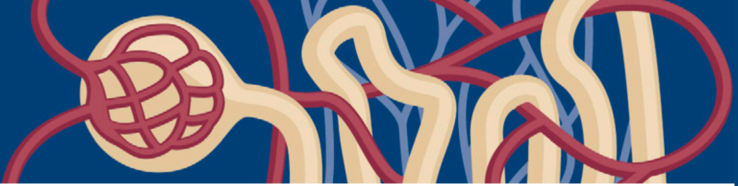
Renal Physiology



# Handling of Drugs, Metabolites, and Uremic Toxins by Kidney Proximal Tubule Drug Transporters

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## Abstract

**The proximal tubule of the kidney plays a crucial role in the renal handling of drugs (e.g., diuretics), uremic toxins (e.g., indoxyl sulfate), environmental toxins (e.g., mercury, aristolochic acid), metabolites (e.g., uric acid), dietary compounds, and signaling molecules. This process is dependent on many multispecific transporters of the solute carrier (SLC) superfamily, including organic anion transporter (OAT) and organic cation transporter (OCT) subfamilies, and the ATP-binding cassette (ABC) superfamily. We review the basic physiology of these SLC and ABC transporters, many of which are often called drug transporters. With an emphasis on OAT1 (SLC22A6), the closely related OAT3 (SLC22A8), and OCT2 (SLC22A2), we explore the implications of recent *in vitro*, *in vivo*, and clinical data pertinent to the kidney. The analysis of murine knockouts has revealed a key role for these transporters in the renal handling not only of drugs and toxins but also of gut microbiome products, as well as liver-derived phase 1 and phase 2 metabolites, including putative uremic toxins (among other molecules of metabolic and clinical importance). Functional activity of these transporters (and polymorphisms affecting it) plays a key role in drug handling and nephrotoxicity. These transporters may also play a role in remote sensing and signaling, as part of a versatile small molecule communication network operative throughout the body in normal and diseased states, such as AKI and CKD.**

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The kidney proximal tubule is the site of elimination of a vast number of small molecules. These include drugs (*e.g.*, antibiotics, antivirals, diuretics, nonsteroidal anti-inflammatory drugs, and antidiabetic agents), physiologically important metabolites (*e.g.*, folate, $\alpha$-ketoglutarate, urate, and carnitine), nutrients (*e.g.*, vitamins and flavonoids), signaling molecules (*e.g.*, odorants, cyclic nucleotides, and prostaglandins), exogenous toxins (*e.g.*, mercurial conjugates and aristolochic acid), gut microbiome products (*e.g.*, kynurenine), and endogenous toxins (so-called uremic toxins, such as indoxyl sulfate) (1–7). Apart from excreting unmodified small molecule drugs, the kidney handles many conjugated metabolites, most of which are produced by phase 1 and phase 2 metabolism in the liver (*e.g.*, products of hydroxylation, sulfation, and glucuronidation reactions) (8). Genes for phase 1 and 2 reactions are also expressed in the kidney and are likely to be very important in metabolic functions of the proximal tubule cells of kidney as well (9), although this area of research is underexplored.

Consider a hypothetical hospitalized patient with stage 3 CKD, who may have slightly elevated levels of circulating uremic toxins (*e.g.*, indoxyl sulfate) and is also being treated with $\beta$-lactam antibiotics, loop diuretics, statins, and antiviral agents. This scenario thus includes a host of drugs, metabolites, and molecules that are handled by proximal tubule transporters, which orchestrate their clearance from the blood and their elimination into the urine. The presence of gene variants of some of these transporters (possibly more common given this patient's ethnic background) could cause differences in expression or function of the transporters in the proximal tubule of this patient compared with others on the ward. Furthermore, perhaps the patient's blood pH has varied considerably, thus altering the net charge of some of the aforementioned organic molecules, and thus their capacity to be transported into different body tissues and fluids or be eliminated. Many of these small molecules tend to be protein bound, and perhaps the patient's albumin concentration is low, which may further affect small molecule distribution and elimination.

While this patient is hypothetical, this type of scenario is not uncommon. The variables that affect serum, tissue, and body fluid levels of a single drug, toxin, or metabolite excreted by the transporters that handle small molecules is quite complicated; much more so if one simultaneously considers several small molecules. Nevertheless, a great deal of progress has been made in the past few decades on the basic biology of drug, toxin, and metabolite handling, including those functioning in the kidney proximal tubule. With these details at hand, integration of this information and application to clinical settings, such as the scenario presented in the preceding paragraph, should eventually be feasible.

Many of the small molecules of clinical interest are charged: organic anions, organic cations, or molecules that have a zwitterionic character (both positive and negative charges). Molecules that are too large or albumin bound have limited glomerular filtration, and excretion instead depends largely on tubular secretion. First, the molecules flow through the peritubular capillaries, where they are extracted by multispecific drug transporters at the basolateral (blood) surface of the proximal tubule cell (Figure 1, A and B). For the most part, these molecules are secreted unchanged into the tubular lumen by a set of transporters at the apical (luminal or urine) surface of the proximal tubule cell. There appear to be more than two dozen types of transporters involved in the net transport of organic anion, organic cation, or organic zwitterions by the proximal tubule. In some cases (*e.g.,* urate), the net transport may be the result of both secretion and absorption, but here we mostly focus on the role of proximal tubule transporters in net secretion in the setting of the normal kidney and in disease states.

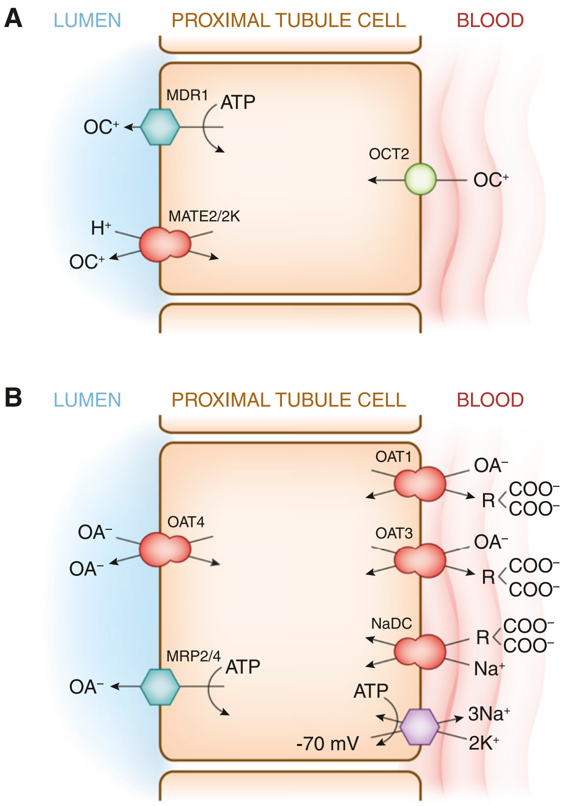

**Figure 1. | Transcellular movement of organic cations (OCs) and organic anions (OAs) in kidney proximal tubule epithelial cells.** (A) Transcellular movement of OCs in a kidney proximal tubule epithelial cell. Movement of OCs are facilitated by the negative potential difference within the cell, which is maintained by the basolateral $Na^+$,$K^+$-ATPase (not shown). OCs enter the cell across the basolateral membrane by organic cation transporters (OCTs), such as OCT2. The authors note that OCT2 is also sometimes depicted as an OC exchanger. Secretion across the apical membrane into the lumen occurs by electroneutral exchange with $H^+$ by solute carrier (SLC) transporters, including multidrug and toxin extrusion (MATE) protein 2/2K, and by ATP-binding cassette (ABC) transporters, such as multidrug-resistant protein 1 (MDR1), which use energy generated by ATP hydrolysis to transport molecules across the apical membrane. Only a fraction of the known transporters are shown. (B) OA transport *via* organic anion transporters (OATs) in a proximal tubule epithelial cell. Primary active transport of sodium out of the cell by the basolateral $Na^+$,$K^+$-ATPase creates the gradient that facilitates sodium dicarboxylate cotransporters (NaDCs) to move sodium and dicarboxylates $[R(COO^-)_2]$, such as $\alpha$-ketoglutarate, into the cell. The resulting high intracellular concentration of dicarboxylates promotes uptake of OA across the basolateral membrane in exchange for $[R(COO^-)_2]$ by organic anion transporters (OAT1 and OAT3). Apical exit involves ABC transporters including multidrug-associated resistance proteins (MRP2, MRP4) and possibly other SLC transporters including OAT4 and urate transporter 1 (URAT1, not shown).

## Classification of Organic Ion Transporters

Organic ion transporters in the proximal tubule are frequently collectively called multispecific drug transporters because of their multispecific nature and their crucial role in drug handling. But depending on the discipline (physiology, biochemistry, or pharmacology), or for historical reasons, a single transporter can sometimes be described by multiple different names in the literature (Table 1) (1). This can make it confusing, even for researchers in the field. These multispecific transporters fall into two general families: solute carrier (SLC) or ATP-binding cassette (ABC) transporters (1,3,10,11). The SLCs generally transport substances either down their concentration gradient or against their concentration gradient coupled with movement of a second substance down its concentration gradient. In the kidney, the most important multispecific SLC transporters appear to be the organic anion transporters (OATs), including OAT1 (SLC22A6, originally described as novel kidney transporter [NKT]) and OAT3 (SLC22A8), which appear to be the main transporters inhibited by the drug probenecid, and the organic cation transporters (OCTs) such as OCT2 (SLC22A2) (1). But increasing attention is being paid to other members of multiple SLC families, including organic anion transporting polypeptides (OATP, or SLCO family), multidrug and toxin extrusion proteins (MATEs or SLC47 family), peptide transporters (SLC15 family), and organic carnitine/zwitterionic transporters (SLC22A4 and SLC22A5) (1,12,13).

ABC transporters use energy generated by the hydrolysis of ATP to transport molecules across cell membranes. Several ABC transporters, including P-glycoprotein (P-gp; ABCB1), also known as multidrug-resistant protein 1 (MDR1), and breast cancer resistance protein (BCRP, also known as ABCG2) play key roles in tubular efflux. Other key family members involved in kidney proximal tubule transport are the multidrug-associated resistance proteins, (MRP2 [also known as ABCC2] and MRP4 [ABCC4]), located on the apical (urinary) surface of the cell (1,10).

Most substrates of medical importance (*e.g.,* drugs, metabolites, and toxins) are eliminated primarily by more than one renal transporter *in vivo*. Para-aminohippurate (PAH), however, is largely extracted from the blood *in vivo* by OAT1, the classic PAH transporter (14–16). Many common drugs (*e.g.,* antivirals) are known to interact with more than one SLC and/or ABC transporter expressed in the kidney, albeit with varying affinities and inhibitory constants (roughly 10 $\mu$M–1 mM); this makes it difficult to pin down the relative contributions of key transporters involved in renal elimination (1,17). Furthermore, while much of the data from *in vitro* transport assays and mouse knockout studies seems relevant to humans, caution must be exercised in extrapolating to human physiology.

**Table 1. Select transporters involved in organic ion transport in the kidney proximal tubule**

| Name | HGNC Gene Symbol[a] | Common Symbol | Alternative Symbol(s) | Other Name |
|---|---|---|---|---|
| Organic Anion Transporter 1 | *SLC22A6* | OAT1 | NKT | Novel Kidney Transporter |
| Organic Anion Transporter 3 | *SLC22A8* | OAT3 | ROCT | Reduced in Osteosclerosis Transporter |
| Organic Anion Transporter 4 | *SLC22A11* | OAT4 | | |
| Urate Anion Exchanger 1 | *SLC22A12* | URAT1 | RST | Renal-Specific Transporter |
| Organic Cation Transporter 2 | *SLC22A2* | OCT2 | | |
| Multidrug and Toxin Extrusion Protein 1 | *SLC47A1* | MATE1 | | |
| Multidrug Resistance Protein 1 | *ABCB1* | MDR1 | P-gp | P-glycoprotein |
| Breast Cancer Resistance Protein | *ABCG2* | BCRP | | |
| Multidrug Resistance Associated Protein 2 | *ABCC2* | MRP2 | | |
| Multidrug Resistance Associated Protein 4 | *ABCC4* | MRP4 | | |

[a] For each solute carrier (SLC), a number (the family series) is followed by the letter A, which serves as a divider, and then the number of the particular transporter within that family; ATP-binding cassette (ABC) nomenclature includes a letter (A–G) to describe the subfamily, and then the number of the transporter member. By convention, transporters are displayed as all uppercase letters when referring to proteins or human genes. HGNC, Human Genome Organisation gene nomenclature committee; OAT, organic anion transporter; URAT1, urate transporter 1; OCT, organic cation transporter; MATE1, multidrug and toxin extrusion protein 1; MDR1, multidrug-resistant protein 1; BCRP, breast cancer resistance protein; MRP, multidrug-associated resistance protein.

## Basic Organic Ion Transporter Physiology

Excretion of organic cations begins with transport on the basolateral surface of the proximal tubular cell (Figure 1A). This is primarily achieved by OCT2, a transporter of organic cations, which takes advantage of the negative potential difference within the cell maintained by the basolateral $Na^+$-$K^+$-ATPase. Several carriers on the apical surface subsequently transport organic cations across the apical membrane through electroneutral transport by exchange with proton ($H^+$), which capitalizes on the electrochemical gradient that favors movement of $H^+$ into the cells. The key apical transporters appear to be the MATEs (SLC47), but other SLC and ABC transporters may be involved.

OATs on the basolateral and apical membranes function in tandem to move organic anions from the blood, across the proximal tubule cells, and into the lumen (Figure 1B). The best-studied transporters on the basolateral membrane are OAT1 and OAT3, which have dozens of well established substrates. These transporters are organic anion/dicarboxylate exchangers, which use a tertiary active transport system on the basolateral side of the proximal tubule cell. The $Na^+$-$K^+$-ATPase pumps sodium out of the cell, while sodium/dicarboxylate cotransporters move sodium and dicarboxylate molecules into the cell. The OAT antiporters move organic anions into the cell and dicarboxylate molecules out of the cell because the gradient favors outward movement of dicarboxylates, such as $\alpha$-ketoglutarate, to the peritubular capillary. The current thinking is that MRP2 (ABCC2) and MRP4 (ABCC4) are the main apical efflux transporters of many of the organic anions taken up by OAT1 and OAT3, but other transporters, such as OAT4 and the urate transporter urate anion exchanger 1 (URAT1 [SLC22A12]), may also be involved (18). These transporters seem to work in concert to control the excretion of organic solutes (1–7,17).

Organic anion secretion has been recognized as an important function of the kidney for more than half a century. In addition, it has long been known that secretion of organic anions by the kidney can be saturated, such that the addition of a second substance can inhibit secretion of the first. Since the mid-to-late 1990s, many of these transporters have been cloned (1,7,15,19,20) and a great deal of knowledge has accumulated as a result of transport studies in microinjected frog oocytes, transfected cells, and *in vivo* as well as *ex vivo* analysis of wild-type and knockout tissues (5,21,22). These data may help explain drug-drug interactions (DDIs) but also may explain differences in pharmacokinetics in different patients, some of whom may have transporter single-nucleotide polymorphisms (SNPs) that lead to more rapid or relatively delayed transport. This represents a large body of work by many investigators, and it is impossible to cover each transporter (1,7,21,23–26). In this review, however, we focus mainly on OAT1, OAT3, and OCT2, which have emerged as the primary transporters for many common drugs, toxins, and metabolites encountered in the clinical renal setting. To illustrate concepts, along the way we also highlight some interesting findings related to some of the other SLC and ABC transporters mentioned earlier. Additional details in the context of the broader field of drug transport can be found elsewhere (1).

## Drugs and Toxins

There are now US Food and Drug Administration (FDA) regulatory guidelines to examine the transport of new drugs with a view toward understanding DDIs at the transporter level (27). Indeed, the FDA recommends that applications for new drugs and biologics in which renal secretion is significant be studied *in vitro* to determine whether these agents are substrates for OAT1, OAT3, or OCT2 (28). When these transporters appear to play a role, additional studies may be required. The list of drugs known to interact with these renal transporters is extensive, and there are data in human, rodents, and other species (5,17). This focus on drug transporters promises to eventually improve understanding of pharmacokinetics in normal and diseased states (5).

The endogenous metabolite, creatinine, is a well described substrate for OCT2 and apical MATEs; competitive inhibition of these carriers can cause increases in serum creatinine by blocking its excretion (Figure 2A) (29–31). Drugs such as cimetidine and trimethoprim are examples. OATs, while mainly transporters of organic anions, can also transport some organic cations, including creatinine (32,33). In addition, newer agents, such as the integrase inhibitor dolutegravir and the novel pharmacoenhancer cobicistat, also block creatinine excretion (34,35). This effect appears to be largely from drug-metabolite interactions due to competition for OCTs, MATEs, and possibly OATs (29,32–35).

OCTs also transport organic cation drugs, such as metformin, and it has been postulated that polymorphisms in these transporters might account for the variability in clinical effect of this medication and the risk of toxicity. In the case of metformin, polymorphisms in OCT1, primarily expressed in hepatic cells, may explain variable efficacy, whereas polymorphisms in renal secretion initiated by OCT2 may contribute to variable pharmacokinetics (13).

The ABC transporter, P-gp (MDR1), which transports a wide range of molecules, is one of the best understood from the viewpoint of protein structure in relation to transport function (1,36,37). P-gp has been widely studied in the context of cancer biology and its contribution to tumor resistance to chemotherapy (with increased expression of MDR1 on cancer cells associated with greater resistance). MDR1 also transports digoxin and interacts with quinidine.

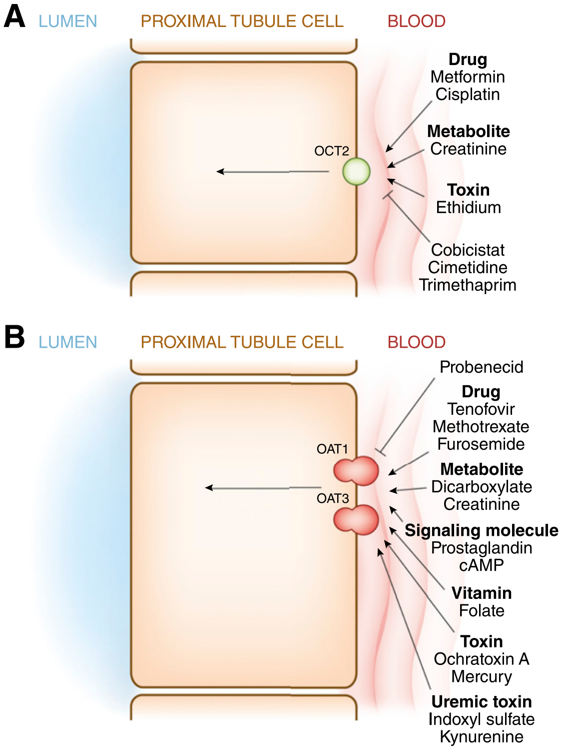

**Figure 2. | Schematic of potential interactions between drugs, metabolites, and toxins due to competition for tubular secretion at the transporter level.** (A) Some of the molecules that are transported by organic cation transporters (*e.g.*, OCT2). Transport is inhibited by cimetidine and trimethoprim and by the novel pharmacoenhancer, cobicistat. (B) Some of the substrates for the organic anion transporters OAT1 and/or OAT3. Transport is inhibited by probenecid.

OAT1, originally described as an NKT (15), and OAT3, also known in mice as reduced in osteosclerosis transporter) (38), are the main transporters of organic anions in the proximal tubule (Figure 2B) (1,7). When the *Oat1* or *Oat3* genes are deleted in mice, the knockout mice have markedly blunted responses to loop and thiazide diuretics (14,39). These diuretics must enter the proximal tubule cell from the blood (basolateral) side and then be secreted into the proximal tubular lumen (apical side) before they can act more distally in the nephron to inhibit sodium transport. In addition, *Oat3* knockout mice are deficient in penicillin excretion (40), and both the *Oat1* and *Oat3* knockout kidney tissue has defective handling of antiviral agents, such as those used to treat HIV (41,42). It is worth noting that the kidney may handle medications in the same class differently. For example, furosemide is filtered and secreted, whereas filtration appears to be the main route of bumetanide entry into the tubular lumen (43,44); these pharmacologic differences may be important when diuretics are used in the setting of certain types of acute kidney disease and CKD.

Recent metabolomics data from *Oat3* knockout mice, together with *in vitro* studies, support the view that OAT3 is a major route of elimination for many compounds that undergo phase 2 modifications by drug-metabolizing enzymes in the liver (*e.g.*, glucuronidation) (8). Although detailed studies need to be performed in knockout mice, such modified compounds might be expected to include dietary plant products along with drugs and toxins. Many of these hepatically metabolized compounds are not routinely measured. In addition, it is possible that competition by drugs (*e.g.*, antibiotics, nonsteroidal anti-inflammatory drugs [NSAIDs], and antivirals) for OATs can affect the levels of many other metabolized compounds in the blood. Historically, probenecid was used to limit renal penicillin elimination where there was a critically small supply (45). It was later used to augment the effect of penicillin in the treatment of gonorrhea and other systemic infections (46). Probenecid is also used to block uric acid reabsorption in the proximal tubule for the treatment of gout (47); as discussed below, uric acid is a substrate of several proximal tubule drug transporters, including BCRP (ABCG2), OAT1, OAT3, and the related OAT family member (URAT1).

Drug transporters also handle environmental toxins, which may contribute to their toxicity to the renal tubules. For example, mercury exists in the blood in thiol conjugates (with glutathione and cystathione), which effectively act like organic anions. OAT1 and OAT3 appear to be the major transporters involved in elimination of mercuric conjugates, which can lead to renal and central nervous system toxicity following mercurial exposure (48–51). The proximal tubule of the kidney of the *Oat1* knockout mouse is largely resistant to nephrotoxic damage that occurs from systemic administration of mercuric chloride (Figure 3) (52). Aristolochic acid (53) and ochratoxin A (54), the putative nephrotoxins involved in Balkan endemic nephropathy, are both transported into the proximal tubule by OATs. In this disorder, patients may present with evidence of tubular dysfunction or simply advanced CKD. The pathology is characterized by tubulointerstitial disease and fibrosis. Although the mechanism is not certain, these substances are clearly toxic to cultured renal epithelial cells (55). There is also a high incidence of uroepithelial cancer in these patients. Environmental toxins, such as the water-repellent polymer, perfluorooctanoic acid, are also substrates of OATs (56).

## Uremic Toxins

Over 100 molecules have been implicated in the pathogenesis of uremic syndrome (57–60). Their precise roles are debated, but many of these are small organic anions, such as indoxyl sulfate, carboxy-methyl-propyl-furanpropionate, p-cresol sulfate, and kynurenine; molecules associated with CKD that can accumulate between dialysis sessions (57,59). These and many other potential uremic toxins are good OAT substrates. In *Oat1* knockout mice, some of these organic anions accumulate (61), although the mice do not appear ill and have normal life spans (14). Putative uremic toxins are also affected to varying degrees in *Oat3* knockout mice and *OATP4C1* transgenic rats (8,62,63). Uremic toxins interact with other SLC and ABC transporters as well. On the basis of *in vitro* transport data, the high levels of circulating organic anion uremic toxins have the possibility of competing, at the level of transporters, for elimination and distribution of drugs, metabolites, and toxins (64). To the extent that transporters such as OAT1 and OAT3 play central roles in the regulation of systemic and local metabolism, this could contribute to the abnormalities in metabolism seen in uremia (1,7,65). How these transporters handle various uremic toxins in the kidney and nonrenal tissues in the setting of both normal physiology and disease is a fertile area for future translational research and may provide important insights into how to both delay and treat the symptoms of uremia.

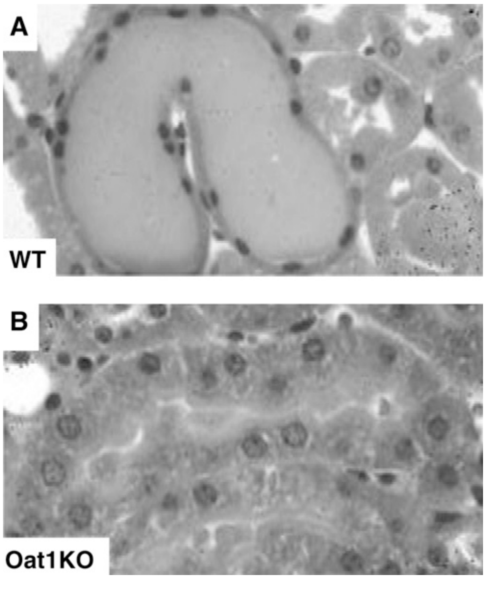

**Figure 3. | Photomicrographs demonstrating tubular and cellular changes in wild-type (WT) and organic anion transporter 1 (*Oat1*) knockout kidneys upon exposure to mercuric chloride, $HgCl_2$.** (A) In the wild-type kidney, substantial tubular damage was seen, as indicated by the dilated tubular lumen and flattened tubule epithelial cells, after a single dose of $HgCl_2$ (4 mg/kg body wt, intraperitoneal). (B) In contrast, the *Oat1* knockout (Oat1KO) mice had normal tubules that appeared unaffected by mercury exposure. Although not shown, serum urea nitrogen levels increased significantly in the wild-type mouse but not in the knockout mouse with preserved tubules. (Adapted with permission from Torres *et al.* 52.)

## Gut Microbiome Products, Nutrients, and Natural Products

There is growing evidence that many of the potential uremic toxins have their origin in the gut microbiome. Indole, for example, is produced by gut bacteria, undergoes sulfation in the liver, and is then excreted as indoxyl sulfate by the kidney (66). One of the primary renal transporters involved appears to be OAT1, which transports several gut microbial products or their metabolites (61,67). OAT1, OAT3, and other SLC and ABC transporters are also necessary for the elimination of dietary natural products, including a wide range of flavonoids, as well as vitamins, and thus might indirectly regulate vitamin-dependent metabolic pathways (8,67).

## Metabolites and Signaling Molecules

Metabolomic studies in knockout animals, particularly *Oat1* and *Oat3* knockout mice, have confirmed a central role for drug transporters in the transport of many important metabolites and signaling molecules (8,14,61,67,68). These include $\alpha$-ketoglutarate, which plays a central role in the Krebs cycle (tricarboxylic acid cycle); vitamins; molecules with antioxidant properties (*e.g.,* urate and flavonoids); and the gut microbial derivatives already described. Signaling molecules, such as cyclic nucleotides, prostaglandins, odorants, and conjugated steroids, are also eliminated *via* the OATs and other SLC and ABC drug transporters. Recent systems biology and omics integration of metabolomics and transcriptomics data from *Oat* knockout mice suggests that OATs and possibly all multispecific drug transporters play a role in the regulation of systemic and tissue metabolic and signaling processes (1,7,8,65,67,69). This type of information has led to the remote sensing and signaling hypothesis discussed below (65,69).

The transporter-mediated regulation of uric acid, mainly by renal transporters but also by nonrenal (*e.g.,* intestinal) transporters, appears quite complex in both humans and mice. Genome-wide association studies, *in vitro* transport data, and studies on knockout mice indicate that earlier models for uric acid handling were oversimplified. Several transporters have been implicated in renal urate handling to date; apical URAT1 (originally known as the renal-specific transporter in mice [70]) and SLC2A9 appear to play important roles in urate reabsorption, whereas apical ABCG2 (BCRP) and basolateral OAT1 and OAT3 are likely to play key roles in urate secretion (71,72). The complex regulation of uric acid may reflect a functional role that is not yet fully appreciated. Further study is clearly needed because uric acid has been implicated as both an antioxidant and a pro-oxidant and, in the case of the latter, a potential culprit in a range of disorders from atherosclerosis to hypertension and renal disease (73).

The details of transcriptional regulation of drug transporters in the proximal tubule are just beginning to unfold. Through a combination of systems-biology approaches involving analysis of transcriptomics and combining chromatin immunoprecipitation with DNA sequencing (ChIP-seq) data, it has been possible to implicate, and then show experimentally, that hepatocyte nuclear factor-4$\alpha$ and hepatocyte nuclear factor-1$\alpha$ are major regulators of OAT and OCT expression, as well as other proximal tubule transporters and drug-metabolizing enzymes (9,74,75). Unlike the extensive modern understanding of drug metabolism in the liver, the role of proximal tubule drug-metabolizing enzymes, in the context of both the proximal tubule cell and systemic physiology, is not well understood and is probably underappreciated. Future studies of the regulation of renal drug transporter expression by pharmaceuticals, uremic toxins, and environmental agents are likely to have a major effect on our understanding of the pathophysiology of CKD, as well as renal pharmacology and toxicology. It is conceivable that the transcription factors in the proximal tubule coordinate the regulation of drug transporter gene expression by sensing levels of signaling molecules, metabolites, drugs, and toxins, and responding by producing and deploying additional transporters as needed (1,7,65,69).

## DDIs and Drug-Metabolite Interactions

Knowledge of DDIs at the level of organic ion transport in the kidney can also influence care. Some DDIs, such as penicillin and probenecid, are well established and have been put to clinical use; the half-life of penicillin is greatly prolonged by coadministration of probenecid, an OAT inhibitor (45–47). In contrast, some DDIs can lead to dire consequences. Methotrexate is taken up from the blood *via* OATs. NSAIDs can inhibit OATs, and when methotrexate and NSAIDs are used together, methotrexate toxicity can occur, manifesting as severe bone marrow suppression (76,77). DDIs at the level of P-glycoprotein (MDR1/ABCB1) in the proximal tubule and elsewhere are thought to explain the well known digoxin-quinidine interaction resulting in digoxin toxicity, including arrhythmias (78). Poor renal function can further complicate the clinical picture (79).

Whereas DDI *via* substrate competition has been well recognized (80), drug-metabolite interaction by a similar mechanism has received comparatively less attention. Considerable *in vitro* and substrate modeling (*e.g.*, pharmacophore) data suggest that drug-metabolite interaction could be a substantial problem because the drugs and metabolites handled by OATs and other drug transporters have structural similarities (61,81). This may be especially important in a setting such as CKD, in which circulating organic anion levels (*e.g.*, uremic toxins such as indoxyl sulfate) are high. With the broader application of metabolomics methods, this should become clearer in the near future.

## Pharmacogenomic and Toxicogenomic Considerations

Early studies of transporter polymorphisms raised the possibility that multiple SNPs in basolateral (*e.g.*, OAT1 and OAT3) (82,83) and apical (*e.g.*, URAT1, OAT4, MRP2, and MRP4) drug transporters may affect the net transport of drugs, toxins, and metabolites from blood to urine (50,84). In addition, noncoding SNPs that regulate drug transporter expression might be particularly important (82,85). These polymorphisms may explain differences in drug response and toxicity among individuals. While emerging data seem to be consistent with these notions, there is much to be done in order to improve understanding of how coding or noncoding SNPs in renal drug transporter genes, and particularly combinations of these SNPs, affect overall renal handling of drugs, metabolites, and toxins (83).

As noted earlier, OAT1 and OAT3 are the major transporters of loop and thiazide diuretics, and secretion into the urinary space by the proximal tubular cells is necessary for these diuretics to induce the desired natriuresis by inhibiting sodium transport in later tubule segments. A noncoding region polymorphism has been identified in patients with diuretic resistance that could affect OAT1 and/or OAT3 expression (86). Polymorphisms in OAT1, OAT3, and MRP2 are more common among miners exhibiting toxicity from mercury-containing vapors (50). Additionally, OAT3 polymorphisms have been associated with altered cephalosporin handling (87), and suggestive evidence indicates the possible involvement of OATs in antiviral and methotrexate elimination (88,89). One of the better-studied examples of drug transporter polymorphisms affecting renal drug elimination is that of OCT2 (SLC22A2). Patients with certain SNPs in this transporter have altered metformin handling (90). In addition, polymorphisms in MATE1, on the apical membrane, may also affect metformin handling (91). OCT2 polymorphisms that affect transport function also may play a role in determining whether cisplatin nephrotoxicity occurs, as cisplatin gains entry into proximal tubule cells from the basolateral membrane (92).

Overall, results from *in vitro* transport studies in cells that overexpress transporters, together with results from studies in knockout animals (or tissues derived from them), and rat experiments (*e.g.*, after probenecid treatment) seem reasonably concordant with available human clinical data. Although there are sure to be many caveats and exceptions, this overall concordance is a very important point from an experimental and translational standpoint; it indicates that the considerable *in vitro*, *ex vivo*, and *in vivo* knockout mouse and rat data can continue to be used to help guide our clinical understanding.

## Pediatric Developmental Pharmacology and Drug Elimination in the Aging Population

Most of our understanding of renal drug elimination comes from analysis of adult patients and adult animals. In addition, most studies have only limited consideration of ethnicity, sex, and the extremes of age (3,93). Human pediatric kidney data remain limited, so what we currently know, especially from a mechanistic standpoint, comes largely from a limited number of rodent studies. For example, it is known that drug transporter expression occurs early in embryogenesis; indeed, several renal drug transporters are expressed transiently in the developing central nervous system and other tissues (94). Late in rodent gestation, the expression is largely limited to the future proximal tubules of the kidney (15,95); thereafter, there is a burst in renal expression around the time of birth, eventually reaching (and perhaps overshooting for a short time) adult expression (9,96). This is paralleled by functional changes, such as increasing PAH clearance (96). There is evidence in animals for a developmental inducibility window, during which renal drug transporters may, during the early postnatal period, be induced by substrates or by hormones (97,98). If this is true in humans, substrate induction could theoretically enhance the ability of a premature infant's kidney to excrete potentially deleterious drugs and toxins. A coordinated response by the postnatal kidneys with the liver is also needed to excrete drugs and metabolites during the continuing period of maturation (3). Furthermore, many OAT1 and OAT3 substrates are drugs, toxins, and metabolites that have been modified by phase 1 (*e.g.*, cytochrome-dependent) or phase 2 (conjugation, such as glucuronidation) reactions. For example, OAT3 is responsible for the elimination of many glucuronidated compounds (8). How this liver-kidney coordination is achieved during postnatal maturation, or during recovery from organ injury, is not yet well understood.

Adverse drug reactions in the elderly are also a major clinical concern. Some of these adverse drug reactions may be partly related to altered expression or function of drug transporters in the aging kidney (99,100). However, data on this important issue are limited.

## AKI and CKD

Following AKI from ischemia or toxins, substantial changes have been observed in transcript and protein expression of many drug transporters. Initially, expression of certain transporters seems to decrease, followed by upregulation during recovery (24). The extent to which this is reflected in functional handling of specific drugs, metabolites, and toxins is not well understood. As discussed earlier, many of the putative uremic toxins, including indoxyl sulfate and kynurenine, are excellent substrates of OATs and other drug transporters, such as OATPs, and they accumulate in the knockout or transgenic models of these transporters. These substances may themselves be toxic to the tubule cell and may also contribute to the progression of CKD (8,61–63,101).

Although drug transporters on the basolateral and apical membranes of the proximal tubule protect from systemic toxicity by enhancing drug and toxin elimination, they are, in some instances, the mechanism by which substances toxic to the proximal tubule gain entry. Cephaloridine, a first-generation cephalosporin that has largely been replaced by newer agents with better bioavailability and less nephrotoxicity, is one such example. Cephaloridine, like other cephalosporins, is excreted unchanged by renal tubular secretion. Yet this agent accumulates in the proximal tubule; after basolateral OAT3 uptake, secretion by apical membrane transporters may not be sufficiently rapid to avoid toxicity, perhaps because of the zwitterionic nature of this agent (102). Toxicity appears to be related to oxidative stress from depletion of reduced glutathione (103). This imbalance in proximal tubular cell entry and exit has also been implicated in the proximal tubule defect produced by the antiviral agent tenofovir. Indeed, *Oat1* knockout mice appear protected from tenofovir-induced proximal tubular damage, whereas those with loss of apical efflux of tenofovir seen in *Mrp4* knockout mice were particularly susceptible (104).

The role of drug transporters in proximal tubule metabolism raises some interesting questions in the context of renal ischemia and other types of injury. The proximal tubule exhibits some unique metabolic characteristics (105). Although glycolytic enzymes are present in the proximal tubule, the cells are largely obligate oxidative in metabolic character; they are relatively incapable of glycolytic metabolism and poorly utilize glucose as a preferred substrate (77,106). Thus, in the setting of cell stress, the proximal tubule cells might be expected to depend more on gluconeogenesis, whereby glucose is synthesized from substrates such as lactate, a compound not produced by proximal tubules but potentially taken up by proximal tubule transporters. Kidney gluconeogenesis then differs from hepatic gluconeogenesis by using lactate as a primary substrate. Because proximal tubules are unable to shift to glycolytic activity, the proximal tubule cells are a potential victim of hypoxic damage in comparison to renal cells located more distally in the nephron, which retain considerable glycolytic capacity, in keeping with the often lower oxygen levels in the environment. The proximal tubule does require substrates and coupling OAT transport with $\alpha$-ketoglutarate as the intracellular counter-transporting molecule, plays a role in regulating the intracellular levels of citric acid cycle molecules involved in oxidative metabolism. This also raises a question as to whether changes in net proximal tubule OAT activity as a result of altered expression levels or competing anions could affect proximal tubular metabolism and ability to maintain normal oxidative substrate supply when the proximal tubule dynamics are altered in the context of extremes of age, stress, injury, or recovery from injury. It may thus be very interesting to study whether resistance to hypoxia and ATP depletion can be conditioned by altering OAT function.

## Remote Sensing and Signaling Hypothesis

In general, the application of multiple omics methods (*e.g.*, metabolomics and transcriptomics in wild-type and knockout animals) combined with systems biology approaches to reconstruct drug transporter–dependent metabolism is beginning to yield a new perspective on the role of renal and nonrenal SLC and ABC multispecific drug transporters. Transport of molecules by various SLC and ABC drug transporters in and out of cells could play a role in remote communication between cells and tissues as well as interfacing body fluids. Because SLC and ABC drug transporters are selectively expressed in different cells (*e.g.*, kidney, choroid plexus, intestine, biliary tract, liver, brain capillary endothelium, olfactory mucosa, placenta, mammary gland, and testes) that interface with body fluids that bathe other cells, these so-called drug transporters may be part of a remote sensing and signaling system (1,7,65,69) involved in cell, tissue, and organ crosstalk. These transporters are also well situated to play a role in mediating and/or restoring homeostasis after injury; certain aspects of the pathophysiology of AKI and CKD may be considered disordered remote sensing and signaling. The broader remote sensing and signaling hypothesis has been explained in more detail in other articles (1,7,65,69).

Several OAT isoforms appear somewhat specialized for transport of classic signaling molecules and antioxidants and therefore well suited for signaling: liver-expressed OAT2 transports cyclic guanosine monophosphate (107), placental-expressed OAT4 transports conjugated estrogens (108), olfactory-expressed OAT6 has high selectivity for odorants (109), and OATPG seems specialized for prostaglandins (110). The drug transporters mediating the influx and efflux through tissues and body fluid compartments (*e.g.*, blood, urine, bile, cerebrospinal fluid, and amniotic fluid) could regulate remote communication *via* transport of key (potentially rate-limiting) metabolites and signaling molecules, hence regulating the movement of these molecules across tissue barriers in the body and also at the cellular level. Various types of data suggest that these SLC and ABC drug transporters are also involved in communication between gut bacteria and the body, and also across the maternal-fetal and maternal-neonate barriers *via* breast milk (1, 7).

In addition to a potential contribution to signaling in their own right, various drug transporters are directly affected by signaling molecules, which may govern transporter density on the cellular membrane. Drug transporters, like other transporters in the cell, may traffic from an internal pool, which might be deployed as needed or internalized (111) under stimulation by signaling pathways. Internalization or abnormal trafficking may affect drug excretion.

## Summary

The hypothetical clinical vignette presented at the outset of this article represents the challenges that can be seen in many patients. The ballooning data regarding handling of small molecule drugs, metabolites, nutrients, and toxins by multispecific transporters expressed in the proximal tubule still do not explain the complexity of organic ion transport at play in the patient. While the discussion here has tended to focus more on renal drug transporters of the SLC22 family (especially OAT1 and OAT3), it is important to emphasize that many of the same principles and considerations are likely to apply to other SLC and ABC drug transporters in the kidney. Moreover, by focusing on the differences in tissue expression patterns, substrate specificities, regulation in development, evolutionary biology, and disease states, the field is just beginning to understand the role these drug transporters play not only in drug and toxin handling but in normal physiology and pathophysiology (24,65,69,112,113). Viewing uremia partly as a disorder of remote sensing and signaling may lead to new avenues of research and, possibly, novel therapeutic approaches.

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### Disclosures

None.

### References

1. Nigam SK: What do drug transporters really do? *Nat Rev Drug Discov* 14: 29–44, 2015
2. Burckhardt G: Drug transport by Organic Anion Transporters (OATs). *Pharmacol Ther* 136: 106–130, 2012
3. Nigam SK, Bhatnagar V: How much do we know about drug handling by SLC and ABC drug transporters in children? *Clin Pharmacol Ther* 94: 27–29, 2013
4. Nigam SK, Bush KT, Bhatnagar V: Drug and toxicant handling by the OAT organic anion transporters in the kidney and other tissues. *Nat Clin Pract Nephrol* 3: 443–448, 2007
5. VanWert AL, Gionfriddo MR, Sweet DH: Organic anion transporters: Discovery, pharmacology, regulation and roles in pathophysiology. *Biopharm Drug Dispos* 31: 1–71, 2010
6. Ahn SY, Bhatnagar V: Update on the molecular physiology of organic anion transporters. *Curr Opin Nephrol Hypertens* 17: 499–505, 2008
7. Nigam SK, Bush KT, Martovetsky G, Ahn SY, Liu HC, Richard E, Bhatnagar V, Wu W: The organic anion transporter (OAT) family: A systems biology perspective. *Physiol Rev* 95: 83–123, 2015
8. Wu W, Jamshidi N, Eraly SA, Liu HC, Bush KT, Palsson BO, Nigam SK: Multispecific drug transporter Slc22a8 (Oat3) regulates multiple metabolic and signaling pathways. *Drug Metab Dispos* 41: 1825–1834, 2013
9. Martovetsky G, Tee JB, Nigam SK: Hepatocyte nuclear factors 4$\alpha$ and 1$\alpha$ regulate kidney developmental expression of drug-metabolizing enzymes and drug transporters. *Mol Pharmacol* 84: 808–823, 2013
10. Masereeuw R, Russel FG: Regulatory pathways for ATP-binding cassette transport proteins in kidney proximal tubules. *AAPS J* 14: 883–894, 2012
11. You G, Morris ME: Overview of drug transporter families. In: *Drug Transporters: Molecular Characterization and Role in Drug Disposition*, 2nd Ed., edited by You G, Morris ME, Hoboken, NJ, John Wiley & Sons, Inc., 2014, pp 1–6
12. Bush KT, Nagle M, Truong DM, Bhatnagar V, Kaler G, Eraly SA, Wu W, Nigam SK: Organic anion transporters. In: *Drug Transporters: Molecular Characterization and Role in Drug Disposition*, 2nd Ed., edited by You G, Morris ME, Hoboken, NJ, John Wiley & Sons, Inc., 2014, pp 25–42
13. Koepsell H: The SLC22 family with transporters of organic cations, anions and zwitterions. *Mol Aspects Med* 34: 413–435, 2013
14. Eraly SA, Vallon V, Vaughn DA, Gangoiti JA, Richter K, Nagle M, Monte JC, Rieg T, Truong DM, Long JM, Barshop BA, Kaler G, Nigam SK: Decreased renal organic anion secretion and plasma accumulation of endogenous organic anions in OAT1 knock-out mice. *J Biol Chem* 281: 5072–5083, 2006
15. Lopez-Nieto CE, You G, Bush KT, Barros EJ, Beier DR, Nigam SK: Molecular cloning and characterization of NKT, a gene product related to the organic cation transporter family that is almost exclusively expressed in the kidney. *J Biol Chem* 272: 6471–6478, 1997
16. Sweet DH, Wolff NA, Pritchard JB: Expression cloning and characterization of ROAT1. The basolateral organic anion transporter in rat kidney. *J Biol Chem* 272: 30088–30095, 1997
17. Morrissey KM, Stocker SL, Wittwer MB, Xu L, Giacomini KM: Renal transporters in drug development. *Annu Rev Pharmacol Toxicol* 53: 503–529, 2013
18. Miyazaki H, Anzai N, Ekaratanawong S, Sakata T, Shin HJ, Jutabha P, Hirata T, He X, Nonoguchi H, Tomita K, Kanai Y, Endou H: Modulation of renal apical organic anion transporter 4 function by two PDZ domain-containing proteins. *J Am Soc Nephrol* 16: 3498–3506, 2005
19. Gründemann D, Gorboulev V, Gambaryan S, Veyhl M, Koepsell H: Drug excretion mediated by a new prototype of polyspecific transporter. *Nature* 372: 549–552, 1994
20. Simonson GD, Vincent AC, Roberg KJ, Huang Y, Iwanij V: Molecular cloning and characterization of a novel liver-specific transport protein. *J Cell Sci* 107: 1065–1072, 1994
21. Burckhardt G, Burckhardt BC: In vitro and in vivo evidence of the importance of organic anion transporters (OATs) in drug therapy. In:*Drug Transporters.*, edited by Fromm MF, Kim RB, Berlin, Heidelberg, Springer, 2011, pp 29–104

22. You G: Membrane transporters in drug disposition. *Pharm Res* 25: 441–443, 2008
23. Eraly SA, Bush KT, Sampogna RV, Bhatnagar V, Nigam SK: The molecular pharmacology of organic anion transporters: From DNA to FDA? *Mol Pharmacol* 65: 479–487, 2004
24. Saito H: Pathophysiological regulation of renal SLC22A organic ion transporters in acute kidney injury: Pharmacological and toxicological implications. *Pharmacol Ther* 125: 79–91, 2010
25. Sweet DH, Bush KT, Nigam SK: The organic anion transporter family: From physiology to ontogeny and the clinic. *Am J Physiol Renal Physiol* 281: F197–F205, 2001
26. Wang L, Sweet DH: Renal organic anion transporters (SLC22 family): Expression, regulation, roles in toxicity, and impact on injury and disease. *AAPS J* 15: 53–69, 2013
27. Lee SC, Zhang L, Huang SM: Regulatory science perspectives on transporter studies in drug development. In: *Drug Transporters: Molecular Characterization and Role in Drug Disposition*, 2n Ed, edited by You G, Morris ME, Hoboken, NJ, John Wiley & Sons, 2014, pp 473–487
28. U.S. Food and Drug Administration: *Guidance for Industry: Drug Interaction Studies—Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations*. Rockville, MD, U.S. Food and Drug Administration, Drug-Drug Interaction Working Group, Center for Drug Evaluation and Research (CDER), 2012, pp 1–75
29. Ciarimboli G, Lancaster CS, Schlatter E, Franke RM, Sprowl JA, Pavenstädt H, Massmann V, Guckel D, Mathijssen RH, Yang W, Pui CH, Relling MV, Herrmann E, Sparreboom A: Proximal tubular secretion of creatinine by organic cation transporter OCT2 in cancer patients. *Clin Cancer Res* 18: 1101–1108, 2012
30. Imamura Y, Murayama N, Okudaira N, Kurihara A, Okazaki O, Izumi T, Inoue K, Yuasa H, Kusuhara H, Sugiyama Y: Prediction of fluoroquinolone-induced elevation in serum creatinine levels: A case of drug-endogenous substance interaction involving the inhibition of renal secretion. *Clin Pharmacol Ther* 89: 81–88, 2011
31. Motohashi H, Inui K: Multidrug and toxin extrusion family SLC47: Physiological, pharmacokinetic and toxicokinetic importance of MATE1 and MATE2-K. *Mol Aspects Med* 34: 661–668, 2013
32. Ahn SY, Eraly SA, Tsigelny I, Nigam SK: Interaction of organic cations with organic anion transporters. *J Biol Chem* 284: 31422–31430, 2009
33. Vallon V, Eraly SA, Rao SR, Gerasimova M, Rose M, Nagle M, Anzai N, Smith T, Sharma K, Nigam SK, Rieg T: A role for the organic anion transporter OAT3 in renal creatinine secretion in mice. *Am J Physiol Renal Physiol* 302: F1293–F1299, 2012
34. Lepist EI, Zhang X, Hao J, Huang J, Kosaka A, Birkus G, Murray BP, Bannister R, Cihlar T, Huang Y, Ray AS: Contribution of the organic anion transporter OAT2 to the renal active tubular secretion of creatinine and mechanism for serum creatinine elevations caused by cobicistat. *Kidney Int* 86: 350–357, 2014
35. Reese MJ, Savina PM, Generaux GT, Tracey H, Humphreys JE, Kanaoka E, Webster LO, Harmon KA, Clarke JD, Polli JW: In vitro investigations into the roles of drug transporters and metabolizing enzymes in the disposition and drug interactions of dolutegravir, a HIV integrase inhibitor. *Drug Metab Dispos* 41: 353–361, 2013
36. Vlaming ML, Teunissen SF, van de Steeg E, van Esch A, Wagenaar E, Brunsveld L, de Greef TF, Rosing H, Schellens JH, Beijnen JH, Schinkel AH: Bcrp1;Mdr1a/b;Mrp2 combination knockout mice: altered disposition of the dietary carcinogen PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) and its genotoxic metabolites. *Mol Pharmacol* 85: 520–530, 2014
37. Aller SG, Yu J, Ward A, Weng Y, Chittaboina S, Zhuo R, Harrell PM, Trinh YT, Zhang Q, Urbatsch IL, Chang G: Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. *Science* 323: 1718–1722, 2009
38. Brady KP, Dushkin H, Förnzler D, Koike T, Magner F, Her H, Gullans S, Segre GV, Green RM, Beier DR: A novel putative transporter maps to the osteosclerosis (oc) mutation and is not expressed in the oc mutant mouse. *Genomics* 56: 254–261, 1999
39. Vallon V, Rieg T, Ahn SY, Wu W, Eraly SA, Nigam SK: Overlapping in vitro and in vivo specificities of the organic anion transporters OAT1 and OAT3 for loop and thiazide diuretics. *Am J Physiol Renal Physiol* 294: F867–F873, 2008
40. Vanwert AL, Bailey RM, Sweet DH: Organic anion transporter 3 (Oat3/Slc22a8) knockout mice exhibit altered clearance and distribution of penicillin G. *Am J Physiol Renal Physiol* 293: F1332–F1341, 2007
41. Nagle MA, Truong DM, Dnyanmote AV, Ahn SY, Eraly SA, Wu W, Nigam SK: Analysis of three-dimensional systems for developing and mature kidneys clarifies the role of OAT1 and OAT3 in antiviral handling. *J Biol Chem* 286: 243–251, 2011
42. Truong DM, Kaler G, Khandelwal A, Swaan PW, Nigam SK: Multi-level analysis of organic anion transporters 1, 3, and 6 reveals major differences in structural determinants of antiviral discrimination. *J Biol Chem* 283: 8654–8663, 2008
43. Bekersky I, Popick AC: Disposition of bumetanide in the isolated perfused rat kidney: effects of probenecid and dose response. *Am J Cardiol* 57: 33A–37A, 1986
44. Tucker BJ, Blantz RC: Effect of furosemide administration on glomerular and tubular dynamics in the rat. *Kidney Int* 26: 112–121, 1984
45. Burnell JM, Kirby WM: Effectiveness of a new compound, benemid, in elevating serum penicillin concentrations. *J Clin Invest* 30: 697–700, 1951
46. Lesiński J, Dzierzanowska D, Szymczak M, Linda H, Wiśniewska C, Nawara A, Winogrodzka A: Treatment of gonorrhoea with procaine penicillin plus probenecid. *Br J Vener Dis* 49: 358–361, 1973
47. Robbins N, Koch SE, Tranter M, Rubinstein J: The history and future of probenecid. *Cardiovasc Toxicol* 12: 1–9, 2012
48. Aslamkhan AG, Han YH, Yang XP, Zalups RK, Pritchard JB: Human renal organic anion transporter 1-dependent uptake and toxicity of mercuric-thiol conjugates in Madin-Darby canine kidney cells. *Mol Pharmacol* 63: 590–596, 2003
49. Di Giusto G, Anzai N, Ruiz ML, Endou H, Torres AM: Expression and function of Oat1 and Oat3 in rat kidney exposed to mercuric chloride. *Arch Toxicol* 83: 887–897, 2009
50. Engström K, Ameer S, Bernaudat L, Drasch G, Baeuml J, Skerfving S, Bose-O'Reilly S, Broberg K: Polymorphisms in genes encoding potential mercury transporters and urine mercury concentrations in populations exposed to mercury vapor from gold mining. *Environ Health Perspect* 121: 85–91, 2013
51. Zalups RK, Ahmad S: Homocysteine and the renal epithelial transport and toxicity of inorganic mercury: Role of basolateral transporter organic anion transporter 1. *J Am Soc Nephrol* 15: 2023–2031, 2004
52. Torres AM, Dnyanmote AV, Bush KT, Wu W, Nigam SK: Deletion of multispecific organic anion transporter Oat1/Slc22a6 protects against mercury-induced kidney injury. *J Biol Chem* 286: 26391–26395, 2011
53. Bakhiya N, Arlt VM, Bahn A, Burckhardt G, Phillips DH, Glatt H: Molecular evidence for an involvement of organic anion transporters (OATs) in aristolochic acid nephropathy. *Toxicology* 264: 74–79, 2009
54. Stefanović V, Polenaković M: Fifty years of research in Balkan endemic nephropathy: Where are we now? *Nephron Clin Pract* 112: c51–c56, 2009
55. Balachandran P, Wei F, Lin RC, Khan IA, Pasco DS: Structure activity relationships of aristolochic acid analogues: Toxicity in cultured renal epithelial cells. *Kidney Int* 67: 1797–1805, 2005
56. Nakagawa H, Hirata T, Terada T, Jutabha P, Miura D, Harada KH, Inoue K, Anzai N, Endou H, Inui K, Kanai Y, Koizumi A: Roles of organic anion transporters in the renal excretion of perfluorooctanoic acid. *Basic Clin Pharmacol Toxicol* 103: 1–8, 2008
57. Toyohara T, Akiyama Y, Suzuki T, Takeuchi Y, Mishima E, Tanemoto M, Momose A, Toki N, Sato H, Nakayama M, Hozawa A, Tsuji I, Ito S, Soga T, Abe T: Metabolomic profiling of uremic solutes in CKD patients. *Hypertens Res* 33: 944–952, 2010
58. Vanholder R, Abou-Deif O, Argiles A, Baurmeister U, Beige J, Brouckaert P, Brunet P, Cohen G, De Deyn PP, Drüeke TB, Fliser D, Glorieux G, Herget-Rosenthal S, Hörl WH, Jankowski J, Jörres A, Massy ZA, Mischak H, Perna AF, Rodriguez-Portillo JM, Spasovski G, Stegmayr BG, Stenvinkel P, Thornalley PJ,

Wanner C, Wiecek A: The role of EUTox in uremic toxin research. *Semin Dial* 22: 323–328, 2009

59. Vanholder R, Van Laecke S, Glorieux G: What is new in uremic toxicity? *Pediatr Nephrol* 23: 1211–1221, 2008
60. Vitetta L, Linnane AW, Gobe GC: From the gastrointestinal tract (GIT) to the kidneys: Live bacterial cultures (probiotics) mediating reductions of uremic toxin levels via free radical signaling. *Toxins (Basel)* 5: 2042–2057, 2013
61. Wikoff WR, Nagle MA, Kouznetsova VL, Tsigelny IF, Nigam SK: Untargeted metabolomics identifies enterobiome metabolites and putative uremic toxins as substrates of organic anion transporter 1 (Oat1). *J Proteome Res* 10: 2842–2851, 2011
62. Masereeuw R, Mutsaers HA, Toyohara T, Abe T, Jhawar S, Sweet DH, Lowenstein J: The kidney and uremic toxin removal: Glomerulus or tubule? *Semin Nephrol* 34: 191–208, 2014
63. Toyohara T, Suzuki T, Morimoto R, Akiyama Y, Souma T, Shiwaku HO, Takeuchi Y, Mishima E, Abe M, Tanemoto M, Masuda S, Kawano H, Maemura K, Nakayama M, Sato H, Mikkaichi T, Yamaguchi H, Fukui S, Fukumoto Y, Shimokawa H, Inui K, Terasaki T, Goto J, Ito S, Hishinuma T, Rubera I, Tauc M, Fujii-Kuriyama Y, Yabuuchi H, Moriyama Y, Soga T, Abe T: SLCO4C1 transporter eliminates uremic toxins and attenuates hypertension and renal inflammation. *J Am Soc Nephrol* 20: 2546–2555, 2009
64. Reyes M, Benet LZ: Effects of uremic toxins on transport and metabolism of different biopharmaceutics drug disposition classification system xenobiotics. *J Pharm Sci* 100: 3831–3842, 2011
65. Wu W, Dnyanmote AV, Nigam SK: Remote communication through solute carriers and ATP binding cassette drug transporter pathways: An update on the remote sensing and signaling hypothesis. *Mol Pharmacol* 79: 795–805, 2011
66. Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, Siuzdak G: Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc Natl Acad Sci U S A* 106: 3698–3703, 2009
67. Ahn SY, Jamshidi N, Mo ML, Wu W, Eraly SA, Dnyanmote A, Bush KT, Gallegos TF, Sweet DH, Palsson BO, Nigam SK: Linkage of organic anion transporter-1 to metabolic pathways through integrated "omics"-driven network and functional analysis. *J Biol Chem* 286: 31522–31531, 2011
68. Eraly SA, Vallon V, Rieg T, Gangoiti JA, Wikoff WR, Siuzdak G, Barshop BA, Nigam SK: Multiple organic anion transporters contribute to net renal excretion of uric acid. *Physiol Genomics* 33: 180–192, 2008
69. Ahn SY, Nigam SK: Toward a systems level understanding of organic anion and other multispecific drug transporters: a remote sensing and signaling hypothesis. *Mol Pharmacol* 76: 481–490, 2009
70. Mori K, Ogawa Y, Ebihara K, Aoki T, Tamura N, Sugawara A, Kuwahara T, Ozaki S, Mukoyama M, Tashiro K, Tanaka I, Nakao K: Kidney-specific expression of a novel mouse organic cation transporter-like protein. *FEBS Lett* 417: 371–374, 1997
71. Sakurai H: Urate transporters in the genomic era. *Curr Opin Nephrol Hypertens* 22: 545–550, 2013
72. Mount DB: The kidney in hyperuricemia and gout. *Curr Opin Nephrol Hypertens* 22: 216–223, 2013
73. Sautin YY, Johnson RJ: Uric acid: The oxidant-antioxidant paradox. *Nucleosides Nucleotides Nucleic Acids* 27: 608–619, 2008
74. Eraly SA, Hamilton BA, Nigam SK: Organic anion and cation transporters occur in pairs of similar and similarly expressed genes. *Biochem Biophys Res Commun* 300: 333–342, 2003
75. Gallegos TF, Martovetsky G, Kouznetsova V, Bush KT, Nigam SK: Organic anion and cation SLC22 "drug" transporter (Oat1, Oat3, and Oct1) regulation during development and maturation of the kidney proximal tubule. *PLoS One* 7: e40796, 2012
76. Maeda A, Tsuruoka S, Kanai Y, Endou H, Saito K, Miyamoto E, Fujimura A: Evaluation of the interaction between nonsteroidal anti-inflammatory drugs and methotrexate using human organic anion transporter 3-transfected cells. *Eur J Pharmacol* 596: 166–172, 2008
77. Eraly SA, Blantz RC, Bhatnagar V, Nigam SK: Novel aspects of renal organic anion transporters. *Curr Opin Nephrol Hypertens* 12: 551–558, 2003
78. Fromm MF, Kim RB, Stein CM, Wilkinson GR, Roden DM: Inhibition of P-glycoprotein-mediated drug transport: A unifying mechanism to explain the interaction between digoxin and quinidine. *Circulation* 99: 552–557, 1999
79. Nigam SK, Burton ME, Vasko MR: Quinidine-induced digoxin toxicity after discontinuing digoxin in a patient with renal failure. *Clin Pharm* 3: 662–664, 1984
80. Giacomini KM, Huang SM, Tweedie DJ, Benet LZ, Brouwer KL, Chu X, Dahlin A, Evers R, Fischer V, Hillgren KM, Hoffmaster KA, Ishikawa T, Keppler D, Kim RB, Lee CA, Niemi M, Polli JW, Sugiyama Y, Swaan PW, Ware JA, Wright SH, Yee SW, Zamek-Gliszczynski MJ, Zhang L; International Transporter Consortium: Membrane transporters in drug development. *Nat Rev Drug Discov* 9: 215–236, 2010
81. Kouznetsova VL, Tsigelny IF, Nagle MA, Nigam SK: Elucidation of common pharmacophores from analysis of targeted metabolites transported by the multispecific drug transporter-Organic anion transporter1 (Oat1). *Bioorg Med Chem* 19: 3320–3340, 2011
82. Bhatnagar V, Xu G, Hamilton BA, Truong DM, Eraly SA, Wu W, Nigam SK: Analyses of 5′ regulatory region polymorphisms in human SLC22A6 (OAT1) and SLC22A8 (OAT3). *J Hum Genet* 51: 575–580, 2006
83. Xu G, Bhatnagar V, Wen G, Hamilton BA, Eraly SA, Nigam SK: Analyses of coding region polymorphisms in apical and basolateral human organic anion transporter (OAT) genes [OAT1 (NKT), OAT2, OAT3, OAT4, URAT (RST)]. *Kidney Int* 68: 1491–1499, 2005
84. Choudhuri S, Klaassen CD: Structure, function, expression, genomic organization, and single nucleotide polymorphisms of human ABCB1 (MDR1), ABCC (MRP), and ABCG2 (BCRP) efflux transporters. *Int J Toxicol* 25: 231–259, 2006
85. Hesselson SE, Matsson P, Shima JE, Fukushima H, Yee SW, Kobayashi Y, Gow JM, Ha C, Ma B, Poon A, Johns SJ, Stryke D, Castro RA, Tahara H, Choi JH, Chen L, Picard N, Sjödin E, Roelofs MJ, Ferrin TE, Myers R, Kroetz DL, Kwok PY, Giacomini KM: Genetic variation in the proximal promoter of ABC and SLC superfamilies: Liver and kidney specific expression and promoter activity predict variation. *PLoS One* 4: e6942, 2009
86. Han YF, Fan XH, Wang XJ, Sun K, Xue H, Li WJ, Wang YB, Chen JZ, Zhen YS, Zhang WL, Zhou X, Hui R: Association of intergenic polymorphism of organic anion transporter 1 and 3 genes with hypertension and blood pressure response to hydrochlorothiazide. *Am J Hypertens* 24: 340–346, 2011
87. Yee SW, Nguyen AN, Brown C, Savic RM, Zhang Y, Castro RA, Cropp CD, Choi JH, Singh D, Tahara H, Stocker SL, Huang Y, Brett CM, Giacomini KM: Reduced renal clearance of cefotaxime in asians with a low-frequency polymorphism of OAT3 (SLC22A8). *J Pharm Sci* 102: 3451–3457, 2013
88. Fujita T, Brown C, Carlson EJ, Taylor T, de la Cruz M, Johns SJ, Stryke D, Kawamoto M, Fujita K, Castro R, Chen CW, Lin ET, Brett CM, Burchard EG, Ferrin TE, Huang CC, Leabman MK, Giacomini KM: Functional analysis of polymorphisms in the organic anion transporter, SLC22A6 (OAT1). *Pharmacogenet Genomics* 15: 201–209, 2005
89. Lopez-Lopez E, Ballesteros J, Piñan MA, Sanchez de Toledo J, Garcia de Andoin N, Garcia-Miguel P, Navajas A, Garcia-Orad A: Polymorphisms in the methotrexate transport pathway: A new tool for MTX plasma level prediction in pediatric acute lymphoblastic leukemia. *Pharmacogenet Genomics* 23: 53–61, 2013
90. Chen Y, Li S, Brown C, Cheatham S, Castro RA, Leabman MK, Urban TJ, Chen L, Yee SW, Choi JH, Huang Y, Brett CM, Burchard EG, Giacomini KM: Effect of genetic variation in the organic cation transporter 2 on the renal elimination of metformin. *Pharmacogenet Genomics* 19: 497–504, 2009
91. Christensen MM, Pedersen RS, Stage TB, Brasch-Andersen C, Nielsen F, Damkier P, Beck-Nielsen H, Brøsen K: A gene-gene interaction between polymorphisms in the OCT2 and MATE1 genes influences the renal clearance of metformin. *Pharmacogenet Genomics* 23: 526–534, 2013
92. Filipski KK, Mathijssen RH, Mikkelsen TS, Schinkel AH, Sparreboom A: Contribution of organic cation transporter 2 (OCT2) to cisplatin-induced nephrotoxicity. *Clin Pharmacol Ther* 86: 396–402, 2009

93. Schreuder MF, Bueters RR, Huigen MC, Russel FG, Masereeuw R, van den Heuvel LP: Effect of drugs on renal development. *Clin J Am Soc Nephrol* 6: 212–217, 2011
94. Pavlova A, Sakurai H, Leclercq B, Beier DR, Yu AS, Nigam SK: Developmentally regulated expression of organic ion transporters NKT (OAT1), OCT1, NLT (OAT2), and Roct. *Am J Physiol Renal Physiol* 278: F635–F643, 2000
95. Sweet DH, Eraly SA, Vaughn DA, Bush KT, Nigam SK: Organic anion and cation transporter expression and function during embryonic kidney development and in organ culture models. *Kidney Int* 69: 837–845, 2006
96. Sweeney DE, Vallon V, Rieg T, Wu W, Gallegos TF, Nigam SK: Functional maturation of drug transporters in the developing, neonatal, and postnatal kidney. *Mol Pharmacol* 80: 147–154, 2011
97. Hirsch GH, Hook JB: Maturation of renal organic acid transport: Substrate stimulation by penicillin and p-aminohippurate (PAH). *J Pharmacol Exp Ther* 171: 103–108, 1970
98. Hook JB, Hewitt WR: Development of mechanisms for drug excretion. *Am J Med* 62: 497–506, 1977
99. Joseph S, Nicolson TJ, Hammons G, Word B, Green-Knox B, Lyn-Cook B: Expression of drug transporters in human kidney: Impact of sex, age, and ethnicity. *Biol Sex Differ* 6: 4, 2015
100. McLean AJ, Le Couteur DG: Aging biology and geriatric clinical pharmacology. *Pharmacol Rev* 56: 163–184, 2004
101. Deguchi T, Ohtsuki S, Otagiri M, Takanaga H, Asaba H, Mori S, Terasaki T: Major role of organic anion transporter 3 in the transport of indoxyl sulfate in the kidney. *Kidney Int* 61: 1760–1768, 2002
102. Takeda M, Babu E, Narikawa S, Endou H: Interaction of human organic anion transporters with various cephalosporin antibiotics. *Eur J Pharmacol* 438: 137–142, 2002
103. Goldstein RS, Smith PF, Tarloff JB, Contardi L, Rush GF, Hook JB: Biochemical mechanisms of cephaloridine nephrotoxicity. *Life Sci* 42: 1809–1816, 1988
104. Kohler JJ, Hosseini SH, Green E, Abuin A, Ludaway T, Russ R, Santoianni R, Lewis W: Tenofovir renal proximal tubular toxicity is regulated by OAT1 and MRP4 transporters. *Lab Invest* 91: 852–858, 2011
105. Kone BC: The metabolic basis of solute transport. In: *Brenner and Rector's The Kidney*, 7th Ed., edited by Brenner BM, Philadelphia, Saunders, 2004, pp 231–260
106. Ruegg CE, Mandel LJ: Bulk isolation of renal PCT and PST. II. Differential responses to anoxia or hypoxia. *Am J Physiol* 259: F176–F185, 1990
107. Cropp CD, Komori T, Shima JE, Urban TJ, Yee SW, More SS, Giacomini KM: Organic anion transporter 2 (SLC22A7) is a facilitative transporter of cGMP. *Mol Pharmacol* 73: 1151–1158, 2008
108. Zhou F, Illsley NP, You G: Functional characterization of a human organic anion transporter hOAT4 in placental BeWo cells. *Eur J Pharm Sci* 27: 518–523, 2006
109. Kaler G, Truong DM, Sweeney DE, Logan DW, Nagle M, Wu W, Eraly SA, Nigam SK: Olfactory mucosa-expressed organic anion transporter, Oat6, manifests high affinity interactions with odorant organic anions. *Biochem Biophys Res Commun* 351: 872–876, 2006
110. Shiraya K, Hirata T, Hatano R, Nagamori S, Wiriyasermkul P, Jutabha P, Matsubara M, Muto S, Tanaka H, Asano S, Anzai N, Endou H, Yamada A, Sakurai H, Kanai Y: A novel transporter of SLC22 family specifically transports prostaglandins and co-localizes with 15-hydroxyprostaglandin dehydrogenase in renal proximal tubules. *J Biol Chem* 285: 22141–22151, 2010
111. Zhang Q, Hong M, Duan P, Pan Z, Ma J, You G: Organic anion transporter OAT1 undergoes constitutive and protein kinase C-regulated trafficking through a dynamin- and clathrin-dependent pathway. *J Biol Chem* 283: 32570–32579, 2008
112. Wu W, Bush KT, Liu HC, Zhu C, Abagyan R, Nigam SK: Shared ligands between organic anion transporters (Oat1 and Oat6) and odorant receptors. *Drug Metab Dispos* 43: 1–9, 2015
113. Zhu C, Nigam KB, Date RC, Springer SA, Bush KT, Saier MH Jr, Wu W, Nigam SK: Evolutionary analysis and classification of OATs, OCTs, OCTNs and other SLC22 transporters: Structure-function implications and analysis of sequence motifs. *PLoS One* 2015, in press

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