Involvement of Human Multidrug and Toxic Compound Extrusion (MATE) Transporters in Testosterone Transport

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Received September 17, 2020; accepted January 8, 2021

Multidrug and toxic compound extrusion (MATE) transporters are primarily expressed in the kidneys and liver, where they contribute to the excretion of organic cations. Our previous study suggested that pig MATE2 (class III) participates in testosterone secretion from Leydig cells. In humans, it is unclear which MATE class is involved in testosterone transport. In this study, we aimed to clarify whether human MATE1 (hMATE1) or human MATE2K (hMATE2K) mediates testosterone transport. To confirm that testosterone inhibits transporter-mediated tetraethylammonium (TEA) uptake, a cis-inhibition assay was performed using cells that stably expressed hMATE1 or hMATE2K. Docking simulations were performed to characterize differences in the binding of hMATE1 and hMATE2K to testosterone. Transport experiments in LLC-PK1 cells that stably expressed hMATE1 were used to test whether hMATE1 mediates testosterone transport. We detected differences between the amino acid sequences of the substrate-binding sites of hMATE1 and hMATE2K that could potentially be involved in testosterone binding. Testosterone and estradiol inhibited TEA uptake mediated by hMATE1 but not that mediated by hMATE2K. Transport experiments in LLC-PK1 cells indicated that testosterone might be transported via hMATE1. This study suggested that hMATE1, but not hMATE2K, is involved in human testosterone transport.

Key words multidrug and toxic compound extrusion (MATE) family; molecular docking; SLC47A; substrate specificity; testosterone transport

INTRODUCTION

The multidrug and toxic compound extrusion (MATE) family of bacterial multidrug transporters is the most recently classified multidrug resistance-conferring protein family.1–3 Although not all of the MATE family properties have been elucidated, some MATE-type proteins are known to mediate H+ - or Na+ -coupled export of cationic drugs in bacteria.1–3

The MATE-type transporters, human MATE1 (hMATE1) (SLC47A1) and human MATE2K (hMATE2K) (SLC47A2), are human MATE orthologs and were the first MATE transporters identified in mammals.5 It has been reported that mammalian MATE-type transporters are responsible for the final stage of excretion of organic cations, using protons as a driving force. Mammalian MATE transporters have been classified into three subgroups, based on phylogenetic analysis.5 Class I transporters, including hMATE1, are mainly expressed in the apical membrane of renal proximal tubules and the canalicular membrane and apical membrane of the bile duct in the liver and are involved in the excretion of organic cations from these tissues.4 Class II transporters, including hMATE2K, have more restricted expression patterns and are primarily expressed in the apical membrane of renal proximal tubules.5 Class III transporters, including mouse MATE2, are predominantly expressed in the Leydig cells of the testes.4 There have been few functional studies on Class III members. Based on the testis-specific expression of mMATE2 and cis-inhibition analysis of testosterone, it has been suggested that mMATE2 is involved in testosterone transport.6 Our previous study suggested that porcine MATE2 (Class III) is involved in testosterone secretion from testicular Leydig cells.7 However, in humans, Class III MATE transporters specifically expressed in the testes have not been discovered. It has been reported that hMATE1 is expressed in the testes and placenta, which are hormone-related organs, in addition to the kidneys and liver, but hMATE2K is explicitly expressed in the kidneys.9 hMATE1 may be involved in testosterone transport, but it remains unknown which human MATE transporter is involved in testosterone transport. Therefore, this study aimed to investigate the involvement of the human MATE transporters hMATE1 and hMATE2K in testosterone transport.

MATERIALS AND METHODS

Materials [14C]Tetraethylammonium bromide (TEA, 5mCi/mmol) and [3H]testosterone (83 mCi/mmol) were purchased from PerkinElmer, Inc. Life Sciences (Boston, MA, U.S.A.). Non-radiolabeled compounds were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.) and were of analytical grade. Cell culture media and reagents were purchased from Invitrogen (Carlsbad, CA, U.S.A.). All other reagents were of the highest commercially available grade.

Homology Modeling, Refinement, and Evaluation of Three-Dimensional (3D) Structures Protein tertiary structures were built using Modeller version 9.2,9–13 which was utilized for backbone generation, loop and side-chain mod-
eling, and cofactor addition. Initially, 100 3D models were generated and ranked based on energy parameters such as discrete optimized protein energy (DOPE) score. The 3D structure of the multidrug transport protein NorM from Vibrio cholerae (NorM-VC, Protein Data Bank ID: 3MKT) was used as a template. The structures generated were refined using the GalaxyWEB server at the Computational Biology Lab in the Department of Biochemistry, Seoul National University (http://galaxy.seoklab.org/).

**Molecular Docking** Information on the compounds used for screening was obtained from the DrugBank database (http://www.drugbank.ca). This database is supported by the Canadian Institutes of Health Research, Alberta Innovates-Health Solutions, and The Metabolomics Innovation Centre (TMIC), Canada. Docking of the compounds was performed using AutoDockTools version 1.5.6 (CLC Bio; Qiagen, Denmark), using a Lamarckian genetic algorithm. Each compound was subjected to 100 iterations.

**Generation of HEK293 Cell Lines Stably Expressing Transporters** The Flp-In system from Invitrogen was used to generate HEK293 cell lines stably expressing hMATE1 or hMATE2K at isogenic locations. cDNAs encoding hMATE1 (SLC47A1) and hMATE2K (SLC47A2) were amplified from human kidney mRNA by RT-PCR. The transporter-coding product was subcloned into the pcDNAs/FRT vector via PCR, restriction enzyme digestion, and ligation. The sequences of all inserts were verified via DNA sequencing and compared with those of reference genes in the National Center for Biotechnology Information database (NM_018242.2 for hMATE1, and NM_001099646.1 for hMATE2K) to ensure fidelity. The constructs were then co-transfected with the pOG44 Flp-recombinase expression vector (Invitrogen) into Flp-In HEK293 cells. Transfected cells were selected on hygromycin B (150 mg/mL) and expanded. pcDNAs/FRT empty vector was co-transfected with pOG44 into Flp-In HEK293 cells to generate control cells.

**Uptake and Inhibition Assays in HEK293 Cells** Control and transporter-expressing cells were seeded in poly-L-lysine-coated 24-well plates and allowed to grow for 2–3 d to reach 80–90% confluence. Uptake assays were performed at 37°C in uptake buffer (5.6 mM glucose, 125 mM NaCl, 4.8 mM KCl, 1.2 mM K2HPO4, 1.2 mM CaCl2, 1.2 mM MgSO4, and 25 mM Tris–HCl, pH 8.0) containing cold substrates and trace amounts of radiolabeled substrates. For uptake studies in hMATE1- and hMATE2K-expressing cells, the uptake buffer was adjusted to pH 8.0 to generate an outwardly directed proton gradient, as these transporters function as proton/organic cation exchangers.1) The cells were then solubilized with 0.1% Triton X-100. The radioactivity in cell lysates was measured using a liquid scintillation counter (LSC–6100; Hitachi Ltd., Tokyo, Japan). For cis-inhibition studies, uptake was performed in the presence of cimetidine (10 μM), testosterone (1, 10 μM), and estradiol (1, 10 μM). Testosterone and estradiol were solubilized in dimethylsulfoxide (DMSO). The protein content of the lysates was measured using Pierce 660 nm Protein Assay Reagent (Thermo Scientific, Waltham, MA, U.S.A.), and cellular uptake was normalized to the corresponding protein concentration. Transporter-specific uptake was calculated by subtracting the background uptake in control cells.

**Generation of LLC-PK1 Cell Lines Stably Expressing hMATE1** The full-length cDNA encoding hMATE1 was subcloned into the pcDNA3.1+ mammalian expression vector (Life Technologies, Carlsbad, CA, U.S.A.), and the construct was transfected into LLC-PK1 cells. Transfected cells were selected on G418 (500 μg/mL) for 3–4 weeks. Colonies with normal cellular morphology and growth rates were isolated and expanded. The cell clones were screened in functional transport assays using [3H]TEA uptake. A control cell line transfected with empty pcDNA3.1+ (mock cells) was generated using similar transfection and selection procedures.

**Transport Studies in hMATE1-Transfected LLC-PK1 Cells** Testosterone flux across LLC-PK1 monolayers expressing hMATE1 was determined using a previously described protocol, with some modifications.14) LLC-PK1 cells were seeded in a 12-well Transwell® (12-well plate insert (0.4-μm pore) at a density of 2 × 10^5 cells/well). Four days after seeding, the transport experiments were performed. After removing the culture medium from both sides of the inserts, the cells were preincubated for 10 min at 37°C in uptake buffer, with the pH adjusted to 6.5 and 8.0 in the apical and basal chambers, respectively. For basal-to-apical transport, 0.5 mL of uptake buffer (pH 6.5) was added to the apical chamber, and 1 mL of uptake buffer (pH 8.0) containing 50 μM [14C]TEA (10kBq per assay) or 10 nM [3H]testosterone (10kBq per assay) was added to the basal chamber. Similar experiments in which the apical buffer had a pH of 8.0 and contained 10 mM ammonium chloride or 1 μM pyrimethamine were also conducted. To measure transcellular transport, a 5-μL aliquot of the incubation medium from the receiving (apical) chamber was periodically collected and replaced with an equal volume of fresh buffer. The radioactivity in the collected medium was measured using a liquid scintillation counter (LSC–6100). The tightness of the LLC-PK1 monolayer was verified by measuring transepithelial electrical resistance (TEER) using a Millicell-ERS system (Millipore Corporation, Bedford, MA, U.S.A.) before and after each experiment to ensure monolayer integrity. Data generated from LLC-PK1 cells with TEER values below 180 V·cm⁻² were not accepted.

**Statistical Analysis** Data are presented as the means ± standard error of the mean (S.E.M.). Significant differences between experimental groups were determined by one-way ANOVA followed by Tukey’s test for multiple comparisons. All analyses were carried out using the JSTAT software package (Version 17.0; SPSS, Chicago, IL, U.S.A.). p < 0.05 was considered significant.

**RESULTS**

**Determination of the Testosterone Transport Pocket** The three-dimensional structures of hMATE1 and hMATE2K were predicted using Modeller, based on the NorM-VC (Protein Data Bank ID: 3MKT) X-ray crystal structure data. We confirmed the predicted hMATE1 and hMATE2K three-dimensional conformation and selected the structure with the highest stability for each protein. Based on docking simulations of hMATE1 and testosterone, the testosterone-binding pocket of hMATE1 was predicted to be in N lobes (TM 1 to TM 7) (Fig. 1). The docking simulation for hMATE2K revealed no binding between hMATE2K and testosterone (data not shown). The amino acid side chains involved in the bind-
Membrane expression of hMATE1 in LLC-PK1 cells with estradiol (Table 1). It has been confirmed that DMSO hMATE1, was not observed for hMATE1 V52T. In contrast, of TEA transport activity by testosterone, as observed for cis-Inhibition was observed for hMATE2K (Table 1). -Inhibition significantly inhibited TEA uptake, but no significant inhibi- tion was observed for hMATE2K T48V. For hMATE1, both testosterone and estradiol port activity of both hMATE1 and hMATE2K was significant compared. In the presence of 10 µM cimetidine, a representative substrate of the MATE-type transporter, the TEA transport activity of both hMATE1 and hMATE2K was significantly suppressed. For hMATE1, both testosterone and estradiol significantly inhibited TEA uptake, but no significant inhibition was observed for hMATE2K (Table 1). cis-Inhibition of TEA transport activity by testosterone, as observed for hMATE1, was not observed for hMATE1 V52T. In contrast, for hMATE2K T48V, significant inhibition was observed with testosterone (10 µM) (Table 1). Similar results were observed with estradiol (Table 1). It has been confirmed that DMSO does not affect the amount of uptake.

Transcellular Transport of Testosterone in hMATE1-LLC-PK1 Cells Membrane expression of hMATE1 in LLC-PK1 cells was confirmed using immunostaining. Polarized cells were then used for the transcellular transport assay. The transcellular transport of TEA from the basal side to the apical side was considerably higher in hMATE1-LLC-PK1 cells than in LLC-PK1 mock cells (data not shown). The transcellular transport of testosterone from the basal side to the apical side between 10 and 30 min was considerably higher in hMATE1-LLC-PK1 cells than in LLC-PK1 mock cells (Fig. 3A). Transport was completely suppressed when pyrimethamine, a representative inhibitor of MATE-type transporters, was added to the apical compartment (Fig. 3A). We investigated the pH dependence of testosterone transcellular transport across cell monolayers from the basal to the apical compartment in our model system after administering the substrates to the basal compartment. Basal-to-apical translocation decreased with an increase in pH in the apical compartment. There was no corresponding pH-dependent change in basal-to-apical transcellular translocation of testosterone in monolayers of LLC-PK1 mock cells (data not shown). Testosterone transport by hMATE1-expressing cells was significantly decreased in the presence of 10 mM ammonium chloride in the apical compartment (Fig. 3B).

**DISCUSSION AND CONCLUSION**

Testosterone is known to be secreted from testicular Leydig cells by passive diffusion. However, it has been suggested that mMATE2 expressed in mouse testicular Leydig cells is involved in active testosterone secretion. Our previous report suggests that pig MATE2 is responsible for testosterone secretion from Leydig cells. However, Class III MATE transporters have not been identified in humans to date, and it is unclear which MATE transporters are involved in the secretion of testosterone in humans. Therefore, in this study, we clarified which of the human MATE transporters is involved in testosterone secretion.

cis-Inhibition analysis showed that TEA uptake by hMATE1, but not hMATE2K, was inhibited by testosterone. Testosterone transcellular transport was significantly higher in hMATE1 expressing LLC-PK1 cells than in wild-type LLC-PK1. These results suggest that lipophilic hormones such as testosterone are substrates for hMATE1, but not hMATE2K. Several studies have reported differences in substrate specificity between hMATE1 and hMATE2K. It has been reported that hMATE1 transports highly lipophilic flavonoids, and in humans, hMATE1 may be responsible for the transport of fat-soluble substrates. To clarify the differences in substrate specificity for testosterone between hMATE1 and hMATE2K, we used modeling and docking simulations. Regarding the known transport mechanism of NorM-VC, a MATE-type transporter in microorganisms, substrate-binding pockets in hMATE1 and hMATE2K were investigated using docking simulations. The results suggested that the binding mode for testosterone is different in hMATE1 and hMATE2K. This result may explain the difference in substrate specificity for testosterone in these proteins and may indicate that hMATE1 recognizes testosterone as a substrate.

Amino acids involved in the binding of hMATE1 with testos- terone were analyzed and compared with the corresponding amino acids in hMATE2K to clarify differences in substrate recognition sites between hMATE1 and hMATE2K. hMATE1 V52 and hMATE2K T48 were identified. A docking simulation revealed that hMATE1 V52T could not recognize testosterone. Furthermore, the cis-inhibition analysis showed that TEA uptake by hMATE1 V52T was not significantly inhibited by testosterone. In contrast, testosterone and estradiol significantly inhibited TEA uptake via hMATE2K T48V. These results suggest that the V52 residue in hMATE1 is required for the binding of testosterone. E273 is known to be a func-
tionally important amino acid in hMATE1. Various single nucleotide polymorphisms that affect the transport activity of hMATE1 have been reported; however, to the best of our knowledge, hMATE1 V52 mutations have not previously been reported.

Transcellular transport was investigated using Transwell assays of LLC-PK1 cells stably expressing hMATE1 to clarify whether hMATE1 actually transports testosterone. LLC-PK1 cells are swine proximal renal tubule-derived cells, known to be polarized depending on the culture conditions, and...
they are often used for transcellular transport studies. Basolateral-to-apical testosterone transport was significantly higher in hMATE1-expressing LLC-PK1 cells than in wild-type LLC-PK1 cells. This increase in testosterone transport was significantly suppressed in the presence of pyrimethamine, a typical hMATE1 inhibitor. It appears that testosterone transport through hMATE1 is driven by a proton gradient. Consistent with our findings, previous reports have suggested that hMATE1 is a H$^+$-coupled organic cation exporter.

The uptake of 50μM radiolabeled TEA by hMATE1, hMATE1 V52T, hMATE2K, or hMATE2K T48V-expressing HEK293 cells at pH 8.0 was determined in the presence or absence of the listed compounds, at the indicated concentrations. Values are expressed as the percentage of radiolabeled TEA uptake under control conditions in each MATE transporter-expressing cell, with no test substance added. The control values for TEA uptake were 79.9, 59.1, 83.1, and 79.7 pmol/mg protein respectively for hMATE1, hMATE1 V52T, hMATE2K, and hMATE2K T48V. Data are means ± S.E.M.s, n = 3–6. *p < 0.05 compared with mock, †p < 0.05 compared with hMATE1 (pH 6.5).

**Figure 3.** Trans-Cellular Transport of Testosterone via hMATE1

(A) Time course of testosterone (10 nM) transport from the basal side to the apical side at pH 6.5 by LLC-PK1 cells expressing hMATE1 or mock controls with or without 1 mM pyrimethamine (PYR). Data are represented as means ± S.E.M.s, n = 6. *p < 0.05; compared with mock. (B) hMATE1 mediates electroneutral H$^+$/testosterone exchange. pH dependence of testosterone transport. LLC-PK1 cells expressing wild-type hMATE1 or a mock control were incubated at apical pH 6.5 or pH 8.0, and testosterone transport was measured. The requirement for membrane potential or pH gradient for testosterone transport was also examined at apical pH 6.5, in the absence or presence of 10mM ammonium chloride in uptake buffer. Data are means ± S.E.M.s, n = 3–6. *p < 0.05, compared with mock, †p < 0.05, compared with hMATE1 (pH 6.5).

*Table 1. Comparison of cis-Inhibition of TEA Transport in hMATE1, hMATE1 V52T, hMATE2K, and hMATE2K T48V*

| Compound (μM) | hMATE1 | hMATE1 V52T | hMATE2K | hMATE2K T48V |
|--------------|--------|-------------|---------|-------------|
| Control      | 100 ± 2.5 | 100 ± 5.4 | 100 ± 4.6 | 100 ± 4.5 |
| Cimetidine   | 18.6 ± 1.8* | 37.6 ± 5.0* | 44.2 ± 4.6* | 21.7 ± 7.3* |
| Estradiol    | 73.9 ± 3.1* | 97.2 ± 4.5 | 114.1 ± 7.8 | 74.3 ± 3.6* |
| Testosterone | 1 ± 2 | 3.4 ± 2.5 | 3.0 ± 2.5 | 2.3 ± 2.5 |

The uptake of 50μM radiolabeled TEA by hMATE1-, hMATE1 V52T-, hMATE2K-, or hMATE2K T48V-expressing HEK293 cells at pH 8.0 was determined in the presence or absence of the listed compounds, at the indicated concentrations. Values are expressed as the percentage of radiolabeled TEA uptake under control conditions in each MATE transporter-expressing cell, with no test substance added. The control values for TEA uptake were 79.9, 59.1, 83.1, and 79.7 pmol/mg protein respectively for hMATE1, hMATE1 V52T, hMATE2K, and hMATE2K T48V. Data are means ± S.E.M.s, n = 3–6. *p < 0.05 compared with mock, †p < 0.05 compared with hMATE1 (pH 6.5).

**Acknowledgments** We thank N. Kobayashi (Setsunan University) for useful discussions. This study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan to M. Goda.

**Author Contributions** Study conception and design: M.G. Performance of the experiments and data acquisition: M.G., M.I., M.S., K.O., K.I., and M.O. Analysis and interpretation of data: M.G., M.I., M.S., K.O., K.I., and M.O. Drafting the work or critically revising it for relevant intellectual content: M.G., M.O.

**Conflict of Interest** The authors declare no conflict of interest.

**REFERENCES**

1) Brown MH, Paulsen I, Skurray R. The multidrug efflux protein NorM is a prototype of a new family of transporters. Mol. Microbiol., 31, 394–395 (1999).
2) Putman M, van Veen H, Konings W. Molecular properties of bacterial multidrug transporters. Microbiol. Mol. Biol. Rev., 64, 672–693 (2000).
3) Hvorup RN, Winnen B, Chang AB, Jiang Y, Zhou XF, Saier MH Jr. The multidrug/oligosaccharidyl-lipid/polysaccharide (MOP) exporter superfamily. Eur. J. Biochem., 270, 799–813 (2003).
4) Otsuka M, Matsumoto T, Morimoto R, Arioza S, Omote H, Moriyama Y. A human transporter protein that mediates the final excretion step for toxic organic cations. Proc. Natl. Acad. Sci. U.S.A., 102, 17923–17928 (2005).
5) Omote H, Hiasa M, Matsumoto T, Otsuka M, Moriyama Y. The...
MATE proteins as fundamental transporters of metabolic and xenobiotic organic cations. *Trends Pharmacol. Sci.*, 27, 587–593 (2006).

6) Hiasa M, Matsumoto T, Komatsu T, Omote H, Moriyama Y. Functional characterization of testis-specific rodent multidrug and toxic compound extrusion 2, a class III MATE-type polytopic H+/organic cation cotransporter. *Am. J. Physiol. Cell Physiol.*, 293, C1437–C1444 (2007).

7) Goda M, Oda K, Oda A, Kobayashi N, Otsuka M. Involvement of the multidrug and toxic compound extrusion transporter in testosterone release from cultured pig Leydig cells. *Pharmacology*, 100, 31–39 (2017).

8) Masuda S, Terada T, Yonezawa A, Tanihara Y, Kishimoto K, Katoh T, Ogawa O, Inui K. Identification and functional characterization of a new human kidney-specific H+/organic cation antiporter, kidney-specific multidrug and toxic compound extrusion 2. *J. Am. Soc. Nephrol.*, 17, 2127–2135 (2006).

9) Webb B, Sali A. Protein structure modeling with MODELLER. *Methods Mol. Biol.*, 1654, 39–54 (2017).

10) Webb B, Sali A. Comparative protein structure modeling Using MODELLER. *Curr. Protoc. Protein Sci.*, 86, 2.9.1–2.9.37 (2016).

11) Martí-Renom MA, Stuart AC, Fiser A, Sánchez R, Melo F, Šali A. Comparative protein structure modeling of genes and genomes. *Annu. Rev. Biophys. Biomol. Struct.*, 29, 291–325 (2000).

12) Eswar N, Webb B, Martí-Renom M, et al. Comparative protein structure modeling using MODELLER. *Curr. Protoc. Protein Sci.*, 50, 2.9.1–2.9.31 (2007).

13) Šali A, Blundell T. Comparative protein modelling by satisfaction of spatial restraints. *J. Mol. Biol.*, 234, 779–815 (1993).

14) Ueda K, Okamura N, Hirai M, Tanigawara Y, Saeki T, Kioka N, Komano T, Hori R. Human P-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone. *J. Biol. Chem.*, 267, 24248–24252 (1992).

15) Tanihara Y, Masuda S, Sato T, Katsuura T, Ogawa O, Inui K. Substrate specificity of MATE1 and MATE2-K, human multidrug and toxic extrusions/H+-organic cation antiporters. *Biochem. Pharmacol.*, 74, 359–371 (2007).

16) Astorga B, Ekins S, Morales M, Wright SH. Molecular determinants of ligand selectivity for the human multidrug and toxin extruder proteins MATE1 and MATE2-K. *J. Pharmacol. Exp. Ther.*, 341, 743–755 (2012).

17) Martinez-Guerrero LJ, Wright SH. Substrate-dependent inhibition of human MATE1 by cationic ionic liquids. *J. Pharmacol. Exp. Ther.*, 346, 495–503 (2015).

18) Lee JH, Lee JE, Kim Y, Lee H, Jun HJ, Lee SJ. Multidrug and toxic compound extrusion protein-1 (MATE1/SLC47A1) is a novel flavonoid transporter. *J. Agric. Food Chem.*, 62, 9690–9698 (2014).

19) Jin Y, Nair A, van Veen H. Multidrug transport protein NorM from *Vibrio cholerae* simultaneously couples to sodium- and proton-motive force. *J. Biol. Chem.*, 289, 14624–14632 (2014).

20) He X, Szewczyk P, Karyakin A, Evin M, Hong WX, Zhang Q, Chang G. Structure of a cation-bound multidrug and toxic compound extrusion transporter. *Nature*, 467, 991–994 (2010).

21) Lu M, Symersky J, Radchenko M, Koide A, Guo Y, Nie R, Koide S. Structures of a Na+-coupled, substrate-bound MATE multidrug transporter. *Proc. Natl. Acad. Sci. U.S.A.*, 110, 2099–2104 (2013).

22) Tanaka Y, Hipolito C, Maturana A, Ito K, Kuroda T, Higuchi T, Kato T, Kato HE, Hattori M, Kumazaki K, Tsukazaki I, Ishitani R, Suga H, Nureki O. Structural basis for the drug extrusion mechanism by a MATE multidrug transporter. *Nature*, 496, 247–251 (2013).

23) Asaka J, Terada T, Tsuda M, Katsuura T, Inui K. Identification of essential histidine and cysteine residues of the H+-organic cation antiporter multidrug and toxin extrusion (MATE). *Mol. Pharmacol.*, 71, 1487–1493 (2007).

24) Yonezawa A, Inui K. Importance of the multidrug and toxin extrusion MATE/SLC47A family to pharmacokinetics, pharmacodynamics/toxicodynamics and pharmacogenomics. *Br. J. Pharmacol.*, 164, 1817–1823 (2011).

25) Meyer zu Schwabedissen HE, Verstuyft C, Kroemer HK, Becquemont L, Kim RB. Human multidrug and toxin extrusion 1 (MATE1/SLC47A1) transporter, functional characterization, interaction with OCT2 (SLC22A2), and single nucleotide polymorphisms. *Am. J. Physiol. Renal Physiol.*, 298, F997–F1005 (2010).

26) Imamura Y, Murayama N, Okudaira N, Kurihara A, Inoue K, Yuasa H, Izumi T, Kusuhara H, Sugiyama Y. Effect of the fluoroquinolone antibacterial agent DX-619 on the apparent formation and renal clearance of tiao-hydroxycortisol, an endogenous probe for CYP3A4 inhibition, in healthy subjects. *Pharm. Res.*, 30, 447–457 (2013).

27) Nozaki Y, Kusuhara H, Endou H, Sugiyama Y. Quantitative evaluation of the drug–drug interactions between methotrexate and nonsteroidal anti-inflammatory drugs in the renal uptake process based on the contribution of organic anion transporters and reduced folate carrier. *J. Pharmacol. Exp. Ther.*, 309, 226–234 (2004).

28) García Rodriguez LA, Jick H. Risk of gynaeomastia associated with cimetidine, omeprazole, and other antiulcer drugs. *BMJ*, 308, 503–506 (1994).

29) Deepinder F, Braunstein GD. Drug-induced gynecomastia, an evidence-based review. *Expert Opin. Drug Saf.*, 11, 779–795 (2012).

30) Bowman JD, Kim H, Bustamante J. Drug-induced gynecomastia. *Pharmacotherapy*, 32, 1123–1140 (2012).