The organic anion transporters (OATs) and organic anion–transporting polypeptides (OATPs) belong to the solute carrier (SLC) transporter superfamily and play important roles in handling various endogenous and exogenous compounds of anionic charge. The OATs and OATPs are often implicated in drug therapy by impacting the pharmacokinetics of clinically important drugs and, thereby, drug exposure in the target organs or cells. Various mechanisms (e.g. genetic, environmental, and disease-related factors, drug-drug interactions, and food-drug interactions) can lead to variations in the expression and activity of the anion drug–transporting proteins of OATs and OATPs, possibly impacting the therapeutic outcomes. Previous investigations mainly focused on the regulation at the transcriptional level and drug-drug interactions as competing substrates or inhibitors. Recently, evidence has accumulated that cellular trafficking, post-translational modification, and degradation mechanisms serve as another important layer for the mechanisms underlying the variations in the OATs and OATPs. This review will provide a brief overview of the major OATs and OATPs implicated in drug therapy and summarize recent progress in our understanding of the post-translational modifications, in particular ubiquitination and degradation pathways of the individual OATs and OATPs implicated in drug therapy.

Membrane transporters are essential proteins that facilitate the directional movement of endogenous solutes and xenobiotics across cell and organelle membranes. Their functions are multi-fold, covering from homeostasis, cell communication, stress resistance, and cellular protection against toxins to drug sensitivity and resistance (1). Transporters are broadly classified into two classes, namely the solute carrier (SLC) and ATP-binding cassette (ABC) transporter superfamilies. The ABC transporters are organized into seven families (ABCA through ABCG) and include the well-known members that function as drug efflux pumps, contributing to chemoresistance (e.g. ABCB1 (P-glycoprotein, Pgp) and ABCG2 (breast cancer–related protein, BCRP)). The ABC transporters have been most extensively investigated for their roles in drug therapy and also with regard to their regulatory and cellular processing mechanisms (2, 3). The SLC transporters are subdivided into 50+ families that can act as either influx or efflux transporters. Among them, the organic anion transporters (OATs) and the organic anion–transporting polypeptides (OATPs) belong to the SLC22A and SLCO superfamily, respectively, and display tissue-dependent expression profiles coordinated by genetic and epigenetic controls (4). By working jointly with the ABC transporters, the OATs and OATPs play important roles in the hepatobiliary transport, renal secretion, intestinal absorption, and brain penetration of various drug molecules (Fig. 1).

As anionic (hydrophilic) drug molecules tend to have a poor membrane permeability via passive diffusion, the transporters can play an important role in determining the cellular entry of anionic drugs and thereby influencing the pharmacokinetics (PKs; the profiles of the absorption, distribution, metabolism, and excretion processes in the body) and altering the drug exposure in the target organs and cells (possibly, the response and toxicity to drug therapies) (5). Accordingly, genetic, environmental, or disease-related factors and drug-drug interactions (DDIs) can influence the expression/activity of the transporters and consequently lead to the occurrence of adverse events in drug therapy and contribute to interindividual variability drug response (6). Severe and even life-threatening side effects of drug therapy may occur by the inhibition or impairment of these transporters (the tragic cases of the lipid-lowering statin drugs and fatalities caused by severe muscle toxicity of rhabdomyolysis (7–9) and many other cases of drug side effects and DDIs via transporter-mediated processes (10, 11)). Thus far, the cases of transporter-mediated DDIs have been mainly the combinations of the drugs that interact with transporters as substrates and/or inhibitors or as inducers and/or repressors (the modulators at the transcriptional level) (12). Changes in the transporters (due to genetic, environmental, or disease-related factors) can also occur via alternative modes that involve the post-translational regulation and processing of transporters (by impacting the localization, trafficking, post-translational modifications, or protein–protein interactions).

This review will focus on the major OATs and OATPs implicated in drug therapy and summarize the recent findings regarding their cellular processing, post-translational regulation, and degradation pathways. We start with a brief overview of the major OATs and OATPs implicated in drug therapy and the general cellular processing and trafficking of transporter proteins (more detailed and comprehensive reviews are available, but they do not focus on the anion drug–transporting proteins (13–15)). A previous review focusing on OATs and
OATPs implicated in drug therapy was published in 2017 (16), and this review will cover some updates in the field.

Overview of the major anionic drug–transporting proteins in the families of OATs and OATPs

The SLC22 and SLCO superfamilies include transporters with broad substrate specificity, covering anionic, zwitterionic, and cationic molecules, and some members are more extensively studied for their pharmaceutical importance. The United States Food and Drug Administration, the European Medicines Agency, and other regulatory agencies recommend testing new drug candidates for possible interactions with selected members in the SLC22 and SLCO superfamilies. The SLC22 superfamily can be divided into at least six subfamilies based on the evolutionary analysis (17), and the SLC22A subfamily includes the major anion drug transporters, such as OAT1 and OAT3 (18). The SLCO superfamily includes 11 human OATPs, among which OATP1B1 and OATP1B3 are best-studied for their role in hepatic handling of the lipid-lowering statin drugs and anticancer drugs. More recently, OATP2B1 has attracted attention as another OATP member of pharmaceutical importance, regarding its role in impacting the intestinal drug absorption (19). Below is a brief overview focusing on the major OATs and OATPs implicated in drug therapy (OAT1, OAT2, OAT3, OAT4; OATP1B1, OATP1B3, OATP1A2, and OATP2B1). Table 1 lists their representative endogenous and exogenous substrates, including clinically important drugs and endogenous probes that are increasingly explored to assess the transporter functions in vivo and the potential for transporter-mediated DDIs (20, 21).

OATs

By far, OAT1 and OAT3 are the most widely recognized drug transporters in the SLC22A subfamily. Both OAT1 (encoded by SLC22A6) and OAT3 (encoded by SLC22A8) are abundantly expressed in the kidney, moving anionic substrates across the basolateral membrane to the proximal tubular cells and enhancing the subsequent excretion into the urine. OAT1 and OAT3 display largely overlapping substrate specificity in handling drug molecules, but the recent analysis from a machine learning–based approach indicated that OAT3 may interact with drugs of a slightly cationic character, differing from OAT1 (22). When a similar approach was applied to the metabolomics data from mice lacking Oat1 or Oat3, a difference emerged in that Oat3 has a propensity for more complex substrates possessing more rings and chiral centers, compared with Oat1 (23).

OAT2 (encoded by SLC22A7) was the first cloned mammalian OAT and initially named as NLT (novel liver-specific transporter) (24). Later found to be expressed in the kidney as well as in the liver and closely related to OAT1, the NLT was renamed as OAT2 (25). A notable aspect of OAT2 is the presence of three splice variants: OAT2-546aa (NM_153320), OAT2-548aa (NM_006672), and OAT2-539aa (AF210455). The sequence difference between the two variants OAT2-546aa and OAT2-548aa is the insertion of 2 amino acids
**Table 1**

**List of the major OATs and OATPs frequently implicated in drug therapy**

This list includes the representative substrates for the assessment of the transporter function in vivo and the risk for drug-drug interactions are underlined; comprehensive lists covering endogenous and exogenous substrates are available in previous reviews (1, 19, 21, 46, 151–154).

| Protein name (gene name) | Location | Example substrates |
|--------------------------|----------|--------------------|
| OAT1 (SLC22A6)           | Kidney, brain, placenta, muscle; basolateral | *Endogenous:* α-ketoglutarate, urate, prostaglandin E₂, taurine  
*Exogenous:* adrenaline, acetylcholine, acyclovir, methotrexate, pravastatin, ciprofloxacin, chlorothiazide, ochratoxin A  
Endogenous: cGMP, creatinine, prostaglandin E₂ |
| OAT2 (SLC22A7)           | Liver, kidney; basolateral | Exogenous: iminocetan, 5-fluorouracil, acyclovir, ganciclovir  
Endogenous: estrone sulfate, bile acids, creatinine, prostaglandin E₂, glycochenodeoxycholate-3-α-sulfate (GCDCA-S), 6β-hydroxyl cortisol  
Exogenous: benzylpenicillin, cimetidine, ranitidine, famotidine, methotrexate, cidofovir, valacyclovir, sitagliptin, pravastatin |
| OAT3 (SLC22A8)           | Kidney, choroid plexus, testis; basolateral | Exogenous: entecavir (an antiviral drug) (26, 27). Many of the OAT2 substrates are also substrates of OAT1 and/or OAT3, but OAT2 appears to use a transport mechanism distinct from OAT1 and OAT3 (28). |
| OAT4 (SLC22A11)          | Kidney (apical), placenta (basolateral) | Exogenous: dehydroepiandrosterone sulfate, urate, estrone sulfate, prostaglandin E₂  
Exogenous: indomethacin, tetracycline, ochratoxin A, methotrexate, olmesartan, levocetirizine  
Endogenous: estrone sulfate, estradiol 17β-glucuronide, dehydroepiandrosterone sulfate, coproporphyrin I and II (CPI and CPII), GCDCA-S, unconjugated and conjugated bilirubin (UCB and CB)  
Exogenous: bosentan, daunorubicin, fexofenadine, pitavastatin, atorvastatin, rosuvastatin, rifampicin, darunavir, mycophenolic acid  
Endogenous: paclitaxel, pitavastatin, rosuvastatin, rifampicin, telmisartan, mycophenolic acid, glucuronide |
| OATP1B1 (SLCO1B1)        | Liver (basolateral) | Exogenous: estrone sulfate, estradiol 17β-glucuronide, dehydroepiandrosterone sulfate, coproporphyrin I and II (CPI and CPII), GCDCA-S, unconjugated and conjugated bilirubin (UCB and CB)  
Exogenous: bosentan, daunorubicin, fexofenadine, pitavastatin, atorvastatin, rosuvastatin, rifampicin, darunavir, mycophenolic acid  
Endogenous: paclitaxel, pitavastatin, rosuvastatin, rifampicin, telmisartan, mycophenolic acid, glucuronide |
| OATP1B3 (SLCO1B3)        | Liver (basolateral) | Exogenous: estrone sulfate, estradiol 17β-glucuronide, dehydroepiandrosterone sulfate, coproporphyrin I and II (CPI and CPII), GCDCA-S, unconjugated and conjugated bilirubin (UCB and CB)  
Exogenous: bosentan, daunorubicin, fexofenadine, pitavastatin, atorvastatin, rosuvastatin, rifampicin, darunavir, mycophenolic acid  
Endogenous: paclitaxel, pitavastatin, rosuvastatin, rifampicin, telmisartan, mycophenolic acid, glucuronide |
| OATP1A2 (SLCO1A2)        | Liver (cholangiocytes), brain capillary, kidney, retinal epithelium | Exogenous: estrone sulfate, retinoids, prostaglandin E₂ |
| OATP2B1 (SLCO2B1)        | Liver, intestine | Exogenous: estrone sulfate, dehydroepiandrosterone sulfate, prostaglandin E₂  
Exogenous: galbenclamide, statins, fexofenadine, atenolol, montelukast |

OATs

Among the 11 OATPs identified in humans, OATP1B1 and OATP1B3 are extensively studied for their roles in hepatic drug disposition and transporter-mediated DDIs. The genes encoding OATP1B1 (SLCO1B1) and OATP1B3 (SLCO1B3) are located in proximity to each other on the chromosome 12, and the two proteins are also highly homologous (80% identity at the amino acid level). OATP1B1 and OATP1B3 display nearly overlapping substrate specificity. The substrates commonly handled by OATP1B1 and OATP1B3 include many clinically important drugs (e.g. lipid-lowering statin drugs, antitumor and antidiabetic drugs), for which their entry to hepatocytes can be rate-determining in the overall hepatic drug elimination process (in which drug molecules are metabolized or excreted out of hepatocytes) (6). In such cases, impaired hepatic drug uptake due to either genetic variations or co-administered drugs can lead to the PK changes, impacting the drug efficacy and toxicity. For lipid-lowering statin drugs such as cerivastatin, impaired hepatic uptake was responsible for the occurrence of myotoxicity (muscle toxicity) ranging from mild cases to fatal rhabdomyolysis (the most severe form of muscle toxicity) (6, 35). To prevent similar tragic consequences, anionic drug candidates are screened for their possible interactions with OATP1B1 and OATP1B3. There is also a continuing effort to enhance our understanding of various mechanisms regulating the expression and function of these important hepatic uptake transporters.

Although OATP1B1 and OATP1B3 are present on the basolateral membrane of hepatocytes, they differ in terms of the zonal expression pattern in the liver: OATP1B1 is expressed throughout the liver parenchyma, but OATP1B3 is expressed in the region surrounding the central vein of hepatic lobules (36–38). Another notable difference was that unlike OATP1B1, OATP1B3 was detected in cancerous cells derived from various nonhepatic organs (38–40). It is now known that the OATP1B3 protein detected in the cancerous cells (called as cancer-type OATP1B3) arises from an alternative mRNA transcript and lacks the N-terminal 28 amino acids compared with the OATP1B3 protein expressed in nonmalignant hepatocytes (called liver-type OATP1B3) (40). Compared with the liver-type OATP1B3, the cancer-type OATP1B3 protein had an
inferior efficiency of membrane trafficking, thus much reduced transport activity (40).

OATP1A2 (encoded by \textit{SLCO1A2}) is another OATP that has been extensively studied for its interactions with a broad range of drugs (e.g. imatinib (an anticancer drug), methotrexate (an anticancer drug), and fexofenadine (an antihistamine drug)), cellular trafficking mechanisms, and differential regulation in disease conditions, including breast cancer (41, 42). OATP1A2 is expressed in the epithelium of various organs, such as the liver (cholangiocytes but not hepatocytes), kidney, brain capillaries, and eye (43, 44). An early study reported the positive immunohistochemical staining of OATP1A2 in the intestine (45), but subsequent studies did not detect the presence of OATP1A2 at either mRNA or protein level (see Ref. 46 and references therein). Those subsequent studies, however, verified the presence of OATP2B1 (encoded by \textit{SLCO2B1}) in the human intestine. Based on its intestinal location and ability to transport clinically drug molecules, OATP2B1 is recognized as an important transporter that influences the intestinal drug absorption and serves as a target for food-drug interactions (19, 47).

Interestingly, the two recent studies reported that the transport activity of OATP2B1 can be inhibited by common food and drug additives (small-molecule excipients added to food and drug products), such as azo dyes (48, 49). The food and drug additives had been presumed to be inactive and inert in the body, but these studies indicated that the food and drug additives can have unintended effects on the intestinal drug absorption and PK profiles by inhibiting OATP2B1 in the intestinal epithelium. Moreover, the OATP2B1-inhibitory additives were converted to metabolites that no longer inhibit OATP2B1 by the gut microbiota (48). These findings highlight the intriguing interplay of OATP2B1 and gut microbiota in influencing the intestinal drug absorption. In addition to the intestinal epithelium, OATP2B1 is also expressed in multiple organs, including the liver (hepatocytes), and its transport activity is pH-dependent with enhanced activity at acidic conditions (50, 51).

Structurally, the members of the SLCO/OATP family share 12 TMDs with an extracellular loop between TMD9 and TMD10 (harboring conserved cysteine residues and glycosylation sites). The highly conserved family signature sequence is located in the region that spans from extracellular loop 3 and TMD6 (52). For OATP1A2 and OATP2B1, their C-terminal regions harbor the PDZ-binding domain (53).

Overview of the general cellular processing of transporters in normal physiology and pathology

In normal physiology, it is generally believed that the translation of a transporter protein is tightly linked to its translocation (i.e. being co-translationally translocated) from ribosomes to the endoplasmic reticulum (ER), as illustrated in Fig. 2. Post-translational modifications play an important role in
coordinating the folding and targeting of newly synthesized transporter proteins to the appropriate cellular organelles and the final destination of the plasma membrane (54–57). The processes that occur in the ER and Golgi apparatus are often referred to as “central quality control,” but the regulatory actions also take place near the plasma membrane. The mechanism is called “peripheral quality control,” and it regulates endosomal recycling and lysosomal degradation near the plasma membrane (54). The central and peripheral quality control pathways are interconnected and work together, shuffling the transporter proteins inside cells and to the plasma membrane, from the ER and Golgi to various intracellular degradation machinery (e.g. endosomes, lysosomes, proteasomes, aggresomes) (58, 59). Post-translational modifications and other protein-protein or protein-lipid interactions are involved in the internalization and recycling of the transporters located at the plasma membrane (15).

In cellular processing of transporter proteins, it is increasingly recognized that the composition of membrane lipids can affect transporter conformation at the plasma membrane, leading to partial misfolding or modifications of cytoplasmic domains and altered susceptibility of the transporter to endocytic turnover processes (13). In particular, lipid rafts (microdomains enriched in cholesterol and glycosphingolipids at the external leaflet of the plasma membrane) have been shown to enhance oligomerization and other protein-protein interactions that impact the activity and trafficking for several transporters (60–63). This aspect is becoming of great interest and is another important layer of the post-translational regulatory mechanisms for the OATs and OATPs.

Defective processing and trafficking of transporters can cause various diseases. Cystic fibrosis is one of the well-known cases, and it is attributed to genetic variations causing misfolding and degradation of the cystic fibrosis transmembrane conductance regulator (CFTR, encoded by ABCC7) (64). With mechanistic understanding of the CFTR trafficking, there now exist therapeutic options for cystic fibrosis patients (e.g. lumacaftor-ivacaftor, tezacaftor-ivacaftor) (65, 66). Correction strategies using trafficking modifiers or chemical chaperones are being explored in preclinical and clinical settings for other transporters (e.g. ABCB11 encoding bile salt export pump (BSEP) and progressive familial intrahepatic cholestasis type 2 (67–69); ABCG2 encoding BCRP and gout (70, 71)). To date, a number of studies have reported alterations in the level and cellular localization of certain OATs and OATPs under disease conditions, such as cancer and liver diseases (72, 73). The OATs and OATPs have been rarely identified as single disease-causing factors, but the altered level/function of the OATs and OATPs may serve as disease-modifying factors and possibly impact the PK profiles of drug molecules in patients (74).

Three common types of post-translational modifications involved in cellular processing of the major anionic drug–transporting proteins in the families of OATs and OATPs

In response to various cellular stresses or stimuli, the transporter protein at the plasma membrane can receive multiple types of post-translational changes and protein-protein interactions that can alter the functional activity, protein internalization, and recycling. Below is a brief description of the three types of post-translational modifications (N-glycosylation, phosphorylation, and ubiquitination) that are commonly involved in the cellular processing and trafficking of the major anion drug transporters, OATs and OATPs.

**N-glycosylation**

In eukaryotic cells, N-glycosylation occurs at Asn residues within the consensus sequences of Asn-Xaa-Thr/Ser, where Xaa cannot be Pro or Asp. The types and linkages of N-glycosylation processed in the ER are diverse and complex, and the extent to which they exert discrete functions is not yet fully understood. Disruption of N-glycosylation can increase the accumulation of misfolded proteins in the ER, enhancing the ER-associated degradation.

**Phosphorylation**

Protein phosphorylation is a process of attaching a phosphate monooester group to a free hydroxyl group of Ser, Thr, or Tyr residues (or far less commonly to other amino acids, such as His, Lys, and Arg). For a single residue modifiable by phosphorylation, the modification can occur by different kinases and phosphatases, leading to different biological outcomes. For many proteins and signaling events, phosphorylation/dephosphorylation often serves as a functional on/off switch, interconnecting and cross-talking with other types of post-translational modifications. Prominent examples include the connection between phosphorylation and ubiquitination (75). Phosphorylation can either promote or inhibit ubiquitination, thereby affecting protein degradation or other molecular events. The activation of protein kinase C (PKC) has been associated with the internalization of the functional transporter from the cell surface, and the underlying mechanisms involve the protein phosphorylation promoting the ubiquitination of the transporter protein residing in a cholesterol-rich microdomain of the plasma membrane (lipid rafts) (76).

**Ubiquitination**

Ubiquitination is a process of attaching ubiquitin by forming an isopeptide bond between a Gly residue of ubiquitin and a specific Lys residue of a target protein. Ubiquitination is carried out in a multistep process involving a cascade of three enzymes: an E1 ubiquitin-activating enzyme, an E2 ubiquitin-conjugating enzyme, and an E3 ubiquitin ligase enzyme (77). The E3 ubiquitin ligases confer substrate specificity and are classified into the two major families: HECT (homology to E6AP C terminus; ~30 ligases) and RING (really interesting new gene; ~600 ligases) (78). Different types (e.g. monoubiquitination (attachment of a single ubiquitin to a single Lys residue of the substrate), multiubiquitination (attachment of several ubiquitin molecules to multiple Lys residues), and polyubiquitination (attachment of polyubiquitin chains)) and linkages of ubiquitination can alter the biological outcomes. For example, Lys-48–linked polyubiquitination (Lys-48 residing in the ubiquitin molecules serves as a base for growing a polyubiquitin chain) commonly serves as the...
 earmark for proteasome-mediated degradation (56). On the other hand, Lys-63–linked polyubiquitination (Lys-63 residing in the ubiquitin molecules also serves as a base for growing a polyubiquitin chain) is used for multiple purposes, including the regulation of the endosomal recycling and degradation events near the plasma membrane (79). Ubiquitination can be reversed by deubiquitinating enzymes (DUBs; >100 enzymes).

**Cellular processing and post-translational modifications of the major anionic drug–transporting proteins in the families of OATs and OATPs**

Below is a summary of current understanding of this topic, and major findings are also depicted in Fig. 3.

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**OAT1**

N-Glycosylation was initially identified to impact substrate binding or membrane trafficking of OAT1 (80). However, the mutation of multiple Asn residues was necessary for disruption of the OAT1 trafficking to the plasma membrane, and the responsible mechanisms or signaling pathways remain unknown.

The PKC activation triggered the internalization of OAT1 from the cell surface, as depicted in Fig. 4A (81). OAT1 was found to undergo the constitutive internalization and recycling at a rate of ~10% of the initial OAT1 protein pool on the cell surface per 5 min, and the PKC activation accelerated the rate of OAT1 internalization without affecting OAT1 recycling (81). Subsequent studies reported that three Lys residues at positions of 297, 303, and 315 play a synergistic role in PKC-
regulated OAT1 ubiquitination, trafficking, and transport activity (82) and that Lys-48–linked polyubiquitination is an important signal for internalization and degradation (83). These findings were further followed up by another observation on the connection between the PKC activation and polyubiquitination (84). The two E3 ubiquitin ligases belonging to the HECT family played an important role in the ubiquitination of OAT1, namely NEDD4-1 (neural precursor cell–expressed, developmentally down-regulated 4-1) and Nedd4-2 (84). In particular, NEDD4-2 was responsible for interconnecting the PKC activation and the internalization of OAT1: PKC activation leads to the phosphorylation and conformational change of NEDD4-2, which then leads to the ubiquitination and internalization of OAT1 (85). In contrast to the PKC activation linked to the OAT1 internalization (and thereby a decrease in the transport activity), the activation of SGK2 (serum- and glucocorticoid-inducible kinase 2) enhanced the surface level of OAT1 and increased the maximal transport activity ($V_{max}$) without affecting the substrate-binding affinity ($K_m$) (86). In either signaling event, OAT1 was not a substrate of direct phosphorylation (by PKC or SGK2 activation).

In a recent study, the two clinically used proteasome inhibitor drugs (bortezomib and carfilzomib) increased the cellular levels of the ubiquitinated OAT1 protein and also enhanced the level of the functional OAT1 at the plasma membrane (87). Interestingly, the ubiquitinated OAT1 protein displayed a molecular size much higher than expected for a monomer (to an extent much higher than poly- or multiubiquitination). These results appear in accordance with the earlier finding that OAT1 can form homooligomers (88). The treatment with a chemical cross-linking agent led to the formation of immunoreactive

Figure 4. Proposed model based on the published findings of signaling pathways regulating the processing and post-translational regulation of OAT1 (A) and OAT3 (B).
OAT1 bands consist with the formation of homooligomers. Immunoprecipitation experiments also revealed that the OAT1 proteins fused with different tags directly interact with each other. It remains to be investigated whether the homooligomerization is impacted by ubiquitination or treatment with proteasome inhibitors. The proteasome inhibitor therapy is often administered for an extended time in combination with other anticancer drugs to suppress the disease’s progression. It is currently unknown whether OAT1 and other transporters’ expression and activity may be impacted in cancer patients receiving the long-term proteasome inhibitor therapy.

Other findings on the post-translational regulation of OAT1 are also related to its oligomerization. The oligomerization of OAT1 was inhibited by co-expressing a short fusion peptide containing the TMD6 of OAT1 (89). Moreover, the membrane-trafficking and transport activity of OAT1 was abolished by the mutation of Gly residues in the TMD2 (within the motif of Gly-Xaa-Xaa-Xaa-Gly) (90). The mutated OAT1 proteins (G144A and G148A) were accumulated in the ER and subsequently degraded by the proteasome. The treatment with the proteasome inhibitor MG132 increased the protein level in total cell lysates but did not improve the trafficking of the proteasome inhibitor therapy.

In terms of the regulation via protein-protein interactions, OAT3 was shown to interact with lipid raft-associated proteins (β-actin, myosin, and caveolin-1) (99). When the authors exposed the rat renal cortical slices to methyl-β-cyclodextrin, which depletes cholesterol from the plasma membrane (thereby disrupting the lipid rafts), there was a dose-dependent reduction in the transport activity of the rat Oat3. In a more recent study, the membrane distribution of the rat Oat1 and Oat3 was assessed in animals that received bile duct ligation (leading to obstructive jaundice) or sham operation (100). The membrane distribution of Oat3 was not shifted in renal cortical cells isolated from the rats that received bile duct ligation. On the other hand, Oat1 displayed a significant shift in its membrane distribution (moving away from lipid raft domains). It remains to be investigated whether the level and function of human OAT1 and OAT3 may be impacted by the cholesterol content in the plasma membrane, which may well vary among individuals and depend on disease types and states.

OAT2

For OAT2, the alternatively spliced transcripts have been identified and investigated for their and function (91, 92). The variant OAT2-548aa contains two additional amino acids in the large extracellular loop (the addition of Ser and Gln between Glu-131 and Trp-132) and displays defective membrane trafficking (91). The previous study reported the presence of the putative sites (the consensus motifs) that could be phosphorylated by protein kinase A (PKA) or PKC (93), but further verification is unavailable yet.

OAT3

In a similar manner to OAT1, the PKC activation decreased the level of the functional OAT3 at the plasma membrane (83, 94) (Fig. 4B). The treatment with angiotensin II enhanced the internalization of OAT3 (resulting in a decrease in the V_{max} value but no change in the K_{m} value), connected to the PKC activation, accelerating the endocytosis of OAT3 (94). Short-term and long-term exposure to a PKC activator led to differential outcomes: the short-term exposure (<30 min) was associated with enhanced OAT3 internalization without affecting the total expression level, but the long-term exposure (>2 h) led to OAT3 degradation by both lysosomes and proteasomes (83). A subsequent study showed a physical interaction between OAT3 and NEDD4-2, indicating that the PKC activation and internalization of OAT3 are interconnected via the ubiquitination by NEDD4-2, like OAT1 (95).

In contrast to the inhibitory effect of PKC on the OAT3 activity, PKA stimulated the OAT3 activity by enhancing its level at the plasma membrane (96, 97) (Fig. 4B). The PKA-mediated stimulation of OAT3 was mediated by SUMOylation (covalent attachment of SUMO (small ubiquitin-related modifier) by SUMO-2 and -3 but not by SUMO-1) (96). Further investigations revealed that SUMOylation could be reversed by the SUMO-specific protease Senp2, whose knockdown by siRNAs increased OAT3 SUMOylation and its transport activity and level. Insulin-like growth factor-1 (IGF-1) activated the PKA, subsequently increasing the SUMOylation of OAT3 (98), but a subsequent study reported that the effect of IGF-1–induced PKA activation was also linked to the phosphorylation of OAT3 (96). A more recent study verified direct phosphorylation of OAT3 by the treatment with Bt2-cAMP (a PKA activator) or IGF-1 (97). Together, the regulatory mechanisms for OAT3 showcase how the post-translational regulation and signaling pathways are interconnected.

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not appear to be mediated by SGK2, another signaling event that enhanced the NEDD4-2 phosphorylation (86, 104). Together, these findings suggest that OAT4 processing and activity are dynamically regulated by the balance among multiple signaling pathways and cellular stimuli.

Another important aspect of OAT4 processing is its interaction with the scaffold proteins PDZK1 (PDZ domain-containing 1) and NHERF1 (Na⁺–H⁺ exchanger regulatory factor 1). Unlike OAT1, OAT2, and OAT3, OAT4 has a protein-protein interaction peptide sequence named the PDZ (PSD-95/Discs Large/ZO-1) motif at the C terminus. The co-immunoprecipitation results verified that OAT4 interacted with the two scaffold proteins PDZK1 and NHERF1 (105, 106). Interestingly, the interactions of OAT4 with PDZK1 and NHERF1 were observed in the LLC-PK cells (of kidney origin) but not in BeWo cells (of placenta origin) (106). These findings suggest that the interaction of OAT4 and the PDZ proteins may be cell- or tissue-specific. The oligomerization ability of scaffold proteins is known to be regulated by phosphorylation and other signaling pathways, via modifications of either scaffold proteins or their partnering proteins, including transporters (107, 108). Thus, it would also be important to consider whether the association of OAT4 with the scaffold proteins is impacted by any of the reported signaling pathways regulating OAT4 on the cell surface.

**OATP1B1**

The **SLCO1B1** gene is highly polymorphic, and commonly occurring genetic variations (in particular, c.521T>C (dbSNP-ID of rs4149056; p.174Val>Ala)) have been firmly associated with PK changes and a high incidence of statin-induced myopathy (6). Most statins rely on the transporters to gain access to the hepatocytes, which becomes the rate-determining step in the overall hepatic elimination processes (6, 109, 110). The decreased hepatic uptake of statins can lower the hepatic elimination of the drug; a drug used for the treatment of malaria and certain autoimmune diseases increased the total OATP1B1 protein levels (based on the band intensities of OATP1B1 immunoblots) in HEK293 cells stably expressing OATP1B1 or human sandwich-cultured hepatocytes (120). Immunofluorescence imaging analysis, however, indicated that the OATP1B1 protein was localized in the cytoplasm and associated with late endosome/lysosome in cells treated with chloroquine. In line with such findings, the chloroquine treatment was associated with a decrease in the Vmax value but no change in the Km value for OATP1B1-mediated transport of estradiol 17β-glucuronide (120). In the same study, the authors included the data from pharmacoepidemiological studies, in which female patients on co-medication of chloroquine and lipid-lowering statin drugs were associated with higher incidence of statin-associated myopathy. Further validation is however necessary, especially to probe the mechanisms for the observed gender differences in the pharmacoepidemiological studies. Treatment with bortezomib (a proteasome inhibitor) led to no changes in the total OATP1B1 protein levels (121), suggesting that the proteasome is likely to play a lesser role in the OATP1B1 degradation than the lysosome.

Regarding the protein-protein interactions, an earlier study reported that OATP1B1 displays immunoreactive bands around 190 kDa in nonreducing conditions and around 75 kDa in reducing conditions (122). The possibility of OATP1B1 homooligomers was further examined using a chemical cross-linking agent or by expressing the OATP1B1 protein fused with different tags in HEK293 cells, and the results verified the oligomer formation of OATP1B1, likely via disulfide bond formation (123). OATP1B1 harbors three Gly-Xaa-Xaa-Gly motifs, among which the motif located in TMD5 was found to be important for oligomerization, based on the results obtained using the mutant G393A (a decreased oligomerization and a reduced uptake of estrone 3-sulfate) (123). Further investigations are however warranted, in particular, using other cellular systems with a more native membrane environment, such as human primary hepatocytes, and the question remains whether the oligomerization of OATP1B1 occurs primarily as a homooligomer or a heterooligomer with other OATP transporters.
and whether the oligomerization of OATP1B1 varies among individuals in healthy or diseased states.

**OATP1B3**

OATP1B3 is highly homologous to OATP1B1 and displays nearly overlapping substrate specificity with OATP1B1. Cholecystokinin-8 (a peptide gastrointestinal hormone) is an exception that is handled uniquely by OATP1B3 and not by OATP1B1. The SLCO1B3 gene is not as polymorphic as the SLCO1B1 gene, but a few nonsynonymous variations have been reported for OATP1B3 (124, 125). For the variants M233I, H520P, and V560A, the OATP1B3 protein levels at the membrane surface were decreased, accompanied by the reduction in the \( V_{\text{max}} \) values for the transport of cholecystokinin-8 (125). In a study that assessed the systemic PK profiles of mycophenolic acid (an immunosuppressant drug), the subjects harboring the haplotype combination of p.233Met>Ile and p.112Ser>Ala (c.699G>A and c.334T>G present in linkage disequilibrium; dbSNP-IDs of rs7311358 and rs4149117) tended to have the elevated systemic levels of mycophenolic acid (126). More importantly, a recent retrospective study reported that the subjects homozygous for the variant haplotype have an improved clinical outcome (in terms of the risk for acute rejection and survival) following lung transplantation (127). Currently, a mechanistic understanding is lacking as to how the genetic variations lead to the reduction in the OATP1B3 level on the plasma membrane and the impaired transport activity.

Unlike OATP1B1 displaying nearly exclusive expression in hepatocytes, OATP1B3 was detected in cancerous cells derived from various nonhepatic organs also expressing OATP1B3, with the predominantly cytoplasmic pattern when probed using the OATP1B3 antibody detecting the C-terminal sequence (38–40). It is now known that the positive cytoplasmic immunostaining was from the cancer-type OATP1B3 (674 aa), lacking the N-terminal 28 amino acids compared with the liver-type OATP1B3 protein of 702 aa) (40). Using the N-terminal truncation mutants, a follow-up study revealed that the N-terminal sequence of OATP1B3 (in particular, the amino acid positions between 12 and 18 within the region lacking in the cancer-type OATP1B3) is important for membrane trafficking of OATP1B3 (128). The structural motifs or individual amino acids responsible for the trafficking of OATP1B3 were not, however, identified in that region. But the importance of the N-terminal sequences of OATP1B3 as well as OATP1B1 was supported by the finding that the N-terminal peptides (50 amino acids) fused with a Myc tag were efficiently localized to the plasma membrane (128). On the other hand, the C-terminal sequences of both OATP1B3 and OATP1B1 were predicted to lack a PDZ-binding motif interacting with the scaffold proteins (53).

For the degradation of the liver-type OATP1B3 protein, the lysosome may play a more prominent role than the proteasome (121). When cells stably expressing the liver-type OATP1B3 were treated with chloroquine (a lysosomal inhibitor) or bortezomib (a proteasome inhibitor), the total OATP1B3 protein (assessed by OATP1B3 immunoblots) was increased only by chloroquine (121). Similar to the case of OATP1B1, the increased level of the total OATP1B3 protein by chloroquine treatment was associated with a decreased transport activity (121, 129). Despite having no impact on the OATP1B3 protein levels in total cell lysates or surface membrane fraction, the bortezomib treatment also led to a modest decrease in the \( V_{\text{max}} \) value but no change in the \( K_m \) value for OATP1B3-mediated transport of cholecystokinin-8 (121). The bortezomib treatment, however, had no impact on the OATP1B3-mediated transport of pitavastatin (a lipid-lowering statin drug) (121). The authors speculated that the bortezomib treatment may change the turnover rate of OATP1B3, but further experiments will be necessary to verify that possibility.

Like OATP1B1, OATP1B3 was found to homo- or heterooligomerize (130). In addition to homooligomers, OATP1B3 oligomerized with OATP1B1 and Na\(^+\)-taurocholate–cotransporting polypeptide (NTCP) in HEK293 cells expressing those transporters. As the rat Ntcp was reported to be located in the lipid rafts of the plasma membrane (131), the question remains whether the heterooligomers of OATP1B3 and NTCP would be also located in the lipid rafts and whether the level and function of OATP1B3 could be affected by the cholesterol content in the membrane.

**OATP1A2**

The decreased OATP1A2 level at the plasma membrane was reported with naturally occurring genetic variations: variations disrupting N-glycosylation (43) and those replacing the negatively charged residues (Asp, Glu) in the intracellular loops or Thr residue in the putative TMD6 (132). A follow-up study focused on the putative TMD6 and narrowed down the amino acids that are important for the OATP1A2 trafficking and degradation via proteasomes or lysosomes (133).

Another important aspect of OATP1A2 processing is its interaction with the scaffold proteins PDZK1 and NHERF1, based on the results from the yeast two-hybrid library screening (53) and the co-immunoprecipitation experiments (134). In the case of PDZK1, no direct interaction was detected by co-immunoprecipitation experiments, but both PDZK1 and NHERF1 enhanced the OATP1A2 level at the membrane surface by reducing OATP1A2 internalization via the clathrin-dependent pathway (134).

A separate study indicated that the clathrin-dependent internalization of OATP1A2 from the cell surface can be accelerated by the PKC activation (135). In addition to PKC, casein kinase 2 (CK2) was involved in regulating the trafficking of OATP1A2 (136). Chemical or genetic inhibition of CK2 led to decreases in the OATP1A2 internationalization and recycling, causing a reduction in the \( V_{\text{max}} \) value but no change in the \( K_m \) value for the OATP1A2-mediated transport of estrone-3-sulfate (136). Interestingly, CK2 is reported to be dysregulated in many disease states, including breast cancer, in which the OATP1A2 level is elevated (42, 137). Another study showed that OATP1A2 trafficking is regulated by 5′-AMP–activated protein kinase signaling associated with an increased incidence of type II diabetes and nonalcoholic fatty liver disease (138). Further investigations will be necessary to assess how and to what extent these multiple kinases are connected and contribute to...
the processing of the functional OATP1A2 in healthy and disease states.

**OATP2B1**

The *SLCO2B1* gene encoding OATP2B1 harbors polymorphic variations whose frequencies vary among different ethnic groups (139). Nonsynonymous genetic variations of OATP2B1 (c.935G>A and c.1457C>T; dbSNP-IDs of rs12422149 and rs230618) have been associated with the altered transport activity and PK changes *in vivo*, with some conflicting results (140–143). However, our understanding is limited as to whether and how these genetic variations differ at the post-translational level.

The OATP2B1 protein contains three consensus sequences for N-glycosylation, two of which are predicted to be intracellular (144). To date, no study has examined specifically whether those N-glycosylation sites play a role in the trafficking mechanism of OATP2B1. The study that examined the human liver tissue sections from patients with nonalcoholic steatohepatitis reported a possible impairment of N-glycosylation for OATP2B1, but to a lesser extent than OATP1B1 and OATP1B3 (115).

Similar to OATP1B1, the PKC activation accelerated the OATP2B1 internalization via the clathrin-dependent pathway and subsequent lysosomal degradation (145). No information is yet available regarding the residue(s) phosphorylated in OATP2B1 and other mediating signaling pathways that interconnect the PKC activation and OATP2B1 processing.

Another study identified that the TMD1 of OATP2B1 is important for its transporter function and stability (146). The replacement of a Phe residue at position 51 enhanced the OATP2B1 degradation, but the functional activity of OATP2B1 was recovered by neither lysosomal nor proteasomal inhibition. Like OATP1A2, OATP2B1 harbors a PDZ-binding motif at its C terminus (53). The localization and function of OATP2B1 was reported to be regulated by the interactions with PDZK1 (147). In HeLa cells stably expressing OATP2B1, the transient transfection of PDZK1 led to an increase in the functional OATP2B1. The N-terminal deletion mutant of OATP2B1 lacking the PDZ-binding motif, however, showed no enhancement by PDZK1 (147). Another intriguing finding was that the membrane localization of OATP2B1 appeared to switch from the apical to basolateral sides in the proximal and distal renal tubules, based on the immunohistochemical staining of the human kidney tissue sections (147). As the PDZK1 was found only on the apical side, the authors cautiously suggested that PDZK1 may be involved in the control of subcellular localization of OATP2B1. Further investigations will be necessary to determine whether similar interactions between OATP2B1 and PDZK1 occur in the intestine and whether variations in the PDZK1 expression may be a source of interindividual variability in the level of the functional OATP2B1.

**Conclusions and future directions**

This review summarized the recent progress in describing the cellular processing and trafficking of the major anion drug-transporting OATs and OATPs. Although some of the SLC transporters have been explored as therapeutic targets, the major anion drug–transporting proteins were not included (148). However, the anion drug–transporting proteins will continue to play a critical part in predicting the PK profiles of clinically important drugs and managing the risk of DDIs during drug development.

A better understanding of post-translational regulation and trafficking of the anion drug–transporting proteins can allow us to obtain an accurate prediction of the transporter-mediated handling of clinical used drugs and new drug candidates. Advances have been made in the quantitative proteomic analysis of transporters, providing increasingly precise measurements of the transporter level across various human tissues and experimental systems and relevant transporter-related parameters that can be scaled up for the prediction of PK profiles *via in silico* modeling–based approaches (149, 150). The more we understand and appreciate the contribution of post-translational mechanisms regulating the transporters, the more we recognize that the protein amounts detected either in cells or at the surface membrane may not necessarily correlate with the functional transporters. Another area that has made significant progress in recent years is the discovery and validation of endogenous probes that can serve as biomarkers for transporter function *in vivo* (20, 21). A number of endogenous probes for the major anion drug–transporting proteins have been identified among the endogenous metabolites or food-derived compounds (as listed in Table 1). The evaluation of the endogenous probes will continue in terms of their transporter selectivity and specificity and sensitivity to detect the intra- and interindividual variations in the transporter function *in vivo*. When discrepancies are found between the transporter level (e.g. the quantitative proteomics data) and the *in vivo* functional outcomes in healthy or diseased subjects (e.g. the assessment using drug probes or endogenous probes), a better understanding of post-translational regulation and trafficking may provide clues to resolving the disconnect. In coming years, more careful investigations will be necessary to examine and understand the changes in the processing and trafficking of transporters over time and in response to various cellular stresses and stimuli.

Protein–protein interactions (including oligomerization) and lipid–protein interactions (e.g. near the lipid rafts of the plasma membrane) are emerging as important players in regulating transporter functioning. In examining the interactions of transporters with drug molecules and the regulatory mechanisms for various transporters, *in vitro* cell line models expressing individual transporters in either a transient or stable manner have been widely employed. In such model systems, the cellular environment may not fully capture protein–protein and protein–lipid interactions that occur in the native environment *in vivo*, including possible variations in the cellular environment among individuals (e.g. gender, age, genetic, environmental, and disease-related). When the transport activity is assessed using commonly available cell line models or primary cells from human donors, there often exist substantial data variabilities within and among laboratories. The observed data variability may be attributable to variations in culturing conditions and possibly factors impacting the processing, trafficking, and degradation of the transporters at the post-translational level.
Thus, careful comparative analysis and cross-validation of the results in different model systems, including primary cells, will be necessary to enhance our understanding of the regulatory mechanisms that have clinical significance.

As covered in this review, the processing and trafficking of the transporters are coordinated by different types of post-translational modification, including ubiquitination and phosphorylation. Ubiquitination and phosphorylation are highly dynamic processes controlled by the balance of the enzymes involved (kinases and phosphatases; E3 ubiquitin ligases and DUBs). Those enzymes are specifically targeted or affected by some approved drugs (e.g., various kinase inhibitors) or drug candidates in clinical and preclinical development. The approved drugs targeting the proteasomes (bortezomib, carfilzomib, and ixazomib) have brought a breakthrough in treating patients with multiple myeloma and other hematological malignancies and are used as long-term therapies. Currently, efforts are ongoing to develop next-generation drugs targeting the proteasomes and E3 ligases as well as drugs targeting DUBs. The lysosomal inhibitors chloroquine and hydroxychloroquine are currently used against some infectious and inflammatory diseases. When these drugs are used to treat patients on a long-term basis, they may have an impact on the cellular proteomic profiles, including transporters. The consequences of such changes may need to be carefully examined in relation to the PK and pharmacodynamic aspects in drug therapy.

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Abbreviations—The abbreviations used are: SLC, solute carrier; ABC, ATP-binding cassette; OAT, organic anion transporter; OATP, organic anion–transporting polypeptide; ER, endoplasmic reticulum; CFTR, cystic fibrosis transmembrane conductance regulator; PK, pharmacokinetic; DDI, drug-drug interaction; TMD, transmembrane domain; BCRP, breast cancer resistance protein; HECT, homology to E6AP C terminus; DUB, deubiquitinating enzymes; PKC, protein kinase C; PKA, protein kinase A; NEDD4-1, neural precursor cell–expressed, developmentally down-regulated 4-1; NEDD4-2, neural precursor cell–expressed, developmentally down-regulated 4-2; SGK2, serum- and glucocorticoid-inducible kinase 2; SUMO, small ubiquitin-like modifier; IGF-1, insulin-like growth factor-1; PDZ, PSD-95/Discs Large/ZO-1; PDZK1, PDZ domain–containing 1; NHERF1, Na+/H+ exchange regulatory cofactor 1; CK2, casein kinase 2.

References
1. Nigam, S. K. (2015) What do drug transporters really do? Nat. Rev. Drug Discov. 14, 29–44 CrossRef Medline
2. Durmus, S., Hendriks, J. I., and Schinkel, A. H. (2015) Apical ABC transporters and cancer chemotherapeutic drug disposition. Adv. Cancer Res. 125, 1–41 CrossRef Medline
3. Crawford, R. R., Potukuchi, P. K., Schuetz, E. G., and Schuetz, J. D. (2018) Beyond competitive inhibition: regulation of ABC transporters by kinases and protein–protein interactions as potential mechanisms of drug-drug interactions. Drug Metab. Dispos. 46, 567–580 CrossRef Medline
4. Hirono, T., Tanaka, T., Takesue, H., and Ieiri, I. (2017) Epigenetic regulation of drug transporter expression in human tissues. Expert Opin. Drug Metab. Toxicol. 13, 19–30 CrossRef Medline
5. Giacomini, K. M., Galetin, A., and Huang, S. M. (2018) The international transporter consortium: summarizing advances in the role of transporters in drug development. Clin. Pharmacol. Ther. 104, 766–771 CrossRef Medline
6. Shiota, Y., Maeda, K., Ikejiri, K., Yoshida, K., Horie, T., and Sugiyama, Y. (2013) Clinical significance of organic anion transporting polypeptides (oatps) in drug disposition: their roles in hepatic clearance and intestinal absorption. Biopharm. Drug Dispos. 34, 45–78 CrossRef Medline
7. Yao, Y., Toshimoto, K., Kim, S. J., Yoshikado, T., and Sugiyama, Y. (2018) Quantitative analysis of complex drug-drug interactions between cerivastatin and metabolism/transport inhibitors using physiologically based pharmacokinetic modeling. Drug Metab. Dispos. 46, 924–933 CrossRef Medline
8. Furberg, C. D., and Pitt, B. (2001) Withdrawal of cerivastatin from the world market. Curr. Control Trials Cardiovasc. Med. 2, 205–207 CrossRef Medline
9. Turner, R. M., and Pirmohamed, M. (2019) Statin-related myotoxicity: A comprehensive review of pharmacokinetic, pharmacogenomic and muscle components. J. Clin. Med. 9, 22 CrossRef Medline
10. Feng, B., and Varma, M. V. (2016) Evaluation and quantitative prediction of renal transporter-mediated drug–drug interactions. J. Clin. Pharmacol. 56, S110–S121 CrossRef Medline
11. Alam, K., Crowe, A., Wang, X., Zhang, P., Ding, K., Li, L., and Yue, W. (2018) Regulation of organic anion transporting polypeptides (oatp) b1b1 and oatp1b3-mediated transport: an updated review in the context of oatp-mediated drug–drug interactions. Int. J. Mol. Sci. 19, 855 CrossRef Medline
12. König, J., Müller, F., and Fromm, M. F. (2013) Transporters and drug–drug interactions: important determinants of drug disposition and effects. Pharmacol. Rev. 65, 944–966 CrossRef Medline
13. Dillellas, G., and Martzoukou, O. (2019) Transporter membrane traffic and function: lessons from a mould. FEBS J. 286, 4861–4875 CrossRef Medline
14. Czuba, L. C., Hillgren, K. M., and Swain, P. W. (2018) Post-translational modifications of transporters. Pharmacol. Ther. 192, 88–99 CrossRef Medline
15. Zhang, Y., and Hagenbuch, B. (2019) Protein–protein interactions of drug uptake transporters that are important for liver and kidney. Biochem. Pharmacol. 168, 384–391 CrossRef Medline
16. Murray, M., and Zhou, F. (2017) Trafficking and other regulatory mechanisms for organic anion transporting polypeptides and organic anion transporters that modulate cellular drug and xenobiotic influx and that are dysregulated in disease. Br. J. Pharmacol. 174, 1908–1924 CrossRef Medline
17. Zhu, C., Nigam, K. B., Date, R. C., Bush, K. T., Springer, S. A., Saier, M. H., Jr., Wu, W., and Nigam, S. K. (2015) Evolutionary analysis and classification of OATs, OCTs, OCTNs, and other SLC22 transporters: structure–function implications and analysis of sequence motifs. PLoS ONE 10, e0140569 CrossRef Medline
18. Burckhardt, G. (2012) Drug transport by organic anion transporters (OATs). Pharmacol. Ther. 136, 106–130 CrossRef Medline
19. Zamek-Gliszczynski, M. J., Taub, M. E., Chothe, P. P., Chu, X., Giacomini, K. M., Kim, R. B., Ray, A. S., Stocker, S. L., Unadkat, J. D., Wittwer, M. B., Xia, C., Yee, S. W., Zhang, L., and Zhang, Y., and International Transporter Consortium (2018) Transporters in drug development: 2018 ITC recommendations for transporters of emerging clinical importance. Clin. Pharmacol. Ther. 104, 890–899 CrossRef Medline
20. Rodrigues, A. D., Taskar, K. S., Kusuhara, H., and Sugiyama, Y. (2018) Endogenous probes for drug transporters: balancing vision with reality. Clin. Pharmacol. Ther. 103, 434–448 CrossRef Medline
21. Chu, X., Liao, M., Shen, H., Yoshida, K., Zur, A. A., Arya, V., Galetin, A., Giacomini, K. M., Hanna, L., Kushbara, H., Lai, Y., Rodrigues, D., Sugiyama, Y., Zamek-Gliszczynski, M. J., and Zhang, L., and International Transporter Consortium (2018) Clinical probes and endogenous biomarkers as substrates for transporter drug-drug interaction evaluation: Perspectives from the international transporter consortium. Clin. Pharmacol. Ther. 104, 836–846 CrossRef Medline

22. Liu, H. C., Goldenberg, A., Chen, Y., Lun, C., Wu, W., Bush, K. T., Balac, N., Rodriguez, P., Abagyan, R., and Nigam, S. K. (2016) Molecular properties of drugs interacting with SLC22 transporters OAT1, OAT3, OCT1, and OCT2: a machine-learning approach. J. Pharmacol. Exp. Ther. 359, 215–229 CrossRef Medline

23. Hagos, Y., Stein, D., Bush, K. T., Bhatnagar, V., Nigam, S. K., and Lee, W. (2008) Organic anion transporting polypeptide 1B3 (OATP1B3) is overexpressed in colorectal tumors and is a predictor of clinical outcome. Clin. Exp. Gastroenterol. 1, 1–7 CrossRef Medline

24. Meyer zu Schwabedissen, H. E., Tirona, R. G., Yip, C. S., Ho, R. H., and Kim, R. B. (2008) Interplay between the nuclear receptor pregnane X receptor and the uptake transporter organic anion transporter polypeptide 1A2 selectively enhances estrogen effects in breast cancer. Cancer Res. 68, 9338–9347 CrossRef Medline

25. Oswald, S. (2019) Organic anion transporting polypeptide (OATP) transporter expression, localization and function in the human intestine. Pharmacol. Ther. 195, 39–53 CrossRef Medline

26. Tamai, I. (2012) Oral drug delivery utilizing intestinal OATP transporters. Adv. Drug Deliv. Rev. 64, 508–514 CrossRef Medline
52. Taylor-Wellis, J., and Meredith, D. (2014) The signature sequence region of the human drug transporter organic anion transporting polypeptide 1B1 is important for protein surface expression. *J. Drug Deliv.* 2014, 129849 CrossRef Medline

53. Kato, Y., Yoshida, K., Watanabe, C., Sai, Y., and Tsuji, A. (2004) Screening of the interaction between xenobiotic transporters and PDZ proteins. *Pharm. Res.* 21, 1886–1894 CrossRef Medline

54. Mózner, O., Bartos, Z., Zámbo, B., Homolya, L., Hegedüs, T., and Sarak, B. (2019) Cellular processing of the abc2g2 transporter-potential effects on gut and drug metabolism. *Cells* 8, 1215 CrossRef Medline

55. Rosnolet, C., Peanne, R., Legrand, D., and Foulquier, F. (2013) Glycosylation disorders of membrane trafficking. *Glycoconj. J.* 30, 23–31 CrossRef Medline

56. Yau, R., and Rape, M. (2016) The increasing complexity of the ubiquitin code. *Nat. Cell Biol.* 18, 579–586 CrossRef Medline

57. Preston, G. M., and Brodsky, J. L. (2017) The evolving role of ubiquitin modification in endoplasmic reticulum-associated degradation. *Biochem. J.* 474, 445–469 CrossRef Medline

58. Hebert, D. N., and Molinari, M. (2007) In and out of the ER: protein folding, quality control, degradation, and related human diseases. *Physiol. Rev.* 87, 1377–1408 CrossRef Medline

59. Markossian, K. A., and Kurganov, B. I. (2004) Protein folding, misfolding, and aggregation. Formation of inclusion bodies and aggresomes. *Biochemistry (Mosc.)* 69, 971–984 CrossRef Medline

60. Coccetti, C., Pyle, E., and Byrne, B. (2019) Transporter oligomerization: roles in structure and function. *Biochem. Soc. Trans.* 47, 433–440 CrossRef Medline

61. Alguer, Y., Cameron, A. D., Dallianas, G., and Byrne, B. (2016) Transporter oligomerization: form and function. *Biochem. Soc. Trans.* 44, 1737–1744 CrossRef Medline

62. Storch, C. H., Hehela, R., Haefeli, W. E., and Weiss, I. (2007) Localization of the human breast cancer resistance protein (BCRP/ABCG2) in lipid rafts/caveolae and modulation of its activity by cholesterol in vitro. *J. Pharmacol. Exp. Thers.* 323, 257–264 CrossRef Medline

63. Szilagyi, J. T., Vetrano, A. M., Laskin, J. D., and Aleksunes, L. M. (2017) Localization of the placental bcrp/abc2g2 transporter to lipid rafts: Role for cholesterol in mediating efflux activity. *Placenta* 55, 29–36 CrossRef Medline

64. Veit, G., Avramescu, R. G., Chiang, A. N., Houck, S. A., Cai, Z., Peters, K. W., Hong, J. S., Pollard, H. B., Guggino, W. B., Skach, W. R., Cutting, G. R., Frizzell, R. A., Beekman, J. M., Naren, A. P., Bridges, R. J., et al. (2019) CFTR modulator theratyping: current status, gaps and future directions. *J. Cyst. Fibros.* 18, 22–34 CrossRef Medline

65. Gonzales, E., Grosse, B., Cassio, D., Davit-Spraul, A., Fabre, M., and Jacquetuin, E. (2012) Successful mutation-specific chaperone therapy with 4-phenylbutyrate in a child with progressive familial intrahepatic cholestasis type 2. *J. Hepatol.* 57, 695–698 CrossRef Medline

66. Hayashi, H., and Sugiyama, Y. (2007) 4-Phenylbutyrate enhances the cell surface expression and the transport capacity of wild-type and mutated bile salt export pumps. *Hepatology* 45, 1506–1516 CrossRef Medline

67. Hayashi, H., Takada, T., Suzuki, H., Akita, H., and Sugiyama, Y. (2005) Two common PFIC2 mutations are associated with the impaired membrane trafficking of BSEP/ABCB11. *Hepatology* 41, 916–924 CrossRef Medline

68. Cleophas, M. C., Joosten, L. A., Stamp, I. K., Dalbeth, N., Woodward, O. M., and Merriman, T. R. (2017) ABCG2 polymorphisms in gout: insights into disease susceptibility and treatment approaches. *Pharmacogenomics Pers. Med.* 10, 129–142 CrossRef Medline

69. Hoque, K. M., Dixon, E. E., Lewis, R. M., Allan, J., Gamble, G. D., Phipps-Green, A. J., Halperin Kuhns, V. L., Horne, A. M., Stamp, L. K., Merriman, T. R., Dalbeth, N., and Woodward, O. M. (2020) The ABCG2 C141K hyperuricemia and gout associated variant illuminates the physiology of human urate excretion. *Nat. Commun.* 11, 2767 CrossRef Medline

70. Thakkar, N., Lockhart, A. C., and Lee, W. (2015) Role of organic anion-transporting polypeptides (OATPs) in cancer therapy. *AAPS J.* 17, 535–545 CrossRef Medline

71. Li, T. T., An, J. X., Xu, J. Y., and Tuo, B. G. (2019) Overview of organic anion transporters and organic anion transporter polypeptides and their roles in the liver. *World J. Clin. Cases* 7, 3915–3933 CrossRef Medline

72. Evers, R., Piquette-Miller, M., Polli, J. W., Russel, F. G. M., Sprowl, J. A., Tohyama, K., Ware, J. A., de Wildt, S. N., Xie, W., Brouwer, K. L. R., and International Transporter Consortium (2018) Disease-associated changes in drug transporters may impact the pharmacokinetics and/or toxicity of drugs: a white paper from the International Transporter Consortium. *Clin. Pharmacol. Ther.* 104, 900–915 CrossRef Medline

73. Hunter, T. (2007) The age of crosstalk: phosphorylation, ubiquitination, and beyond. *Mol. Cell* 28, 730–738 CrossRef Medline

74. Mayati, A., Moreau, A., Le Vee, M., Steiger, B., Denizot, C., Parmantier, Y., and Fardel, O. (2017) Protein kinases C-mediated regulations of drug transporter activity, localization and expression. *Int. J. Mol. Sci.* 18, 764 CrossRef Medline

75. Ciechanover, A. (2005) Proteolysis: from the lysosome to ubiquitin and the proteasome. *Nat. Rev. Mol. Cell Biol.* 6, 79–87 CrossRef Medline

76. Zheng, N., and Shabek, N. (2017) Ubiquitin ligases: structure, function, and regulation. *Annu. Rev. Biochem.* 86, 129–157 CrossRef Medline

77. MacGurn, J. A., Hsu, P. C., and Emr, S. D. (2012) Ubiquitin and membrane protein turnover: from cradle to grave. *Annu. Rev. Biochem.* 81, 231–259 CrossRef Medline

78. Tanaka, K., Xu, W., Zhou, F., and You, G. (2004) Role of glycosylation in the organic anion transporter OAT1. *J. Biol. Chem.* 279, 14961–14966 CrossRef Medline

79. Zhang, Q., Hong, M., Duan, P., Pan, Z., Ma, J., and You, G. (2008) 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 CrossRef Medline

80. Li, S., Zhang, Q., and You, G. (2013) Three ubiquitination sites of organic anion transporter-1 synergistically mediate protein kinase c-dependent endocytosis of the transporter. *Mol. Pharmacol.* 84, 139–146 CrossRef Medline

81. Zhang, Q., Li, S., Patterson, C., and You, G. (2013) Lysine 48-linked polyubiquitination of organic anion transporter-1 is essential for its protein kinase C-regulated endocytosis. *Mol. Pharmacol.* 83, 217–224 CrossRef Medline

82. Xu, D., Wang, H., Zhang, Q., and You, G. (2016) Nedd4-2 but not Nedd4-1 is critical for protein kinase c-regulated ubiquitination, expression, and transport activity of human organic anion transporter 1. *Am. J. Physiol. Renal Physiol.* 310, F821–F831 CrossRef Medline

83. Xu, D., Zhang, J., Zhang, Q., Fan, Y., Liu, C., and You, G. (2017) PKC/Nedd4-2 signaling pathway regulates the cell surface expression of drug transporter HOAT1. *Drug Metab. Dispos.* 45, 887–895 CrossRef Medline

84. Wang, H., Xu, D., Tohill, M. F., Pao, A. C., and You, G. (2016) Serum- and glucocorticoid-inducible kinase SGK2 regulates human organic anion transporters 4 via ubiquitin ligase Nedd4-2. *Biochem. Pharmacol.* 102, 120–129 CrossRef Medline

85. Fan, Y., and You, G. (2020) Proteasome inhibitors bortezomib and carfilzomib stimulate the transport activity of human organic anion transporter 1. *Mol. Pharmacol.* 97, 384–391 CrossRef Medline

86. Hong, M., Xu, W., Yoshida, T., Tanaka, K., Wolf, D. J., Zhou, F., Inouye, M., and You, G. (2005) Human organic anion transporter hOAT1 forms homooligomers. *J. Biol. Chem.* 280, 32285–32290 CrossRef Medline

87. Duan, P., Li, S., and You, G. (2011) Transmembrane peptide as potent inhibitor of oligomerization and function of human organic anion transporter 1. *Mol. Pharmacol.* 79, 569–574 CrossRef Medline
90. Duan, P., Wu, J., and You, G. (2011) Mutational analysis of the role of GXXG motif in the function of human organic anion transporter 1 (hOAT1). *Int. J. Biochem. Mol. Biol.* 2, 1–7 CrossRef Medline

91. Cropp, C. D., Komori, T., Shimaa, J. E., Urban, T. J., Yee, S. W., More, S. S., and Giacomini, K. M. (2008) Organic anion transporter 2 (SLC22A7) is a facilitative transporter of cGMP. *Mol. Pharmacol.* 73, 1151–1158 CrossRef Medline

92. Hotchkiss, A. G., Berrigan, L., and Pelis, R. M. (2015) Organic anion transporter 2 transcript variant 1 shows broad ligand selectivity when expressed in multiple cell lines. *Front. Pharmacol.* 6, 216 CrossRef Medline

93. Srimaroeng, C., Cecile, J. P., Walden, R., and Pritchard, J. B. (2013) Regulation of renal organic anion transporter 3 through activation of protein kinase Ca: accelerating endocytosis of the transporter. *Eur. J. Pharmacol.* 627, 49–55 CrossRef Medline

94. Xu, D., Wang, H., and You, G. (2016) An essential role of Nedd4-2 in the ubiquitination, expression, and function of organic anion transporter-3. *Mol. Pharm.* 13, 621–630 CrossRef Medline

95. Xu, D., Wang, H., and You, G. (2019) Regulation of human organic anion transporter 3 through activation of protein kinase Ca: accelerating endocytosis of the transporter. *Eur. J. Pharmacol.* 627, 49–55 CrossRef Medline

96. Zhang, J., Yu, Z., and You, G. (2020) Insulin-like growth factor 1 modulates sumoylation, expression and function of human organic anion transporter 1 (OAT1) and OAT3 through protein kinase a signaling pathway. *Acta Pharm. Sin. B* 10, 186–194 CrossRef Medline

97. Zhang, J., Yu, Z., and You, G. (2020) Integrates the phosphorylation, expression, and activity of organic anion transporter-3 through protein kinase a signaling pathway. *Acta Pharm. Sin. B* 10, 186–194 CrossRef Medline

98. Wang, H., and You, G. (2019) The SUMO-specific protease Senp2 regulates SUMOylation, expression and function of human organic anion transporter 3. *Biochim. Biophys. Acta Biomembr.* 1861, 1293–1301 CrossRef Medline

99. Duan, P., Li, S., and You, G. (2012) Angiostatin ii inhibits activity of human organic anion transporter 3 through activation of protein kinase Ca: accelerating endocytosis of the transporter. *Eur. J. Pharmacol.* 627, 49–55 CrossRef Medline

100. Zhou, F., Hong, M., and You, G. (2007) Regulation of human organic anion transporters. *Xenobiotica* 38, 889–935 CrossRef Medline

101. Wang, H., and You, G. (2019) The SUMO-specific protease Senp2 regulates SUMOylation, expression and function of human organic anion transporter 3. *Biochim. Biophys. Acta Biomembr.* 1861, 1293–1301 CrossRef Medline

102. Zhang, J., Yu, Z., and You, G. (2020) Insulin-like growth factor 1 modulates the phosphorylation, expression, and activity of organic anion transporter 3 through protein kinase a signaling pathway. *Acta Pharm. Sin. B* 10, 186–194 CrossRef Medline

103. Wang, H., Zhang, J., and You, G. (2019) Activation of protein kinase a stimulates sumoylation, expression, and transport activity of organic anion transporter 3. *AAPS J.* 21, 30 CrossRef Medline

104. Zhou, F., Hong, M., and You, G. (2007) Regulation of human organic anion transporters. *Xenobiotica* 38, 889–935 CrossRef Medline

105. Xu, D., Wang, H., and You, G. (2016) An essential role of Nedd4-2 in the ubiquitination, expression, and function of organic anion transporter-3. *Mol. Pharm.* 13, 621–630 CrossRef Medline

106. Wang, H., Zhang, J., and You, G. (2019) Activation of protein kinase a stimulates sumoylation, expression, and transport activity of organic anion transporter 3. *AAPS J.* 21, 30 CrossRef Medline

107. Zhang, J., Yu, Z., and You, G. (2020) Insulin-like growth factor 1 modulates the phosphorylation, expression, and activity of organic anion transporter 3 through protein kinase a signaling pathway. *Acta Pharm. Sin. B* 10, 186–194 CrossRef Medline

108. Centonze, M., Saponaro, C., and Mangia, A. (2018) Nherf1 between promises and hopes: overview on cancer and prospective openings. *Transl. Oncol.* 11, 374–390 CrossRef Medline

109. Gillette, J. R., and Pang, K. S. (1977) Theoretic aspects of pharmacokinetic drug interactions. *Clin. Pharmacol. Ther.* 22, 623–639 CrossRef Medline

110. Roberts, M. S., and Rowland, M. (1986) A dispersion model of hepatic elimination: 2. Steady-state considerations—influence of hepatic blood flow, binding within blood, and hepatocellular enzyme activity. *J. Pharmacokinet. Biopharm.* 14, 261–288 CrossRef Medline

111. Tirona, R. G., Leake, B. F., Merino, G., and Kim, R. B. (2001) Polymorphisms in OATP-C: identification of multiple allelic variants associated with altered transport activity among European- and African-Americans. *J. Biol. Chem.* 276, 35669–35675 CrossRef Medline

112. Kameyama, Y., Yamashita, K., Kobayashi, K., Hosokawa, M., and Chiba, K. (2005) Functional characterization of SLCO1B1 (OATP-C) variants, SLCO1B1*5, SLCO1B1*15 and SLCO1B1*15+CI007G, by using transient expression systems of HeLa and HEK293 cells. *Pharmacogenet. Genomics* 15, 513–522 CrossRef Medline

113. Crowe, A., Zheng, W., Miller, J., Pahwa, S., Alam, K., Fung, K. M., Rubin, E., Yin, F., Ding, K., and Yue, W. (2019) Characterization of plasma membrane localization and phosphorylation status of organic anion transporting polypeptide (OATP) 1B1 c521 T>C nonsynonymous single-nucleotide polymorphism. *Pharm. Res.* 36, 101 CrossRef Medline

114. Yao, J., Hong, W., Huang, J., Zhan, K., Huang, H., and Hong, M. (2012) N-Glycosylation dictates proper processing of organic anion transporting polypeptide 1B1. *PLoS ONE* 7, e32563 CrossRef Medline

115. Clarke, J. D., Novak, P., Lake, A. D., Hardwick, R. N., and Cherrington, N. J. (2017) Impaired N-linked glycosylation of uptake and efflux transporters in human non-alcoholic fatty liver disease. *Liver Int.* 37, 1074–1081 CrossRef Medline

116. Hong, M., Hong, W., Ni, C., Huang, J., and Zhou, C. (2015) Protein kinase C affects the internalization and recycling of organic anion transporting polypeptide 1B1. *Biochim. Biophys. Acta* 1848, 2022–2030 CrossRef Medline

117. Mayati, A., Le Vee, M., Moreau, A., Jouan, E., Bucher, S., Stieger, B., Denizot, C., Parmentier, Y., and Fardel, O. (2015) Protein kinase C-dependent regulation of human hepatic drug transporter expression. *Biochem. Pharmacol.* 98, 703–717 CrossRef Medline

118. Bian, Y., Song, C., Cheng, K., Dong, M., Wang, F., Huang, J., Sun, D., Wang, L., Ye, M., and Zou, H. (2014) An enzyme assisted RP-RPLC approach for in-depth analysis of human liver phosphoproteome. *J. Proteomics* 96, 253–262 CrossRef Medline

119. Farasyn, T., Crowe, A., Hatley, O., Neuhoff, S., Alam, K., Kanyo, J., Lam, T. T., Ding, K., and Yue, Y. (2019) Precinuculation with everolimus and sirolimus reduces organic anion-transporting polypeptide (OATP)1B1 and 1B3-mediated transport independently of mTOR kinase inhibition: Implication in assessing OATP1B1- and OATP1B3-mediated drug-drug interactions. *J. Pharm. Sci.* 108, 3443–3456 CrossRef Medline

120. Alam, K., Pahwa, S., Wang, X., Zhang, P., Ding, K., Abuznait, A. H., Li, L., and Yue, W. (2016) Downregulation of organic anion transporting polypeptide (OATP) 1B1 transport function by lysosomalotropic drug chloroquine: implication in OATP-mediated drug-drug interactions. *Mol. Pharm.* 13, 839–851 CrossRef Medline

121. Alam, K., Farasyn, T., Crowe, A., Ding, K., and Yue, W. (2017) Treatment with proteasome inhibitor bortezomib decreases organic anion transporting polypeptide (OATP) 1B3-mediated transport in a substrate-dependent manner. *PLoS ONE* 12, e0189694 CrossRef Medline

122. Wang, P., Kim, R. B., Chowdhury, J. R., and Wolffk, A. W. (2003) The human organic anion transport protein SLC21A6 is not sufficient for bili-rubin transport. *J. Biol. Chem.* 278, 20695–20699 CrossRef Medline

123. Ni, C., Yu, X., Fang, Z., Huang, J., and Hong, M. (2017) Oligomerization study of human organic anion transporting polypeptide 1B1. *Mol. Pharm.* 14, 359–367 CrossRef Medline

124. Lutschert, K., Keppler, D., and Konig, J. (2004) Mutations in the SLC21B3 gene affecting the substrate specificity of the hepatocellular uptake transporter OATP1B3 (OATP8). *Pharmacogenet.* 14, 441–452 CrossRef Medline
