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The role of organic anion transporting polypeptides in drug absorption, distribution, excretion and drug-drug interactions

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Abstract

Introduction: The *in vivo* fate and effectiveness of a drug depends highly on its absorption, distribution, metabolism, excretion and toxicity (ADME-Tox). Organic anion transporting

polypeptides (OATPs) are membrane proteins involved in the cellular uptake of various organic compounds, including clinically used drugs. Since OATPs are significant players in drug absorption and distribution, modulation of OATP function via pharmacotherapy with OATP substrates/inhibitors, or modulation of their expression, affects drug pharmacokinetics. Given their cancer-specific expression, OATPs may also be considered anticancer drug targets.

Areas covered: We describe the human OATP family, discussing clinically relevant consequences of altered OATP function. We offer a critical analysis of published data on the role of OATPs in ADME and in drug–drug interactions, especially focusing on OATP1A2, 1B1, 1B3 and 2B1.

Expert opinion: Four members of the OATP family, 1A2, 1B1, 1B3 and 2B1, have been characterized in detail. As biochemical and pharmacological knowledge on the other OATPs is lacking, it seems timely to direct research efforts towards developing the experimental framework needed to investigate the transport mechanism and substrate specificity of the poorly described OATPs. In addition, elucidating the role of OATPs in tumor development and therapy response are critical avenues for further research.

Keywords: Drug-drug interaction, hepatic clearance, intestinal absorption, organic anion transporting polypeptides, pharmacokinetics, pharmacogenetics, ADME, GWAS

Article highlights box

- OATPs 1A2, 1B1, 1B3 and 2B1 are multi-specific transporters involved in the absorption, distribution and elimination of widely used drugs
- The function of these OATPs can be altered by genetic variations and drug interactions that result in altered pharmacokinetics (PK) and toxicity

- Based on their expression in barrier tissues (blood-brain barrier, placenta) and in detoxifying organs, lesser known members of the OATP family may also influence PK
- Research efforts should be directed at the development of the experimental toolkit needed to elucidate the role of the less described OATPs in ADME
- Increased expression of selected OATPs in cancer may be exploited by novel anti-cancer therapy

Abbreviations

ABC: ATP-binding cassette, **ADME-Tox**: absorption, distribution, metabolism, excretion and toxicity, **atROL**: all-*trans*-retinol, **AUC**: area under the curve, **BBB**: blood-brain barrier, **BPS**: Beraprost Sodium, **BSP**: Bromsulphthalein/ sulfobromophthalein, **CCK-8**: cholecystokinin, **CD**: Crohn's Disease, **CKD**: chronic kidney disease, **COX**: cyclooxygenase, **CML**: Chronic Myeloid Leukemia**, CsA**: cyclosporin A, **DBF**: 4′,5′-dibromofluorescein, **DCF**: 2′,7′- dichlorofluorescein, **DCF-AG:** diclofenac acyl glucuronide, **DDI**: drug-drug interaction, **DHEAS-**dehydroepiandrosterone sulfate, **DPDPE**: [D-penicillamine^{2,5}]encephalin, **EMA:** the European Medicines Agency, **ES**: estrone-3-sulphate, **E17βG**: estradiol-17-β-glucuronide, **FDA:** the US Food and Drug Administration **Fl-MTX**: fluorescein-methotrexate, **GWAS**: genome-wide association study, **ITS**: International Transporter Consortium **LTC4**: leukotriene C4, **MSS**: Mesomelia-syntoses syndrome, **MTX**: methotrexate, **Na-Fluo**: sodium-fluorescein, **OATP**: Organic Anion Transporting Polypeptide, **PGE**: prostaglandin E, **PHO**: Primary hypertrophic osteoarthropathy, **PK**: pharmacokinetics, **PSP**: progressive supranuclear palsy, **RS**: Rotor syndrome, **SLC**: solute carrier superfamily, **SP**: substance P, **SR101**: sulforhodamine 101, **SQV**: saquinavir mesylate, **TB**: tuberculosis, **TBPM-PI**: tebipenem pivoxil, **TC**: taurocholate, **TCL**: trospium chloride,

TKI: tyrosine kinase inhibitors, **T3**: 3,3',5-triiodothyronine, **T4**: thyroxine, **VIP**: vasoactive intestinal peptide

1. Introduction

According to a 2012 survey, one in four Americans over the age of 40 is taking statins [1]. Prescribed to reduce the risk of heart disease, statins lower the serum levels of low density lipoproteins by inhibiting the activity of HMG-CoA reductase, the rate-limiting enzyme of cholesterol synthesis [2]. As is the case with every drug, the efficacy of the treatment largely depends on the fate of the statins in the body. Studies on large patient populations have found significant inter-individual differences in the pharmacokinetics (PK) of statins, and suggested the relevance of drug-drug interactions. Since many statins are substrates of uptake transporters of the Organic Anion Transporting Polypeptide (OATP) family, it is not surprising that co-administration of cholesterol-lowering drugs with other OATP substrates has been associated with serious side effects, including potentially fatal rhabdomyolysis [3][4]. Expressed in barrier tissues and detoxifying organs, OATPs transport a wide variety of endogenous and exogenous compounds into the cell. OATPs are members of the solute carrier superfamily (SLC), a large group of transporters that facilitate the cellular mobility of various compounds. Similar to the efflux pumps of the ATP-binding cassette (ABC) family, uptake transporters of the SLC superfamily are now recognized as major determinants of the absorption, distribution, excretion and toxicity (ADME-Tox) properties of clinically important drugs (Figure 1) [5].

Acknowledging the importance of transporters to the PK of drugs, the International Transporter Consortium (ITS), the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have recommended investigating the interaction of new molecular entities with several ABC (ABCB1, ABCG2) and SLC transporters (OATP1B1, OATP1B3, OCT2, OAT1, OAT3) [6–8].

The dramatic rise in the number of reviews on the role of OATPs in drug absorption, distribution and drug-drug interactions is reflective of the increasing recognition of these transporters as determinants of PK. Compared to these reviews, we give an additional overview of other members of the OATP family that are potentially involved in ADME and drug-drug interaction (DDI). We also provide a critical overview of the *in vitro* and *in vivo* methods that are used to identify clinically relevant molecules as potential OATP substrates or inhibitors. We discuss disease association of OATPs and single nucleotide polymorphisms (SNPs) that are relevant in PK. Finally, we review the *in vitro* and *in vivo* models that are currently available to interrogate OATP-drug interactions.

2. The human OATP family

2.1. OATP-mediated transport

The 11 human OATPs, encoded by the SLCO genes, are membrane proteins that mediate the sodium and ATP-independent uptake of large (usually >300 Da) organic molecules. It is generally accepted that OATPs act as electroneutral exchangers, coupling substrate uptake to the efflux of a counter ion, such as glutathione, conjugated glutathione, bicarbonate or glutamate [9,10]. Other lines of evidence suggest that OATP-mediated uptake may be driven by a proton gradient [11], although, the pH sensitivity of transport appears to be OATP- and

substrate-dependent [12,13]. It is unclear whether OATPs are obligate uptake transporters or whether they have additional functions as efflux transporters [14].

2.2. Substrate recognition by OATPs

The substrates of these transporters are primarily organic anions, though OATPs are also capable of transporting uncharged (e.g. digoxin (4C1); oubain (1B3)), zwitterionic (e.g. fexofenadine (1A2, 1B3, 2B1)) and positively charged molecules (e.g. doxorubicin (1A/1B) and triptans (1A2)) [11,15–18]. Among the endogenous substrates of OATPs are bile acids, bilirubin, eicosanoids, prostaglandins, hormones and their sulfated and glucuronated conjugates (summarized in Table 1). Hence, under physiological conditions, OATPs are important in bile acid homeostasis (1A2, 1B, 1C1, 2B1, 4A1, 4C1), bilirubin elimination (1A2, 1B), inflammatory processes (4C1) and the regulation of hormonal levels (1A2, 1B, 1C1, 2A1, 2B1, 3A1, 4A1, 4C1) [11,15]. Many OATPs also recognize exogenous compounds such as statins, cardiac glycosides, antidiabetic agents, immune suppressants, antibiotics, antivirals (e.g. HIV protease inhibitors) and anticancer medications. The extensive body of literature on the OATP-mediated transport of chemotherapy drugs has been recently reviewed by Sprowl and Sparreboom [19].

Based on their substrate recognition pattern, OATPs can be divided into two groups. The first group includes OATP1A2, 1B1, 1B3 and 2B1, which have partially overlapping substrate specificities, similar to ABC efflux transporters (e.g. ABCB1, ABCG2 and ABCC2/3) [6]. The other members of the family (1C1, 2A1, 3A1, 4A1, 4C1, 5A1 and 6A1) recognize a much smaller set of compounds. This latter set of transporters has been less characterized; therefore, our current knowledge about their substrates may be incomplete. Nevertheless, the increasing number of genome-wide association studies (GWAS) and expanding repertoire of *in vitro* and *in vivo* assays are rapidly enhancing our knowledge on potential substrates. OATP substrates with the greatest clinical relevance are summarized in Tables 1 and 2. For a more exhaustive

list of substrates, the reader is referred to excellent reviews in the literature [11,15]. Because most of the OATP-interacting compounds have been identified *in vitro*, often using concentrations that exceed those occurring *in vivo*, substrate recognition data should be carefully interpreted. Additionally, interacting compounds identified by indirect *in vitro* studies do not necessarily distinguish between a transported substrate and an inhibitor.

2.3. Tissue distribution and localization

OATPs are present in the cell membrane of epithelial and endothelial cells and display distinct expression patterns; some OATPs are broadly expressed while others are expressed in specific organs. The characterization of the tissue distribution of OATPs relies heavily on mRNA data. For example, mRNAs for OATP2A1, 3A1 and 4A1 have been detected in a broad number of tissues, while OATP1B1 and 1B3 are restricted to the liver and OATP6A1 is expressed in the testes [11]. A number of recent immunofluorescence analyses suggest unexpected localization patterns for some OATPs, such as the prostaglandin transporter OATP2A1, which was detected within the lysosomes of normal macrophages [20], and OATP2B1, 3A1 and the poorly characterized OATP5A1, which localized to the intracellular spaces within tumorous breast tissues [21]. As OATP expression has been thoroughly discussed in recent reviews [11,15] we discuss this information only in the context of ADME properties.

2.3.1. OATPs in hepatic clearance:

OATP1A2 was the first human OATP isolated. Expressed in cholangiocytes, OATP1A2 is involved in bile acid, unconjugated bilirubin and xenobiotic reabsorption [11]. The key role of OATP1B1 in hepatic drug uptake was recognized when it was realized that plasma statin levels increase in the presence of OATP1B1 inhibitors, such as cyclosporin A or gemfibrozil [3,22,23]. Several *in vitro* and *in vivo* experiments confirmed the relevance of OATP1B transporters in hepatic clearance [24,25]. OATP1B1 and 1B3 are almost exclusively

expressed in the sinusoidal membranes of hepatocytes and are involved in the uptake of bilirubin, bile acids and various drugs from the blood into hepatocytes. OATP2B1, which is ubiquitously expressed, may also be important in hepatic clearance [11]. It is difficult to estimate the relative contribution of OATP1B1, 1B3 and 2B1 to drug uptake *in vivo* due to their overlapping substrate/inhibitor specificity. However, based on mRNA and protein expression data, OATP1B1 is the most abundant and most relevant OATP in the liver [26].

2.3.2. OATPs in the kidney:

In addition to the liver, the kidney is a relevant site of drug elimination. OATP4C1 is a kidney-specific transporter localized to the basolateral membranes of proximal tubules. OATP4C1 is involved in uremic toxin elimination [27,28] and mediates the uptake of certain heart medications (digoxin, ouabain), and anticancer drugs (methotrexate; MTX), from the blood [29,30]. Kidney-specific expression of the human OATP4C1 provided protection against hypertension and inflammation in a rat renal failure model, demonstrating the role of OATP4C1 in renal toxin elimination [27,28]. In a recent study, bupropion (an anti-depressant) decreased the area under the plasma concentration-time curve (AUC, a measure of drug exposure) of digoxin via the activation of OATP4C1-mediated renal clearance [29].

OATP1A2 is also expressed in the kidney, though it localizes to the distal tubules of the nephrons. OATP1A2 may play a role in the active tubular reabsorption of MTX and in MTX-induced toxicities [31]. Knauer et al. demonstrated that mRNA expression levels of OATP2B1 in the kidney were comparable to expression levels in the small intestine [32]. However, OATP2B1 protein expression in the kidney has not yet been confirmed.

2.3.3. OATPs in the intestine:

Several ubiquitously expressed OATPs (1A2, 2B1, 3A1 and 4A1) have been detected in the intestine. Based on quantitative mRNA data, OATP2B1 is the most abundantly expressed OATP in the intestine [33] and the expression of this transporter on the apical side of

enterocytes has also been confirmed by immunofluorescent labeling [34]. Based on these data, OATP2B1 is the dominant OATP involved in first line drug absorption and a significant determinant of the oral availability of drugs.

2.3.4. Other blood-tissue barriers:

The blood-brain barrier (BBB) provides a tight control of the cerebral entry of molecules. Due to many medications aimed at targeting the brain, the BBB is the most extensively investigated blood-tissue barrier. OATP1A2 and 2B1 are expressed on the apical surface of brain capillary endothelial cells [35] with similar mRNA expression levels. A recent study demonstrated that both 1A2 and 2B1 are present in the retina, mediating neurotransmitter and neurosteroid uptake in this tissue [35]. OATP1A2 is also expressed in neurons and may influence neuronal statin and MTX levels [36]. In the choroid plexus, OATP1C1 and OATP3A1 protein expression has been detected [37,38].

OATPs may also be involved in drug transport across the blood-testes (1A2, 1C1, 3A1, 6A1) [39][11,15], blood-ocular (1A2, 1C1, 2B1, 1A2, 3A1, 4A1) [40,41] and maternal-fetal barriers (1A2, 1B1, 1B3, 2B1, 2A1, 4A1) [15,42]. OATPs that are present in the placenta are important for steroid sulfate (2B1) [43] and thyroid hormone (4A1) [44] transport but the role of placental OATPs in fetal exposure to drugs is poorly understood. OATP expression may be significantly altered in tumor tissues compared to healthy cells (see chapter 2.4.); however, the functional consequences of this phenomenon are not yet well understood.

2.4. The role of OATPs in disease

To date, few diseases have been associated with mutations in OATP genes. Rotor syndrome (RS) is a rare, benign disorder marked by elevated levels of bilirubin in the blood and coproporphyrin in the urine [45]. The role of OATP1B1/1B3 in bilirubin transport has been indicated in a number of GWAS, including families with RS whose GWAS results

revealed simultaneous mutations in OATP1B1 and 1B3 that rendered both transporters nonfunctional [45,46]. These data were further confirmed in mice harboring mutations in genes for the 1A/1B family of OATPs, which resulted in hyperbilirubinemia [47].

Mesomelia-syntoses syndrome (MSS) is a rare, autosomal-dominant disease characterized by limb shortening and various congenital malformations. A study of five patients in four families identified an interstitial deletion in chromosome 8q13 spanning two genes: SULF1 (heparan sulfate 6-O-sulfatase 1) and SLCO5A1 (OATP5A1) [48]. OATP5A1 is expressed in the adult heart and in fetal brain but its function is currently unknown. The contribution of OATP5A1 to MSS requires further investigation, as a partial deletion of SLCO5A1 was reported in a healthy individual.

Primary hypertrophic osteoarthropathy (PHO) is a rare genetic disease affecting skin and bone formation. A study of three individuals with PHO indicated that inactivating mutations in SLCO2A1 cause PHO by impairing prostaglandin E2 (PGE_2) transport [49]. Loss of SLCO2A1 function has also been implicated in a form of hereditary enteropathy that is characterized by chronic ulcers in the small intestine [50]. Furthermore, a study using a mouse model of pulmonary fibrosis suggested that SLCO2A1 may also be critical to lung tissue restoration [51]. Given the multiple roles of PGE_2 in the body, prostaglandin transport-inactivating SLCO2A1 mutations will likely remain intensely investigated.

A GWA study of over 1100 patients with progressive supranuclear palsy (PSP), a rare neurodegenerative movement disorder similar to Parkinson's disease, revealed a putative association with SLCO1A2 [52]. OATP1A2 is located in the brain, eyes, kidney, liver and intestine. Bile acids, bilirubin and dehydroepiandrosterone sulfate (DHEAS), a precursor of steroid hormones, are among the physiological substrates of OATP1A2 [11]. The possible role of this OATP in PSP has not been investigated. Another GWA study of Crohn's disease within an Ashkenazi Jewish population found a variant of SLCO6A1 [53].

OATPs have become the focus of considerable attention because of the altered expression of these transporters in various types of cancer (Table 3). The liver-specific transporters 1B1 and 1B3 were found to be down-regulated in liver cancers and significantly upregulated in tumors of the ovaries (1B1, 1B3), colon (1B1, 1B3), breast (1B3), prostate (1B3) and lung (1B3) [54]. Similarly, OATP6A1 expression, normally limited to the testes, was detected in tumors of the brain, bladder and lung [54]. Many of the widely distributed OATPs have also been reported to be upregulated in certain malignant cells.

Because OATPs are able to transport a wide variety of substrates, including hormones, one would hypothesize that an upregulated or atypical OATP expression could lead to the proliferation of estrogen- and androgen-dependent tumors. Indeed, OATP expression levels correlate with tumor growth. Estrone-3-sulfate uptake by OATPs 1A2 [55], 1B3 [56], 3A1 and 4A1 has been implicated in the survival of hormone-dependent breast cancer cells [57]. These data suggest that targeting these transporters in the treatment of hormone-responsive breast cancer may have beneficial effects and improved survival [55,57].

OATPs also influence disease progression in androgen-dependent prostate cancers (PC). OATP1B3 transports testosterone and the 334T allelic variant of 1B3, which efficiently transports testosterone, is associated with decreased patient survival [58]. In another study of prostate cancer patients, presence of a testosterone transport-deficient variant of OATP1B3 (haplotype 334GG/699AA) was associated with better survival over 10 years [59]. Similarly, an OATP2B1 variant, with increased DHEAS transport, was correlated with increased patient mortality [58].

In summary, changes in OATP expression have been demonstrated in numerous cancers. However, conflicting reports on the tumor-specific expression of OATP1A2 and 2B1 suggest that the therapeutic or prognostic value of expression changes should be cautiously

interpreted. Nevertheless, mounting evidence supports the hypothesis that OATPs are upregulated in tumors, potentially to meet the increased nutritional demand of cancer cells.

2.5. Methods and models to investigate OATP-drug interactions

2.5.1. Test substrates of OATPs

OATP function is commonly investigated in whole cell-based systems by measuring the uptake of radioactively labeled substrates. Estrone-3-sulfate, bromosulfophthalein and estradiol 17 β-D-glucuronide are among the most extensively used tritiated substrates and have been used to investigate the function of multiple OATPs [15]. However, due to the cost and limited availability of radiolabeled substrates, their utility in large-scale substrate-screening experiments is impeded and recent efforts have focused on fluorescent substrates as safe, simple and cost-effective alternatives. A multitude of fluorescent probes (Na-fluorescein, fluorescein-methotrexate, fluorescein-cAMP, various fluorescent bile acids [60–63]) have been used to uncover interacting compounds of OATP1B transporters; however, until recently no fluorescent assay has been available for other OATPs. Recently, expression of the 11 human OATPs in insect cells revealed that, under acidic conditions, Na-fluorescein is a general OATP substrate, suitable for the characterization of the entire human OATP family [13]. A pan-OATP substrate is of particular importance for the characterization of the poorly characterized members of the OATP family, 5A1 and 6A1. The advantage of fluorescein derivatives in developing substrate inhibition assays for OATP1B and 2B1 transporters was also demonstrated in mammalian cells [64]. Typical and newly developed test substrates of OATPs are listed in Table 1.

Because indirect transport assays cannot reveal the nature of the interaction between molecule and OATP, the transport of candidate substrates should be confirmed by direct transport measurements, such as mass spectrometry or direct labeling.

2.5.2. *In vitro* models

2.5.2.1. Engineered cell lines: The preferred model systems for the investigation of OATPs are mammalian cell lines with exogenous OATP expression, although transiently transfected Xenopus oocytes and insect cells have also been used [6,13]. While many stable OATP-expressing cell lines have been generated to date, evidence suggests that the overexpression of certain OATPs in standard mammalian laboratory cell lines is not straightforward (our own unpublished results). This may be due to metabolic perturbation of the cells, although the exact mechanism behind this phenomenon is still unclear.

2.5.2.2. Pharmacological models: The individual role of a transporter in the transmembrane movement of drugs is most easily assessed in cell lines engineered to express a single OATP. Additionally, co-transfected cell lines with simultaneous OATP and ABC expression have also been established [65]. However, because the transport of drugs occurs in an elaborate network of uptake and efflux transporters as well as drug metabolizing enzymes, a closer approximation of the *in vivo* environment requires more complex *in vitro* model systems. Caco-2 cells, which form monolayers resembling the intestinal epithelium, are currently considered the "gold standard" in studying intestinal absorption. Nonetheless, Caco-2 cells do not fully reflect the transporter profile of the natural intestinal environment and are unable to recapitulate *in vivo* organization at a tissue level [66]. These limitations led to the proposal of stem cell-derived organoids [67] and precision cut intestinal slices[68] as ADME models; however, the application of these methods to the investigation of drug transport is limited [68]. Polarized cells (e.g. MDCKII or LLCPK) have been successfully used to model renal processes. However, establishing *in vitro* models that recapitulate the complexity of the liver has proved challenging. Several hepatic models exist, ranging from immortalized cell lines (HepG2, HepaRG), liver slices and stem cell-derived hepatocytes to 3D cell cultures and bioreactors [69,70]. These models vary in maintenance costs, accessibility and transporter

expression pattern [71]; therefore, the appropriate models should be selected based on these considerations and the pharmacological goal.

2.5.3. OATP-mediated ADME *in vivo*

To predict DDI during the preclinical phase is of major importance, however the extrapolation of *in vitro* data to more relevant *in vivo* processes is a difficult task [25]. Therefore, *in vivo* data gained from pharmacogenetic/pharmacogenomic studies, clinical trials involving volunteers and animal models are crucial in modeling the *in vivo* fate of a drug.

2.5.3.1. Animal studies:

Recognizing the importance of liver-specific transporters in drug disposition, Oatp1a/1b knockout (KO) mice have been widely used to study the pharmacokinetics of clinically applied drugs [72] as well as natural OATP substrates [72,73]. For example, Oatp1b2 (a homolog of OATP1B1/1B3) single knockout mice have been used to study the liver and plasma distribution of toxins (phalloidin, microcystin-LR), cholesterol-lowering drugs (cerivastatin, lovastatin acid, pravastatin, and simvastatin acid) and antibiotics (rifampicin and rifamycin SV) [72,74,75]. Mice with a deletion of the 1a/1b locus (missing all established mouse 1a/1b transporters) were used to elucidate the hepatic clearance of bilirubin, bile acids and drugs from the blood [47]. In addition, 1a/1b KO mice have been used to establish coproporphyrin (CP) I and III as endogenous biomarkers for the assessment of transporter activity during early drug development [73]. The applicability of CPs as endogenous probes for liver transport was also confirmed in cynomologous monkeys by administering oatp1a/1b inhibitors [73].

There are significant species differences that hinder the interpretation of data from mouse models. OATP1Bs and 1A2 have no rodent orthologs and the homology between OATP2B1 and its mouse ortholog is only 77% [76]. As exemplified by the rat Oatp4c1, which localizes to the apical, instead of the basolateral, membrane of the proximal tubules of

the kidney, the localization of some rodent OATP orthologs may also differ [77]. To address these issues, van de Steeg et al. generated humanized mice with liver-specific expression of human OATP1B1, 1B3 and 1A2 in a mouse oatp1a/1b knockout background [78,79]. OATP1A2-humanized mice do not mimic normal conditions in the liver as OATP1A2 is expressed in hepatocytes [79], not cholangiocytes. Further limiting the *in vivo* assessment of hepatic clearance, a knockout mouse model for OATP2B1 has not been established.

Nevertheless, humanized mice are an invaluable tool for studying the *in vivo* disposition of drugs and have been used to study the pharmacokinetics of anticancer medications (e.g. methotrexate, paclitaxel and docetaxel [79,80]) and to detect drug-drug interactions (e.g. between methotrexate and the antibiotic rifampicin, or the antihypertensive drug, telmisartan [81]).

2.5.3.2. Human studies:

The majority of *in vivo* data on the role of OATPs in drug PK arose from unexpected toxicity due to either co-administration of OATP substrates/inhibitors or altered OATP function/expression caused by SNPs.

2.5.3.2.1. Drug interaction studies:

A striking example of OATP-mediated DDIs is the potentially lethal interaction between cerivastatin and gemfibrozil (used to treat hypercholesterolemia and hypertriglyceridemia, respectively), which led to the withdrawal of cerivastatin from the market [24]. Retrospective *in vitro* analyses revealed that the major mechanism of cerivastatin-mediated toxicity was the inhibition of both OATP1B1 and the metabolizing enzyme CYP2C8 by gemfibrozil glucuronide [22]. Many additional clinical data indicated statin-mediated toxicity upon the simultaneous administration of OATP substrates/inhibitors (cyclosporin A, rifampicin, lopinavir) and statins [3,24,25]. The role of OATP2B1 in muscular toxicity of statins was proposed due to its expression in skeletal muscle [82]. In addition to statins, the AUC of

bosentan, an endothelin receptor antagonist, is influenced by the OATP1B inhibitors rifampicin, cyclosporin A and sildenafil [83].

Considering the physiological role of OATPs, drugs inhibiting the transport of endogenous substrates may disrupt bile acid or hormone homeostasis. Indeed, it has been documented that the administration of tyrosine kinase inhibitors or high-doses of cyclosporine A result in hyperbilirubinemia, probably due to the inhibition of bilirubin uptake by OATP1B1/3 [84,85].

2.5.3.2.2. GWA and genotype panel studies:

Pharmacogenetic studies have made an enormous contribution to our understanding of the role of OATPs in PK and revealed various SNPs in OATP genes (SLCO) that cause inter-individual differences in drug efficacy and safety. While GWAS and genotype panels highlighted the importance of certain SLCO polymorphisms, detailed functional analyses required *in vitro* follow-up studies.

The most clinically relevant SLCO SNPs are summarized in Table 4.

SLCO1B1: Given its recognized role in hepatic transport, the pharmacological consequences of SLCO1B1 SNPs have been extensively investigated. The two most common polymorphisms of SLCO1B1 are c.521T>C (p.174V>A, rs4149056), and c.388A>G (p.130N>D, rs2306283), though more than 14 SNPs in SLCO1B1 have been analyzed.

The c.521T>C variant (allele *5) results in decreased OATP1B1 activity [86], leading to increased plasma levels of various OATP1B1 substrates including drugs used in the treatment of high cholesterol (statins), high blood pressure (olmesartan), diabetes (atrasentan), heart disease (torsemide), HIV (lopenavir), cancer (SN-38), allergy (fexofenadine) and immune diseases (tacrolimus) [5,87,88]. Accordingly, elevated plasma levels of these medications may increase the risk of toxicity. Indeed, a GWA study of 85 patients with myopathy and 90 matched controls indicated that an SLCO variant in near complete linkage disequilibrium with

the *SLCO1B1**5 allele is the most important predictor of myopathy in patients taking high doses of simvastatin [88]. The association between the *SLCO1B1**5 allele and adverse drug reactions upon statin treatment (simvastatin, pravastatin, lovastatin) was confirmed in multiple GWA studies [89,90] and genotype panels revealed that the *SLCO1B1**5 allele may markedly affect the PK of various statins (simvastatin, atorvastatin, rosuvastatin, pravastatin) [23,90,91]. However, the c.521T>C variant did not influence *in vivo* fluvastatin clearance, indicating a substrate-specific transport alteration by this variant [90]. Alternatively, minor effects of the c.521T>C variant on fluvastatin clearance were not detected due to study power limitations.

While *in vitro* and *in vivo* data on the c.388A>G polymorphism are controversial (haplotype *b), this SNP was associated with decreased AUC of several drugs including the non-statin cholesterol-lowering medication ezetimibe, the antidiabetic repaglinide [92,93] and lovastatin acid (the active metabolite of lovastatin) [5,94,95]. Contrastingly, the c.388A>G polymorphism did not alter response to statin therapy in a study of 386 adults of Greek origin [96]. The, c.388A>G polymorphism is often linked to c.521T>C, resulting in the *15 haplotype (the most frequent of the 18 documented haplotypes). Similarly to the effect of haplotype *5, *15 is associated with increased plasma levels of pravastatin and lovastatin [5,6,95,97]. In addition, lower methotrexate clearance has been associated with variations in non-coding regions of SLCO1B1.

In summary, based on the extensive clinical data available for SLCO1B1, haplotype information can be a good predictive marker in personalized medication.

SLCO1B3: The two most common mutations of SLCO1B3 are c.334T>G (p.112S>A, rs4149117) and c.699G>A (p.233M>I, rs7311358). Allele frequency data indicate that 334G and 699A are the most frequent variants in the Caucasian and Asian populations. Because the 334G and 699A polymorphisms are in near complete linkage disequilibrium, with an allele

frequency above 70% (Table 4), the haplotype encoding 112A and 233I should be regarded as dominant in these populations [98]. *In vitro* studies show that the single variants have no effect on transporter function, while the 112A/233I variant has reduced activity compared to the reference sequence [59,98].

Likely due to the compensatory effect of other OATPs, clinical data about the effect of SLCO1B3 SNPs are scarce and controversial (summarized in [25]). While the c.699G>A variant was associated with decreased docetaxel clearance in Chinese nasopharyngeal cancer patients [99], the c.334T>G polymorphism increased the clearance of imatinib in chronic myeloid leukemia patients in a Japanese population [100]. As described in the disease section, prostate cancer patients harboring the 334GG/699AA haplotype showed longer median survival than patients carrying the TT/AA and TG/GA haplotypes [59]. Interestingly, an intronic variant, harboring an extra intron, was found to be associated with increased AUC of telmisartan and docetaxel [99].

SLCO2B1: The expression pattern and pH sensitivity of OATP2B1 suggest that it contributes to intestinal drug absorption although, current data are insufficient to firmly support this hypothesis. The c.1457C>T variant (p.S486F), which has a 31% frequency in the Japanese population, decreases *in vitro* transport activity [101] and results in a decreased AUC of the beta-blocker celiprolol [5,102]. These data indicate that OATP2B1 contributes to intestinal absorption, rather than hepatic uptake. OATP2B1 variants also influence the progression of androgen-dependent prostate cancer as a function of DHEAS transport activity [58,103]. Accordingly, time to progression was increased in patients with the androgen transport deficient variant c.935G>A (rs12422149) [103].

SLCO1A2: Although several SLCO1A2 SNPs have been characterized *in vitro*, allele frequency data suggest that the clinical significance of these polymorphisms may be limited. The only allele with potential *in vivo* significance is c.38T>C (p.13I>T). Based on *in vitro*

analyses, the c.38T>C variant exhibits normal transporter function [31,104]. However, a two-fold increase in methotrexate uptake was documented *in vivo*, supporting increased transport by this variant [36]. Additionally, a mutation in the promoter region of SLCO1A2 (c.361G>A) resulted in increased imatinib clearance in chronic myeloid leukemia patients [105].

3. Conclusions

The role of OATPs in pharmacokinetics is increasingly recognized. OATPs transport large, primarily anionic, compounds into cells and are known to influence the absorption and elimination of common medications, such as statins, antivirals, anti-diabetic and anti-cancer molecules. The four OATPs that are proven to have a major impact on the *in vivo* fate of drugs are 1A2, 1B1, 1B3 and 2B1. Hepatic OATPs 1B3 and, the more abundant, 1B1 have a key role in the hepatic clearance of drugs, bile acids and bilirubin. OATP2B1 is also expressed in the liver. However, the exact contribution of this transporter to hepatic clearance is not yet elucidated. Increasing evidence suggests that OATP2B1 is involved in the intestinal absorption of orally administered drugs. In addition, cerebral and muscular drug levels may be determined by OATP1A2 and OATP2B1, respectively. Recently, the digoxin transporter, OATP4C1, has emerged as a determinant of the renal elimination of drugs, although the substrate recognition pattern of this transporter is not fully mapped.

Until now, OATP research has focused on OATP1A2, 1B1, 1B3 and 2B1 because of the profound pharmacological significance of these transporters. The rest of the OATP family, however, received less attention, despite emerging evidence that OATPs in the blood-testes (1A2, 1C1, 3A1, 6A1 [11,15,39] and maternal-fetal barrier (2A1, 4A1) are also involved in hormone transport and drug absorption [15,42–44]. The hiatus in our knowledge about the other members of the OATP family arises from the following: 1) the lack of established

expression systems and suitable functional assays and, 2) the scarcity of *in vivo* data. Therefore, to uncover the substrate recognition pattern of the poorly investigated OATPs, research efforts should focus on developing novel *in vitro* methods that allow for high-throughput substrate screening and the further collection of *in vivo* data.

Finally, as many OATPs show *de novo* expression in tumors, they may be important in influencing local, intra-tumor concentrations of therapeutic compounds. Thus, the mapping of drug-OATP interactions would be critical to tumor-specific drug delivery.

4. Expert opinion

OATPs 1A2, 1B1, 1B3 and 2B1 participate in the absorption and distribution of various medications and are sites of DDI leading to altered drug efficacy or unexpected toxicity. Altered transporter function, as a result of inter-individual variations in OATP-encoding genes (i.e. polymorphisms), may lead to altered drug exposure over time. Food components and solubilizing agents, such as polysorbate 80 [19], may also affect transporter function. Finally, drugs may alter the OATP-mediated transport of endogenous compounds (bilirubin, bile salts or hormones). Therefore, the International Transporter Consortium (ITS) recognizes OATP1A2, 1B1, 1B3 and 2B1 as major determinants of drug pharmacokinetics and recommends the investigation of these transporters during drug development.

To investigate OATP-drug interactions, various *in vitro* methods have been established. OATP function is commonly investigated using radioactively labeled test substrates, although the use of fluorescently labeled compounds would be simpler, safer and more cost-effective. Indeed, several fluorescence-based OATP1B assays have been established. For OATP1A2 and 2B1, however, fluorescent assays have been described only recently, and there are no established assays for the large-scale measurement of drug interactions with the other members of the OATP family. A potential solution would be to

screen the available library of fluorescent molecules for OATP substrates with low passive cell permeability. Alternatively, known OATP substrates could be conjugated to fluorescent molecules. Appropriate and well-characterized *in vitro* assays would aid the characterization of the entire OATP family by allowing for the reproducible comparison of OATP variants and the further mapping of OATP-mediated DDIs.

When designing *in vitro* assays to determine OATP-mediated DDIs, the following should be considered: 1) because of the complexity of the substrate binding site of the transporter, the function of each OATP should be tested using multiple substrates 2) due to the promiscuous nature of OATPs, it is almost impossible to measure all potential OATP-mediated DDIs, 3) substrates/inhibitors should be used at physiologically relevant concentrations.

In the body, OATPs are part of a complex system of influx and efflux transporters as well as metabolizing enzymes; therefore, the effect of these transporters on PK should be interpreted in the context of the entire organism. Attempts at mimicking the *in vivo* environment varied from the development of pharmacological models to the use of humanized mice. While these models have been profoundly useful in studying the function of OATPs, they still suffer from major limitations. Pharmacological models, however complex, cannot fully recapitulate the *in vivo* environment and data acquired from Oatp K.O. mice are limited by species differences. One solution to these problems would be to rely on pharmacogenetic/pharmacogenomic data to evaluate the relevance of OATPs, however, with the exception of OATP1B1, these studies are scarce. In addition, although results obtained from pharmacogenetic studies do faithfully represent the *in vivo* environment, these data should be interpreted by considering inter-individual genetic differences and the potential compensatory effect of other transporters.

Because OATPs also influence local drug concentrations, the differential expression of OATPs may be exploited in several ways. Liver-specific OATPs may be exploited in hepatic

drug targeting or in non-invasive diagnostic techniques (e.g. positron emission tomography) [106]. In addition, OATPs that show cancer-specific expression could be used for tumor-selective drug delivery. However, tumor-selective drug delivery would require the use of selective substrates to minimize systemic toxicity. In addition, tumors could also be targeted using a different approach: as the physiological function of OATPs is hormone and nutrient transport, cancer-cells could be deprived of these factors using OATP-specific inhibitors.

OATPs 1A2, 1B1, 1B3 and 2B1 are relatively well-characterized; however, less is known about the other members of the OATP family including a liver-specific OATP2B1 variant [32], and a cancer-specific 1B3 isoform [107]. An increasing number of GWAS is likely to elucidate which members of the OATP family are most critical to ADME. However, discovering OATP-specific substrates for targeted drug delivery requires the establishment of *in vitro* assays suitable for large-scale substrate screening experiments.

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Declaration of interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Tables

Table 1: List of major endogenous and OATP test substrates*

OATP	Physiological substrates	Fluorescent or radioactive substrates
1A2	• atROL [40] •bile salts (taurocholate, cholate, ursodeoxycholic acid) [15,108] •bilirubin [15] •hormones (T4, DHEAS, ES)[15] •PGE_2 [15] •neuropeptides: SP, VIP [35]	• [^{3}H] atROL [40] • [^{3}H] BSP [109] • [^{35}S] BSP [108] • [tyrosyl-3,5-3H] deltrophin II [110] • [^{3}H] digoxin [111] • [^{3}H] docetaxel [80] • [tyrosyl-2,6-3H(N)] DPDPE [110] • [^{3}H] ES [111] • Fl-MTX [13] • [^{3}H] MTX [31] • [^{3}H] nadolol [109] • Na-Fluo [13] • [^{3}H] PGE2 [111] • [^{3}H] quercetin [112] • [^{3}H] quinidine [113] • Rhodamine 123 [114] • [^{3}H] N- methyl-quinine [111] • [^{14}C] SQV [115] • [^{3}H] TCL [116]
1B1	•bile salts (taurocholate, tauroursodeoxycholate) [15] • bilirubin [15] •eicosanoids (LTC4, LTE4, PGE_2, thromboxane B2) [76] •hormones (ES, E17βG, T3, T4, DHEAS [15]	• [^{3}H] BSP [117] • [^{3}H] BPS [118] • DCF and DBF [64] • [^{3}H] docetaxel [119] • [^{3}H] E17βG [120] • [^{3}H] ES [117] • Fl-MTX [63] • Fluo-3 [121] • Flutax-2 (Oregon Green 488-Paclitaxel) [15] • Na-Fluo [62] • Oregon green [64] • [^{3}H] TC [120]

1B3	•bile salts (cholate, glycocholate, taurocholate, taurochenodeoxycholate, taurodeoxycholate, tauroursodeoxycholate) [15] • bilirubin [15] •CCK-8 [122] •hormones (T3, T4, ES, DHEAS, testosterone) [15] •LTC4 [15] •steroid conjugates [54]	• [^{3}H] BSP [123] • [^{3}H] BPS [118] • [^{3}H] CCK-8 [122] • DBF [64] • [^{3}H] docetaxel [119] • [^{3}H] E17βG [123] • [^{3}H] ES [111] • Fl-MTX [63] • Fluo-3 [64] • Na-Fluo [62] • Oregon green [64] • [^{3}H] TC [120] • [^{125}I]-T3 [111] • [^{125}I]-T4 [111]
1C1	•hormones (ES, E17βG, thyroid hormones) [15,54]	• [^{3}H] BSP [37] • [^{3}H] docetaxel [119] • [^{3}H] E17βG [37] • [^{3}H] ES [37] • Na-Fluo [13] • SR101 [124]
2A1	•PGs (PGE_1, PGE_2, PGD_2 ,$PGF_{2\alpha}$) [125]	• Na-Fluo [13] • [^{3}H] PGE2 [125] • [^{3}H] PGE1 [125] • [^{3}H] quercetin [112]
2B1	•DHEAS [126] •ES [15] •LTC4 [126] •neuropeptides: SP, VIP [35] •PGE_2 [15] •taurocholate [126,127] •thromboxane B2 [125]	• [^{3}H] BSP [111] • DCF and DBF [64] • [^{3}H]-ES [128] • Fl-MTX [13] • Na-Fluo [13] • Oregon green [64] • [^{3}H] quercetin [112] • [^{3}H] PGE2 [128] • [^{3}H]TC [127]
3A1	•ES [11] •PGE_1, PGE_2 [11] •T4 [126] •vasopressin [11]	• [prolyl-3,4(N)-^{3}H]-BQ-123 [38] • [^{3}H]-ES [128] • Na-Fluo [13] • [^{3}H] PGE2 [38] • [^{3}H] PGE1 [38] • [tyrosyl-3,5(N)-^{3}H]-vasopressin [38]
4A1	•E17βG [15] •ES [15] •PGE_2 [128] •thyroid hormones (T4, rT3(weak), T3, Taurocholate [129])	•[^{3}H] ES [128] •[^{3}H] PGE2 [128] • Na-Fluo [13] •[^{3}H] taurocholate [129] •[^{125}I] T4 [129]
4C1	•cAMP [30] •ES [130] •thyroid hormones [30]	• [^{3}H] digoxin [30] • [^{3}H] ES [12,130] • Na-Fluo [13] • [^{14}C] and [^{3}H] sitagliptin [131]
5A1		• Na-Fluo [13] • [^{3}H] quercetin [112]
6A1		• Na-Fluo [13]

* See footnotes for a list of abbreviations.

Table 2: OATP-drug interactions

OATP (human)	Substrates and inhibitors *in vitro*	Drug interactions *in vivo* *
1A2	**Antibiotics** •direct **TBPM-PI** (β -lactam antibiotic) uptake [132] **Anaesthetics and analgesics** •direct deltrophin II and **DPDPE** transport [110] •direct rocuronium transport inhibited by **APM**, taurocholate, K-strophantoside, **QD**, and **NMQD** [113] **Anticancer drugs** •**ES** uptake inhibited by **MTX** [31] • imatinib transport inhibited by naringin [105] **Antihypertensive drugs** •direct nadolol uptake inhibited by green tea, naringin, verapamil [109] •direct talinolol uptake [133] **Antihistaminic drugs** •direct fexofenadine uptake [134] •direct fexofenadine uptake inhibited by naringin and hesperidin [135] **Antiretroviral drugs** •direct **SQV** uptake [115] **Statins** •direct pravastatin uptake [136] **Toxins** •direct microcystin transport [137] **Others** •direct **ES** uptake inhibited by **atROL**, direct **atROL** transport [40] •direct **TCL** uptake [116] •direct uptake measurements with triptans [17] For further list of interacting molecules see [5,15,138]	•reduced fexofenadine AUC by citrus juices [134,139] •imatinib pharmacokinetics affected by SLCO1A2 SNPs in CML patients [105] •green tea ingestion decreases plasma concentrations of nadolol in humans, presumably in part by inhibition of OATP1A2-mediated intestinal absorption of nadolol [109] •docetaxel transport in humanized mice [80]
1B1	**Antibiotics** •**ES** uptake inhibited by several anti-**TB** drugs [140] •**E17βG** uptake inhibited by novobiocin [141] **Anticancer** •direct docetaxel uptake [119] •direct flavopiridol uptake and increased toxicity [142] •involved in toxicity and disposition of platinum anticancer drugs [143] •**TKI**s as 1B substrates (eg. direct sorafenib transport)[144] **Antihypertensive drugs** •direct bosentan uptake inhibited by **CsA**	•rifampicin as an inhibitor of OATP1B1 and OATP1B3 •Oral or intravenous dose of rifampicin increases exposure of rosuvastatin and pitavastatin [149] •docetaxel transport (humanized mice) [80] •role for OATP1Bs in the elimination of sorafenib (humanized mice)[144]

	and rifampicin [83] •direct valsartan uptake [145] **Anti-inflammatory drugs** •direct mesalazine transport inhibited by budesonide, cyclosporine A, rifampin [146] **Statins** •transport inhibitors: lovastatin acid, pravastatin acid, and simvastatin acid [141] •direct cerivastatin uptake inhibited by **CsA** [3] •cerivastatin mediated toxicity caused by 1B1 inhibition with gemfibrozil [22] **Toxins** •direct microcystin transport and cytotoxicity[137,147] For further interacting molecules, see: [5,15,126,138,141,148]	
1B3	**Antibiotics** •**ES** uptake inhibited by several **anti-TB** drugs [140] •direct **E17βG** uptake inhibited by novobiocin [141] **Anticancer drugs** •direct paclitaxel transport [150] •transport inhibitors: mitoxantrone and vincristine 27 [141] •direct docetaxel transport [119] •direct flavopiridol uptake and increased toxicity [142] •1B3 linked toxicity and disposition of cisplatin, carboplatin, and oxaliplatin [143] •**TKI**s as 1B substrates (eg. direct sorafenib transport)[144] **Anti-inflammatory drugs** •direct mesalazine transport inhibited by budesonide, cyclosporine, rifampin [146] **Antihypertensive drugs** •direct bosentan uptake inhibited by **CsA** and rifampicin [83] •direct valsartan uptake [145] **Toxins** •direct microcystin transport and cytotoxicity [137,147] For an exhaustive list of interacting molecules see:[5,15,126,138,141]	•imatinib pharmacokinetics affected by SNPs in **CML** patients [151] •paclitaxel pharmacokinetics affected by SNPs [150] •docetaxel transport (humanized mice) [80] •role for OATP1Bs in the elimination of sorafenib (humanized mice) [144] •rifampicin as an inhibitor of OATP1B1 and OATP1B3 [149] •rifampicin as an inhibitor of OATP1B1 and OATP1B3 •Oral or intravenous dose of rifampicin increases exposure of rosuvastatin and pitavastatin [149]
1C1	•direct docetaxel transport [119]	
2A1	**Anti-inflammatory drugs** •direct **PGE_2** uptake inhibited by diclofenac and lumiracoxib [152] •direct **PGE_2** uptake induced by indomethacin, ketoprofen, and naproxen [152] **Flavonoids** •direct quercetin transport [112] **Prostaglandin analogs** •direct latanoprost acid uptake [153]	

2B1	**<u>Antibiotics</u>** •direct **ES** uptake inhibited by several **anti-TB** drugs [140] •direct **TBPM-PI** (β -lactam antibiotic) uptake [132] •direct **ES** uptake inhibited by novobiocin [141] **<u>Anticancer drugs</u>** •transport inhibitor: erlotinib [141] •direct flavopiridol uptake and increased toxicity [142] **<u>Anti-inflammatory drugs</u>** •direct mesalazine transport inhibited by budesonide, cyclosporine, rifampin [146] •direct **DCF-AG** transport and toxicity [154] **<u>Antihypertensive drugs</u>** •direct talinolol uptake [133] **<u>Prostaglandin analogs</u>** •direct latanoprost acid uptake [153] **<u>Statins</u>** •transported by 2B1 [5] •involved in increased cytotoxicity of statins [82] For further interacting molecules see: [5,15,126,138,141]	
3A1	**<u>Antibiotics</u>** •direct benzylpenicillin transport [128] **<u>Antihypertensive drugs</u>** •direct BQ-123 transport [38]	•3A1 as a novel **CD**-associated gene, results higher incidence of bowel perforation in **CD** patients [155]
4A1	**<u>Antibiotics</u>** •direct benzylpenicillin transport [128]	
4C1	**<u>Antidiabetics</u>** •direct sitagliptin transport [131] **<u>Cardiac glycosides</u>** •direct digoxin transport [30,131] •direct digoxin transport increased by bupropion [29] **<u>Statins</u>** •statins increase the expression and function of OATP4C1[28]	•SLCO4C1 overexpression reduced hypertension, cardiomegaly, and inflammation in a rat renal failure model [28]
5A1	**<u>Anticancer drugs</u>** •5A1 expressing cells showed higher resistance to satraplatin [156] **<u>Flavonoids</u>** •direct quercetin uptake [112]	
6A1		

* Human OATP transporter activity and OATP-related disposition of drugs measured *in vivo* (human clinical or rodent data)

Table 3: OATP expression in normal and cancerous tissues.

Most data are based on mRNA expression. Protein data are indicated by bold letters.

OATP	normal expression		cancer	
		localization	**downregulated**	**upregulated**
1A2	ubiquitous: **BBB** [104,157] eye (retina) [35,40] intestine [33] **kidney (distal tubule)** [104] **liver (cholangiocytes)** [104] **neurons** [35]	apical	breast* [158] colon [159] gliomas [157]	bone [160] **breast*** [54,55] prostate [161]
1B1	**liver (hepatocytes)** [128]	basolateral (sinusoidal)	liver cancer [54]	colon [54,162] ovaries [162]
1B3	**liver (hepatocytes)** [123] **pancreas** (Langerhans islets) [163]	basolateral (sinusoidal)	liver cancer [54]	breast [162] **colon** [54,162] **lung** [54,162] **pancreas** [54,162] **prostate** [54,162] ovaries [107,162]
1C1	brain (choroid plexus) **testis** (Leydig cells) [11,37]	basolateral		malignant bone cysts [160]
2A1	ubiquitous: **eye (retina, ciliary epithelium)** [153] **endometrium** [164] **neurons** [165]			bile duct [166] bone [160] breast [158] **liver** [166]
2B1	ubiquitous: **BBB** [157] **intestine** [34] **liver (hepatocytes)** [111] **skeletal muscle** [82]	apical (enterocytes) basolateral (hepatocytes)	breast* [158]	colon [128] bone [160] **breast*** [167] gliomas [157]
3A1	**brain** (choroid plexus, neurons) [38] **testis** [38]	apical (3A1_v2) basolateral (3A1_v1)		colon [167] bone [160] breast (altered localization) [21,57,168,169] liver [166] lung [167] pancreas [167]

4A1	ubiquitous: **eye (ciliary body)** [41] kidney [129] pancreas [129] **placenta** [170]	apical		**lung** [171] **liver** [166] colon [128] pancreas [128] breast [57,168] bone [160]
4C1	**kidney** (human OATP4C1 expressed in rats localizes to proximal tubule cells) [28]	basolateral		breast (altered localization) [21,158] lung [171]
5A1	heart[172] fetal brain [172] **breast** [21]			**breast (altered localization)** [21] liver [166] lung [171]
6A1	testis (Sertoli cells) [173]			bladder, brain and esophagus [173] **lung** [54,171]

*: controversial reports

Table 4: List of the most relevant SLCO SNPs altering *in vivo* PK

Gene	dbSNP ID	allele	mutant allele frequency (%)			nucleotide change	aa change	functional consequences	
			Caucasian	Afro-American	Asian			*in vitro*	*in vivo*
SLCO1A2	rs10841795	*2	13-16	2-4	<1	c.38T>C	I13T	increased ^{3}H-MTX and ^{3}H-E1S uptake[31] unaltered ^{3}H-E1S transport [104]	increased AUC of methotrexate [36]
	rs3764043		2	9	17	c.361G>A	promoter region		increased imatinib clearance [105]
SLCO1B1	rs2306283	*1b	30-45	72-83	59-86	c.388A>G	N130D	unaltered transport function [174]	decreased AUC of repaglinide, ezetimibe and simvastatin acid [92,93] no alteration in statin response [96]
	rs4149056	***5**	**8-20**	**1-8**	**8-16**	**c.521T>C**	**V174A**	**decreased function** [86]	**increased AUC of statins, sartans, torsemide, lopinavir, fexofenadine and tacrolimus** [5,87,88]
		***15**	**16-25**	**2-16**	**12**	**c.388A>G, c.521T>C**	**N130D + V174A**	**decreased function** [174]	**increased plasma levels of pravastatin and simvastatin** [95,97] **and increased risk of rifampin-induced liver injury** [175]
SLCO1B3	rs4149117		65-80	<50%	75-86	c.334T>G	S112A	unaltered transport function [59,98]	increased the clearance of imatinib [100]
	rs7311358		81-84	<50%	64-81	c.699G>A	M233I	unaltered transport function [59,98]	decreased docetaxel clearance [99]
		***1**	**>70%**		**>70%**	**c.334G, c.699A**	**S112A + M233I**	**decreased function** [59,98]	**better survival in prostate cancer** [59]
	rs11045585		14	22	18	IVS12-5676A>G	intronic		increased AUC of docetaxel and telmisartan [99]

SLCO2B1	rs12422149		8-14	13	37	c.935G>A	R312Q		increased survival in prostate cancer [103] decreased AUC of montelukast [176]
	rs2306168	*3	3	19	31	c.1457C>T	S486F	decreased transport of ^{3}H-ES [101]	increased AUC of a beta blocker celiprolol [5]

Figure 1. OATPs involved in drug uptake. Members of the OATP/SLCO (OATP1A2, 1B1, 1B3 and 2B1) and SLC22 (OCT and OAT1 and OAT3) transporter families are key determinants of drug uptake. Within the cell, drugs may undergo modifications by CYP (cytochrome P450), UDP (uridine diphospho-glucuronosyltransferase), GST (glutathione S-transferase) and SULT (sulphotransferase) enzymes. The most relevant transporters involved in the efflux of drugs and toxins are the ATP Binding Cassette proteins (ABCB1, ABCG2, ABCC2 and 3) and member of the SLC47 family (MATE1 and MATE2-K).