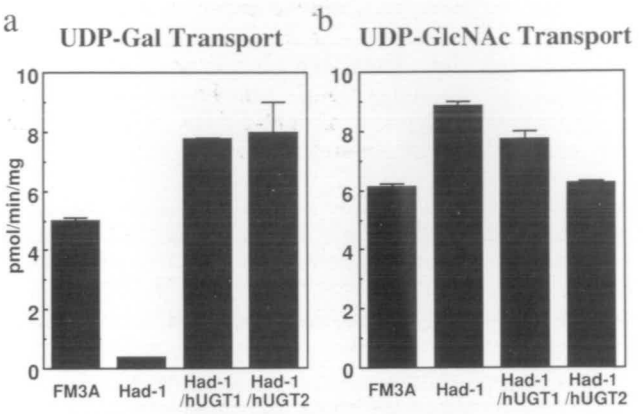

Fig. 3. **Recovery of the UDP-galactose transporting activity of microsomal vesicles derived from Had-1 cells on the introduction of human UGT isozyme cDNAs.** Cells grown in ES medium supplemented with 2% FCS were harvested, washed with PBS, and then suspended in 2 volumes of a solution comprising 0.25 M sucrose, 10 mM Tris-HCl buffer (pH 7.5), 1 mM EDTA, and 1 μg each of leupeptin and pepstatin A. The cells were homogenized with a homogenizer with a rotor stator generator probe (Physcotron Micro Homogenizer NS-310E; NITI-ON, Chiba) at the speed setting of 5 for 30 s. The post-mitochondrial supernatant obtained through successive centrifugation of the homogenate at $1,000\times g$ for 5 min and $7,700\times g$ for 30 min was further centrifuged at $105,000\times g$ for 30 min to recover microsomal membrane vesicles in the pellet. The microsomal membranes were suspended in a solution comprising 0.25 M sucrose, 10 mM Tris-HCl (pH 7.5), and 1 mM $MgCl_2$ at a concentration of about 25 mg protein/ml, and stored frozen at −80°C. The uptake of UDP-Gal and UDP-GlcNAc was measured according to the procedure described by Waldman and Rudnick (*12*). The transport reaction was started by adding an appropriate radiolabeled substrate, and the samples were incubated for 1 min at 30°C. As a control for nonspecific adsorption, the reaction was stopped immediately after the start, and the radioactivity remaining on the nitrocellulose membrane filter under these conditions was subtracted as the background value.

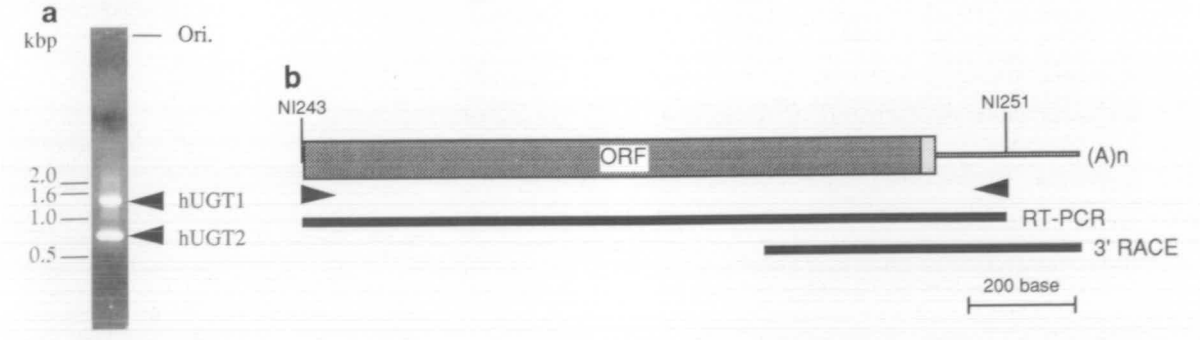

Fig. 1. **Cloning of hUGT2 cDNA.** a: 3′-RACE products obtained from poly(A)$^+$ RNA of TIG-1 cells. The 3′-RACE procedure including the design of the RACE primers was as described (*11*). b: The sequence information in the 3′-region was obtained by 3′-RACE. Arrowheads indicate the positions of the RT-PCR primers used to prepare the hUGT2 ORF fragment. The lightly shaded portion, constituting the 3′ end of the cDNA, indicates the sequence unique to hUGT2 cDNA, while the shaded region represents the sequence shared by hUGT1 and hUGT2.

surface glycoconjugates was assessed by determining the lectin-sensitivity of the transformants (*3*). Approximately 80% of the G418 resistant transformants could form colonies on agar plates containing 20 μg/ml GS-II (EY Laboratories, San Mateo, CA), indicating that the recombinant plasmid conferred resistance to GS-II on Had-1 mutant cells (data not shown).

We further investigated whether or not the GS-II-resistant clones exhibited a lectin-sensitivity spectrum similar to that of FM3A, as would be expected if the hUGT2 cDNA complemented the genetic defect of Had-1, as the hUGT1 cDNA did. Thus, ten clones were isolated on agarose plates containing GS-II from the pool of pMKIT-neo-hUGT2 transformants of Had-1. The isolated clones were examined as to their lectin-sensitivity, and were shown to exhibit practically the same lectin-sensitivity spectra. The results obtained with a representative clone, Had-1-hUGT2-GS-II-G5, are shown in Fig. 2. In parallel experiments, the lectin-sensitivity of Had-1-neo-2, FM3A-neo-2, and Had-1-hUGT1-GS-II-3 (formerly designated as Had-1-hUGT-GS-II-3) (*3*) was examined for reference purposes. The Had-1-hUGT2-GS-II-G5 cells were shown to exhibit a lectin-resistance spectrum similar to that of the parental FM3A and almost identical to that of Had-1-hUGT1-GS-II-3 (Fig. 2). This indicates that the defect of the Had-1 mutant, namely the inability to transport UDP-Gal from the cytosol to the Golgi lumen, was complemented by the genetic information carried by the hUGT2 cDNA, as was the case with hUGT1 cDNA.

The transport of nucleotide-sugars was directly examined with a microsomal fraction consisting mainly of endoplasmic reticulum and Golgi membranes obtained from these cells. As shown in Fig. 3, the membrane vesicles from Had-1 cells lacked the ability to transport UDP-Gal (Fig. 3a), while their UDP-GlcNAc transporting ability was comparable to or even higher than that of vesicles derived from the parent FM3A cells (Fig. 3b). Upon introduction of either hUGT1 or hUGT2 cDNA into Had-1 cells, the membrane vesicles derived from the transformants recovered the UDP-Gal transporting activity, it reaching a level a little higher than that of FM3A (Fig. 3a).

Several cDNA clones showing significant degrees of

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          1                                                          60
hUdpGalTr1  MAAVGAGGST AAPGPGAVSA GALEPGTASA AHRR.....L KYISLAVLVV QNASLILSI.
hUdpGalTr2  MAAVGAGGST AAPGPGAVSA GALEPGTASA AHRR.....L KYISLAVLVV QNASLILSI.
  hCmpSaTr  .......... .......... ........MA APRDNVTLLF KLYCLAVMTL MAAVYTIAL.
  hUGTrel1  .......... .......... ........MA SSSSLVPDRL RL.PLCFLGV FVCYFYYGIL
  mUGTrel1  .......... .......... ........MA ASRSLVPDRL RL.PLCFLGV FVCYFYYGIL
  rUGTrel1  .......... .......... ........MA ASRSLVPDRL RL.PLCFLGV FVCYFYYGIL

          61                                                         120
hUdpGalTr1  .......RYA RTLPGDRF.F ATTAVVMAEV LKGLTCLLLL FAQKRGNVKH LVLFLHEAVL
hUdpGalTr2  .......RYA RTLPGDRF.F ATTAVVMAEV LKGLTCLLLL FAQKRGNVKH LVLFLHEAVL
  hCmpSaTr  .......RYT RTSDKELY.F STTAVCITEV IKLLLSVGIL .AKETGSLGR FKASLRENVL
  hUGTrel1  QEKITRGKYG EGAKQETFTF ALTLVFIQCV INAVFAKILI QFFDTARVDR TRSWLYAACS
  mUGTrel1  QEKITRGKYG EGPKQETFTF ALTLVFIQCV INAMFAKILI QFFDTARVDR TRTWLYAACS
  rUGTrel1  QEKITRGKYG EGPKQETFTF ALTLVFIQCV INAMFAKILI QFFDTARVDR TRTWLYAACS

          121                                                        180
hUdpGalTr1  VQYVDTLKLA VPSLIYTLQN NLQYVAISNL PAATFQVTYQ LKILTTALFS VLMLNRSLSR
hUdpGalTr2  VQYVDTLKLA VPSLIYTLQN NLQYVAISNL PAATFQVTYQ LKILTTALFS VLMLNRSLSR
  hCmpSaTr  GSPKELLKLS VPSLVYAVQN NMAFLALSNL DAAVYQVTYQ LKIPCTALCT VLMLNRTLSK
  hUGTrel1  ISYLGAM... VSS.....NS ALQFV...NY PTQVLGKSC. .KPIPVMLLG VTLLKKKYPL
  mUGTrel1  VSYVGAM... VSS.....NS ALQFV...NY PTQVLGKSC. .KPIPVMLLG VTLLKKKYPL
  rUGTrel1  VSYVGAM... VSS.....NS ALQFV...NY PTQVLGKSC. .KPIPVMLLG VTLLKKKYPL

          181                                                        240
hUdpGalTr1  LQWASLLLLF TGVAIVQAQQ AGGGGPRPLD QNPGAGLAAV VASCLSSGFA GVYFEKILKG
hUdpGalTr2  LQWASLLLLF TGVAIVQAQQ AGGGGPRPLD QNPGAGLAAV VASCLSSGFA GVYFEKILKG
  hCmpSaTr  LQWVSVFMLC AGVTLVQWKP AQATKV.VVE QNPLLGFGAI AIAVLCSGFA GVYFEKVLKS
  hUGTrel1  AKYLCVLLIV AGVALFWYKP KKVVGIE..E HTVGYGELLL LLSLTLDGLT GVSQDHWRAH
  mUGTrel1  AKYLCVLLIV AGVALFWYKP KKVVGIE..E HTVGFGELLL LWSLTLDGLT GVSQDHWRAH
  rUGTrel1  AKYLCVLLIV AGVALFWYKP KKVVGIE..E HTVGFGELLL LLSLTLDGLT GVSQDHWRAH

          241                                                        300
hUdpGalTr1  SSGSVWLRNL QLGLFGTALG LVGLWWAEG. .TAVATRGFF FGYTPAVWGV VLNQAFGGLL
hUdpGalTr2  SSGSVWLRNL QLGLFGTALG LVGLWWAEG. .TAVATRGFF FGYTPAVWGV VLNQAFGGLL
  hCmpSaTr  SDTSLWVRNI QWYLSGIIVT LAGVYLSDG. .AEIKEKGFF YGYTYYVWFV IFLASVGGLY
  hUGTrel1  YQTGSNHWWL NINLWSTLLL GWGILFTGEL WEFLSFAERY PAIIYNILLF GLTSALGQSF
  mUGTrel1  YQTGSNHWWL NINLWSTFLL GAGILFTGEL WEFLSFAERY PTIIYNILLF GLTSALGQSF
  rUGTrel1  YQTGSNHWWL NINLWSTVLL GAGILFTGEL WEFLSFAERY PTIIYNILLF GLTSALGQSF

          301                                                        360
hUdpGalTr1  VAVVVKYADN ILKGFATSLS IVLSTVASIR LFGFHVDPLF ALGAGLVIGA VYLYS.LPRG
hUdpGalTr2  VAVVVKYADN ILKGFATSLS IVLSTVASIR LFGFHVDPLF ALGAGLVIGA VYLYS.LPRG
  hCmpSaTr  TSVVVKYTDN IWKGFSAAAA IVLSTIASVM LFGLQITLTF ALGTLLVCVS IYLYG.LPRQ
  hUGTrel1  IFWTVVYFGP LTCSIITTTR KFFTILASVI LFANPISPWQ WVGTVLVFLG LGLDAKFGKG
  mUGTrel1  IFWTVVYFGP LTCSIITTTR KFFTILASVI LFANPISSWQ WVGTVLVFLG LGLDAKFGKG
  rUGTrel1  IFWTVVYFGP LTCSIITTTR KFFTILASVI LFANPISSWQ WVGTVLVFLG LGLDAKFGKG

          361                                                       415
hUdpGalTr1  AAKAIASASA SASGPCVHQQ PPGQPPPPQL SSHRGDLITE PFLPKSVLVK *....
hUdpGalTr2  AAKAIASASA SASGPCVHQQ PPGQPPPPQL SSHRGDLITE PFLPK.LLTK VKGS*
  hCmpSaTr  DTTSIQQGET ASKERVIGV* .......... .......... .......... .....
  hUGTrel1  AKKTSH*... .......... .......... .......... .......... .....
  mUGTrel1  TKKTSH*... .......... .......... .......... .......... .....
  rUGTrel1  TKKTSH*... .......... .......... .......... .......... .....
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Fig. 4. **Deduced amino acid sequences of human UGT isozymes and UGT-related genes.** The nucleotide sequences were determined with an ABI 377 DNA sequencer (Perkin-Elmer, Norwalk, CT) using a Taq DyeDeoxy Thermal Cycle Sequencing Kit (Perkin-Elmer). Alignment was carried out with the aid of the PILEUP program (Genetic Computer Group, Madison, WI). The nucleotide sequences of hUdpGalTr2, hCmpSaTr, hUGTrel1, mUGTrel1, and rUGTrel1, including their 5′- and 3′-untranslated regions, have been submitted to the DDBJ/GenBank™/EMBL Data Bank under accession numbers D88146, D87969, D87989, D87990, and D87991, respectively.

TABLE I. **Similarity among the nucleotide-sugar transporter genes.** The percent similarity was calculated with the BESTFIT program (Genetic Computing Group). The accession numbers of data from the DDBJ/GenBank™/EMBL for the amino acid sequences are: hUdpGalTr1 (hUGT1), D84454; hUdpGalTr2 (hUGT2), this paper; hCmpSaTr, this paper; mCmpSaTr, Z71268; ZK370.7, M98552; human, mouse, and rat UGTrel1, this paper; yUdpGlcNAcTr (UDP-GlcNAc transporter from *Kluyveromyces lactis*), U48413; yel004p, U18530; CB53B4.6, Z68215; and CDC91, U22383.

	hUdpGalTr1	hUdpGalTr2	hCmpSaTr	mCmpSaTr	ZK370.7	hUGTrel1
hUdpGalTr1	100	99.7	64.7	64.8	66.1	48.2
hUdpGalTr2	–	100	64.7	64.8	66.1	48.2
hCmpSaTr	–	–	100	**95.5**	66.4	48.7
mCmpSaTr	–	–	–	100	67.2	47.7
ZK370.7	–	–	–	–	100	51.0
hUGTrel1	–	–	–	–	–	100
mUGTrel1	–	–	–	–	–	–
rUGTrel1	–	–	–	–	–	–
yUdpGlcNAcTr	–	–	–	–	–	–
yel004p	–	–	–	–	–	–
C53B4.6	–	–	–	–	–	–

	mUGTrel1	rUGTrel1	yUdpGlcNAcTr	yel004p	C53B4.6	CDC91
hUdpGalTr1	45.8	45.8	41.9	43.8	47.7	43.0
hUdpGalTr2	45.8	45.8	41.9	43.8	47.7	43.9
hCmpSaTr	46.6	46.6	46.3	41.2	49.1	48.6
mCmpSaTr	45.9	46.3	43.3	44.4	49.8	46.2
ZK370.7	53.1	51.4	45.0	44.4	48.4	54.2
hUGTrel1	97.8	97.8	**48.8**	45.9	46.8	46.5
mUGTrel1	100	99.7	**49.3**	43.6	47.1	46.8
rUGTrel1	–	100	**49.0**	43.3	47.1	46.5
yUdpGlcNAcTr	–	–	100	70.5	51.9	49.5
yel004p	–	–	–	100	50.8	44.3
C53B4.6	–	–	–	–	100	43.5

similarity with human UGT were found on reference to a database of expressed sequence tags (dbEST) (*6*) by means of a BLAST search (*7*). Some of these clones were clearly derived from the same gene, because their available sequences showed various degrees of overlapping with exact matching. A representative clone that was estimated to have the longest cDNA among each group of cDNA clones derived from one gene was selected. The following clones, I.M.A.G.E. Consortium CloneID 44875, 295714, and 333687 (*8*), and clone EST105806 (*9*), were thus selected as representative products of the four separate genes. The former three were provided by the I.M.A.G.E. Consortium, and the last by the Institute for Genomic Research. Characterization of these clones by nucleotide sequence analysis showed that clones 44875, 295714, and 333687 lacked the 5′-proximal information. 5′-RACE was then performed using a human adult liver cDNA pool (Clontech Human Liver 5′-RACE-Ready cDNA), human adult and fetal liver cDNA pools (Clontech Human Liver 5′-RACE-Ready cDNA and Clontech Human Fetal Liver Marathon-Ready cDNA), and a mouse liver cDNA pool (Clontech Mouse Liver Marathon-Ready cDNA), respectively, and the complete nucleotide sequences of these clones were obtained (refer to the DDBJ/GenBank™/EMBL Data-Bank, accession numbers D87969, D87989, and D87990, for the sequences). Clones 295714, 333687, and EST-105806 represent a set of homologous genes in man, mouse, and rat, and are named tentatively UGT-related isozymes 1 (UGTrel1) genes. Clone 44875 codes for the human homologue for the mouse CMP-sialic acid transporter (*2*), as judged from its close similarity to the latter (Table I).

The members of the nucleotide-sugar transporter gene family so far identified are summarized in Table I. The similarities of the UGTrel1 genes with other mammalian nucleotide-sugar transporter genes are rather low. In this context, it is interesting that the UGTrel1 genes are more similar to the gene of the UDP-GlcNAc transporter from *Kluyveromyces lactis* (*1*) than to other mammalian genes of the family, the similarity being around 49%. It is intriguing that hUGTrel1 gene might represent human counterpart of the yeast UDP-GlcNAc transporter. Alternatively, it is also possible that it may code for a specific transporter for other nucleotide-sugars, including UDP-*N*-acetylgalactosamine, UDP-xylose, UDP-glucuronic acid, UDP-glucose, and GDP-fucose (*10*). Obviously there must be more genes coding for transporters for these nucleotide-sugars, and they should exhibit significant similarity with each other. In fact, candidate cDNAs that may be members of the nucleotide-sugar transporter gene family have been noted in dbEST, and are currently being characterized in our laboratory.

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