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Organic anion transporting polypeptide 2B1 – More than a glass-full of drug interactions

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ABSTRACT

The importance of uptake transporters in determining drug disposition is increasingly appreciated. While the focus of regulatory agencies worldwide has been on the hepatic organic anion transporting polypeptides (OATPs)—1B1 and—1B3, there is another isoform of the OATP sub-family, OATP2B1, which should be considered equally relevant. Unlike the other members of the OATP sub-family, OATP2B1 is expressed in multiple organs in humans, including in the intestine and the liver. Similar to other OATPs, OATP2B1 mediates the hepatic and intestinal uptake of many drugs and endogenous compounds. The importance of OATP2B1 in the disposition of many drugs is highlighted by the growing recognition of its role in significant *in vivo* drug-drug or food-drug interactions. The dramatic changes in drug exposure attributable to inhibition of OATP2B1 highlight the importance of developing a better understanding of the clinical role of OATP2B1. This review aims to provide a thorough summary of the current understanding of the pharmacogenetics, regulation, expression and abundance of OATP2B1 in humans, as well as its clinical relevance in drug-drug and food-drug interactions.

Contents

1. Introduction

Organic anion transporting polypeptides (OATPs) belong to the solute carrier (SLC)-superfamily of transporters and are involved in the cellular uptake of both endogenous and exogenous compounds (Köck et al., 2010). The high conservation of these transporters across many species has been attributed to their central and critical role in detoxification processes (Meier-Abt, Mokrab, & Mizuguchi, 2006). OATP2B1, initially referred to as OATP-B, was originally isolated from

Abbreviations: AUCR, area under the curve ratio (inhibited control); BSP, bromosulfophthalein; DDI, drug-drug interaction; FDI, food-drug interaction; E3S, estrone-3-sulfate; OATP, organic anion transporting polypeptide..

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human brain tissue (Hagenbuch & Meier, 2003; Kullak-Ublick et al., 2001; Tamai et al., 2000). OATP2B1 is one of the main members of the OATP family, along with OATP1B1 and OATP1B3. In contrast to the liver-specific expression of OATP1B1 and 1B3, OATP2B1 has a wider expression profile (Liu & Li, 2014; Nakanishi & Tamai, 2012; Obaidat, Roth, & Hagenbuch, 2012). While research on OATP2B1 is relatively new compared to other transporters, OATP2B1 has been shown to play an important role in not only drug disposition, but also drug-drug interactions (DDIs) and food-drug interactions (FDIs). Since 2001 when OATP2B1 was first identified, the number of publications regarding the function and structure of OATP2B1 has steadily increased. Furthermore, the number of DDIs and FDIs attributed to OATP2B1 continues to rise, with a 400% increase in publications evaluating the role of OATP2B1 drug-drug interactions between 2006 and 2017 (Fig. 1) (National Center for Biotechnology Information, 2018). However, because there is significant overlap in the substrate specificities of OATP2B1 and the hepatic OATPs, the fraction of drug transported by OATP2B1 (relative to other OATPs, other transporters or passive diffusion), f_t, must be considered when evaluating the role of OATP2B1 in these interactions, as well as in translating *in vitro* data *to in vivo* predictions. Even with this increasing body of knowledge and strong evidence of clinically-significant interactions, regulatory agencies do not currently require evaluation of OATP2B1 during the drug development process. This review focuses on an in-depth evaluation of the current literature on OATP2B1 as it relates to drug disposition and interactions, and provides insight into the growing evidence of the clinical importance of this transporter.

2. Expression and transcriptional regulation

OATP2B1, encoded by the *SLCO2B1* gene, is a 709-amino acid glycoprotein containing 12 putative transmembrane-spanning domains (Hagenbuch, 2010). As mentioned above, OATP2B1 is widely expressed in humans and has been detected in various normal tissues (Table 1). While the transporter was initially identified in the brain, expression in the tissue is relatively low compared to the liver and other tissues (Hagenbuch & Meier, 2004). OATP2B1 has also been identified in multiple tumor cell lines and interestingly, the abundance of the transporter increases with tumor grade. This increase in abundance may indicate that the transporter is involved in tumor promotion and the proliferation of cancer cells by facilitating the uptake of hormones and steroid sulfates such as estrone sulfate (E3S) (Al Sarakbi et al., 2006; Bronger et al., 2005).

Immunolocalization studies have shown that OATP2B1 is primarily expressed on the plasma membrane (Kindla et al., 2011; Kleberg et al., 2012; Knauer et al., 2010; Le Vee, Noel, Jouan, Stieger, & Fardel, 2013). OATP2B1 can undergo clathrin-dependent internalization leading to significant intracellular accumulation of the protein (Köck et al., 2010; Mayati et al., 2015). In both *in vitro* cell culture and *ex vivo* placental perfusion, membrane expression of the transporter decreased following treatment with an activator of protein kinase C (known to participate in localization and regulation of transporters), leading to a decrease in transport. Following internalization, the protein can be redirected to the membrane to restore transport activity or subjected to lysosomal degradation (Köck et al., 2010). This internalization may be a rapid response mechanism to assist in maintaining cellular homeostasis, as many endogenous steroids and hormones are substrates of OATP2B1 (Köck et al., 2010).

In almost all tissues, OATP2B1 is assumed to facilitate the uptake of compounds into the tissue. In the intestine, some researchers have suggested that OATP2B1 may be expressed on the basolateral membrane of the enterocytes (Keiser et al., 2017; Kobayashi et al., 2003; Mooij et al., 2016; Sai et al., 2006). The proteomic analysis completed by Keiser et al. showed enrichment of the transporter at the basolateral membrane of enterocytes in the jejunum (17-fold higher than the apical abundance), yet immunofluorescence staining was inconclusive. The basolateral localization of OATP2B1 was confirmed in Caco-2 cells, however this was inconsistent between apical and basolateral membranes in human intestinal samples (Keiser et al., 2017). These paradoxical findings indicates that OATP2B1 may not only play a role in the absorption of compounds, as has historically been assumed, but may also play a regulatory role in removing bile acids and toxic metabolites from the circulation. Additionally, there are conflicting reports within those showing basolateral expression on the direction of transport. To illustrate, while Keiser et al. found that the transport was from the blood into the enterocyte, Mooij et al. classified the transporter as facilitating movement of compounds from the enterocyte to the blood (Keiser et al., 2017; Mooij et al., 2016). These findings create an interesting question of the *in vivo* role of OATP2B1 – does uptake to or from the blood predominate? These findings and their contradictory and

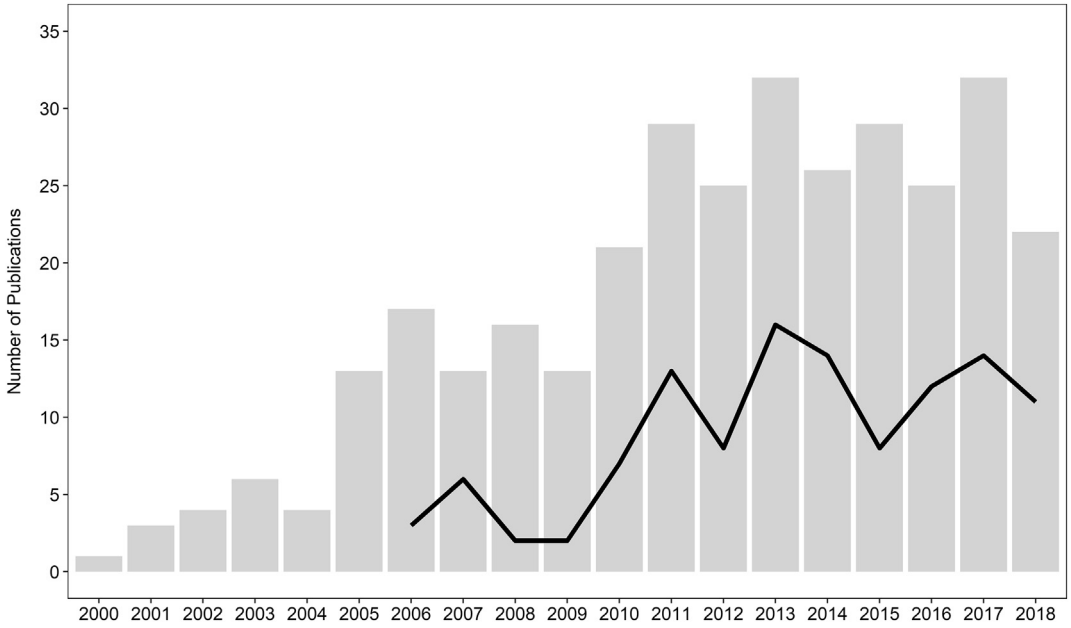

Fig. 1. Number of PubMed citations for OATP2B1 and OATP2B1-associated drug-drug interactions per year. Bars – number of citations appearing for "OATP2B1" or "OATP-B" query per year; line – number of citations for "OATP2B1 drug-drug interaction" per year. PubMed query performed 23 May 2018. [National Center for Biotechnology Information, 2018]

Table 1
Tissue expression and localization of OATP2B1.

Organ	Cell type	Direction of transport	Cellular localization	Detection method	Reference
Normal					
liver	hepatocyte sinusoidal membrane	blood - > liver	plasma membrane, basolateral	IHC, western blot	(Kullak-Ublick et al., 2001)
small intestine	enterocyte	intestine - > blood	plasma membrane, apical	IHC, mRNA	(Kobayashi et al., 2003; Sai et al., 2006)
		blood - > intestine	plasma membrane, basolateral	IHC, LC-MS/MS	(Keiser et al., 2017; Mooij et al., 2016)
placenta	syncytiotrophoblast	fetal blood - > placenta	plasma membrane, basolateral	IHC, western blot	(St-Pierre, Hagenbuch, Ugele, Meier, & Stallmach, 2002)
brain	capillary endothelial cells	blood - > brain	plasma membrane, apical	IHC, mRNA	(Bronger et al., 2005; Kovacsics, Patik, & Özvegy-Laczka, 2016)
heart	vascular endothelium	blood - > heart	plasma membrane, apical	mRNA, western blot	(Grube et al., 2006)
skeletal muscle	sarcolemmal membrane	blood - > muscle	plasma membrane	IHC, mRNA	(Knauer et al., 2010)
mammary gland	myoepithelium	blood - > duct	plasma membrane	IHC, northern blot	(Pizzagalli et al., 2003)
lung	epithelial cells	blood - > lung	plasma membrane	LC-MS/MS	(Sakamoto et al., 2013)
eye	retina	blood - > retina	ND	IHC, western blot	(Gao, Vavricka, Meier, & Stieger, 2015)
prostate	ND	blood - > tissue	ND	northern blot	(Tamai et al., 2000)
Tumor					
breast tumor	blood-tumor barrier	blood - > tumor	plasma membrane	IHC, mRNA	(Al Sarakbi et al., 2006; Pizzagalli et al., 2003)
glioma	blood-tumor barrier	blood - > tumor	plasma membrane, luminal	IHC, mRNA	(Bronger et al., 2005)
prostate carcinoma	blood-tumor barrier	blood - > tumor	ND	--[a]	(Yang et al., 2011)

[a] The role of OATP2B1 in prostate cancer was postulated by preexisting knowledge that the transporter is expressed in prostate tissue and that hormones involved in disease progression are substrates of the transporter. Variations in the *SLCO2B1* gene are associated with time to disease progression (Yang et al., 2011). IHC – immunohistochemical detection; plasma membrane (apical or basolateral) is indicated based on author's conclusion or figures shown. Note that many of these figures also showed some intracellular localization, LC-MS/MS – liquid chromatography tandem mass spectrometry, ND – no data available.

controversial nature further illustrate the need for additional research into the localization and function of OATP2B1.

Quantitative proteomics studies completed by our laboratory have determined the relative abundance of transporters in human liver tissue using liquid chromatography-tandem mass spectrometry (LC-MS/MS), including the abundance of OATP2B1 (Billington et al., 2018; Wang et al., 2015; Wang et al., 2016). While initial experiments underestimated the abundance of the OATPs, subsequent experiments using an optimized methodology have determined that OATP2B1 accounts for approximately 9% of the total transporter protein quantified in the liver. The abundance of OATP2B1 is approximately half that of OATP1B1, yet approximately equal in abundance to OATP1B3 (Fig. 2A). A meta-analysis of transporter abundance data completed in 2017 using seven available studies confirmed this finding for healthy Caucasian subjects (Burt et al., 2016). This analysis also found that there is slightly higher abundance of OATP2B1 in males compared to females (1.3-fold, $P < 0.05$) and that there is a weak correlation between abundance and age ($r_s = 0.268$, $P < 0.05$). The absolute abundance of OATP2B1 in human intestinal tissue has also been determined. Drozdzik et al. found that OATP2B1 was the primary OATP expressed in the intestine, accounting for approximately 6% and 12% of the total transporter protein quantified in the small intestine and colon, respectively (Fig. 2B, C) although this longitudinal variation in protein abundance was not significant ($P > .05$) (Drozdzik et al., 2014).

To date, there has been limited research on the regulation of OATP2B1 expression. Multiple transcription factors have been shown to possibly play a role, but a definitive answer is still lacking. Specificity protein 1 (Sp1), a general transcription factor that plays a role in the regulation of many cellular processes such as cell differentiation, has been shown to be required for OATP2B1 expression in the liver and small intestine (Maeda, Hirayama, Higashi, Sato, & Tamai, 2006). This transcription factor is ubiquitously expressed, which may partly explain the broad expression of OATP2B1 compared to other liver-specific OATPs (O'Connor, Gilmour, & Bonifer, 2016; Vizcaíno, Mansilla, & Portugal, 2015). Additionally, Knauer et al. demonstrated that hepatocyte nuclear factor 4α (HNF4α), a liver-specific transcription factor, may also take participate in the regulation of hepatic OATP2B1 expression (Knauer, Girdwood, Kim, & Tirona, 2013). *In vitro*, knockdown of HNF4α resulted in a significant decrease in *SLCO2B1* transcription, supporting the hypothesis that HNF4α expression affects OATP2B1 expression in the liver. Interestingly, it has also been shown that treatment with two chemical activators of transcription - phenobarbital, a constitutive androstane receptor (CAR) activator, and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), an aryl hydrocarbon receptor (AhR) activator – down-regulate SLCO2B1 mRNA *in vitro*, although the exact mechanism of these interactions is still unknown (Jigorel, Le Vee, Boursier-Neyret, Parmentier, & Fardel, 2006). Furthermore, when human hepatocytes were incubated with rifampin, a PXR ligand, the mRNA expression of *SLCO2B1* was increased 1.73-fold (Benson et al., 2016). While these studies are a promising start to understanding the regulation of OATP2B1, more data are needed (such as quantitative proteomics) since mRNA expression does not often correlate well with protein expression.

3. Function

Like other OATPs, OATP2B1 facilitates the sodium-independent transport of a variety of amphipathic organic compounds. The presumed physiological role of OATP2B1 is to assist in the distribution and elimination of regulatory compounds, as a majority of the endogenous substrates of the transporter are steroid hormones and bile acids. OATP2B1, along with OATP1A2 and −1B3, shows pH-dependent transport activity (Leuthold et al., 2008). So far, the detailed mechanism of this phenomenon is unclear, however, all of the pH-sensitive OATPs have a conserved histidine residue in the third putative transmembrane domain that may be contributing to the effect (Leuthold et al., 2008). Studies have shown greater OATP2B1-mediated uptake of E3S at pH 5.0 compared to pH 7.4, corresponding to a 7-fold increase in V_{max} and 1.5-fold increase in K_m (Kobayashi et al., 2003; Nozawa, Imai, Nezu, Tsuji, & Tamai, 2004; Sai et al., 2006; Varma et al., 2011). Similar observations have been made for pravastatin, where OATP2B1-mediated uptake was only observed under acidic conditions

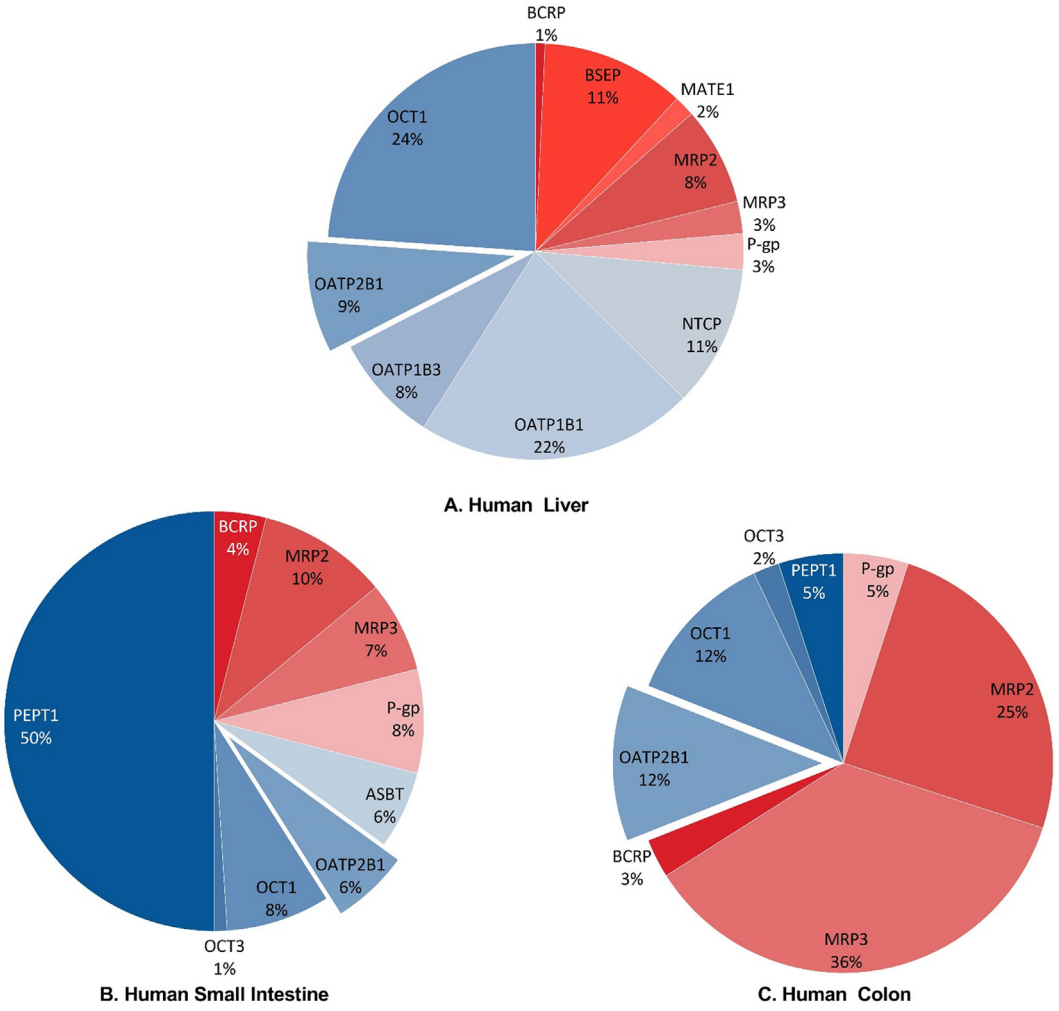

Fig. 2. Abundance of OATP2B1 relative to other transporters in the human liver and intestine. Blue- uptake transporters, red- efflux transporters. Expressed as percentage of total tissue transporter abundance. Adapted with permission from Drozdzik et al., 2014. Copyright 2014 American Chemical Society.

(Kobayashi et al., 2003; Nozawa et al., 2004; Varma et al., 2011). An increase in transport activity, as well as an increase in substrates recognized by OATP2B1 at acidic pH, likely plays a physiological role in the uptake of drugs from the intestinal lumen where the microclimate pH is weakly acidic (Tamai, 2012). However, the effect of pH on OATP2B1-mediated transport is substrate-dependent. The OATP2B1-mediated uptake of substrates such as bromosulfophthalein (BSP) and rosuvastatin, common OATP probe substrates, appear to be independent of pH (Varma et al., 2011; Visentin, Chang, Romero, Zhao, & Goldman, 2012). This may be partly explained by the observation that OATP2B1 likely has multiple binding sites. Shirasaka et al. showed that uptake of E3S into OATP2B1-transfected oocytes showed saturable biphasic kinetics. Further kinetic evaluation identified high and low affinity binding sites, with a 100-fold difference in K_m between the two (Shirasaka, Mori, Shichiri, Nakanishi, & Tamai, 2012). These sites were also found to show different pH sensitivity which could contribute to the observed substrate-dependent differences.

4. Importance of the concept of f_t in interpreting *in vivo* OATP2B1 data

To understand the *in vivo* relevance of OATP2B1 through pharmacogenetic or DDI/DFI studies, the importance of the concept of fraction of drug transported (f_t) by OATP2B1, *in vivo*, needs to be appreciated. For example, if OATP2B1 is responsible for only 5% of the total hepatic uptake of the drug (relative to that mediated by diffusion or other transporters such as OATP1B1), then complete inhibition of hepatic OATP2B1 activity by OATP2B1 inhibitor drugs, food constituents or due to pharmacogenetics variants of OATP2B1 is unlikely to result in a clinically significant change in the plasma concentration of the drug (after IV or oral administration). Also, in the narrative below, the f_t by OATP2B1 by the liver or intestine may significantly differ due to the presence of other transporters in the respective organ capable of transporting the OATP2B1 drug (*e.g.* OATP1B1 is present in the liver but not in the intestine). What determines the f_t of an OATP drug substrate and how can it be estimated? F_t is the ratio of the uptake clearance of the drug by OATP2B1 relative to the uptake clearance of the drug by other transporters plus diffusion. The OATP2B1-mediated intrinsic uptake clearance of the drug will be determined by the ratio of the *in vivo* OAT2B1 V_{max}/K_m for the drug (assuming linear kinetics). Because it is difficult to determine the *in vivo* OATP2B1 K_m of a drug, for simplicity it can be assumed to be the same as that in the *in vitro* system expressing only OATP2B1 (*e.g.* transfected cell line). In contrast, the same cannot be said for the V_{max} of OATP2B1-mediated drug transport since the V_{max} is determined by the level of OATP2B1 expression in the *in vitro* system used. Parenthetically, for this reason, V_{max} values are not reported in Table 2. To overcome this problem, and to extrapolate the *in vitro* V_{max} to that *in vivo*, one can use either the relative expression factor (REF) or the relative activity factor (RAF) approach. The REF approach relies on quantifying the abundance of OATP2B1 (and other relevant

Table 2
In vitro substrates of OATP2B1.

Substrate	K_m (μM)[a]	Uptake Ratio[a]	Cell System	Also a substrate for OATP1B1/1B3?[c]
Selective for OATP2B1				
Aliskiren	72 (Vaidyanathan et al., 2008)	ND[b]	HEK293-transfected	No
Celiprolol	ND[b]	Uptake Observed (Ieiri et al., 2012)	*X. laevis* oocytes-injected	No
Erlotinib	0.324 (Bauer et al., 2017)	ND[b]	A431 cells	No
Pemetrexed	307 (Visentin et al., 2012)	ND[b]	HeLa-transfected	No
Some selectivity over OATP1B1/1B[c]				
Eltrombopag	ND[b]	Uptake Observed (Takeuchi et al., 2011)	HEK293-transfected	Yes/No (Takeuchi et al., 2011)
Ethinyl estradiol sulfate	10.7 (Han et al., 2010)	ND[b]	HEK293-transfected	Yes*/No (Han et al., 2010)
Glyburide	6.3 (Satoh et al., 2005)	2.7 (Koenen et al., 2012)	HEK293-transfected	Yes*/No (Koenen et al., 2012)
Telmisartan glucuronide	1.1 (Ishiguro et al., 2008)	ND[b]	HEK293-transfected	No/Yes+ (Ishiguro et al., 2008)
Substrates of OATP2B1 and OATP1B1/1B3[c]				
Asunaprevir	ND[b]	1.8 (Eley et al., 2015)	HEK293-transfected	Yes‡ (Eley et al., 2015)
Atorvastatin	2.8 (Karlgren et al., 2012)	5.0 (Koenen et al., 2012)	HEK293-transfected	Yes* (Karlgren et al., 2012)
Bromosulfophthalein (BSP)	0.7 (Kullak-Ublick et al., 2001)	ND[b]	*X. laevis* oocytes-injected	Yes‡ (Kullak-Ublick et al., 2001)
	ND[b]	10.3 (Koenen et al., 2012)	HEK293-transfected	
Coproporphyrin III	0.31 (Bednarczyk & Boiselle, 2016)	3.2 (Bednarczyk & Boiselle, 2016)	CHO-transfected	Yes‡/+ (Bednarczyk & Boiselle, 2016)
Estrone-3-sulfate (E3S)	8.1/13.1 (pH 5.0) (Nozawa et al., 2004)	106 (Shen et al., 2013)	HEK293-transfected	Yes* (Cui et al., 2001; Kullak-Ublick et al., 2001)
Fexofenadine	0.14 (Shirasaka et al., 2014)	ND[b]	*X. laevis* oocytes-injected	Yes+ (Matsushima et al., 2008; Shimizu et al., 2005)
	ND[b]	2.8 (Ming et al., 2011)	MDCK-transfected	
Fluvastatin	0.75 (Noé et al., 2007)	ND[b]	HEK293-transfected	Yes+/ND (Noé et al., 2007)
Mesalazine	188.9 (König et al., 2011)	ND[b]	HEK293-transfected	Yes* (König et al., 2011)
Montelukast	ND	1.9 (Varma et al., 2017)	HEK293-transfected	Yes (Varma et al., 2017)
Pitavastatin	1.2 (Hirano et al., 2006)	< 2.0 (pH 5.5-6.5) (Varma et al., 2011)	HEK293-transfected	Yes‡ (Hirano et al., 2006)
Pravastatin	6.3 (Shirasaka et al., 2014)	ND[b]	*X. laevis* oocytes-injected	Yes+/ND (Glaeser et al., 2010; Hsiang et al., 1999)
	ND[b]	1.3 (pH 5.0) (Nozawa et al., 2004)	HEK293-transfected	
Rosuvastatin	2.4 (Ho et al., 2006)	ND[b]	HeLa-transfected	Yes*/+ (Ho et al., 2006)
	ND[b]	14.0 (Kimoto et al., 2015)	HEK293-transfected	
Taurocholic acid	71.8 (pH 5.9) (Nozawa et al., 2004)	1.3 (Nozawa et al., 2004)	HEK293-transfected	Yes* (Briz et al., 2006; Hsiang et al., 1999)
SN-38 carboxylate	0.76 (pH 6.5) (Fujita et al., 2015)	ND[b]	*X. laevis* oocytes-injected	ND
SN-38 lactone	1.3 (pH 6.5) (Fujita et al., 2015)	ND[b]	*X. laevis* oocytes-injected	ND
Sulfasalazine	1.7 (Kusuhara et al., 2012)	ND[b]	HEK293-transfected	ND
Tebipenem pivoxil	>1000 (pH 6.0) (Kato et al., 2010)	7.0 (pH 6.0) (Kato et al., 2010)	*X. laevis* oocytes-injected	ND
Unoprostone carboxylate	91 (Gao et al., 2005)	2.8 (Gao et al., 2005)	*X. laevis* oocytes-injected	ND

[a] Experiments were conducted at pH 7.4 unless otherwise stated. Reported values are the lowest K_m or highest uptake ratio (transfected/mock) reported in literature. V_{max} Is not reported as it depends on the abundance of transporter which may vary between expression systems.
[b] No data available.
[c] Substrate affinity for OATP2B1 relative to OATP1B1/1B3 is as follows: *The OATP2B1 K_m is ≥ 2-fold higher than OATP1B1 or 1B3; +The OATP2B1 K_m is ≥ 50% lower than OATP1B1 or 1B3; ‡The K_m for OATP2B1 and OATP1B1 or 1B3 are within 100% of each other. If the relative affinity differs between isoforms, the K_m relative to OATP1B1 is listed first followed by OATP1B3. When no symbol is indicated, only an uptake ratio was reported. Not determined (ND) if K_m values or uptake ratios were not available for OATP1B1/1B3 in the literature. Data retrieved from the UW DIDB 02 Jan 2018.

transporters) in the *in vitro* system used to measure the intrinsic uptake clearance of the drug relative to that in the tissue of interest (*e.g.* the liver and/or the intestine). In contrast, the RAF relies on availability of data on *in vitro* and *in vivo* uptake clearance of a model substrate which is selectively transported by OATP2B1 (both *in vitro* and *in vivo*). However, since many OATP2B1 substrates are also substrates of other transporters (*e.g.* OATP1B1) *in vivo*, the RAF approach is not a viable approach. Therefore, we have proposed the use of REF and have successfully shown its application in prediction of the *in vivo* uptake clearance of rosuvastatin in the rat (Ishida, Ullah, Toth, Juhasz, & Unadkat, 2018). In addition, we have elaborated on the concept of f_t in Prasad and Unadkat (Prasad & Unadkat, 2015). We have used both the REF approach and the concept of f_t to successfully predict the magnitude of DDI between rosuvastatin and rifampin in the rat where rosuvastatin is transported into the liver by multiple Oatp transporters (please see Ishida et al., 2018). Finally, for *in vitro* to *in vivo* extrapolation of OATP2B1-mediated clearance of drugs, one should keep in mind that uptake by OATP2B1 may not be the only rate-determining step in the clearance of the drug (see Patilea-Vrana & Unadkat, 2016 for detailed discussion).

5. Substrates

OATP2B1 mediates the uptake of many drugs, hormones, and natural products (Table 2). While there is some substrate overlap with other members of the OATP family, compounds with preferential uptake by OATP2B1 have been identified. For example, drugs such as aliskiren, celiprolol, erolitinib and pemetrexed are transported by OATP2B1 but not by OATP1B1/1B3. The reverse is also true as estradiol-17β-glucuronide, a prototypical substrate of OATP1B1/1B3, is not transported by OATP2B1 (Nozawa et al., 2004). Some drugs, including tebipenem pivoxil and unaprostone, have greater *in vitro* affinity for OATP2B1 compared to OATP1B1/1B3 (Koenen, Kroemer, Grube, & Meyer Zu Schwabedissen, H. E., 2011; Vaidyanathan et al., 2008); however of the OATP substrates tested, the majority of drugs and compounds that are substrates of OATP2B1 are also substrates of OATP1B1/1B3. Despite this significant overlap with other OATPs, OATP2B1 has fewer substrates at standard physiological pH. However, its substrate selectivity broadens at acidic pH and becomes similar to the other transporters in the family. Under acidic conditions, such as in the intestine, OATP2B1 is able to facilitate the uptake of a broader

spectrum of compounds including taurocholate, which suggests that it may play a role in the enterohepatic circulation of bile acids (Nozawa et al., 2004).

Statins, including fluvastatin, pitavastatin, pravastatin, rosuvastatin and atorvastatin are also substrates of OATP2B1 (Hirano, Maeda, Shitara, & Sugiyama, 2006; Ho et al., 2006; Karlgren et al., 2012; Noé, Portmann, Brun, & Funk, 2007; Nozawa et al., 2004). While the hepatic uptake of these drugs is most likely mediated primarily by OATP1B1, OATP2B1 likely contributes to the hepatic uptake as well as intestinal absorption and bioavailability of these drugs. Some researchers have suggested that OATP2B1 may contribute to statin-related muscle toxicity, as overexpression of OATP2B1 in primary human skeletal muscle myoblast cells results in a significant increase in intracellular concentrations of both atorvastatin and rosuvastatin *in vitro* (Knauer et al., 2010). Despite these *in vitro* data, the low abundance of OATP2B1 in muscle suggests that its contribution to the *in vivo* myotoxicity of the statins is likely to be low (Knauer et al., 2010).

6. Inhibitors

A number of compounds, both drugs and natural products, have been found to inhibit OATP2B1 uptake activity *in vitro* (Table 3). While most of these compounds have not been investigated *in vivo* at this time, many show high inhibitory potency towards the transporter including erlotinib (IC_{50} = 0.03 μM), cyclosporine (IC_{50} = 0.07 μM), and antiviral drugs such as asunaprevir (IC_{50} = 0.27 μM) and ritonavir (IC_{50} = 0.55 μM) (Eley, Garimella et al. 2015; Ho, Leake, Kim, & Wang, 2006; Johnston, Rawling, Chan, Zhou, & Murray, 2014; U.S. Food and Drug Administration, 2014). Several natural products have been identified that also inhibit the transport activity of OATP2B1 resulting in DFIs. In fact, one of the earliest DFIs attributed to transporters was the inhibition of OATP2B1 by grapefruit juice (Glaeser et al., 2007; Kirby & Unadkat, 2007). Incubation with grapefruit, orange, and apple juices resulted in a significant decrease in transport activity of OATP2B1 *in vitro* (Shirasaka, Shichiri, Mori, Nakanishi, & Tamai, 2013). Further investigation into which ingredients of the fruit juices could cause the observed interactions identified naringin and hesperidin as the most likely perpetrators (Shirasaka et al., 2013). Additionally, compounds found in herbal medicines and natural products including catechins (commonly found in teas), and flavonols such as scutellarin, silymarin and silybin (components of milk thistle extract), and phloretin are also potent inhibitors of OATP2B1 (Fuchikami et al., 2006; Köck, Xie, Hawke, Oberlies, & Brouwer, 2013; Shirasaka et al., 2013; Wen, Shi, Bian, Zhang, & Gui, 2015). With the high abundance of OATP2B1 in the intestine, it is a likely site for a variety of DFIs affecting the absorption of drugs.

To date, no selective inhibitors of OATP2B1 have been identified, though aliskerin, celiprolol and premetrexed could be candidates as they are selective substrates of OATP2B1. If a drug has the ability to potently inhibit OATP2B1 while showing only weak or moderate inhibition of OATP1B1/1B3 (*e.g.* 10-fold difference in IC_{50} or Ki values), such a drug could be used *in vivo* as a selective inhibitor of OATP2B1, provided the *in vivo* unbound plasma concentration of the drug does not approach the unbound IC_{50} for inhibition of OATP1B1/1B3 and it does not inhibit other confounding transporters. For example, the OATP2B1 IC_{50} values for erlotinib (0.03 μM (Johnston et al., 2014)) and glimepiride (2.4 μM (Klatt, Fromm, & König, 2013)) are >10-fold lower than that of OATP1B1 or 1B3 (erlotinib – 21 μM and 1.19 μM, respectively, and glimepiride – 52 μM and 40.9 μM, respectively (Johnston et al., 2014; Karlgren et al., 2012; Klatt et al., 2013)) which could allow for dosing to selectively inhibit OATP2B1. Additionally, determination of expected inhibitor concentrations at the intestine and liver inlet show that it is possible to selectively inhibit intestinal OATP2B1 without inhibiting hepatic OATP2B1 (Table 3). While compounds such as ronacaleret and naringenin are potent inhibitors of intestinal OATP2B1, systemic concentrations are well below the reported IC_{50} values, indicating that inhibition of hepatic OATP2B1 is unlikely. This difference in concentration, and therefore inhibitory potential, can facilitate the determination of the primary site of inhibition, *i.e.* intestine *vs.* liver.

Another complicating factor regarding understanding inhibitors of OATP2B1 is that there can be a large substrate-specific difference in potency. For example, inhibition of OATP2B1-mediated transport by rifampin varies from no observed inhibition of atorvastatin transport (IC_{50} > 630 μM) to inhibition of E3S transport with an IC_{50} value of 40.1 μM (Shen et al., 2013; Vildhede et al., 2014). While the exact reason for this difference is unknown, the presence of multiple-binding sites, as described earlier, may provide an explanation. Some inhibitors, including naringin, taurocholate, and pravastatin, only inhibit the high-affinity site, while other inhibitors, including testosterone and penicillin G, preferentially inhibit the low-affinity site (Shirasaka et al., 2012). Given this substrate-specific inhibition, care should be taken to make sure that *in vitro* inhibition studies use multiple substrate/inhibitor combinations to ensure accurate prediction of the degree of OATP2B1 inhibition *in vivo*.

7. Pharmacogenetics

Genetic polymorphisms have the potential to influence the uptake activity of OATP2B1 (Table 4). While many variants of the *SLCO2B1* gene have been identified, little is currently known about the effect of these variants on OATP2B1 transport activity. One of the most studied variants, *SLCO2B1**3 (rs2306168), describes the nonsynonymous mutation c.1457C > T, resulting in the amino acid change Ser486Phe (Namgoong et al., 2015). While this variant has been frequently studied, the precise effect on function has yet to be fully elucidated. Studies conducted *in vitro* showed that *SLCO2B1*3* exhibited similar affinity for E3S compared to wild-type *SLCO2B1*1*, but demonstrated a 50% decrease in transport activity when the V_{max} was adjusted for OATP2B1 protein content (Nozawa et al., 2002). Similarly, a decrease in the uptake of both rosuvastatin and atorvastatin in cells transfected with *SLCO2B1*3* relative to *SLCO2B1*1* has also been observed (Nies et al., 2013). Clinical studies investigating the effect of this variant, however, have shown inconsistent results. A genotype-dependent decrease in activity has been observed for the beta-blocker celiprolol after oral administration, with a significant decrease in celiprolol AUC of 49.9% for homozygous individuals (with a corresponding 58% decrease in C_{max}) and a non-significant decrease of 29.1% for heterozygous individuals compared to wild-type (Ieiri et al., 2012). Imanaga et al. observed significantly decreased intestinal absorption of fexofenadine (35.5% decrease in AUC) in *SLCO2B1*3* carriers compared to those with the *SLCO2B1*1/*1* genotype (Imanaga et al., 2011). However, in another report, *SLCO2B1*3* carriers (either *SLCO2B1*1/*3* or **3/*3*) exhibited a 51% increase in AUC_{0-24} for *(S)*-fexofenadine ($P < 0.05$), but no significant change in C_{max}, suggesting predominately decreased hepatic uptake by OATP2B1 (Akamine et al., 2010). Additionally, there was no significant change in *(R)*-fexofenadine or montelukast exposure between those with *SLCO2B1*1* and *SLCO2B1*3* genotypes, indicating that the effect of this variant may be **drug- and stereo-specific** (Akamine et al., 2010; Kim, Lee, Joo, Park, & Park, 2013).

Other variants have also been shown to influence OATP2B1 transport function. The c.601G > A variant showed a decrease in OATP2B1-mediated uptake of rosuvastatin *in vitro*, although no effect was observed *in vivo* using montelukast as the substrate (Kim, Joo, Lee, & Park, 2013; Kim, Lee, et al., 2013). Similarly, the c.935G > A variant showed substrate-specific effects on OATP2B1-mediated uptake *in vivo*, with no change in AUC observed between genotypes for montelukast but a significant increase was observed for rosuvastatin (112% and 41.7% increases in *SLCO2B1* 935A/A and *SLCO2B1* 935G/935A individuals, respectively) (T.-E. Kim et al., 2017; Tapaninen, Karonen, Backman, Neuvonen, & Niemi, 2013). In contrast, Mougey et al. showed significantly lower montelukast concentrations (36.5% decrease in C_{max} and 45.7% decrease in AUC) in individuals heterozygous

Table 3
In vitro inhibition of OATP2B1.

Inhibitor	Substrate	IC_{50}(μM)[a]	Expression system	Interaction Expected[b]		Reference
				Intestine	Liver	
afatinib	E3S	2.08	HEK293-transfected	yes	yes	(Johnston et al., 2014)
apigenin	atorvastatin	13.9	HEK293-transfected	yes	no	(Mandery et al., 2010)
	BSP	20.8[c]				
asunaprevir[i]	E3S	0.27	HEK293-transfected	yes	yes	(Eley et al., 2015)
atazanavir	E3S	5.2	HEK293-transfected	yes	yes	(Karlgren et al., 2012)
atorvastatin	E3S	0.7[c]	MDCK II - transfected	yes	yes	(Grube et al., 2006)
BSP	E3S	0.28	HEK293-transfected	ND[d]		(Sai et al., 2006)
clarithromycin	E3S	384	HEK293-transfected	no	no	(Peters et al., 2012)
cyclosporine	rosuvastatin	2.2	HEK293-transfected	yes	yes	(Varma et al., 2011)
	E3S	37				(Karlgren et al., 2012)
DHEAS	E3S	12	Caco-2 cells	yes	yes	(Sai et al., 2006)
doxorubicin[e]	E3S	240	HEK293-transfected	NA	no	(Karlgren et al., 2012)
eltrombopag	E3S	8.5[c]	HEK293-transfected	yes	yes	(Takeuchi et al., 2011)
erlotinib[f,g]	E3S	0.03	HEK293-transfected	yes	yes	(Johnston et al., 2014)
gefitinib[f]	E3S	0.65	HEK293-transfected	yes	yes	(Johnston et al., 2014)
gemfibrozil[i]	rosuvastatin	8	Hela-transfected	yes	yes	(Ho et al., 2006)
glimepiride[f,g]	BSP	2.4	HEK293-transfected	yes	no	(Klatt et al., 2013)
glyburide	BSP	2	HEK293-transfected	yes	no	(Klatt et al., 2013)
glycyrrhetinic acid	E3S	13	HEK293-transfected	yes	yes	(Wen et al., 2015)
hesperetin	E3S	67.6 (pH 6.5)	*X. laevis* oocytes-injected	yes	no	(Shirasaka et al., 2013)
hesperidin	E3S	1.9 (pH 6.5)	*X. laevis* oocytes-injected	yes	yes	(Shirasaka et al., 2013)
		72.3	HEK293-transfected			(Wen et al., 2015)
hyperoside	E3S	29.4	HEK293-transfected	NA		(Wen et al., 2015)
icariin	E3S	6.4	HEK293-transfected	yes	no	(Li et al., 2014)
indinavir	E3S	3.9	Caco-2 cells	yes	yes	(Annaert et al., 2010)
montelukast[f]	BSP	1	MDCK-transfected	yes	yes	(Letschert et al., 2006)
mulberrin	E3S	1.8	HEK293-transfected	NA		(Wen et al., 2015)
naringenin[h]	E3S	49.2 (pH 6.5)	*X. laevis* oocytes-injected	yes	yes	(Shirasaka et al., 2013)
naringin[g,h]	E3S	4.6 (pH 6.5)	*X. laevis* oocytes-injected	NA		(Shirasaka et al., 2013)
nateglinide	BSP	28.5	HEK293-transfected	yes	yes	(Klatt et al., 2013)
neratinib	E3S	2.7	HEK293-transfected	yes	yes	(Johnston et al., 2014)
nilotinib	E3S	2.7	HEK293-transfected	yes	yes	(Johnston et al., 2014)
pelitinib	E3S	2	HEK293-transfected	NA		(Johnston et al., 2014)
phloretin	E3S	1.31 (pH 6.5)	*X. laevis* oocytes-injected	NA		(Shirasaka et al., 2013)
phloridzin	E3S	23.2 (pH 6.5)	*X. laevis* oocytes-injected	NA		(Shirasaka et al., 2013)
quercetin[h]	atorvastatin	14.1	HEK293-transfected	yes	yes	(Mandery et al., 2010)
	BSP	8.7[c]				
	E3S	7.5				(Wen et al., 2015)
rifampin[i]	BSP	80.5	HEK293-transfected	yes	yes	(Leonhardt et al., 2010)
	E3S	40.1	HEK293-transfected			(Shen et al., 2013)
rifamycin	E3S	3.6 (pH 6.0)	HEK293-transfected	yes	yes	(Varma et al., 2011)
	rosuvastatin	0.96 (pH 6.0)				
ritonavir	E3S	6.1	HEK293-transfected	yes	yes	(Karlgren et al., 2012)
ronacaleret[h]	rosuvastatin	12 (pH 6.0)	HEK293-transfected	yes	yes	(Johnson et al., 2017)
		16				
rutin	E3S	60.7	HEK293-transfected	NA		(Wen et al., 2015)
scutellarin[g]	E3S	2	HEK293-transfected	NA		(Wen et al., 2015)
silybin A	E3S	4.5	HEK293-transfected	yes	yes	(Köck et al., 2013)
silybin B[g]	E3S	0.8	HEK293-transfected	yes	yes	(Köck et al., 2013)
silychristin	E3S	3.6	HEK293-transfected	yes	yes	(Köck et al., 2013)
silymarin[g]	E3S	0.3	HEK293-transfected	NA		(Köck et al., 2013)
sulfasalazine	E3S	3	HEK293-transfected	yes	yes	(Karlgren et al., 2012)

[a] Lowest literature values are reported; experiments were conducted at pH 7.4 unless otherwise specified.
[b] Expected intestinal interaction and hepatic interaction determined by $I_{gut}/IC_{50} \geq 10$ and $1 + I_{inlet}/IC_{50} \geq 1.1$, respectively. Full calculations are presented in Supplemental Table 1.
[c] K_i value, no IC_{50} reported in literature.
[d] Only used as an *in vitro* inhibitor.
[e] IV formulation, unbound C_{max} used in hepatic interaction prediction.
[f] IC_{50} for OATP2B1 is ≥90% less than OATP1B1.
[g] IC_{50} for OATP2B1 is ≥90% less than OATP1B3.
[h] Inhibitor has been tested *in vivo* and the interaction is likely attributable to OATP2B1, see Table 5 for more detail.
[i] Inhibitor has been tested *in vivo* and while it is possible that OATP2B1 plays a role in the observed interaction, the primary mechanism is likely inhibition of OATP1B1/1B3, see Table 5 for more detail. E3S, estrone-3-sulfate; BSP, bromosulfophthalein; NA – not calculated, no dose or C_{max} data available. Data retrieved from the UW DIDB on or before 02 Jan 2018.

Table 4
Selected single nucleotide polymorphisms in the *SLCO2B1* gene and their impact on *in vivo* pharmacokinetics of drugs.

rs number	Nucleotide change	Amino acid variation	MAF[a]	Allelic frequency (%)[b]			Functional change *In Vitro* or *In Vivo* (substrate, % change AUC)[c]
				Caucasian	Asian	African-American	
rs56837383	C·43C > T	P15S	0.0028	0	0	1.6	Decrease (rosuvastatin)[d] (Ho, Leake, et al., 2006)
rs35199625	C.601G > A	V201 M	0.0258	0	4.2	0.8	Decrease (rosuvastatin)[d] (Ho, Leake, et al., 2006)
rs12422149	C.935G > A	R312Q	0.2099	8.5	37.5	10.5	−45.7% (montelukast)[e] to 112.39% (rosuvastatin)[f] (T.-E. Kim et al., 2017; Mougey et al., 2011)
rs1621378 (*SLCO2B1*2*)	C.1175C > T	T392I	0.0000	NA	NA	NA	−20.7% (E3S)[d,g] (Nozawa et al., 2002)
rs2306168 (*SLCO2B1*3*)	C.1457C > T	S486F	0.1803	3.9	19.2	34.1	−38.5% (fexofenadine)[e] to 51.3% (fexofenadine)[e] (Akamine et al., 2010; Imanaga et al., 2011) Increase (rosuvastatin)[d] (Ho, Leake, et al., 2006)
rs2712807	g.-282G > A	–	0.2346	86	90.7	72.9	−3.3% (montelukast)[e,h] to −10.3% (montelukast)[f,h] (K.-A. Kim et al., 2013)
rs145875125	C.1638C > A	N546K	0.0004	0	0	0.8	NA

MAF, minor allele frequency; NA, not available.
[a] Global minor allele frequency, obtained from 1000 Genomes Project Consortium et al., 2015.
[b] Allelic frequencies from the dbSNP database (Sherry et al., 2001).
[c] Changes are statistically significant relative to the control (wild type) group as determined by the authors unless otherwise stated.
[d] Change in function observed *in vitro*.
[e] Heterozygous for variant.
[f] Homozygous for variant.
[g] Change in CL_{int} relative to OATP2B1*1.
[h] Observed change is not statistically significant ($P > 0.05$).

for the variant compared to wild-type (Mougey, Feng, Castro, Irvin, & Lima, 2009; Mougey, Lang, Wen, & Lima, 2011). While the *SLCO2B1*2* variant c.1175C > T, which causes the amino acid change Thr392Ile, has not been evaluated *in vivo*, a decrease in uptake of E3S was observed in HEK293 cells expressing the variant compared with the wild-type (Nozawa et al., 2002). By conducting studies with am OATP2B1 selective substrate such as aliskiren, the clinical effect of these variants may be determined more definitively. However, as substrate-specific effects of some variants have been identified, studies with multiple substrates should be considered to form a complete picture of the effect of these variants.

8. Food-drug and drug-drug interactions

With high abundance in both the intestine and liver, OATP2B1 plays a critical role in the uptake of many compounds, making it a likely site for DDIs and FDIs. This has been demonstrated by multiple studies *in vivo* showing changes in exposure following treatment with known inhibitors of OATP2B1 (Table 5). Considering the high degree of substrate and inhibitor overlap with OATP1B1/1B3, as well as the broad expression profile of OATP2B1, it is likely that these interactions have intestinal as well as hepatic components. However, because of the observed decreases in AUC, it is likely that the effect of hepatic inhibition is minor compared to inhibition at the intestine. Additionally, the inhibitory potency of the inhibitor for each transporter relative to the intestinal and hepatic concentrations will determine the predominant site of inhibition. The intestinal concentration is usually significantly higher than the hepatic, possibly explaining the higher frequency of OATP2B1-mediated interactions resulting in a decreased AUC due to intestinal inhibition.

A review of clinical DDIs mediated by intestinal OATPs found that fruit juices are the most well-studied inhibitors of intestinal OATP2B1 activity *in vivo*, showing marked decreases in the plasma concentrations of several OATP2B1 substrates including fexofenadine, aliskiren, and celiprolol (Yu, Zhou, Tay-Sontheimer, Levy, & Ragueneau-Majlessi, 2017). Apple juice decreased the AUC of fexofenadine by approximately 85% and that of aliskiren by 63% (Imanaga et al., 2011; Tapaninen, Neuvonen, & Niemi, 2011), while grapefruit juice decreased the AUC of fexofenadine up to 52%, aliskiren by 61%, and celiprolol by 84% (Akamine et al., 2015; Ieiri et al., 2012; Tapaninen, Neuvonen, & Niemi, 2010). Likewise, orange juice decreased the AUC of aliskiren by approximately 62% (Tapaninen et al., 2011). In these studies, however, the typical volume of juice is much higher than normal consumption. For example, a total volume of 1.5 L over 3 h was administered to participants in the aforementioned apple juice/fexofenadine study (Imanaga et al., 2011) and 200 mL grapefruit juice was given three times a day for multiple days in the grapefruit juice/celiprolol study (Ieiri et al., 2012). For multiple juices, a volume-dependent effect has been observed. While significant interactions can be observed with high volumes of juice, little to no effect is observed at low doses that are similar to a standard beverage volume (Dresser et al., 2002; Dresser, Kim, & Bailey, 2005; Glaeser et al., 2007). To illustrate, Luo et al. showed that the observed interaction between fexofenadine and apple juice increased from 10% to 61% when the volume was increased from 150 mL to 600 mL (Luo et al., 2016). Studies such as this indicate that significant DFIs with juices can likely be avoided by reducing the volume of juice administered at a time. Additionally, it is likely that interactions can also be avoided by staggering the administration of juices and affected drugs. Delaying administration of fexofenadine by two hours following ingestion of grapefruit juice resulted in a lower level of inhibition than concomitant dosing (38% *versus* 52% decrease in AUC_{0-8}) and no inhibition was observed when the drug was given four hours after (Glaeser et al., 2007; Tanaka et al., 2013).

In addition to FDIs mediated by fruit juices, some DDIs have been attributed almost exclusively to OATP2B1. The now terminated drug ronacaleret, which was identified *in vitro* as an inhibitor of OATP2B1 (IC_{50} = 12 μM, estimated C_{max} of 5.2 μM following a clinical dose of 400 mg (Johnson et al., 2017)) decreased rosuvastatin exposure by approximately 50%, with the interaction mainly ascribed to inhibition of intestinal OATP2B1 (Johnson et al., 2017). Another example of a DDI mediated by OATP2B1 is the interaction of voxilaprevir with sofosbuvir/velpatasvir. All three drugs are part of the fixed-dose combination treatment Vosevi®, a recently approved treatment for chronic hepatitis C infection (FDA, 2017b; Gilead Sciences, 2017). *In vitro*, voxilaprevir is a proposed substrate of OATP2B1, and velpatasvir is an inhibitor of OATP2B1 (FDA, 2017b). When coadministered with sofosbuvir/velpatasvir, the AUC of voxilaprevir decreased by 63%, which is attributed to a decrease in intestinal absorption due to inhibition of OATP2B1. However, that no change in the AUC of voxilaprevir was observed following administration of grapefruit juice (European

Table 5
Examples of DDIs/FDIs likely mediated primarily by OATP2B1.

Intestinal OATP2B1 interaction[a]					
Substrate	Substrate dose[b]	Inhibitor	Inhibitor dose[b]	AUCR	Reference
aliskiren	150 mg	apple juice	200 mL [TID, 5 days]	0.37	(Tapaninen et al., 2011)
		grapefruit juice	200 mL [TID, 5 days]	0.39	(Tapaninen et al., 2010)
		orange juice	200 mL [TID, 5 days]	0.38	(Tapaninen et al., 2011)
atenolol	50 mg	apple juice	1200 mL (total)	0.17–0.21	(Jeon et al., 2013)
			600 mL (total)	0.41	
celiprolol	100 mg	grapefruit juice	200 mL [TID, 3 days]	0.16–0.25	(Ieiri et al., 2012; Tanaka et al., 2013)
	1200 μg	grapefruit juice	200 mL [TID, 3 days]	0.57	(Kashihara et al., 2017)
fexofenadine	60 mg	apple juice	1200 mL (total)	0.15	(Imanaga et al., 2011)
			600 mL	0.39	(Luo et al., 2016)
			300 mL	0.62	
(*R*)-fexofenadine[c]	60 mg	apple juice	400 mL	0.47	(Akamine et al., 2014)
		grapefruit juice	250 mL	0.59	(Akamine et al., 2015)
(*S*)-fexofenadine[c]	60 mg	apple juice	400 mL	0.35	(Akamine et al., 2014)
		grapefruit juice	250 mL	0.43	(Akamine et al., 2015)
glyburide	300 μg	grapefruit juice	200 mL [TID, 3 days]	0.55	(Kashihara et al., 2017)
rosuvastatin	20 mg	epigallocatechin gallate	300 mg	0.84	(T.-E. Kim et al., 2017)
	250 μg	grapefruit juice	200 mL [TID, 3 days]	0.70	(Kashihara et al., 2017)
	10 mg	ronacaleret	400 mg [QD, 10 days]	0.51	(Johnson et al., 2017)
sulfasalazine	300 μg	grapefruit juice	200 mL [TID, 3 days]	0.66	(Kashihara et al., 2017)
sumatriptan	600 μg	grapefruit juice	200 mL [TID, 3 days]	0.73	(Kashihara et al., 2017)
talinolol	100 mg	quercetin	20 mg [BID, 7 days]	0.77	(Nguyen, Staubach, Wolffram, & Langguth, 2014)
			500 mg [TID, 7 days]	0.79	
ticlopidine	250 mg	ergoloid mesylates	1.5 mg [TID, 3.5 days]	0.70	(Lu, Huang, & Lai, 2006)
voxilaprevir	100 mg	grapefruit juice	300 mL	0.98	(FDA, 2017b)
	200 mg	sofobuvir/velpatasvir	400 mg / 100 mg [QD, 4 days]	0.39	

Hepatic OATP2B1 Interaction[a,d]					
Substrate	Substrate Dose[a]	Inhibitor	Inhibitor Dose[a]	AUCR	Reference
asunaprevir	200 mg	rifampin	600 mg	14.82	(Eley, Garimella et al., 2015)
montelukast	10 mg	gemfibrozil	600 mg [BID, 3 days]	4.54	(Karonen et al., 2010)
rosuvastatin	10 mg	asunaprevir	200 mg [BID, 11 days]	1.41	(Eley, Garimella et al., 2015)

[a] Current research supports that these interactions are likely driven by inhibition of intestinal or hepatic OATP2B1, however it cannot be ruled out that other unidentified pathways could be contributing to the observed changes in AUC.
[b] Administered as a single oral dose unless otherwise noted.
[c] Administered as a racemic mixture.
[d] It is likely that the hepatic interactions are primarily driven by inhibition of OATP1B1/1B3, with minor contribution from OATP2B1. For rifampin and gemfibrozil, the IC_{50} values for OATP1B1 are approximately half that of OATP2B1 indicating that inhibition of OATP1B1 is more likely the primary interaction given the estimated liver inlet concentrations. The increase in AUCR indicates that inhibition of hepatic uptake is the dominant interaction over intestinal inhibition of OATP2B1, however the magnitude of the hepatic interaction could be blunted from decreased absorption. Data retrieved from the UW DIDB 02 Jan 2018.

Medicines Agency, 2006). Thus, while there is strong evidence that OATP2B1 is the primary site of inhibition in these interactions, clinical research into the role of OATP2B1 is still in its infancy compared to other transporters and the contribution of other pathways to these interactions cannot be ruled out at this time.

Identifying the primary pathway contributing to transporter-based DDIs is challenging. For most drugs, multiple enzymes and/or transporters contribute to their *in vivo* disposition. For example, both the AUC and C_{max} of glyburide, a substrate of OATP2B1 as well as OATP1B1, were significantly increased (125% and 81%, respectively) after a single intravenous infusion of rifampicin, a recommended OATP inhibitor (Zheng, Huang, Frassetto, & Benet, 2009). While intravenous dosing removes the potential for intestinal inhibition, there is currently no way to determine the contribution of each hepatic transporter involved *in vivo* due to the lack of selective inhibitors. The calculation of the liver inlet concentration relative to the IC_{50} for each transporter can give some insight into the uptake pathway that is likely driving the interaction, but this method is not definitive as it is a static prediction based on estimated concentrations. Determination of the fraction transported by each of the relevant uptake transporters, *via* techniques such as proteomics, would allow for a more accurate determination of the primary site of inhibition and a better understanding of the underlying mechanisms of interactions.

Transporter-based DDIs are also complicated by cases where the interaction can occur at multiple organs and tissues in addition to involving multiple transporters. A study investigating the effect of asunaprevir on rosuvastatin exposure also highlights the difficulty in determining the mechanism of transport inhibition *in vivo*. Asunaprevir is a potent *in vitro* inhibitor of OATP2B1 (0.27 μM), OATP1B1 (0.3 μM), and OATP1B3 (3.0 μM); however, when asunaprevir was coadministered with rosuvastatin, only a modest increase in rosuvastatin exposure was observed (1.4-fold). Although it appears that the main effect of asunaprevir is inhibition of hepatic uptake (affecting OATP1B1/1B3 and OATP2B1) it is quite possible that there is incomplete inhibition or, since both drugs were administered orally, that the observed increase in exposure could be somewhat blunted by reduced absorption due to inhibition of intestinal OATP2B1 (Eley, Garimella et al. 2015).

9. Conclusions and perspective

OATP2B1 is ubiquitously expressed in humans, showing the highest abundance in the liver and intestine, the principal organs that determine the absorption, metabolism, and elimination of drugs. OATP2B1 is the primary OATP expressed in the intestine and its abundance in the liver is equal to that of OATP1B3 (Drozdzik et al., 2014; Wang et al., 2016). Therefore, OATP2B1 likely contributes to drug absorption and hepatic clearance to a much greater extent than has been previously considered. In fact, accumulating evidence suggests that it is crucial for the efficient uptake of many drugs with variable chemical structures and clinical uses.

A growing body of data indicates that studies should be undertaken to elucidate the role of OATP2B1 in intestinal absorption of drugs and

DDI/FDI during the approval process. The most recent FDA draft guidance defines eight transporters that should be studied during drug discovery including P-gp and OATP1B1/1B3, but not OATP2B1 (2017a; FDA, 2017c). However, current research has shown that there are significant changes in exposure, equivalent or surpassing changes observed for other OATPs, following inhibition of OATP2B1. While many of these drugs are also substrates of hepatic OATPs, the decrease in AUCR indicates that intestinal uptake by OATP2B1 plays a key role in disposition of these substrates. This significant contribution to disposition can be confirmed by determination of the fraction transported by OATP2B1. Similar to the concept of fraction metabolized, understanding the contribution of each transporter can assist in interpretation of complex interactions (Prasad & Unadkat, 2015). For those compounds with a high affinity for OATP2B1 but a low f_t relative to other transporters or passive diffusion, the likelihood of a significant clinical interaction by OATP2B1 is low. However, for those compounds with a high f_t, inhibition of OATP2B1 is likely the predominant site of inhibition and the driving force for observed changes in exposure. In this regard, there is an urgent need to identify selective *in vivo* substrates of OATP2B1, those with high f_t by the transporter, and selective *in vivo* inhibitors of OATP2B1. *In vivo* studies with such selective substrates and inhibitors will allow for a more complete understanding of OATP2B's role in *in vivo* drug disposition and DDIs/FDIs.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pharmthera.2018.12.009.

References

Akamine, Y., Miura, M., Komori, H., Saito, S., Kusuhara, H., Tamai, I., et al. (2014). Effects of one-time apple juice ingestion on the pharmacokinetics of fexofenadine enantiomers. *European Journal of Clinical Pharmacology 70*(9), 1087–1095.

Akamine, Y., Miura, M., Komori, H., Tamai, I., Ieiri, I., Yasui-Furukori, N., et al. (2015). The change of pharmacokinetics of fexofenadine enantiomers through the single and simultaneous grapefruit juice ingestion. *Drug Metabolism and Pharmacokinetics 30*(5), 352–357.

Akamine, Y., Miura, M., Sunagawa, S., Kagaya, H., Yasui-Furukori, N., & Uno, T. (2010). Influence of drug-transporter polymorphisms on the pharmacokinetics of fexofenadine enantiomers. *Xenobiotica; the Fate of Foreign Compounds in Biological Systems 40*(11), 782–789.

Al Sarakbi, W., Mokbel, R., Salhab, M., Jiang, W. G., Reed, M. J., & Mokbel, K. (2006). The role of STS and OATP-B mRNA expression in predicting the clinical outcome in human breast cancer. *Anticancer Research 26*(6 C), 4985–4990.

Bauer, M., Matsuda, A., Wulkersdorfer, B., Philippe, C., Traxl, A., & O'Zvegy-Laczka, C., Et Al. (2017). Influence of OATPs on Hepatic Disposition of Erlotinib measured with Positron Emission Tomography. *Clinical Pharmacology and Therapeutics 104*(1), 139–147.

Bednarczyk, D., & Boiselle, C. (2016). Organic anion transporting polypeptide (OATP)-mediated transport of coproporphyrins I and III. *Xenobiotica 46*(5), 457–466.

Benson, E. A., Eadon, M. T., Desta, Z., Liu, Y., Lin, H., Burgess, K. S., et al. (2016). Rifampin regulation of drug transporters gene expression and the association of microRNAs in human hepatocytes. *Frontiers in Pharmacology 7*(APR), 1–13.

Billington, S., Ray, A. S., Salphati, L., Xiao, G., Chu, X., Humphreys, W. G., et al. (2018). Transporter expression in noncancerous and cancerous liver tissue from donors with hepatocellular carcinoma and chronic hepatitis C infection quantified by LC-MS/MS proteomics. *Drug Metabolism and Disposition 46*(2), 189–196.

Bronger, H., König, J., Kopplow, K., Steiner, H. H., Ahmadi, R., Herold-Mende, C., et al. (2005). ABCC drug efflux pumps and organic anion uptake transporters in human gliomas and the blood-tumor barrier. *Cancer Research 65*(24), 11419–11428.

Burt, H. J., Riedmaier, A. E., Harwood, M. D., Crewe, H. K., Gill, K. L., & Neuhoff, S. (2016). Abundance of hepatic transporters in Caucasians: A meta-analysis. *Drug Metabolism and Disposition 44*(10), 1550–1561.

Dresser, G. K., Bailey, D. G., Leake, B. F., Schwarz, U. I., Dawson, P. A., Freeman, D. J., et al. (2002). Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine. *Clinical Pharmacology and Therapeutics 71*(1), 11–20.

Dresser, G. K., Kim, R. B., & Bailey, D. G. (2005). Effect of grapefruit juice volume on the reduction of fexofenadine bioavailability: Possible role of organic anion transporting polypeptides. *Clinical Pharmacology and Therapeutics 77*(3), 170–177.

Drozdzik, M., Gröer, C., Penski, J., Lapczuk, J., Ostrowski, M., Lai, Y., et al. (2014). Protein abundance of clinically relevant multidrug transporters along the entire length of the human intestine. *Molecular Pharmaceutics 11*(10), 3547–3555.

Eley, T., Garimella, T., Li, W., & Bertz, R. J. (2015). Asunaprevir: A review of preclinical and clinical pharmacokinetics and drug–drug interactions. *Clinical Pharmacokinetics 54*(12), 1205–1222.

Eley, T., Han, Y. H., Huang, S. P., He, B., Li, W., Bedford, W., et al. (2015). Organic anion transporting polypeptide-mediated transport of, and inhibition by, asunaprevir, an inhibitor of hepatitis C virus NS3 protease. *Clinical Pharmacology and Therapeutics 97*(2), 159–166.

European Medicines Agency (2006). *Vosevi: EPAR - Public Assessment Report44. (pp. 153), 153.*

FDA (2017a). Clinical Drug Interaction Studies — Study Design. *Data Analysis , and Clinical Implications: Guidance for Industry.*

FDA (2017b). Drug Approval Package: VOSEVI (sofosbuvir/velpatasvir/voxilaprevir). *FDA Application NDA 209195.*

FDA (2017c). *In Vitro Metabolism- and Transporter- Mediated Drug-Drug Interaction Studies: Guidance for Industry.*

Fuchikami, H., Satoh, H., Tsujimoto, M., Ohdo, S., Ohtani, H., & Sawada, Y. (2006). Effects of Herbal Extracts on the Function of Human Organic Anion-Transporting Polypeptide OATP-B. *Drug Metabolism and Disposition 34*(4), 577–582.

Gao, B., Huber, R. D., Wenzel, A., Vavricka, S. R., Ismair, M. G., Remé, C., et al. (2005). Localization of organic anion transporting polypeptides in the rat and human ciliary body epithelium. *Experimental Eye Research 80*(1), 61–72.

Gao, B., Vavricka, S. R., Meier, P. J., & Stieger, B. (2015). Differential cellular expression of organic anion transporting peptides OATP1A2 and OATP2B1 in the human retina and brain: Implications for carrier-mediated transport of neuropeptides and neurosteriods in the CNS. *Pflugers Archiv European Journal of Physiology 467*(7), 1481–1493.

Genomes Project Consortium, Auton, A., Brooks, L. D., Durbin, R. M., Garrison, E. P., Kang, H. M., et al. (2015). A global reference for human genetic variation. *Nature 526*(7571), 68–74.

Gilead Sciences, I. (2017). *VOSEVI (sofosbuvir/velpatasvir/voxilaprevir) Prescribing Information, 1–35.*

Glaeser, H., Bailey, D. G., Dresser, G. K., Gregor, J. C., Schwarz, U. I., McGrath, J. S., et al. (2007). Intestinal drug transporter expression and the impact of grapefruit juice in humans. *Clinical Pharmacology and Therapeutics 81*(3), 362–370.

Grube, M., Köck, K., Oswald, S., Draber, K., Meissner, K., Eckel, L., et al. (2006). Organic anion transporting polypeptide 2B1 is a high-affinity transporter for atorvastatin and is expressed in the human heart. *Clinical Pharmacology and Therapeutics 80*(6), 607–620.

Hagenbuch, B. (2010). Drug uptake systems in liver and kidney: A historic perspective. *Clinical Pharmacology and Therapeutics 87*(1), 39–47.

Hagenbuch, B., & Meier, P. J. (2003). The superfamily of organic anion transporting polypeptides. *Biochimica et Biophysica Acta - Biomembranes 1609*(1), 1–18.

Hagenbuch, B., & Meier, P. J. (2004). Organic anion transporting polypeptides of the OATP/SLC21 family: Phylogenetic classification as OATP/SLCO super-family, new nomenclature and molecular/functional properties. *Pflugers Archiv European Journal of Physiology 447*(5), 653–665.

Hirano, M., Maeda, K., Shitara, Y., & Sugiyama, Y. (2006). Drug-drug interaction between pitavastatin and various drugs via OATP1B1. *Drug Metabolism and Disposition: The Biological Fate of Chemicals 34*(7), 1229–1236.

Ho, R. H., Leake, B. F., Kim, R. B., & Wang, Y. (2006). OATP2B1 Allelic Variants Differentially Transport Rosuvastatin in Vitro. *Drug Metabolism Reviews 38*(Suppl. 2), 240–241.

Ieiri, I., Doi, Y., Maeda, K., Sasaki, T., Kimura, M., Hirota, T., et al. (2012). Microdosing clinical study: pharmacokinetic, pharmacogenomic (*SLCO2B1*), and Interaction (Grapefruit Juice) profiles of celiprolol following the oral microdose and therapeutic dose. *The Journal of Clinical Pharmacology 52*(7), 1078–1089.

Imanaga, J., Kotegawa, T., Imai, H., Tsutsumi, K., Yoshizato, T., Ohyama, T., et al. (2011). The effects of the SLCO2B1 c.1457C > T polymorphism and apple juice on the pharmacokinetics of fexofenadine and midazolam in humans. *Pharmacogenetics and Genomics 21*(2), 84–93.

Ishida, K., Ullah, M., Toth, B., Juhasz, V., & Unadkat, J. D. (2018). Transport Kinetics, Selective Inhibition, and successful Prediction of in Vivo Inhibition of Rat Hepatic Oatps. *Drug Metabolism and Disposition 12.*

Ishiguro, N., Maeda, K., Saito, A., Kishimoto, W., Matsushima, S., Ebner, T., et al. (2008). Establishment of a set of double transfectants coexpressing organic anion transporting polypeptide 1B3 and hepatic efflux transporters for the characterization of the hepatobiliary transport of telmisartan acylglucuronide. *Drug Metabolism and Disposition 36*(4), 796–805.

Jeon, H., Jang, I. -J., Lee, S., Ohashi, K., Kotegawa, T., Ieiri, I., et al. (2013). Apple juice greatly reduces systemic exposure to atenolol. *British Journal of Clinical Pharmacology 75*(1), 172–179.

Jigorel, E., Le Vee, M., Boursier-Neyret, C., Parmentier, Y., & Fardel, O. (2006). Differential regulation of sinusoidal and canalicular hepatic drug transporter expression by

xenobiotics activating drug-sensing receptors in primary human hepatocytes. *Drug Metabolism and Disposition 34*(10), 1756–1763.
Johnson, M., Patel, D., Matheny, C., Ho, M., Chen, L., & Ellens, H. (2017). Inhibition of intestinal OATP2B1 by the calcium receptor antagonist ronacaleret results in a significant drug-drug interaction by causing a 2-fold decrease in exposure of rosuvastatin. *Drug Metabolism and Disposition 45*(1), 27–34.
Johnston, R. A., Rawling, T., Chan, T., Zhou, F., & Murray, M. (2014). Selective inhibition of human solute carrier transporters by multikinase inhibitors. *Drug Metabolism and Disposition 42*(11), 1851–1857.
Karlgren, M., Vildhede, A., Norinder, U., Wisniewski, J. R., Kimoto, E., Lai, Y., et al. (2012). Classification of inhibitors of hepatic organic anion transporting polypeptides (OATPs): Influence of protein expression on drug-drug interactions. *Journal of Medicinal Chemistry 55*(10), 4740–4763.
Karonen, T., Filppula, A., Laitila, J., Niemi, M., Neuvonen, P. J., & Backman, J. T. (2010). Gemfibrozil markedly increases the plasma concentrations of montelukast: A previously unrecognized role for CYP2C8 in the metabolism of montelukast. *Clinical Pharmacology and Therapeutics 88*(2), 223–230.
Kashihara, Y., Ieiri, I., Yoshikado, T., Maeda, K., Fukae, M., Kimura, M., et al. (2017). Small-dosing clinical study: pharmacokinetic, pharmacogenomic (SLCO2B1 and ABCG2), and interaction (Atorvastatin and Grapefruit juice) profiles of 5 probes for OATP2B1 and BCRP. *Journal of Pharmaceutical Sciences 106*(9), 2688–2694.
Kato, K., Shirasaka, Y., Kuraoka, E., Kikuchi, A., Iguchi, M., Suzuki, H., et al. (2010). Intestinal absorption mechanism of tebipenem pivoxil, a novel oral carbapenem: Involvement of human OATP family in apical membrane transport. *Molecular Pharmaceutics 7*(5), 1747–1756.
Keiser, M., Kaltheuner, L., Wildberg, C., Müller, J., Grube, M., Partecke, L. I., et al. (2017). The organic anion transporting peptide (OATP) 2B1 is localized in the basolateral membrane of the human jejunum and Caco-2 monolayers. *Journal of Pharmaceutical Sciences 106*, 1–7.
Kim, K. -A., Joo, H. -J., Lee, H. -M., & Park, J. -Y. (2013). SLCO2B1 genetic polymorphisms in a Korean population: Pyrosequencing analyses and comprehensive comparison with other populations. *Molecular Biology Reports 40*(7), 4211–4217.
Kim, K. -A., Lee, H. -M., Joo, H. -J., Park, I. -B., & Park, J. -Y. (2013). Effects of polymorphisms of the SLCO2B1 transporter gene on the pharmacokinetics of montelukast in humans. *Journal of Clinical Pharmacology 53*(11), 1186–1193.
Kim, T. -E., Ha, N., Kim, Y., Kim, H., Lee, J. W., Jeon, J. -Y., et al. (2017). Effect of epigallocatechin-3-gallate, major ingredient of green tea, on the pharmacokinetics of rosuvastatin in healthy volunteers. *Drug Design, Development and Therapy 11*, 1409–1416.
Kimoto, E., Li, R., Scialis, R. J., Lai, Y., & Varma, M. V. S. (2015). Hepatic disposition of gemfibrozil and its major metabolite gemfibrozil 1-O-β-glucuronide. *Molecular Pharmaceutics 12*(11), 3943–3952.
Kindla, J., Rau, T. T., Jung, R., Fasching, P. A., Strick, R., Stoehr, R., et al. (2011). Expression and localization of the uptake transporters OATP2B1, OATP3A1 and OATP5A1 in non-malignant and malignant breast tissue. *Cancer Biology & Therapy 11*(6), 584–591.
Kirby, B. J., & Unadkat, J. D. (2007). Grapefruit juice, a glass full of drug interactions? *Clinical Pharmacology & Therapeutics 81*(5), 631–633.
Klatt, S., Fromm, M. F., & König, J. (2013). The influence of oral antidiabetic drugs on cellular drug uptake mediated by hepatic OATP family members. *Basic and Clinical Pharmacology and Toxicology 112*(4), 244–250.
Kleberg, K., Jensen, G. M., Christensen, D. P., Lundh, M., Grunnet, L. G., Knuhtsen, S., et al. (2012). Transporter function and cyclic AMP turnover in normal colonic mucosa from patients with and without colorectal neoplasia. *BMC Gastroenterology 12*, 78.
Knauer, M. J., Girdwood, A. J., Kim, R. B., & Tirona, R. G. (2013). Transport function and transcriptional regulation of a liver-enriched human organic anion transporting polypeptide 2B1 transcriptional start site variant. *Molecular Pharmacology 83*(6), 1218–1228.
Knauer, M. J., Urquhart, B. L., Meyer Zu Schwabedissen, H. E., Schwarz, U. I., Lemke, C. J., Leake, B. F., et al. (2010). Human skeletal muscle drug transporters determine local exposure and toxicity of statins. *Circulation Research 106*(2), 297–306.
Kobayashi, D., Nozawa, T., Imai, K., Nezu, J., Tsuji, A., & Tamai, I. (2003). Involvement of human organic anion transporting polypeptide OATP-B (SLC21A9) in pH-dependent transport across intestinal apical membrane. *The Journal of Pharmacology and Experimental Therapeutics 306*(2), 703–708.
Köck, K., Koenen, A., Giese, B., Fraunholz, M., May, K., Siegmund, W., et al. (2010). Rapid modulation of the organic anion transporting polypeptide 2B1 (OATP2B1, SLCO2B1) function by protein kinase C-mediated internalization. *Journal of Biological Chemistry 285*(15), 11336–11347.
Köck, K., Xie, Y., Hawke, R. L., Oberlies, N. H., & Brouwer, K. L. R. (2013). Interaction of silymarin flavonolignans with organic anion-transporting polypeptides. *Drug Metabolism and Disposition 41*(5), 958–965.
Koenen, A., Köck, K., Keiser, M., Siegmund, W., Kroemer, H. K., & Grube, M. (2012). Steroid hormones specifically modify the activity of organic anion transporting polypeptides. *European Journal of Pharmaceutical Sciences 47*(4), 774–780.
Koenen, A., Kroemer, H. K., Grube, M., & Meyer Zu Schwabedissen, H. E. (2011). Current understanding of hepatic and intestinal OATP-mediated drug-drug interactions. *Expert Review of Clinical Pharmacology 4*(6), 729–742.
Kovacsics, D., Patik, I., & Özvegy-Laczka, C. (2016). The role of organic anion transporting polypeptides in drug absorption, distribution, excretion and drug-drug interactions. *Expert Opinion on Drug Metabolism & Toxicology 13*(4), 1–16.
Kullak-Ublick, G. A., Ismair, M. G., Stieger, B., Landmann, L., Huber, R., Pizzagalli, F., et al. (2001). Organic anion-transporting polypeptide B (OATP-B) and its functional comparison with three other OATPs of human liver. *Gastroenterology 120*(2), 525–533.
Le Vee, M., Noel, G., Jouan, E., Stieger, B., & Fardel, O. (2013). Polarized expression of drug transporters in differentiated human hepatoma HepaRG cells. *Toxicology in Vitro : An International Journal Published in Association with BIBRA 27*(6), 1979–1986.
Leuthold, S., Hagenbuch, B., Mohebbi, N., Wagner, C. A., Meier, P. J., & Stieger, B. (2008). Mechanisms of pH-gradient driven transport mediated by organic anion polypeptide transporters. *AJP: Cell Physiology 296*(3), C570–C582.
Liu, T., & Li, Q. (2014). Organic anion-transporting polypeptides: A novel approach for cancer therapy. *Journal of Drug Targeting 22*(1), 14–22.
Lu, W. -J., Huang, J. -D., & Lai, M. -L. (2006). The effects of ergoloid mesylates and ginkgo biloba on the pharmacokinetics of ticlopidine. *Journal of Clinical Pharmacology 46*(6), 628–634.
Luo, J., Imai, H., Ohyama, T., Hashimoto, S., Hasunuma, T., Inoue, Y., et al. (2016). The pharmacokinetic exposure to fexofenadine is volume-dependently reduced in healthy subjects following oral administration with apple juice. *Clinical and Translational Science 9*(4), 201–206.
Maeda, T., Hirayama, M., Higashi, R., Sato, M., & Tamai, I. (2006). Characterization of human OATP2B1 (SLCO2B1) gene promoter regulation. *Pharmaceutical Research 23*(3), 513–520.
Mandery, K., Bujok, K., Schmidt, I., Keiser, M., Siegmund, W., Balk, B., et al. (2010). Influence of the flavonoids apigenin, kaempferol, and quercetin on the function of organic anion transporting polypeptides 1A2 and 2B1. *Biochemical Pharmacology 80*(11), 1746–1753.
Mayati, A., Le Vee, M., Moreau, A., Jouan, E., Bucher, S., Stieger, B., et al. (2015). Protein kinase C-dependent regulation of human hepatic drug transporter expression. *Biochemical Pharmacology 98*(4), 703–717.
Meier-Abt, F., Mokrab, Y., & Mizuguchi, K. (2006). Organic anion transporting polypeptides of the OATP/SLCO superfamily: Identification of new members in nonmammalian species, comparative modeling and a potential transport mode. *Journal of Membrane Biology 208*(3), 213–227.
Mooij, M. G., de Koning, B. E. A., Lindenbergh-Kortleve, D. J., Simons-Oosterhuis, Y., van Groen, B. D., Tibboel, D., et al. (2016). Human intestinal PEPT1 transporter expression and localization in preterm and term infants. *Drug Metabolism and Disposition 44*(7), 1014–1019.
Mougey, E. B., Feng, H., Castro, M., Irvin, C. G., & Lima, J. J. (2009). Absorption of montelukast is transporter mediated: A common variant of OATP2B1 is associated with reduced plasma concentrations and poor response. *Pharmacogenetics and Genomics 19*(2), 129–138.
Mougey, E. B., Lang, J. E., Wen, X., & Lima, J. J. (2011). Effect of citrus juice and SLCO2B1 genotype on the pharmacokinetics of montelukast. *Journal of Clinical Pharmacology 51*(5), 751–760.
Nakanishi, T., & Tamai, I. (2012). Genetic polymorphisms of OATP transporters and their impact on intestinal absorption and hepatic disposition of drugs. *Drug Metabolism and Pharmacokinetics 27*(1), 106–121.
Namgoong, S., Cheong, H. S., Kim, J. O., Kim, L. H., Na, H. S., Koh, I. S., et al. (2015). Comparison of genetic variations of the SLCO1B1, SLCO1B3, and SLCO2B1 genes among five ethnic groups. *Environmental Toxicology and Pharmacology 40*(3), 692–697.
National Center for Biotechnology Information (2018). *N. L. of M. (n.d.). PubMed.*
Nguyen, M. A., Staubach, P., Wolffram, S., & Langguth, P. (2014). Effect of single-dose and short-term administration of quercetin on the pharmacokinetics of talinolol in humans - Implications for the evaluation of transporter-mediated flavonoid-drug interactions. *European Journal of Pharmaceutical Sciences: Official Journal of the European Federation for Pharmaceutical Sciences 61*, 54–60.
Nies, A. T., Niemi, M., Burk, O., Winter, S., Zanger, U. M., Stieger, B., et al. (2013). Genetics is a major determinant of expression of the human hepatic uptake transporter OATP1B1, but not of OATP1B3 and OATP2B1. *Genome Medicine 5*(1), 1.
Noé, J., Portmann, R., Brun, M. E., & Funk, C. (2007). Substrate-dependent drug-drug interactions between gemfibrozil, fluvastatin and other organic anion-transporting peptide (OATP) substrates on OATP1B1, OATP2B1, and OATP1B3. *Drug Metabolism and Disposition 35*(8), 1308–1314.
Nozawa, T., Imai, K., Nezu, J. -I., Tsuji, A., & Tamai, I. (2004). Functional characterization of pH-sensitive organic anion transporting polypeptide OATP-B in human. *The Journal of Pharmacology and Experimental Therapeutics 308*(2), 438–445.
Nozawa, T., Nakajima, M., Tamai, I., Noda, K., Nezu, J. -I., Sai, Y., et al. (2002). Genetic polymorphisms of human organic anion transporters OATP-C (SLC21A6) and OATP-B (SLC21A9): allele frequencies in the Japanese population and functional analysis. *The Journal of Pharmacology and Experimental Therapeutics 302*(2), 804–813.
Obaidat, A., Roth, M., & Hagenbuch, B. (2012). The expression and function of organic anion transporting polypeptides in normal tissues and in cancer. *Annual Review of Pharmacology and Toxicology 52*, 135–151.
O'Connor, L., Gilmour, J., & Bonifer, C. (2016). The role of the ubiquitously expressed transcription factor Sp1 in tissue-specific transcriptional regulation and in disease. *Yale Journal of Biology and Medicine 89*(4), 513–525.
Patilea-Vrana, G., & Unadkat, J. D. (2016). Transport *vs.* Metabolism: What Determines the Pharmacokinetics and Pharmacodynamics of Drugs? Insights From the Extended Clearance Model. *Clinical Pharmacology and Therapeutics 100*(5), 413–418.
Pizzagalli, F., Varga, Z., Huber, R. D., Folkers, G., Meier, P. J., & St-Pierre, M. V. (2003). Identification of steroid sulfate transport processes in the human mammary gland. *The Journal of Clinical Endocrinology & Metabolism 88*(8), 3902–3912.
Prasad, B., & Unadkat, J. D. (2015). The concept of fraction of drug transported (ft) with special emphasis on BBB efflux of CNS and antiretroviral drugs. *Clinical Pharmacology and Therapeutics 97*(4), 320–323.
Sai, Y., Kaneko, Y., Ito, S., Mitsuoka, K., Kato, Y., Tamai, I., et al. (2006). Predominant contribution of organic anion transporting polypeptide OATP-B (OATP2B1) to apical uptake of estrone-3-sulfate by human intestinal Caco-2 cells. *Drug Metabolism and Disposition 34*(8), 1423–1431.
Sakamoto, A., Matsumaru, T., Yamamura, N., Uchida, Y., Tachikawa, M., Ohtsuki, S., et al. (2013). Quantitative expression of human drug transporter proteins in lung tissues: Analysis of regional, gender, and interindividual differences by liquid

chromatography-tandem mass spectrometry. *Journal of Pharmaceutical Sciences 102*(9), 3395–3406.
Shen, H., Yang, Z., Mintier, G., Han, Y. -H., Chen, C., Balimane, P., et al. (2013). Cynomolgus monkey as a potential model to assess drug interactions involving hepatic organic anion transporting polypeptides: *in vitro*, *in vivo*, and *in vitro*-to-*in vivo* extrapolation. *The Journal of Pharmacology and Experimental Therapeutics 344*(3), 673–685.
Sherry, S. T., Ward, M. H., Kholodov, M., Baker, J., Phan, L., Smigielski, E. M., et al. (2001). dbSNP: the NCBI database of genetic variation. *Nucleic Acids Research 29*(1), 308–311.
Shirasaka, Y., Mori, T., Murata, Y., Nakanishi, T., & Tamai, I. (2014). Substrate- and dose-dependent drug interactions with grapefruit juice caused by multiple binding sites on OATP2B1. *Pharmaceutical Research 31*(8), 2035–2043.
Shirasaka, Y., Mori, T., Shichiri, M., Nakanishi, T., & Tamai, I. (2012). Functional pleiotropy of organic anion transporting polypeptide OATP2B1 due to multiple binding sites. *Drug Metabolism and Pharmacokinetics 27*(3), 360–364.
Shirasaka, Y., Shichiri, M., Mori, T., Nakanishi, T., & Tamai, I. (2013). Major active components in grapefruit, orange, and apple juices responsible for OATP2B1-mediated drug interactions. *Journal of Pharmaceutical Sciences 102*(1), 280–288.
St-Pierre, M. V., Hagenbuch, B., Ugele, B., Meier, P. J., & Stallmach, T. (2002). Characterization of an organic anion-transporting polypeptide (OATP-B) in human placenta. *Journal of Clinical Endocrinology and Metabolism 87*(4), 1856–1863.
Takeuchi, K., Sugiura, T., Umeda, S., Matsubara, K., Horikawa, M., Nakamichi, N., et al. (2011). Pharmacokinetics and hepatic uptake of eltrombopag, a novel platelet-increasing agent. *Drug Metabolism and Disposition 39*(6), 1088–1096.
Tamai, I. (2012). Oral drug delivery utilizing intestinal OATP transporters. *Advanced Drug Delivery Reviews 64*(6), 508–514.
Tamai, I., Nezu, J., Uchino, H., Sai, Y., Oku, A., Shimane, M., et al. (2000). Molecular identification and characterization of novel members of the human organic anion transporter (OATP) family. *Biochemical and Biophysical Research Communications 273*(1), 251–260.
Tanaka, S., Uchida, S., Miyakawa, S., Inui, N., Takeuchi, K., Watanabe, H., et al. (2013). Comparison of inhibitory duration of grapefruit juice on organic anion-transporting polypeptide and cytochrome P450 3A4. *Biological & Pharmaceutical Bulletin 36*(12), 1936–1941.
Tapaninen, T., Karonen, T., Backman, J. T., Neuvonen, P. J., & Niemi, M. (2013). SLCO2B1 c. 935G > A single nucleotide polymorphism has no effect on the pharmacokinetics of montelukast and aliskiren. *Pharmacogenetics and Genomics 23*(1), 19–24.
Tapaninen, T., Neuvonen, P. J., & Niemi, M. (2010). Grapefruit juice greatly reduces the plasma concentrations of the OATP2B1 and CYP3A4 substrate aliskiren. *Clinical Pharmacology and Therapeutics 88*(3), 339–342.
Tapaninen, T., Neuvonen, P. J., & Niemi, M. (2011). Orange and apple juice greatly reduce the plasma concentrations of the OATP2B1 substrate aliskiren. *British Journal of Clinical Pharmacology 71*(5), 718–726.
U.S. Food and Drug Administration (2014). Drug Approval Package: VIKIRA PAK (ombitasvir/paritaprevir/ritonavir/dasabuvir). *FDA application NDA 206619*.
Vaidyanathan, S., Camenisch, G., Schuetz, H., Reynolds, C., Yeh, C. -M., Bizot, M. -N., et al. (2008). Pharmacokinetics of the oral direct renin inhibitor aliskiren in combination with digoxin, atorvastatin, and ketoconazole in healthy subjects: the role of P-glycoprotein in the disposition of aliskiren. *The Journal of Clinical Pharmacology 48*(11), 1323–1338.
Varma, M. V., Kimoto, E., Scialis, R., Bi, Y., Lin, J., Eng, H., et al. (2017). Transporter-mediated hepatic uptake plays an important role in the pharmacokinetics and drug-drug interactions of Montelukast. *Clinical Pharmacology and Therapeutics 101*(3), 406–415.
Varma, M. V., Rotter, C. J., Chupka, J., Whalen, K. M., Duignan, D. B., Feng, B., et al. (2011). pH-sensitive interaction of HMG-CoA reductase inhibitors (statins) with organic anion transporting polypeptide 2B1. *Molecular Pharmaceutics 8*(4), 1303–1313.
Vildhede, A., Karlgren, M., Svedberg, E. K., Wisniewski, J. R., Lai, Y., Norén, A., et al. (2014). Hepatic uptake of atorvastatin: influence of variability in transporter expression on uptake clearance and drug-drug interactions. *Drug Metabolism and Disposition: The Biological Fate of Chemicals 42*(7), 1210–1218.
Visentin, M., Chang, M. -H., Romero, M. F., Zhao, R., & Goldman, I. D. (2012). Substrate- and pH-specific antifolate transport mediated by organic anion-transporting polypeptide 2B1 (OATP2B1-SLCO2B1). *Molecular Pharmacology 81*(2), 134–142.
Vizcaíno, C., Mansilla, S., & Portugal, J. (2015). Sp1 transcription factor: A long-standing target in cancer chemotherapy. *Pharmacology and Therapeutics 152*, 111–124.
Wang, L., Collins, C., Kelly, E. J., Chu, X., Ray, A. S., Salphati, L., et al. (2016). Transporter expression in liver tissue from subjects with alcoholic or hepatitis C cirrhosis quantified by targeted quantitative proteomics. *Drug Metabolism and Disposition 44*(11), 1752–1758.
Wang, L., Prasad, B., Salphati, L., Chu, X., Gupta, A., Hop, C. E. C. A., et al. (2015). Interspecies variability in expression of hepatobiliary transporters across human, dog, monkey, and rat as determined by quantitative proteomics. *Drug Metabolism and Disposition 43*(3), 367–374.
Wen, F., Shi, M., Bian, J., Zhang, H., & Gui, C. (2015). Identification of natural products as modulators of OATP2B1 using LC-MS/MS to quantify OATP-mediated uptake. *Pharmaceutical Biology 0209*(November), 1–10.
Yang, M., Xie, W., Mostaghel, E., Nakabayashi, M., Werner, L., Sun, T., et al. (2011). SLCO2B1 and SLCO1B3 may determine time to progression for patients receiving androgen deprivation therapy for prostate cancer. *Journal of Clinical Oncology 29*(18), 2565–2573.
Yu, J., Zhou, Z., Tay-Sontheimer, J., Levy, R., & Ragueneau-Majlessi, I. (2017). Intestinal drug interactions mediated by OATPs: A systematic review of preclinical and clinical findings. *Journal of Pharmaceutical Sciences*, 1–14.
Zheng, H. X., Huang, Y., Frassetto, L. A., & Benet, L. Z. (2009). Elucidating rifampin's inducing and inhibiting effects on glyburide pharmacokinetics and blood glucose in healthy volunteers: unmasking the differential effects of enzyme induction and transporter inhibition for a drug and its primary metabolite. *Clinical Pharmacology and Therapeutics 85*(1), 78–85.