Human multdrug and toxin extrusion 1 (MATE1/SLC47A1) transporter: functional characterization, interaction with OCT2 (SLC22A2), and single nucleotide polymorphisms

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Both the kidney and liver play a major role in the elimination of exogenous and endogenous compounds. The combination of drug metabolism and transport contributes significantly to the clearance of potentially harmful compounds. While transport as a mechanism determining drug disposition has been extensively studied in the liver (e.g., OATP1B1; Ref. 23), transporter-mediated handling of xenobiotics in kidney is less well characterized. It is widely appreciated that renal drug elimination is determined not only by glomerular filtration but also by active transport processes that facilitate tubular reabsorption and secretion of drugs. Indeed, the mechanisms governing the renal elimination of organic anions have been studied in detail, and the transporters facilitating the tubular uptake and/or secretion of organic anions have been identified (1, 6, 12, 31, 43). Similarly, a separate transport system has been noted for the elimination of organic cations (OCs; Ref. 20). Specifically, endogenous or xenobiotic cations are taken up from the circulation by organic cation transporters OCT2 (15) expressed on the basolateral domain of renal tubular cells. This transporter has been extensively studied both for its genetic variability as well as the potential role of this transporter in certain drug-drug interactions (19, 40, 57). Although, the luminal exit of OCs had been proposed to be mediated by an OC/H exchanger dating back to 1981 by Holohan and Ross (14), linkage of this function to a specific transporter at the luminal membrane of the tubular cells had not been made until the recent molecular cloning and functional characterization of MATE1 transporter. There is now emerging evidence to support that the recently identified multdrug and toxic compound extrusion (MATE)-type transporters, namely SLC47A1 (MATE1) and SLC47A2 (MATE2; Refs. 24, 30) expressed on the apical domain of tubular cells, perform the key OC efflux function. Indeed the prototypical OCT2 substrate metformin (42) and other nephrotoxic cationic agents (53) were reported to be substrates of MATE1 supporting a functional link between these two transporters in the kidney.

It should be noted that although MATE1 has been detected in several tissues including liver, skeletal muscle, and heart, the main organ of expression appears to be the kidney, specifically the brush border (apical) membrane of the proximal and distal convoluted tubules (30). Due to the bidirectional transport activity dependent on a proton gradient (45, 47), the role of MATE1 in tissues other than kidney remains to be clarified. However, MATE1 has been described to mediate electroneutral exchange of several prototypical cations, including tetraethylammonium (TEA), 1-methyl-4-phenylpyridinium, paraquat (9), cimetidine (26, 34, 42), procainamide (42), fluoroquinolones (28), oxaliplatin (8, 53), and metformin (42).

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Among the MATE1 substrates, the biguanide antidiabetic agent metformin has been the most widely characterized in terms of interaction with cationic transporters such as the liver-enriched transporter OCT1 and the kidney-specific transporter OCT2. Indeed loss of function single nucleotide polymorphisms (SNPs) in these transporters have been associated with altered metformin disposition in humans (38, 41, 50). However, as mentioned above net elimination not only depends on the uptake mechanism but also on the transporter mediating the cellular efflux. With regard to metformin, more recent findings (3, 46) suggest that MATE1 is as an important determinant of the in vivo elimination and hence both OCT2 and MATE1 can modify renal clearance of metformin.

Accordingly, we hypothesized that the interplay between OCT2 and MATE1 is critical to the vectorial movement of shared substrates such as metformin across the renal tubular cells. We therefore systematically determined the role of OCT2 and MATE1 in terms of differential drug-drug interaction potential and also identified functional genetic polymorphisms in MATE1 using a heterologous gene expression system (10, 13). We now note that a number of compounds in clinical use exhibit differential inhibitory potency for OCT2 vs. MATE1.

**MATERIAL AND METHODS**

**Cloning of MATE1 (Slc47a1).** MATE1 (GenBank accession) was cloned by RT-PCR from human kidney RNA using the primers Slc47a1-for 5'-CGCAGGGCGGATCTACAGG-3' and Slc47a1-rev 5'-GGCCTGTGAAATGGTGTTAAGC-3' and the Phusion-PCR kit (Fermentas/NEB). The amplification product was cloned into pEF6-V5-His-TOPO (Invitrogen, Burlington, ON, Canada) after introducing an A-overhang using AmpliTaqGold DNA polymerase (Applied Biosystems, Streetsville, ON, Canada). The confirmed reference sequence was used to introduce the published single nucleotides using the Stratagene multisite directed mutagenesis kit and the following primers: C404T 5'-CCCAACCTCATGTGATCT-TCATTCCAGCTC-3', G1012T 5'-GGCAGGGGATCTCTCAGCATTTCCTGTG-3', and G1490T CCGTCTACGGTCTCTG-3'.

**Western blot analysis.** Western blot analysis of MATE1 was performed by 10% SDS-PAGE using 5-100 μg of protein lysate per lane for detection of the MATE1-vector and the Phusion-PCR product. The blots were probed with polyclonal antiserum against MATE1, and visualized using a chemiluminescence detection kit (ECL; Amersham Biosciences). The bands were quantified using a densitometer (Kodak Gel Logic System; GE Healthcare, Baie d'Urfe, QC, Canada). The confirmed reference sequence was used to introduce the published single nucleotides using the Stratagene multisite directed mutagenesis kit and the following primers: C404T 5'-CCCAACCTCATGTGATCT-TCATTCCAGCTC-3', G1012T 5'-GGCAGGGGATCTCTCAGCATTTCCTGTG-3', and G1490T CCGTCTACGGTCTCTG-3'.

**Eulerian and expression transport experiments.** The produced vectors were used for heterologous expression in HeLa cells. Therefore, HeLa cells were seeded in 12-well plates. After reaching 80% confluency, the cells were transfected with 1 μg of the MATE1-vector or vector control using 3 μl Lipofectin (Invitrogen) in 400 μl Opti-MEM medium (Invitrogen). Expression was driven by a T7 RNA-polymerase that is introduced into the cell system by infection with a recombinant attenuated vaccinia virus (vTF7-3; ATCC no. VR-2153) (13). For transport experiments, the cells were washed with Opti-MEM and exposed to radiolabeled [3H]CTEA or [14C]metformin (1 μM/200,000 dpm/well) diluted in pH-controlled Krebs-Henseleit bicarbonate buffer containing 118 mM NaCl, 1.2 mM MgSO4, 1.2 mM KH2PO4, 4.7 mM KCl, 26 mM NaHCO3, and 2.5 mM CaCl2. Uptake transport kinetics was determined using data obtained at 5 min after initiation of transport at multiple concentrations. Cells were washed three times with ice-cold PBS, and the amount of accumulated radioactivity was determined after cell lysis in 1% SDS using UltimagOLD-scintillation fluid and a scintillation counter (Liquid Scintillation Counter, Tri-Carb 2900TR; Perkin Elmer Life Sciences).

Kinetic parameters were determined using the Michaelis-Menten Model using the GraphPad Prism software (GraphPad Prism Software, San Diego, CA). For determination of the IC50 values of inhibitors of MATE1 transport, 10 concentrations of inhibitor were included in the experiment ranging from 0.1 to 100 μM. Extent of inhibition was determined after a 10-min incubation with the radiolabeled substrate in the presence of the inhibitor.

**Substrates.** [14C]-labeled TEA bromide (specific activity of 3.5 mCi/mmol) and [14C]-labeled metformin hydrochloride (specific activity of 100 mCi/mmol) were purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA) and American Radiolabeled Chemicals (St. Louis, MO), respectively. Rapamycin was obtained from Calbiochem (EMD Chemicals, Gibbstown, NJ). The compounds ritonavir, tipranavir, and amprenavir were obtained from Toronto Research Chemicals (Toronto, CA). Other compounds used in the study were purchased from Sigma-Aldrich (St. Louis, MO).

**Western blot analysis.** Western blot analysis of MATE1 was performed using cell lysate of HeLa cells. Briefly, transfected HeLa cells were collected in 5 mM Tris · HCl after several times shock freezing in liquid nitrogen followed by 20 strokes using a potter homogenizer, and the protein content was determined using a BCA-protein quantification system (Thermo Fisher Scientific, Nepean, ON, Canada). Microtubular proteins were separated and electrotransferred to a nitrocellulose membrane. Western blots were visualized with a chemiluminescence detection kit (ECL; Amersham Biosciences). The bands were quantified using a densitometer (Kodak Gel Logic System; GE Healthcare, Baie d'Urfe, QC, Canada). The confirmed reference sequence was used to introduce the published single nucleotides using the Stratagene multisite directed mutagenesis kit and the following primers: C404T 5'-CCCAACCTCATGTGATCT-TCATTCCAGCTC-3', G1012T 5'-GGCAGGGGATCTCTCAGCATTTCCTGTG-3', and G1490T CCGTCTACGGTCTCTG-3'.

**Human DNA samples.** We tested the frequencies of two SNPs on 253 DNA controls of healthy volunteers aged 18 to 65 yr in this study. They had been enrolled in a previous pharmacogenetic study (5). The details of eligibility criteria for selection of healthy volunteers have been defined previously. No selection on ethnic origin was realized, and 222 white subjects and 31 nonwhite subjects were included in the study. Written informed consent to participate in the genotyping part of the study was obtained from the volunteers, and the studies themselves were approved by the Comité Consultatif pour la Protection des Personnes Participant à la Recherche Biomédicale of Pitie Salpêtrière Hospital (Assistance Publique des Hôpitaux de Paris local ethics committee). The recommendations of the declaration of Helsinki for biomedical research involving human subjects were followed. The DNA samples of Japanese and Tanzanian Africans were kindly provided by Dr. D. Rosskopf (32, 33). A randomly chosen subset of the originally collected DNA samples has been included in this study to determine allele frequencies.
MECHANISMS INFLUENCING MATE1 TRANSPORT ACTIVITY

Fig. 1. Transport of $[^{14}C]$ metformin was performed using heterologous transporter expression in HeLa cells. T7 polymerase-driven overexpression of organic cation transporter 2 (OCT2; ▲ and ○) or multidrug and toxic compound extrusion-type transporter 1 (MATE1; ■) using a recombinant vaccinia virus (vTF7-3) revealed time dependency (A) and concentration dependency (B) of metformin uptake by both transporters. Data are expressed as means ± SD.

Results

Metformin transport mediated by OCT2 (SLC22A2) and MATE1 (SLC47A1). Transport of $[^{14}C]$ metformin was assessed using a heterologous expression system. MATE1 is a proton-dependent extrusion transporter that functions in an export or uptake mode dependent on the intravesicular proton gradient. Transport experiments employing the T7 RNA polymerase-driven protein expression system for MATE1 were performed in the uptake mode (pH 8.0) revealing time- and concentration-dependent accumulation of the biguanide in MATE1-transfected HeLa cells (Fig. 1, A and B). The MATE1-specific metformin uptake (5 min) was saturable with a $K_{m}$ value of 202 ± 31 μM/L. Similar results were obtained for OCT2-specific uptake (at 5 min; $K_{m}$ value of 680 ± 175 μM/L). This is in accordance with previously reported $K_{m}$ values for OCT2 (ranging from 393 μM/L to 1.38 mM/L) and MATE1 (227 μM/L) assessed using other types of gene expression systems (9, 17, 20, 40).

Analysis of MATE1 (SLC47A1) and OCT2 inhibitors (SLC22A2).

To test the potential of MATE1 as target of drug-drug interactions, we tested a wide range of potential inhibitors. As mentioned before, the net effect of MATE1 in renal tubular cells depends on a basolateral uptake mechanism; therefore, we also determined the effect of those compounds on OCT2. Included were substances with major renal elimination routes, such as azidothymidine, captopril, cimetidine, ciprofloxacin, metronidazole, ofloxacin, omeprazole, probenecid, pyrimethamine, rapamycin, ranitidine, tolbutamide, and trimethoprim. As MATE1 was also reported to be expressed in the liver, a selection of drugs predominantly eliminated by the liver was also included in the screening of potential inhibitors.

As summarized in Table 1, both MATE1- and OCT2-mediated cellular uptake of metformin after a 10-min incubation was significantly inhibited in the presence of 25 μM amprinavir, cimetidine, ciprofloxacin, clotrimazole, dipyridamole, ketoconazole, mitoxantrone, probenecid, pyrimethamine, ranitidine, rapamycin, sildenafil, and zidovudine. These substances were also identified as OCT2 inhibitors (data not shown).

Table 1. Inhibition screening of OCT2 or MATE1 in vitro

<table>
<thead>
<tr>
<th>Substance</th>
<th>MATE1, % control†</th>
<th>OCT2, % control†</th>
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<tbody>
<tr>
<td>Amprenavir</td>
<td>70.91 ± 7.71 *</td>
<td>34.17 ± 4.80 *</td>
</tr>
<tr>
<td>Captopril</td>
<td>166.67 ± 14.82</td>
<td>69.30 ± 15.21 *</td>
</tr>
<tr>
<td>Cinacalcet</td>
<td>100.62 ± 13.97</td>
<td>64.60 ± 5.44 *</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>21.08 ± 0.81 *</td>
<td>47.01 ± 6.77 *</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>34.57 ± 0.70 *</td>
<td>83.31 ± 4.78</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>30.20 ± 3.19 *</td>
<td>57.33 ± 2.19</td>
</tr>
<tr>
<td>Cyclosporine A</td>
<td>99.44 ± 12.89</td>
<td>50.04 ± 0.54</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>35.87 ± 1.35 *</td>
<td>44.84 ± 2.91 *</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>114.87 ± 14.35</td>
<td>111.52 ± 15.33</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>25.85 ± 0.56 *</td>
<td>38.52 ± 2.02 *</td>
</tr>
<tr>
<td>Metamizolazone</td>
<td>162.16 ± 43.76</td>
<td>103.77 ± 4.38</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>28.50 ± 0.68 *</td>
<td>97.48 ± 3.26</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>85.56 ± 10.98</td>
<td>53.48 ± 9.52 *</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>79.37 ± 2.65 *</td>
<td>43.80 ± 5.76 *</td>
</tr>
<tr>
<td>Pachitaxel</td>
<td>89.27 ± 15.87</td>
<td>124.73 ± 15.29</td>
</tr>
<tr>
<td>Probenecid</td>
<td>43.19 ± 2.37 *</td>
<td>43.71 ± 2.63 *</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>32.17 ± 0.66 *</td>
<td>40.29 ± 5.84 *</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>40.57 ± 0.58 *</td>
<td>74.89 ± 0.73 *</td>
</tr>
<tr>
<td>Rapamycin</td>
<td>30.43 ± 1.91 *</td>
<td>109.46 ± 1.63</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>25.75 ± 9.19 *</td>
<td>77.35 ± 5.70 *</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>145.33 ± 18.23</td>
<td>112.91 ± 8.28</td>
</tr>
<tr>
<td>Tolbutamide</td>
<td>157.14 ± 5.66 *</td>
<td>137.81 ± 14.78</td>
</tr>
<tr>
<td>Trimetazin</td>
<td>53.15 ± 14.01 *</td>
<td>44.01 ± 2.91 *</td>
</tr>
<tr>
<td>Trimethoprin</td>
<td>25.35 ± 0.49 *</td>
<td>59.52 ± 3.95 *</td>
</tr>
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</table>

Data are expressed as means ± SD. Inhibition of metformin uptake was assessed in a heterologous expression system expressing the renal uptake transporter organic cation transporter 2 (OCT2) or the apical efflux transporter multidrug and toxic compound extrusion-type transporter 1 (MATE1). Transport experiments involving MATE1 were performed in uptake mode at pH 8.0. *$P < 0.05$ by Mann-Whitney-U test. †Inhibitor concentration of 25 μM.

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mycin, tipranavir, and trimethoprim (%uptake of DMSO control; \( P < 0.05 \)). Subsequently, the IC\(_{50}\) of MATE1 mediated for uptake of metformin and of TEA was determined for ritonavir (15.4 ± 2.5 and 13.9 ± 1.2, respectively), ranitidine (18.9 ± 7.3 and 17.5 ± 4.4, respectively), rapamycin (3.27 ± 0.46 and 3.51 ± 0.61, respectively), and mitoxantrone (4.4 ± 1.3 and 5.2 ± 0.9, respectively). It should be noted that in vivo peak concentrations for the here tested compounds have been reported to be 11 \( \mu \)g/ml (~15 \( \mu \)M) for ritonavir, 462 ± 54 ng/ml (~1.30 \( \mu \)M) for ranitidine, 94–210 ng/ml (~0.1–0.22 \( \mu \)M) for rapamycin, and 308 ± 133 ng/ml (~0.6 \( \mu \)M) for mitoxantrone (7).

**Effect of inhibitors on metformin accumulation in cells expressing both OCT2 (SLC22A2) and MATE1 (SLC47A1).**

Several compounds exhibited divergent inhibitory effects for OCT2 vs. MATE1 (Table 1) when the transporters were studied separately. To be more reflective of the in vivo situation, we also tested cells expressing both OCT2 and MATE1 in terms of net effect on metformin cellular levels. In the control experiments, MATE1-mediated export (experiments conducted at pH 6.5) significantly reduced the cellular accumulation of \([^{14}\text{C}]\)metformin in the presence of the uptake transporter OCT2; therefore, we tested cells expressing both OCT2 and MATE1 for effects on the accumulation of metformin (%OCT2-expressing cells, OCT2-transfected cells: 100.00 ± 14.65 and double-transfected cells: 23.78 ± 2.06 by Mann-Whitney \( U \)-test, \( P < 0.05 \); data not shown). In further experiments conducted at pH 6.5, the influence of compounds identified as differential inhibitors was tested. Treatment of cells with compounds mainly reducing OCT2 transport activity such as cyclosporine A or captopril (25 \( \mu \)M) resulted in no change of the cellular metformin accumulation in OCT2-MATE1 double transfectants compared with OCT2-only expressing cells treated with the inhibitor (26.62 ± 2.92 and 26.28 ± 3.41% of OCT2-expressing cells, respectively, by Students \( t \)-test; \( P < 0.05 \); Fig. 2A). However, treatment with ritonavir and ranitidine (25 \( \mu \)M), compounds that had similar potency of inhibition for both transporters, resulted in a modest but statistically significant increase in cellular levels of metformin (37.88 ± 0.90 and 31.97 ± 2.56% of OCT2-expressing cells, respectively, by Mann-Whitney \( U \)-test \( P < 0.05 \); Fig. 2B). Subsequent experiments were conducted using compounds that were identified as more specific inhibitors of MATE1, such as rapamycin and mitoxantrone (52.33 ± 15.76 and 52.32 ± 5.12% of OCT2, respectively, by Mann-Whitney \( U \)-test; \( P < 0.05 \); Fig. 2C).

**SLC47A1 (MATE1) SNPs.** Inspection of the NCBI SNP database revealed the presence of three missense mutations located in the coding sequence of SLC47A1. The SNP
c.404T>C (rs35646404) with an unknown frequency results in an amino acid exchange in position 159 (p.159T>M). In addition, the coding polymorphisms c.1012G>A (p.338V>I, rs35790011) and c.1490G>T (p.497C>F; rs35395280) with the following heterozygosity frequencies of 0.065 and 0.023, respectively, were published in the database. With the use of the TMpred program, a software to predict orientation and protein structure (http://www.ch.embnet.org/software/TMPRED_form.html), the potential secondary structure of human MATE1 was calculated (Fig. 3A). In contrast, to the first published predicted structure of the protein (30), but in common with following reports (44) 13 transmembrane domains were predicted. This topology model is supported by recent experimental findings by Zhang and Wright (54). The missense mutations were not located in any highly conserved regions of the transporter (29) and did not include glutamate residues, which are thought to be essential for transport activity (Fig. 3A; Ref. 25).

Influence of genetic variants of MATE1 on transport and protein localization. The functional role for the nonsynonymous SNPs in MATE1 was assessed in vitro through the study of

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**Fig. 3.** Influence of coding single nucleotide polymorphisms (SNPs) on MATE1 transport activity. SNPs previously deposited in the NCBI SNP database were evaluated for their effect on transport activity of MATE1. To determine location of the SNP-associated amino acids in the protein, the secondary structure of MATE1 was predicted using TMpred. SNPs resulting in T159M, V338I, and C479F are shown (A), and their effects on the transport of the prototypical substrates tetraethylammonium (TEA; B) and metformin (C) were determined by performing the experiments in the uptake mode at pH 8.0. Similar results were obtained by assessing transport in the efflux mode (pH 6.5) in double-transfected cells (D and E). pEF, pEF6-V5/His-TOPO.
[14C]metformin and [14C]TEA uptake as prototypical substrates. We noted a significant reduction in transport activity in cells expressing the p.159T>M variant (c.404T>C, rs35646404) or the p.338V>I (c.1012G>A, rs35790011), whereas no statistically significant difference was seen for the p.497C>F (c.1490G>T, rs3595280) variant (Fig. 3, inset). To determine if similar effects are seen for the efflux mode of MATE1, the influence of the MATE1 SNPs was determined using double-transfected cells expressing OCT2 and MATE1 (Fig. 3, inset). As shown in Fig. 3D, cells expressing the variants p.T159V and p.V338I exhibited significantly increased cellular accumulation of TEA in the presence of the uptake transporter OCT2, compared with wild type and C497F variant (TEA uptake %vector control, pEF-control: 100.00 ± 11.75%; OCT2-control: 1.229.06 ± 151.64%; OCT2&MATE1: 329.06 ± 21.81%; OCT2&MATE1-T159V: 803.42 ± 221.35%; OCT2&MATE1-V338I: 835.90 ± 130.11%; and OCT2&MATE1-C497F: 313.67 ± 23.27%). Similar results were obtained for metformin (Fig. 3E). The kinetics of [14C]metformin uptake (5 min) was determined for wild-type and MATE1-variants showing significant changes in Vmax and Km values as shown in Table 2.

**Determination of cell surface expression of MATE1 and its genetic variants.** One potential reason for the observed reduction in transport activity of MATE1 variants is reduced protein synthesis and/or impaired cell surface sorting. To determine whether one of these mechanisms is involved, we carried out cell surface expression analysis. Surprisingly, Western blot analysis revealed higher total expression in cells transfected with the impaired function variants T159M and V338I, respectively (MATE1-to-wild type ratio ± SD, MATE1-wild type: 0.68 ± 0.25; MATE1-T159M: 1.42 ± 0.48; MATE1-V338I: 1.37 ± 0.53; and MATE1-C497F: 0.51 ± 0.19; Fig. 4B). Immunofluorescent staining suggested that the variants might be associated with intracellular accumulation of the expressed transporter (Fig. 4A). This is in accordance with recent findings by Chen et al. (8). Assessment of MATE1 expression after performing a biotinylatation-streptavidin enrichment of surface proteins did not reveal any differences in membrane expressed MATE1-T159M, whereas the V338I variant exhibited higher protein levels in the membrane (Fig. 4C). Therefore, our findings would suggest that the assessed MATE1 variants do not result in a significant change in the cell surface trafficking of the transporter. Thus the observed reduction in activity likely represents SNP-associated loss of transport function rather than expression.

**Determination of allele frequencies in different populations.** The frequency of the impaired function alleles was assessed in populations of different ethnicity such as European Caucasians (n = 253), Japanese Asians (n = 95), Tansanian Africans (n = 95), and African Americans (n = 95). The c.1012G>A (p.338Val>Ile, rs35790011) polymorphism was seen in one heterozygous subject of European Caucasian descent, thus quite rare with a minor allele frequency of 0.004. However, this variant was much more common among those of African ancestry with a minor allele frequency of 0.05 and 0.10 in African-Americans and Tansanian Africans. Among Japanese Asians, the minor allele frequency was 0.01. The nucleotide polymorphism c.404T>C (p.159T>M, rs35646404) was only seen in the Japanese Asians (minor allele frequency of 0.01) (Table 3).

**DISCUSSION**

MATE1 is a recently identified electroneutral OC/H+ exchanger localized in the apical membrane of renal tubule cells (30). Importantly, the class of MATE-type transporters, is the first described to function as mediators of the cellular elimination of OCs into the renal tubular lumen. The notion that MATE1 is pivotal for the renal elimination of OCs is not only supported by its unique function but also by the finding that much of its expression is localized to the proximal tubule, the part of the nephron where secretion of xenobiotics is assumed to take place. The pH-driven function of MATE1 (45) suggests that the majority of cationic export mediated by this transporter occurs towards the end of the proximal tubule, as micropuncture experiments have shown the luminal pH is 7.4 at the beginning of the proximal tubule, while it is thought to be much lower at the distal portion (21, 49). However, in the context of previous studies showing that excretion of OCs is highest in the proximal portion of the renal proximal tubule (35), which correlates with axial heterogeneity of OC/H exchange activity along the length of the proximal tubule (27), the function of MATE1 would be predicted to be modest in the elimination of OCs. However, a significant functionally relevant role for MATE1 to OC secretion is supported by the recent findings (46) that show that the targeted deletion of murine Mate1 results in a significant reduction in the renal clearance of substrate drugs in vivo. Therefore, electroneutral OC exchange may be a major contributor of the observed OC efflux by MATE1. This is consistent with the obligatory cation exchanger exchange process as shown by Wright and Wunz (51). Thus it is likely that MATE1 can establish a luminal concentration of cationic substrates that is similar to that of the intracellular concentration even in the absence of a pronounced pH gradient.

However, with regard to the renal clearance of OCs from the circulation, the overall impact of MATE1 is also likely to depend on the processes that mediate the uptake of cationic compounds at the basolateral membrane of the tubular cell. OCT2/SLC22A2 has been previously described as the major transporter facilitating this process (20). In fact, OCT2 has been a focus of a variety of studies concerning the renal transport of OCs (15, 48, 56). However, basolateral uptake function of OCT2 means that a separate efflux transport process must be in place on the apical domain of tubular cells for the efficient vectorial movement of cationic drugs into urine. Indeed since OCT2 can mediate the intracellular accumulation of cationic substrates, efflux of shared substrates by MATE1 would likely continue even in the absence of a proton gradient through an obligatory cation exchange process. In this study, we addressed

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**Table 2.** Kinetic parameters of [14C] metformin uptake of MATE1 variants

<table>
<thead>
<tr>
<th>Variant</th>
<th>Vmax ± SE, nmol·mg⁻¹·min⁻¹</th>
<th>Km ± SE, μM</th>
<th>Vmax/Km ± SE, protein⁻¹·min⁻¹/μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>MATE1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>1.808 ± 0.151</td>
<td>238 ± 44.62</td>
<td>7.549 ± 0.421</td>
</tr>
<tr>
<td>V199M</td>
<td>0.518 ± 0.041*</td>
<td>94.66 ± 31.07*</td>
<td>5.472 ± 0.206*</td>
</tr>
<tr>
<td>V338I</td>
<td>0.692 ± 0.053*</td>
<td>123.15 ± 48.09*</td>
<td>5.619 ± 0.178*</td>
</tr>
<tr>
<td>C597F</td>
<td>1.750 ± 0.53;</td>
<td>179.0 ± 40.95</td>
<td>9.6819 ± 0.709</td>
</tr>
</tbody>
</table>

Data are means ± SE. Kinetic parameters of metformin uptake (5 min) were determined using HEK cells transiently expressing wild-type or variants of MATE1. For determination of the kinetic parameters Vmax and Km, the Michalis-Menten model was used. *P < 0.05 by Student’s t-test.
key aspects of mechanisms that govern the interplay between renal OC uptake and efflux transporters with a specific focus on the recently described human MATE1 transporter.

A key feature that supports the coordinated function of OCT2 and MATE1 is the remarkable overlap in substrate specificity, which also suggests that both transporters would exhibit similar propensity for drug inhibitors. However, to verify this assumption, we tested the effects of an array of compounds on both OCT2 and MATE1 transport activity and found that while some drugs can inhibit both transporter with similar inhibitory potency, there were drugs such as rapamycin, mitoxantrone, and ciprofloxacin that exhibited differential inhibitory potency on metformin transport when tested using in single-transporter expressing cells (Table 1).

To evaluate the functional impact of differential inhibition on the interplay of both transporters, we assessed the impact on drug accumulation in double-transfected cells. Preferential inhibition of MATE1 resulted in enhanced cellular retention of the tested substrate compound (Fig. 2). This effect can be of clinical consequence especially for cationic drugs with known nephrotoxicity like fluoroquinolones, platinum compounds, or the herbicide paraquat, which have been reported as substrates of both OCT2 and MATE1 transporters (9, 28, 53).

Compounds such as cimetidine, ranitidine, and probenecid inhibited both OCT2 and MATE1 (Fig. 2), suggesting that in vivo drug-drug interactions involving these compounds would likely result in higher circulating plasma level of the substrate drug and thereby the risk of side effects in nonrenal tissues. One example is metformin, a small cationic compound that is widely used in the treatment of type 2 diabetes. Metformin targets the liver in terms of the glucose-lowering effect, but it is actually eliminated pre-

<table>
<thead>
<tr>
<th>Minor allele frequencies of reduced function MATE1-SNPs</th>
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<td><strong>SNPs, single nucleotide polymorphisms; NE, nucleotide exchange; AA, amino acid position.</strong></td>
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Table 3. Minor allele frequencies of reduced function MATE1-SNPs

<table>
<thead>
<tr>
<th>dbs SNP ID</th>
<th>NE</th>
<th>AA</th>
<th>Exchange</th>
<th>European Caucasians</th>
<th>Japanese Asians</th>
<th>African Americans</th>
<th>Tansanian Africans</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs35646404</td>
<td>404T&gt;C</td>
<td>159 T&gt;M</td>
<td>0.00</td>
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<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
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<td>1012G&gt;A</td>
<td>338 V&gt;I</td>
<td>0.00</td>
<td>0.01</td>
<td>0.05</td>
<td>0.10</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4. Influence of MATE1 genetic variants to subcellular and localization. Localization of MATE1 wild type and variants (green) was determined performing immunofluorescence (A). Western blot analysis of cell lysates was performed to determine overall expression of the variant proteins compared with the wild type (B). Extent of cell surface sorting of the variant proteins is shown in C.
dominantly through the kidney. In general, it is assumed that the glucose-lowering efficacy of metformin is governed by its pharmacokinetic profile. Indeed, plasma concentration and tissue distribution are considered to be key determinants of the glucose-lowering response to metformin (37). Among various pharmacokinetic-related genes, drug transporters governing renal tubular secretion and/or hepatic accumulation are of particular interest. Previously, it has been estimated that 93% of the interindividual variability of metformin clearance is determined by genetic factors (22, 52) and that OCT2, the kidney enriched transporter, has been suggested to play a pivotal role in this process (11, 17, 18). This is supported by findings showing that naturally occurring SNPs of the OCT2 encoding gene (SLC22A2), which are associated with reduced cellular uptake in vitro (40), translate into changes in renal tubular clearance in individuals harboring a function impairing variant allele (39, 50). However, attempts to translate those findings into altered response to metformin of diabetic patients have not been successful (36, 55). Very recently a noncoding SNP (rs2289669 G>A SNP) of MATE1 has been reported to influence the pharmacodynamic response to metformin, suggesting reduced transport activity of the transporter being associated with a higher reduction in HbA1c (4). In the current study, we assessed for the effect of coding region nonsynonymous SNPs noted in the NCBI SNP database in terms of MATE1 transport activity in vitro. We show that the SNPs resulting in amino acid exchanges at position p.159T>M and p.338V>I, respectively, exhibited significant changes in transport activity in uptake and efflux mode for prototypical substrates of MATE1. This is consistent with a very recent study (8) that showed that p.338V>I significantly reduced transport activity in the uptake mode at pH 8.0. Kajiwara et al. (16) sequenced 89 healthy Japanese volunteers identifying SNPs located in the same domain as the latter, also showing significant reduction in the transport activity of MATE1. The reduced function polymorphisms p.310A>V, p.328D>A, and p.338V>I are located in or in vicinity of phylogenetically highly conserved regions in transmembrane domain 7, 8, or 10 of the transporter (29). It had been suggested before that this region may be of major importance for substrate recognition and transport activity as shown by mutations of glutamate residues (Glu273, Glu278, Glu300, and Glu389; Refs. 25, 30). Similar results were obtained for mutants of His 385 (2). The clinical relevance of the here reported SNPs needs to be further elucidated. Although it has been reported very recently that mice lacking the expression of MATE1 do not show any obvious physiologic phenotype in several tissues tested (46), the low allele frequencies of genetic variants (Table 3) that have been associated with impaired transport function of MATE1 [also compare the recent report by Kajiwara et al. (16)] would suggest that a fully functional protein is important for the maintenance of normal physiological processes. Finally, it seems noteworthy that the above-mentioned mouse model exhibited significant changes in metformin elimination. In fact, lack of the transporter was associated with a markedly decreased renal clearance of metformin (18% of wild-type animals; Ref. 46).

In summary, we have systematically assessed the role of OCT2 and MATE1 in the renal elimination of shared substrates and have been able to identify several MATE1- and OCT2-selective drug inhibitors. The effect of such inhibitors was assessed in both single and double transporter-transfected model systems. Therefore, our findings provide additional data on the interplay of uptake and efflux transporters needed for the prediction of the in vivo situation. In addition, we tested for the functional relevance of the known nonsynonymous polymorphisms in MATE1 using an in vitro heterologous gene expression system and note that a number of the genetic variants significantly reduced its transport activity. Since MATE1 has only been recently cloned and functionally studied, our finding of its modulation by drugs in clinical use has the potential to better predict renal drug-drug interactions and contribute to our understanding of the influence of drug transporters such as MATE1 and OCT2 to xenobiotic disposition by the kidney.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

REFERENCES


