Polymorphisms in Human Organic Anion-transporting Polypeptide 1A2 (OATP1A2)

**IMPLICATIONS FOR ALTERED DRUG DISPOSITION AND CENTRAL NERVOUS SYSTEM DRUG ENTRY***

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Organic anion-transporting polypeptide 1A2 (OATP1A2) is a drug uptake transporter known for broad substrate specificity, including many drugs in clinical use. Therefore, genetic variation in SLCO1A2 may have important implications to the disposition and tissue penetration of substrate drugs. In the present study, we demonstrate OATP1A2 protein expression in human brain capillary and renal distal nephron using immunohistochemistry. We also determined the extent of single nucleotide polymorphisms in SLCO1A2 upon analyses of ethnically defined genomic DNA samples (n = 95 each for African-, Chinese-, European-, and Hispanic-Americans). We identified six nonsynonymous polymorphisms within the coding region of SLCO1A2 (T38C (I13T), A516C (E172D), G559A (A187T), A382T (N128Y), A404T (N135I), and C2003G (T668S)), the allelic frequencies of which appeared to be ethnic-ity-dependent. In vitro functional assessment revealed that the A516C and A404T variants had markedly reduced capacity for mediating the cellular uptake of OATP1A2 substrates, estrone 3-sulfate and two δ-opioid receptor agonists, deltorphin II, and [d-penicillamine2,5]-enkephalin. On the other hand, the G559A and C2003G variants appeared to have substrate-dependent changes in altered plasma membrane expression of the transporter may contribute to reduced transport activity associated with the A516C, A404T, and C2003G variants. The A404T (N135I) variant also showed a shift in the apparent molecular size, indicative of alterations in glycosylation status. Taken together, these data suggest that SLCO1A2 polymorphisms may be an important yet unrecognized contributor to inter-individual variability in drug disposition and central nervous system entry of substrate drugs.

During the past decade, there has been an increasing recognition of the critical interplay between drug transporters and drug-metabolizing enzymes as determinants of drug disposition and response. Indeed, the extent of targeted tissue entry for many drugs may be facilitated by drug transporters, which are often expressed in a tissue-specific manner with broad substrate specificities. Among the uptake transporters, members of the organic anion-transporting polypeptides (human, OATPs) have been shown to be expressed in organs such as the central nervous system, liver, and intestine and mediate the cellular uptake of a large number of structurally divergent compounds (1). Within this family, OATP1A2 (SLCO1A2, also known as human OATP-A or OATP1) was the first human OATP to be cloned and characterized (2). OATP1A2 mRNA has been detected in various tissues including the brain, liver, and kidney (2, 3). Substrates of OATP1A2 include endogenous compounds such as bile acids, steroid hormones, and their conjugates, thyroid hormones, as well as drugs including fexofenadine, ouabain, peptides (e.g. deltorphin II, [d-penicillamine2,5]-enkephalin, DPDPE), and the toxin, microcystin (2, 4–7). In humans, OATP1A2 has been reported to be the only OATP transporter detected in the brain capillary endothelium at present, suggesting that OATP1A2 may play a critical role in the central nervous system penetration of many drugs and hormones across the blood-brain barrier (BBB) (7, 8). Given the extent of its substrate specificity and expression in organs of importance to drug disposition and response, genetic variations in SLCO1A2 may have significant pharmacologic and toxicologic consequences.

In the present study, we were able to confirm the expression of OATP1A2 at the level of the brain capillaries that make up the BBB. Moreover, we describe the identification and functional characterization of SNPs in SLCO1A2 from a population of European-, Chinese-, Hispanic-, and African-Americans. Genotypic frequencies of six nonsynonymous polymorphisms within the coding region of SLCO1A2 were dependent on ethnicity, and some of the genetic variants were associated with markedly reduced uptake transport activity for estrone 3-sulfate and two δ-opioid receptor agonists, [d-penicillamine2,5]-enkephalin and deltorphin II. Our data indicate that SLCO1A2 polymorphisms may contribute to inter-individual variability in drug disposition and may be a heretofore unrecognized factor governing the central nervous system entry of some drugs.

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Materials—Radioabeled [3H]estrone 3-sulfate (53 Ci/mmole), [tyrosyl-3,5-H]deltorphin II (2-n-Ala, 38.5 Ci/mmole), [tyrosyl-2,6-3H]enkephalin (p-tert-butylinamine), DPDPE, 34.0 Ci/mmole), and unlabeled estrone-sulfate were purchased from PerkinElmer Life Science (Boston, MA). [3H]Deltorphin II (5 µCi) and DPDPE were obtained from Bachem (King of Prussia, PA). Recombinant vaccinia virus containing the T7 RNA polymerase gene (T7-7) was a gift provided by Dr. Bernard Moss (National Institutes of Health, Bethesda, MD). The pEF6/V5-His-TOPO® expression vector and the monoclonal mouse anti-V5 antibody were purchased from Invitrogen. Genomic DNA isolated from peripheral blood lymphocytes of healthy European-, African-, Chinese-, and Hispanic-Americans was obtained from Coriel cell repositories (Camden, NJ). An enzymatic deglycosylation kit was obtained from Glyko (San Leandro, CA), and tunicamycin was purchased from Calbiochem. Immunohistochemistry reagents were obtained from Bio- genes (San Ramon, CA). All other chemicals and reagents, unless stated otherwise, were obtained from Sigma and were of the highest grade available.

Identification of SNPs in SLCO1A2 and Determination of Genotypic Frequencies—Initially, total genomic DNA was isolated from blood samples obtained from healthy volunteer subjects (46 European- and 32 African-Americans) residing in middle-Tennessee who were judged to be healthy on the basis of medical history, physical examination, and laboratory test indicative of normal cardiac, renal, and liver function. The protocol was approved by the Vanderbilt University Institutional Review Board, and informed consent was obtained. Further analyses of genetic variations in the human SLCO1A2 gene used ethnically defined SNPs databases, including PharmGKB (www.pharmgkb.org), IMS-JST (http://www.iwpc.org/imsst), and the SNP database from AEIC (http://www.aeic.co.jp). SNPs were determined by genotypic frequencies of the nonsynonymous SNPs identified by single/nucleotide polymorphism databases included PharmGKB (www.pharmgkb.org), IMS-JST (http://www.iwpc.org/imsst) and the SNP database from AEIC (http://www.aeic.co.jp).

Expression of Variant SLCO1A2 Alleles—For transport studies, HeLa cells were grown in 12-well plates (0.8 × 10^6 cells/65 µl) and subsequently incubated with anti-OATP1A2 antisera diluted in blocking buffer (1:100–1:500 dilutions) for 2 h at room temperature. After three washes, sections were incubated with the avidin and biotin blocking reagents (avidin-biotin blocking kit, Biogenex) to reduce nonspecific background staining due to endogenous biotin. After three washes with PBS containing 0.4% Triton X-100, sections were incubated with biotinylated anti-rabbit IgG (Biogenex) for 20 min and then with streptavidin–horseradish peroxidase conjugate (Biogenex) for 20 min. After three washes, the immune reaction was visualized using 3-aminoo-9-ethylcarbazole (Biogenex), and nuclei were counterstained with hematoxylin (Biogenex). The specificity of immunoreactive signals for OATP1A2 was verified by various negative controls, which were incubated with the rabbit preimmune serum, blocking buffer, or polyclonal antiserum that did not contain an insert as used as vector control. OATP1A2 staining was then evaluated using labeled drug substrates as outlined previously (9). To measure estrone 3-sulfate, deltorphin II, and DPDPE transport kinetics, radioabeled drug uptake during the linear phase (the first 3 min) was assessed in the presence of varying concentrations of unlabeled respective compounds. Passive diffusion was determined by carrying out parallel experiments using the parental plasmid DNA lacking the antisense cDNA insert and this value was used as the control. Transepithelial transport was measured from the total uptake rate seen in the presence of the transporter cDNA. Michaelis-Menten-type nonlinear curve fitting was carried out to obtain estimates of the maximal uptake rate (U_max) and the concentration at which half the maximal uptake occurs (K_s). OATP1A2 Cell Surface Expression—HeLa cells were grown on 6-well plates and transfected with wild-type or variant SLCO1A2 cDNA packed into either pEF6/V5-His-TOPO® or pSPORT® vector (Invitrogen), along with LipofectTM (Invitrogen), and incubated at 37 °C for 16 h. The parental plasmid lacking any insert was used as vector control. Transepithelial transport was then evaluated using labeled drug substrates as outlined previously (9).

Functional OATP1A2 Polymorphisms and Drug Transport

EXPERIMENTAL PROCEDURES

Identification of SNPs in SLCO1A2 and Determination of Genotypic Frequencies—Initially, total genomic DNA was isolated from blood samples obtained from healthy volunteer subjects (46 European- and 32 African-Americans) residing in middle-Tennessee who were judged to be healthy on the basis of medical history, physical examination, and laboratory test indicative of normal cardiac, renal, and liver function. The protocol was approved by the Vanderbilt University Institutional Review Board, and informed consent was obtained. Further analyses of genetic variations in the human SLCO1A2 gene used ethnically defined SNPs databases, including PharmGKB (www.pharmgkb.org), IMS-JST (http://www.iwpc.org/imsst), and the SNP database from AEIC (http://www.aeic.co.jp). SNPs were determined by genotypic frequencies of the nonsynonymous SNPs identified by single/nucleotide polymorphism databases included PharmGKB (www.pharmgkb.org), IMS-JST (http://www.iwpc.org/imsst) and the SNP database from AEIC (http://www.aeic.co.jp).
1 h at room temperature. Cells were then incubated with monoclonal antibody against V5 epitope (diluted 1:500 in blocking buffer) for 2 h at room temperature. After three washes in PBS containing 0.05% Tween 20, cells were incubated with secondary goat anti-mouse antibody labeled with the fluorescent dye, Texas Red (Molecular Probes, Eugene, OR) for 30 min at 37 °C. During the final washes in PBS containing 0.05% Tween 20, SYTOX® Green (Molecular Probes, Eugene, OR) was added for nuclei acid staining. Confocal microscopy was performed with a Zeiss Axiovert 100-M inverted microscope equipped with a LSM510 laser scanning unit. A Zeiss 63 × 1.4 numerical aperture plan Apochromat oil immersion objective was used for all experiments. Confocal images were obtained using single excitation (585 nm) and emission (610–630 nm Texas Red) filter sets. Cells transfected with the parental plasmid lacking any insert were used as control. Cells transfected with V5-tagged SLC01A2*1 plasmid without incubation with primary antibody were also used as an additional control. For slow frame scanning, confocal images were obtained by scanning either laterally (top view, x-y scans) or axially (side view, x-z scans) across the cell. Image analysis and processing were performed with Zeiss LSM and Adobe Photoshop software.

Deglycosylation of Total and Cell Surface-expressed OATP1A2—

HeLa cells were grown on 6-well plates and transfected with V5-tagged SLC01A2*1 and *6 using a protocol similar to that for the transport assays described above. Total and cell surface-expressed OATP1A2 variants (*1 and *6) were isolated as described previously and subjected to enzymatic deglycosylation according to the instructions provided by the manufacturer (Glyko, San Leandro, CA). Briefly, total or cell surface-expressed OATP1A2 protein samples were subjected to denaturation and enzymatic deglycosylation by N-glycanase, sialidase A, and O-glycanase. Samples were separated by 7.5% SDS-PAGE and analyzed by Western blotting for detection of immunoreactive proteins with monoclonal anti-V5 antibody (1:5000 dilution). In separate experiments, tunicamycin, an inhibitor of the N-linked glycosylation of proteins, was added to a final concentration of 0.3 µg/ml during a 16-h transient transfection period. Total and cell surface-expressed OATP1A2 protein samples were isolated and analyzed by Western blotting as described previously.

Statistical Analysis—Determination of the statistical differences between various group parameters was performed using analysis of variance (using Newman-Keuls multiple comparison test). p values of <0.05 were considered to reflect statistical significance.

RESULTS

OATP1A2 Immunohistochemistry—

Immunohistochemical staining of OATP1A2 was performed to define the tissue distribution of OATP1A2 in the human brain and kidney using rabbit polyclonal antiserum against human OATP1A2. Different dilutions of the antiserum (1:100–1:500) were used to optimize the detection of different expression levels in immunohistochemical analyses. Nonspecific background staining was absent even when immunohistochemical staining was performed with the highest antiserum concentration utilized (1: 100). In normal human brain cortex sections, immunohistochemistry detected OATP1A2 expression almost exclusively in endothelial capillary cells (Fig. 1A). Preincubation of the polyclonal antiserum against OATP1A2 with the antigenic peptide abolished positive staining in brain capillary (Fig. 1A, inset). OATP1A2 expression in human kidney was localized to the apical domain of distal nephrons (Fig. 1B). In human liver sections, OATP1A2 expression appeared to be localized to the cholangiocytes (Fig. 1C).

Genetic Variations in Human SLC01A2 Gene—

Initial single-strand conformational polymorphism analyses of all 14 exons of SLC01A2 (using genomic DNA samples from 46 European- and 32 African-Americans) and a search of available SNP databases identified 11 nonsynonymous SNPs. Based on the reported genotypic frequencies and putative location of amino acid changes, we determined the allelic frequencies of six nonsynonymous SNPs (T38C, A516C, G559A, A382T, A404T, and C2003G) using ethnically defined genomic DNA (European-, African-, Chinese-, and Hispanic-American, n = 95 each) (Table 1). T38C and A516C polymorphisms were more common in European-Americans (11.1 and 5.3%, respectively) than Hispanic-Americans, whereas these SNPs were not observed with Chinese-Americans. G559A variation was observed only in Hispanic-Americans (0.5%), whereas A382T was found only in African-Americans (1.0%). However, A404T polymorphism was not detected in genomic DNA samples used in the present study. C2003G variation was observed in African- and Hispanic-Americans with varying allelic frequencies of 3.7 and 1.0%, respectively. None of the six nonsynonymous SNPs tested were observed in Chinese-Americans.

Transport Properties of SLC01A2 Variant Alleles—

An array of expression plasmids comprising seven SLC01A2 allelic variants (*1–*7) was transiently overexpressed using the recombinant vaccinia system (vtf-7). OATP1A2*1 was capable of transporting known substrates, such as estrone 3-sulfate (Fig. 2A), deltorphin II (Fig. 2B), and DPDPE (Fig. 2C). To determine the relative transport efficiencies of the OATP1A2 variants, we compared the total cellular uptake (0.5 µM) at 30 min. Transport activities associated with OATP1A2*3 (A516C) and *6 (A404T) variants were significantly lower than the reference allele (*1) for all three substrates tested. The OATP1A2*4 (A404T) variant exhibited markedly reduced transport activity toward estrone 3-sulfate but not toward deltorphin II and DP-
TABLE I
Allele frequencies of nonsynonymous SNPs in OATP1A2 among various ethnic populations

Samples were taken from African-, European-, Chinese-, and Hispanic-Americans, n = 95 each. PhamGKB and dbSNP represent “Pharmacogenetics and Pharmacogenomics Knowledge Base” and “SNP database,” respectively.

| OATP1A2 variants | Base pair change | Exon | Amino acid change | Allelic frequency |
|------------------|------------------|------|-------------------|------------------|
|                  |                  |      |                   | African- | European- | Chinese- | Hispanic- | PharmGKB | dbSNP |
| OATP1A2*2        | T38C             | 1    | I13T              | 2.1      | 11.1     | 0        | 5.7       | 6.3       | 11.9   |
| OATP1A2*3        | A516C            | 5    | E172D             | 2.1      | 5.3      | 0        | 2.1       | 1.1       | 2.2    |
| OATP1A2*4        | G559A            | 5    | A187T             | 0        | 0        | 0        | 0.5       | 0         | 0      |
| OATP1A2*5        | A382T            | 4    | A128N             | 1.0      | 0        | 0        | 0         | 0.5       | 1.1    |
| OATP1A2*6        | A404T            | 4    | N135S             | 0        | 0        | 0        | 0         | 0.4       | 0.7    |
| OATP1A2*7        | C2003G           | 14   | T668S             | 3.7      | 0        | 0        | 1.0       | 1.5       | 2.9    |

FIG. 2. Uptake transport activity of the OATP1A2 variants following recombinant vaccinia-mediated expression. Left panels, uptake transport of radiolabeled estrone 3-sulfate (A), deltorphin II (B), and DPDPE (C) using the initial concentrations of 0.5 μM over time by HeLa cells transfected with the wild-type SLCO1A2*1 (closed circles) and vector only control (open circles). Right panels, uptake transport of radiolabeled estrone 3-sulfate (A), deltorphin II (B), and DPDPE (C) using the initial concentrations of 0.5 μM at 30 min by HeLa cells transfected with the SLCO1A2 variants (*2–*7) was assessed relative to the wild-type SLCO1A2*1. (Values are expressed as the percentage of cellular uptake by OATP1A2*1 (mean ± S.E., n = 4–6)). **, p < 0.01; ***, p < 0.001, analysis of variance followed by Newman-Keuls test for multiple comparisons.
DPE. In contrast, the OATP1A2*7 (C2003G) variant appeared to have the reduced cellular uptake of deltorphin II and DPDPE but normal uptake activity for estrone 3-sulfate.

For a more comprehensive characterization, concentration-dependent uptake kinetics, utilizing uptake data during the linear phase (first 3 min), of estrone 3-sulfate and deltorphin II were examined for all seven allelic variants (Figs. 3 and 4). Consistent with initial screening results, some of the OATP1A2 variants (e.g., *3, A516C and *6, A404T) possessed significantly lower \( V_{\text{max}} \) values for both estrone 3-sulfate and deltorphin II than the reference allele (OATP1A2*1). On the other hand, the OATP1A2*4 (A404T) variant showed significantly altered \( V_{\text{max}} \) values with deltorphin II. Due to low \( V_{\text{max}} \) values, it was not feasible to obtain reliable \( K_m \) values for those variants.

**Total and Cell Surface Expression of OATP1A2 Variants**—Immunoblot analyses of total OATP1A2 protein expression (Fig. 5A) indicated that the reduced transport activity of some OATP1A2 variants (*6, A404T and *7, C2003G) may be associated with alterations in total protein expression. In addition to the band corresponding to the expected molecular size of \(~74\text{-kDa} \) (2), the blots showed multiple band patterns, sugges-
cell surface-expressed OATP1A2 *1 and *6 variants. Enzymatic N-deglycosylation was carried out on the total and cell surface-expressed OATP1A2*1 and *6, and Western blot analyses were subsequently performed to probe immunoreactive bands and shifts in molecular sizes. Chemical deglycosylation experiments were carried out to specifically capture plasma membrane-associated OATP1A2 transporters. Cell surface biotinylation experiments were performed to specifically capture plasma membrane-associated OATP1A2 (Fig. 5B). Similar to that seen with total cell lysate, OATP1A2*6 (A404T) and *7 (C2003G) variants showed significantly lower cell surface-associated expression of immunoreactive proteins. For OATP1A2*3 and *5, a lower intensity of bands of ∼71 and 100 kDa was noted (Fig. 5B). The enrichment of cell surface proteins within the biotinylated fractions was evidenced by the lack of immunodetectable calnexin (an intracellular protein) in the samples (Fig. 5B). In addition, the OATP1A2*6 (A404T) variant showed altered mobility of the immunoreactive protein, indicative of possible changes in glycosylation status.

Deglycosylation of Total and Cell Surface-expressed OATP1A2—When expressed in HeLa cells, the total and cell surface-expressed OATP1A2 protein appeared to possess multiple molecular masses including the predicted (unglycosylated) size of ∼59 kDa, as well as larger molecular size forms of ∼71, 100, and 150 kDa (Fig. 5, A and B). We performed enzymatic deglycosylation experiments to determine whether this apparent difference reflects various the glycosylation status of total and cell surface-expressed OATP1A2. In addition, we also compared the deglycosylation pattern of the OATP1A2*6 (A404T) variant (which showed altered mobility in Fig. 5A) with that of OATP1A2*1. When subjected to enzymatic N-deglycosylation, both total and cell surface-expressed fractions of OATP1A2*1 and *6 showed a significant shift in mobility, resulting in similar apparent molecular sizes (Fig. 6). These observations are consistent with the presence of additional N-glycosylation sites in OATP1A2 protein as predicted by the NetNGlyc (www.cbs.dtu.dk/services/NetNGlyc) and ScanProsite algorithms (us.expasy.org/tools/scanprosite); five putative N-glycosylation sites were identified, including Asn residues at the amino acid positions of 62, 124, 412, and 483 as well as the 135 position, corresponding to the OATP1A2*6 variant. Chemical deglycosylation experiments were also carried out using tunicamycin, an inhibitor of Asn-linked glycosylation. Similar to enzymatic deglycosylation results, tunicamycin treatment shifted the molecular masses of the immunoreactive bands of both total and cell surface-expressed OATP1A2*1 and

![Figure 5](http://www.jbc.org/)

**Fig. 5.** Total and cell surface expression of OATP1A2 variants. Total cell lysate proteins (A, 0.5 μg of total protein) containing both biotinylated and nonbiotinylated proteins and cell surface-expressed proteins (B, 50% of biotinylated proteins captured on streptavidin beads) were subjected to SDS-PAGE, transferred into nitrocellulose membranes, and probed with anti-V5 antibody (top panels) and anti-calnexin antibody (bottom panels). Immunoblots for total and cell surface proteins were exposed to x-ray film for identical periods of time. In addition to the band corresponding to the expected (unglycosylated) molecular size of ∼59 kDa of OATP1A2 (indicated by the arrow a) and 74 kDa (glycosylated, indicated by the arrow b), additional band patterns (indicated by the arrows c and d) suggest various glycosylation statuses as well as possible dimer formation. The OATP1A2*6 (A404T) variant exhibited a shift in the mobility of immunoreactive bands.

![Figure 6](http://www.jbc.org/)

**Fig. 6.** Enzymatic and chemical deglycosylation of total and cell surface-expressed OATP1A2 *1 and *6 variants. Enzymatic N-deglycosylation was carried out on the total and cell surface-expressed OATP1A2*1 and *6, and Western blot analyses were subsequently performed to probe immunoreactive bands and shifts in molecular sizes. Chemical deglycosylation experiments were carried out by adding tunicamycin (0.3 μg/ml) during the post-transfection period after recombinant vaccinia-mediated transfection.

![Figure 7](http://www.jbc.org/)

**Fig. 7.** Immunofluorescence confocal microscopy of OATP1A2 variants in HepG2 (A and B) and HeLa cells (C). Texas red (red fluorescence) was used to detect V5-tagged OATP1A2 variants. OATP1A2*1 was targeted to the cell surface in transiently transfected HepG2 and HeLa cells as demonstrated by lateral (x-y) and axial (x-z) confocal imaging across the cell. Certain OATP1A2 variants (*3, *6, and *7) appeared to have substantial alterations in plasma membrane trafficking in both HepG2 and HeLa cells.
*6 protein to a similar extent as that observed with enzymatically N-deglycosylated samples (Fig. 6). These results confirm that Asn-135 is a key glycosylation site in OATP1A2.

**Plasma Membrane Localization of OATP1A2 Variants Using Immunofluorescence Analysis**—Cell surface biotinylation experiments and Western blot analysis of total cell lysates in transfected HeLa cells suggested that the reduced transport activities of certain OATP1A2 variants may be due to altered trafficking of the protein to the cell surface. To further assess our findings, we performed immunofluorescence confocal microscopy experiments from the liver-derived HepG2 as well as our findings, we performed immunofluorescence confocal microscopy experiments from the liver-derived HepG2 as well as HeLa cells following transient transfection with the V5-tagged SLCO1A2 variants (+1–+7). Using primary antibody against V5 epitope and secondary antibody labeled with Texas Red, we observed that wild-type OATP1A2*1 protein was targeted to the plasma membrane of both HepG2 and HeLa cells when viewed axially (x-z scans) and laterally (x-y scans) (Fig. 7). The expression patterns of certain OATP1A2 variants, including OATP1A2*3 (A516C), *6 (A404T), and *7 (C2003G), suggested substantial alterations in cell surface trafficking in both HepG2 and HeLa cells (Fig. 7).

**DISCUSSION**

Drug absorption, elimination, and targeted tissue penetration are now widely recognized to be significantly dependent on drug uptake and efflux transporters (11, 12). Although the extent of our knowledge in terms of efflux transporters such as MDR1 (P-glycoprotein) is quite significant, only recently has there been the appreciation of the presence and importance of drug uptake transporters to the overall drug disposition process. A family of solute carriers, broadly referred to as OATP, appears to have particular relevance to drug disposition. Targeted expression of OATPs in organs such as the liver, intestine, kidney, and brain seems to alter the extent of drug bioavailability and possibly central nervous system penetration. Recently, a new classification and nomenclature system (OATPS/SLCO superfamily) has been implemented to permit an unequivocal and species-independent identification of OATP genes and gene products (1). Clearly, a better understanding of OATP transporters at the molecular level is likely to aid the development of drugs with more predictable bioavailability and efficacy profiles.

In this report, we describe the differential expression pattern of OATP1A2 in human brain and kidney and the identification and functional characterization of nonsynonymous SNPs in SLCO1A2. We first assessed the expression of OATP1A2 using multiple human organ tissues via immunohistochemistry. We utilized an antibody raised against the C-terminal of OATP1A2, which appeared to be specific when compared with antibodies generated against OATP1B1 (OATP-C) and OATP1B3 (OATP-8) (data not shown). In the liver, OATP1A2 expression was localized specifically to cholangiocytes but not in hepatocytes. In the kidney, OATP1A2 was expressed specifically on the apical side of distal nephrons. Consistent with the reported literature (7), we detected OATP1A2 mainly in the brain capillary.

Genotypic analyses of subjects from various ethnic populations revealed that the allelic frequencies of six identified SNPs in SLCO1A2 are dependent on ethnicity (Table I), although the extent of genetic variation appeared modest when compared with other members of OATPs such as OATP1B1 and OATP2B1 (10, 13). When the transport function of the wild-type and six SLCO1A2 allelic variants was assessed in vitro, the OATP1A2*3 (A516C, E172D) and *6 (A404T, N135I) variants demonstrated markedly reduced transport activity toward all three substrates tested, i.e., estrone 3-sulfate, deltorphin II, and DPDE (Figs. 2–4). Other OATP1A2 variants showed altered transport activity in a substrate-specific manner (Figs. 3 and 4). For example, OATP1A2*4 (G559A, A187T) exhibited reduced uptake transport activity with estrone 3-sulfate but not with deltorphin II and DPDE. However, the opposite was found to be the case with OATP1A2*5 (A382T, N128Y) and *7 (C2003G, T668S).

Upon analysis of transport kinetic data, it was evident that mutations at codons 172 (OATP1A2*3), 187 (OATP1A2*4), 128 (OATP1A2*5), 135 (OATP1A2*6), and 668 (OATP1A2*7) have functional consequences. An OATP1A2 membrane topology map based on hydropathy analysis suggested that mutations associated with OATP1A2*3 (A516C) and *6 (A404T) are located in the transmembrane domain 4 and the second external loop, respectively (Fig. 8). Both mutations appear to be located in relatively conserved amino acid sequence regions in comparison with other related OATP members. These regions have been suggested to have a potentially important function in terms of substrate specificity based on sequence homology with an evolutionarily primitive Oatp cloned from skate liver (14). Reduced transport activities of these OATP1A2 variants (OATP1A2*6 and *7) may relate in part to reduced total OATP1A2 protein expression. In addition, the OATP1A2*6 variant exhibited significantly reduced cell surface-associated expression in HepG2 and HeLa cells when assessed using cell
surface biotinylation or immunofluorescence confocal microscopy. Similarly, confocal microscopy revealed significant loss of cell surface-associated expression for OATP1A2*3 and *7. It should be noted that there may be a degree of cell type-dependent differences in the extent of cell surface expression for variants such as OATP1A2*7. However, for the most part, there appeared to be good concordance in terms of SNP-induced differences in cell surface expression between the two cell lines. In addition, for OATP1A2*6 (A404T, N135I), loss of asparagine at this position appeared to result in altered glycosylation at a predicted consensus sites (Asn-X-(Ser/Thr)) mutation, putatively located in the second external loop, is at a predicted consensus sites (Asn-X-(Ser/Thr)) for N-glycosylation (Fig. 8). The N-glycosylation of membrane-bound proteins has been demonstrated to play a number of important roles including modulation of biological activity, regulation of intracellular targeting, protein folding, and maintenance of protein stability. A recent study demonstrated that N-glycosylation also plays an important role in OAT1 transport activity (15).

When tissue expression pattern and genetic variation in OATP1A2 are considered together, a number of novel insights regarding organ-specific elimination or drug entry become apparent. First, the presence of OATP1A2 in the distal tubules of the nephrons suggests a potentially important role for this transporter to reabsorption of drugs that are filtered or secreted at the level of the proximal tubule. Its expression at the level of the BBB is of particular clinical relevance given the broad substrate specificity for this transporter, including opioid peptide analogues such as deltorphin II and DPDPE (Fig. 2). The BBB, formed by the tight intracellular junctions of brain capillary endothelial cells, express a number of transporter proteins on the apical and basolateral membranes. Expression of transporters has been shown to be critical to the regulated and sometimes limited entry of xenobiotic compounds into the brain (16). Among multiple transporters expressed at the BBB, OATP1A2 may be one of the major uptake transporters that therapeutic drugs may use to gain the access to the brain (7). The efficacy of analgesics and peptide-mimetic compounds may depend critically on OATP1A2-mediated transport across membrane barriers to their cellular target in the central nervous system. Accordingly, genetic variations associated with OATP1A2 may not only affect the disposition of endogenous and xenobiotic compounds in organs such as the kidney but also alter the extent of drug delivery to key tissue compartments such as the brain, thereby contributing to inter-individual variability in drug responsiveness.

In summary, we report the identification and functional characterization of polymorphisms in human SLCO1A2. To our knowledge, this represents the first detailed examination of tissue-selective expression and characterization of polymorphisms in human OATP1A2 and creates the framework for further investigations of the consequences of SLCO1A2 polymorphisms in vivo. Considering its substrate specificity and expression in organs such as the brain and kidney, genetic variations in SLCO1A2 may be an important contributor to inter-individual variability in drug disposition and central nervous system entry of substrate drugs.

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Polymorphisms in Human Organic Anion-transporting Polypeptide 1A2 (OATP1A2): IMPLICATIONS FOR ALTERED DRUG DISPOSITION AND CENTRAL NERVOUS SYSTEM DRUG ENTRY

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