The human organic anion transporter 3 (OAT3; SLC22A8): genetic variation and functional genomics

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Erdman, Andrew R., Lara M. Mangravite, Thomas J. Urban, Leah L. Lagpacan, Richard A. Castro, Melanie de la Cruz, Wendy Chan, Conrad C. Huang, Susan J. Johns, Michiko Kawamoto, Douglas Struyke, Travis R. Taylor, Elaine J. Carlson, Thomas E. Ferrin, Claire M. Brett, Esteban G. Burchard, and Kathleen M. Giacomini. The human organic anion transporter 3 (OAT3; SLC22A8): genetic variation and functional genomics. Am J Physiol Renal Physiol 290: F905–F912, 2006. First published November 15, 2005; doi:10.1152/ajprenal.00272.2005.—The human organic anion transporter, OAT3 (SLC22A8), plays a critical role in renal drug elimination, by mediating the entry of a wide variety of organic anions, including a number of commonly used pharmaceuticals, into the renal proximal tubular cells. To understand the nature and extent of genetic variation in OAT3, and to determine whether such variation affects its function, we identified OAT3 variants in a large, ethnically diverse sample population and studied their transport activities in cellular assays. We identified a total of 10 distinct coding-region variants, which altered the encoded amino acid sequence, in DNA samples from 270 individuals (80 African-Americans, 80 European-Americans, 60 Asian-Americans, and 50 Mexican-Americans). The overall prevalence of these OAT3 variants was relatively low among the screened population, with only three variants having allele frequencies of >1% in a particular ethnic group. Clones of each variant were created by site-directed mutagenesis, expressed in HEK-293 cells, and tested for function using the model substrates, estrone sulfate (ES) and cimetidine (CIM). The results revealed a high degree of functional heterogeneity among OAT3 variants, with three variants (p.Arg1498Ser, p.Glu239Stop, and p.Ile260Arg) that resulted in complete loss of function, and several others with significantly reduced function. One of the more common variants (p.Ile260Phe), found in 3.5% of Asian-Americans, appeared to have altered substrate specificity. This variant exhibited a reduced ability to transport ES, but a preserved ability to transport CIM. These data suggest that genetic variation in OAT3 may contribute to variation in the disposition of drugs.

anion transporters; SNP; estrone sulfate; cimetidine; kinetics; confocal microscopy; pharmacogenetics

INTERINDIVIDUAL VARIATION in drug response, especially unexpected drug toxicity or reduced efficacy, represents a major problem in rational pharmacotherapy. Inherited differences in the expression level or function of various drug-metabolizing enzymes and drug transporters probably account for a significant portion of this variation (5, 10, 14). Transporters and enzymes in the intestine, liver, and kidney, which play a crucial role in systemic drug absorption and disposition, are thought to account for the greatest source of variability.

One critical drug transport system in humans is the group of organic anion transporters known as OATs, members of the solute carrier family 22 (SLC22), which mediate the cellular uptake of a wide variety of organic compounds and commonly used drugs (14, 27). Human OAT3 (SLC22A8) is an important member of this group. It is highly expressed in the kidney, with mRNA expression greater than eightfold higher than OAT1, the next most abundant SLC22 transcript in the kidney (16, 21). OAT3 is expressed specifically in renal proximal tubule cells, where it facilitates the uptake of a broad array of endogenous and exogenous anions from the blood, across the tubular basolateral membrane, into the tubular cells (15, 23–25, 28). The cellular uptake of organic anions is coupled, via anionic exchange, to the efflux of dicarboxylates down their concentration gradient, a gradient maintained, indirectly, through the action of the sodium-potassium pump (2, 22). OAT3, in particular, plays a crucial role in the elimination of a variety of substrates, especially those anionic and hydrophilic compounds that rely heavily on renal secretion. It operates in concert with other anion transporters on the tubular basolateral membrane, including OAT1 (SLC22A6), but whereas the two transporters share overlapping substrate spectra, they often display unique preferences for certain compounds. Given their important location and function, variation in the activity of OAT3 and OAT1 may be a significant source of interindividual variation in drug response.

In this study, we describe the identification of genetic OAT3 variants in a large, ethnically diverse sample population. The functional characteristics of the identified nonsynonymous variants were examined using an in vitro cellular expression system.

MATERIALS AND METHODS

Identification of SLC22A8 variants. Genomic DNA samples were collected from a cohort of unrelated healthy individuals in the San Francisco Bay Area as part of the Studies of Pharmacogenetics in Ethnically Diverse Populations (SOPHIE) project. The SOPHIE project was reviewed and approved by the UCSF Committee on Human Research, and informed consent was obtained from all sub-

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jects enrolled. The cohort was composed of 270 individuals, including 80 African-Americans, 80 European-Americans, 60 Asian-Americans (50 Chinese-Americans and 10 Japanese-Americans), and 50 Mexican-Americans. Variants of SLC22A8 were identified by direct sequencing of individual DNA samples, as has been previously described elsewhere (12). Briefly, the reference cDNA sequence was obtained from GenBank (http://www.ncbi.nlm.nih.gov, accession number NM004254.2). Sequencing primers were designed manually, to span the exonic as well as 50–200 bp of flanking intronic, regions of SLC22A8. The primer sequences are listed at http://www.pharmgkb.org.

Construction of OAT3 variants. Human OAT3 cDNA (GenBank accession number NM004254.2) was obtained by homology cloning and then subcloned into the mammalian expression vector pcDNA5/FRT (Invitrogen, Carlsbad, CA) to obtain an OAT3 reference-containing plasmid. The reference sequence corresponds to the OAT3 coding sequence that had the highest frequency in all ethnic groups. Each of the 10 nonsynonymous OAT3 variants identified in the population sample was then constructed by site-directed mutagenesis of the OAT3 reference-containing plasmid using the QuickChange mutagenesis protocol (Stratagene, La Jolla, CA). The sequence of each variant was confirmed by complete DNA sequencing, to ensure that the appropriate nucleotide change had occurred, and to confirm that no other alterations had been introduced. After confirmation, each OAT3 variant in plasmid was transformed into Subcloning Efficiency DH5α Escherichia coli cells (Invitrogen). These bacterial cells were grown in selective media overnight, and the OAT3-containing plasmid DNA was then isolated using the QIAprep Miniprep Kit (QIA-GEN, Valencia, CA) and following the manufacturer’s protocol.

Expression of OAT3 variants in mammalian cells. Each of the 10 nonsynonymous OAT3 variants was tested for function by measuring the uptake of selected radiolabeled OAT3 substrates in human cells transiently expressing each variant (i.e., after the cells had been transfected with the appropriate OAT3 variant in plasmid). Cells transfected with empty vector (EV) served as a negative control.

The transfection process was performed as follows. Human embryonic kidney cells (Flp-In-293; Invitrogen) were obtained and propagated per the manufacturer’s recommendations. Cells were plated at 25% confluence on Biocoat poly-D-lysine-coated 24-well plates (Becton Dickinson, Bedford, MA) 18–24 h before transfection. At the time of transfection, cells had typically reached a confluence of ~70–90%. The cells in individual wells were then transfected with OAT3 reference-containing plasmid or with OAT3 variant-containing plasmid using a lipid vehicle, Lipofectamine 2000 (Invitrogen), and Opti-MEM I Reduced Serum Media (UCSF Cell Culture Facility), following the manufacturer’s protocol, which had been optimized to a total DNA content of 1 μg/well and a Lipofectamine content of 3 μl/well. Cells were incubated overnight in the transfection mixture, and then the process was stopped by removal of the mixture and replacement with fresh media. The cells were again incubated overnight after the media change. Transfection efficiency using this procedure was consistently very high (>95%); therefore, it was assumed that differences in transfection efficiency between variants would not contribute significantly to differences in functional expression.

Uptake studies in OAT3 variant-expressing cells. Cellular uptake studies were performed with two different substrates, estrone sulfate (ES) and cimetidine (CIM), in separate experiments. Uptake studies were performed 36–48 h after transfection as follows: the media was removed and the cells were incubated in a solution containing 5 mM glutaric acid in PBS (UCSF Cell Culture Facility) for 30 min at 37°C. This solution was then removed and replaced with a buffered Na+-containing solution (128 mM NaCl, 4.73 mM KCl, 1.25 mM CaCl₂, 1.25 mM MgSO₄, and 5 mM HEPES or Tris, in H₂O, pH 7.4), and the cells were incubated for another 5 min. This second solution was then removed and replaced with a third solution, the uptake solution, in which the cells were incubated for 1–10 min, depending on the substrate and study. The incubation time in the uptake reaction mixture was determined, based on previously conducted time course studies, to coincide with the linear portion of the uptake vs. time curve. The two uptake solutions used in our studies contained measured quantities of either ES or CIM dissolved in buffered Na+-containing solution. Specifically, the ES uptake solution contained 20 nM [³⁵S]ES (specific activity 50 Ci/mmol; American Radiolabeled Chemicals, St. Louis, MO) and 20 nM unlabeled ES (Sigma, St. Louis, MO), whereas the CIM solution contained 250 nM [³⁵S]CIM (specific activity 21 Ci/mmol; Amersham Biosciences, Piscataway, NJ) and 1.75 μM unlabeled CIM (Sigma). The uptake reactions were stopped at various times by washing the cells three times with ice-cold choline-containing buffer (128 mM choline, 4.73 mM KCl, 1.25 mM CaCl₂, 1.25 mM MgSO₄, and 5 mM HEPES or Tris, in H₂O, pH 7.4). The cells were then lysed by incubation in a solution of 0.1 N NaOH/0.1% SDS. Radioactivity in an aliquot of the resultant mixture was then determined using a scintillation counter. The resultant uptake values for each variant were normalized for both protein content (to correct for differences in cell number from well to well) and uptake incubation time (to compare the results of uptake experiments using different incubation times). Protein assays were conducted on a spectrophotometric plate reader, using a BCA Protein Assay Kit (Pierce, Rockford, IL) and following the manufacturer’s recommended protocol.

Studies of each variant, and with each substrate, were performed in triplicate or sextuplicate (i.e., 3 to 6 wells for each variant) in each experiment, and the means ± SE uptake were calculated for each variant. Experiments were repeated at least twice for all variants, including mock-transfected and OAT3 reference-transfected cells as controls in each experiment. Statistical analysis for the functional studies was carried out by comparing the mean uptake for each variant (normalized to protein content) to that of both EV-transfected cells (negative control) and OAT3 reference-transfected cells (positive control) using the unpaired Student’s t-test.

Common OAT3 variants with reduced function were further tested by reversing the mutation back to the reference sequence, again by site-directed mutagenesis, to confirm that any observed differences in uptake function were solely the result of a single nucleotide change at the intended position (and not, for example, due to mutations inadvertently created outside the coding region by the mutagenesis process).

Kinetic studies of a common reduced function OAT3 variant. To elucidate the reason for reduced substrate uptake by the common variant, p.Ile305Phe, detailed kinetic studies were performed with ES and CIM on cells transfected with this variant as well as the common variant p.Val448Ile.

For these kinetic studies, varying amounts of unlabeled ES or CIM were added to the usual respective uptake solutions to give increasing total (radiolabeled plus unlabeled) substrate concentrations, typically ranging from 100 nM to 100 μM for ES, and 1.25 to 750 μM for CIM. Studies were performed in triplicate (i.e., 3 wells for each variant), and the means ± SD uptake were calculated for each variant at each substrate concentration in the experiment. Nonspecific cell-associated radioactivity was determined by measuring substrate uptake in EV-transfected cells at each substrate concentration, and these values were then subtracted from the results in OAT3-transfected cells to give the final kinetic data. These data were plotted using GraphPad Prism 4.03 (GraphPad Software), and a best-fit curve was drawn through the data points to fit the following equation: \( V = V_{\text{max}} [S]/(K_m + [S]) \), where \( V \) refers to the rate of substrate transport, \( V_{\text{max}} \) refers to the maximum rate of substrate transport, \([S]\) refers to the concentration of substrate, and \(K_m\) is defined as the concentration of substrate at the half-maximal transport rate. Kinetic parameters (\( V_{\text{max}} \) and \( K_m \)) for the different substrates were estimated for both variant and reference OAT3. Statistical differences in parameter estimates between variants and OAT3 reference were tested by comparing the distributions of estimates for each variant obtained from replicate experiments using the unpaired Student’s t-test.
Confocal microscopic studies of OAT3 reference and a common reduced function variant. Confocal microscopic studies were performed on cells that had been transfected with a plasmid containing a modified copy of the OAT3 reference or c.-913A>T (p.Ile305Phe) OAT3 variant cDNA, in frame with green fluorescent protein (GFP) for visualization by confocal microscopy. GFP-labeled reference and variant OAT3 cDNAs were constructed in the expression vector pcDNA5/FRT. These GFP-labeled OAT3 plasmids were then transfected into HEK cells grown on 24-well plates using the same transfection protocol as detailed above. In addition, the same vector containing a copy of the GFP coding sequence only, but no OAT3 gene, was transfected into separate wells of HEK cells to serve as a negative control. After transfection (24–48 h later), the coverslips containing transfected HEK cells were removed, washed in PBS, fixed with 4% paraformaldehyde, and stained using an Image-iT LIVE Plasma Membrane and Nuclear Labeling Kit (Invitrogen), which specifically stains the plasma membrane. Cells were then examined under a confocal microscope (Leica), using the manufacturer’s suggested protocols for optimal imaging of the GFP label and cellular stains.

RESULTS

SLC22A8 variants. We identified 10 nonsynonymous, coding-region variants in the SLC22A8 gene, all of them nucleotide substitutions, from the DNA samples of 270 individuals (540 chromosomes; Table 1). The nature and position of each SLC22A8 variant and its population frequency information have been deposited in the online databases dbSNP (http://www.ncbi.nlm.nih.gov/SNP). Positions are relative to the ATG start site and are based on the cDNA sequence from GenBank accession number NM004254.2.

Loss-of-function (with respect to estrone sulfate transport) variants are shown in bold. Reduced function variants are shown in italics. Data available at http://www.pharmgkb.org. *Positions are relative to the ATG start site and are based on the cDNA sequence from GenBank accession number NM004254.2. †Grantham values range from 5 to 215, with lower values indicating chemical similarity between amino acid residues, and higher values indicating chemical dissimilarity. ‡EC/EU classifies the amino acid at this location as being evolutionarily conserved (EC) or evolutionarily unconserved (EU) to other mammalian OAT3 orthologs; evolutionarily conserved residues are identical across multiple mammalian species, in this case—human, rat, mouse, and rabbit. §Some samples contained amplicons that could not be sequenced. Allele frequencies were calculated from actual DNA samples sequenced. AA, African-American; EA, European-American; AS, Asian-American; ME, Mexican-American; n, number of chromosomes.

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Fig. 1. Putative secondary structure of organic anion transporter (OAT3) embedded in the cellular plasma membrane and showing locations of the 10 identified nonsynonymous variants. The transmembrane topology diagram was rendered using TOPO2 [S. J. Johns (UCSF, San Francisco, CA) and R. C. Speth (Washington State University, Pullman, WA), transmembrane display software available at the UCSF Sequence Analysis Consulting Group website, http://www.sacs.ucsf.edu/TOPO/topo.html]. Individual amino acids are represented by circles, with variant amino acids represented by enlarged red circles and labeled with the corresponding nucleotide and amino acid positions and substitutions. Loss-of-function variants are highlighted with dark blue boxes, and reduced function variants are highlighted with light blue boxes. The specificity variant is noted with a light blue ellipse.
www.ncbi.nlm.nih.gov/SNP) and PharmGKB (http://www.pharmgkb.org). Figure 1 shows the location of these coding-region SNPs in the proposed protein topology of OAT3. Five of 10 variants were located in the intracellular loop between transmembrane domains (TMDs) 6 and 7.

Six variants (c. 387C>A, c. 715C>T, c. 779T>G, c. 829C>T, c. 929C>T, c. 1195G>T) were singletons, identified in only one of the 540 chromosomes sequenced. Four variants (c. 445C>A, c. 842T>C, c. 913A>T, c. 1342G>A) were found in more than one chromosome, and three variants (c. 842T>C, c. 913A>T, c. 1342G>A) were found to be polymorphic, having allele frequencies of >1% in a particular ethnic group. Notably, one variant (c. 842T>C) occurred at an allele frequency of 6% in African Americans, and another variant (c. 913A>T) was identified at a frequency of 3.5% among Asian Americans. One variant (c. 715C>T) resulted in the generation of a premature stop codon instead of coding for a glutamine amino acid residue.

**Uptake studies in OAT3 variant-expressing cells.** The results of uptake experiments for all of the OAT3 variants, using ES and CIM, are shown in Fig. 2, A and B, respectively.

With respect to ES, cells transfected with the OAT3 reference-containing plasmid displayed an uptake over 40 times that of EV-transfected cells (negative controls). This uptake was significantly reduced by the addition of known OAT3 inhibitors, such as probenecid, to the uptake media (data not shown). Of the 10 OAT3 variants, five had significantly reduced function (P < 0.005 by unpaired Student’s t-test) relative to OAT3 reference. Three of the variants (p. Arg149Ser, p. Gln239Stop, p. Ile260Arg) were discovered to result in complete loss of function, with an uptake similar to EV controls. As expected, the variant coding for a premature stop codon (p. Gln239Stop) was among the loss-of-function variants. Two additional variants (p. Arg277Trp and p. Ile305Phe) exhibited reduced function. Of note, one of these reduced function variants was the common variant (p. Ile305Phe), identified at an allele frequency of 3.5% in the Asian-American sample. Reversal of this mutation back to the reference OAT3 sequence (i.e., c. 913T>A) resulted in significantly reduced (P < 0.005) uptake compared with OAT3 reference. CIM uptake by this variant dropped back to reference. Based on the results of our initial screen, we further probed the activity of the p.Ile305Phe polymorphism, because this variant showed an apparent selectivity difference in our initial screen, and because this was the most common nonsynonymous variant with altered function in this screen. The results of detailed kinetic studies for OAT3 reference and the common variants p. Ile305Phe and p. Val448Ile, using ES and CIM as substrates, are shown in Fig. 3, A and B, respectively. With respect to ES, the Km and Vmax were 9.4 ± 0.4 pmol·min⁻¹·mg protein⁻¹ and 4.1 ± 0.2 pmol·min⁻¹·mg protein⁻¹, respectively. With respect to CIM, the Km and Vmax were 9.4 ± 0.4 pmol·min⁻¹·mg protein⁻¹ and 4.1 ± 0.2 pmol·min⁻¹·mg protein⁻¹, respectively, for OAT3 reference; 14 ± 4.6 μM and 630 ± 87 pmol·min⁻¹·mg protein⁻¹, respectively, for OAT3

![Fig. 2. Uptake studies in OAT3 variant-expressing cells.](http://ajpregnal.physiology.org/)
respectively, for the p. Ile305Phe variant; and 6.0 ± 0.9 µM and 628 ± 85 pmol·min⁻¹·mg protein⁻¹ for the p. Val448Ile variant. With respect to CIM, the data revealed a $K_m$ and $V_{max}$ of 174 ± 67 µM and 994 ± 52 pmol·min⁻¹·mg protein⁻¹, respectively, for OAT3 reference; 120 ± 26 µM and 955 ± 92 pmol·min⁻¹·mg protein⁻¹, respectively, for the p. Ile305Phe variant; and 92 ± 0.8 µM and 903 ± 120 pmol·min⁻¹·mg protein⁻¹ for the p. Val448Ile variant. Kinetic parameter estimates here are given as means ± SE from estimates obtained from multiple repeat experiments. The p. Ile305Phe variant showed a trend toward higher $K_m$ toward ES and lower $K_m$ toward CIM, consistent with the results of the initial screen; however, these differences were not statistically significant.

Representative confocal microscopic images of HEK cells expressing GFP-labeled OAT3 reference and GFP-labeled variant, p. Ile305Phe, are shown in Fig. 4. These, and other, confocal studies did not show an appreciable difference between the two variants with respect to overall expression level and subcellular localization.

**DISCUSSION**

We report the results of a comprehensive genetic and functional screen of variation in human OAT3, a major renal transporter involved in the elimination of a wide variety of drugs.

**Genetic variation in human OAT3.** Within a large and ethnically diverse sample population, we identified 10 nonsynonymous SNPs in SLC22A8, variants that altered the amino acid sequence of the resulting translated OAT3 protein. Among these, only one variant had an allele frequency of >1% in the overall population sample, and only three had ethnic-specific allele frequencies of >1%. The most common variant was identified at an allele frequency of 6% in the African-American subset of our sample. The overall amino acid diversity of OAT3 is low compared with other SLC transporters, such as the nucleoside transporter CNT1 (with ethnic-specific allele frequencies up to 49% for certain nonsynonymous variants) and OATP-C (with ethnic-specific allele frequencies up to 26%). Furthermore, amino acid variation is low compared with less functionally consequential genetic variations (e.g., synonymous or intronic OAT3 variants) (9, 11, 26). In this context, our findings suggest an intolerance of amino acid variation in OAT3, which may suggest an important physiological role of OAT3 in human fitness.

**Functional heterogeneity of OAT3 variants.** In our cellular assays, we found a high degree of functional heterogeneity among the 10 identified nonsynonymous OAT3 variants. Such functional heterogeneity has been reported for transporters in the SLC22 and SLCO families, such as OCT1, OATP1A2, and OATP-C, but is in distinct contrast to transporters like ENT1, CNT2, and CNT3 in the SLC28 and SLC29 families, which have, to date, shown little or no functional differences between nonsynonymous variants (1, 12, 13, 17, 18, 20, 26).

In general, the three OAT3 variants we identified as resulting in complete loss of function were rarer than the OAT3 variants that retained at least some function. They were also found at lower allele frequencies than reduced function variants of OCT1, OATP1A2, and OATP-C (13, 20, 26). Furthermore, all three of the loss-of-function OAT3 variants were observed to be population specific, that is, they were identified in only one ethnic group. These findings are consistent with the suggestion that OAT3 plays an important role in vivo, because mutations which deleteriously affect an organism’s viability or homeostasis are unlikely to spread into multiple populations (6).

We also identified one OAT3 variant (p. Ile305Phe) with altered substrate specificity, namely a reduced ability to transport ES but not CIM. This behavior by p.Ile305Phe was consistently observed in replicate experiments using the assay described here, as well as in another expression system (T7-vaccinia virus infection of transfected HeLa cells, unpublished data). Such specificity variants have been identified among other transporters in the SLC superfamily (e.g., CNT2, OATP-C, OATP1A2) and ABC (ATP-binding cassette) superfamily (e.g., MRP1) (13, 18, 26, 29). Although we have studied the activity of OAT3 variants using only two prototypical substrates, the anion ES and the cation CIM, it is possible that this specificity difference may extend to other substrates; thus p. Ile305Phe may have reduced activity with respect to other anionic compounds, but increased activity with respect to other cations. Variants that alter a transporter’s substrate specificity may be particularly important determinants of variation in drug response.

**Potential mechanism for reduced OAT3 function.** Of the three loss-of-function OAT3 variants (p. Arg149Ser, p. Gln239Stop, p. Ile260Arg), two had significant amino acid changes (i.e., those with a relatively large chemical distance) in conserved residues (i.e., residues that are the same across mammalian orthologs of OAT3) (8). This suggests that the loss...
of transport by these two variants is due to the substitution of a chemically different amino acid at a functionally crucial location (amino acid 149 or 260). However, further studies will need to be performed to rule out differences in mRNA or protein expression as a cause or contributory factor to differences in function. The third variant coded for the premature placement of a stop codon, resulting in a truncated OAT3 protein, which explains its lack of function.

Of the two hypomorphic (i.e., partial loss-of-function) variants with respect to ES transport (p. Arg277Trp and p. Ile305Phe), one, p. Ile305Phe, occurred at an evolutionarily conserved amino acid residue. The other, p. Arg277Trp, resulted in the substitution of an amino acid with a large chemical change. In the case of the relatively common specificity variant (p. Ile305Phe), it appears that alterations in the local chemical environment, resulting from the switch to an amino acid with a bulkier hydrophobic side chain (phenylalanine), significantly reduced the transporter’s ability to transport ES, but increased, or at least preserved, its ability to transport CIM. The location of the specificity variant also suggests that the intracellular loop between TMDs 6 and 7 may play a crucial role in substrate recognition or binding. Alternatively, because OAT3 is thought to function as an organic anion/dicarboxylate exchanger (2, 22), the alterations in activity observed for this and other OAT3 variants may be explained by disruption of dicarboxylate recognition or translocation. Further studies will be required to differentiate between these and other mechanisms for reduced transport function.

The kinetic studies performed on the relatively common variant p. Ile305Phe did not clearly establish the mechanism for reduced ES transport by p. Ile305Phe under nonsaturating conditions. Michaelis-Menten parameter estimates suggest that the differences in substrate selectivity of p. Ile305Phe may be due to small differences in the transporter’s affinity for ES (represented by an increased K_m toward this substrate) and its affinity for CIM (represented by a decreased K_m for CIM). However, the differences in these parameter estimates did not reach statistical significance. Confocal imaging revealed no gross localization differences between this variant and reference OAT3. The other polymorphism, p. Val448Ile, did not exhibit significant differences in transport kinetics for either ES or CIM.

The amino acid residues identified as affecting OAT3 function in our studies should be added to the growing list of functionally important residues or regions of OAT3 (27).

Implications of findings. The broad functional diversity in OAT3 that we identified has potentially significant clinical implications. OAT3 is highly expressed in the human kidney, where it appears to serve as the rate-limiting step in the renal secretion of many widely used drugs, such as NSAIDs, antibiotics, antivirals, chemotherapeutic agents, statins, and others (15, 16, 19, 23–25, 28). Functional genetic variation has also

Fig. 4. Digital confocal microscopic images of HEK cells expressing OAT3 reference and the common reduced function OAT3 variant, Ile305Phe, each tagged with green fluorescence protein (GFP). A: representative cluster of individual cells expressing GFP-tagged OAT3 reference (green color) and showing localization primarily to the plasma membrane. Inset: location of the plasma membrane (red color), by staining, in the same group of cells. B: representative cluster of individual cells expressing the GFP-tagged variant Ile305Phe. Inset: staining of the plasma membrane in the same cells. HEK cells were plated on lysine-coated coverslips at the bottom of 24-well plates and transfected with 1 μg of either GFP-tagged OAT3 reference or GFP-tagged OAT3 variant DNA. After 24–48 h, the cells were fixed and stained, then visualized at ×100 power using a confocal microscope (Leica), and images were taken with an attached digital camera. C: representative cluster of individual cells expressing GFP-tagged empty plasmid vector and showing localization throughout the cytoplasm.
been described for OAT1, a close homolog of OAT3 that is also expressed abundantly in the kidney (3, 7). OAT1 may compensate for reduced OAT3 activity, and vice versa; however, depending on the relative specificity for OAT1 and OAT3, functional genetic variation in OAT1 may also be additive or synergistic with reduced function OAT3 variants. If uncompensated, a reduced ability to transport a particular drug could result in altered pharmacokinetics, systemic drug accumulation, and the development of drug-related toxicity. Alternatively, reduced cellular uptake of nephrotoxic compounds by a reduced or nonfunctional OAT3 variant could decrease the intracellular exposure to such toxins and hence ameliorate the risk of renal tubular injury. In addition to its critical role in transporting anions into renal tubular cells, OAT3 has also been identified in human skeletal muscle and within the blood-brain barrier (4, 25). Functional alterations might, therefore, significantly alter the tissue distribution and central nervous system penetration of certain drugs. Future studies in humans will be needed to confirm whether these cellular data translate into clinical differences.

In summary, we discovered an intermediate degree of genetic variation, but a high degree of functional diversity, in the human transporter OAT3. Despite the observation that OAT3 appears to be under fairly strong negative evolutionary selection pressure, we identified a significant number of rare variants with no function whatsoever, and several more with reduced function. One common variant altered the substrate specificity of OAT3. While clinical studies are clearly needed, our observations suggest that genetic variants of OAT3 may be associated with reduced renal elimination of certain important drugs.

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DISCLOSURES

The authors have no specific conflicts of interest to disclose.

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